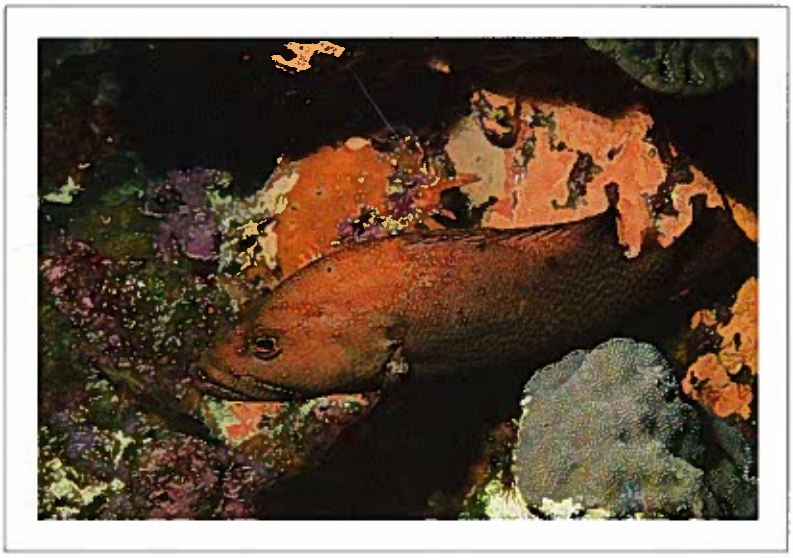
Adaptive Coloration in Invertebrates



Animal Behavior

Compiled by
Dr. Mary Wicksten
Department of Biology
Texas A&M University

TEXAS A&M SEA GRANT COLLEGE PROGRAM
TAMU-SG-90-106

Adaptive Coloration in Invertebrates

Proceedings of a Symposium
Sponsored by the American Society of Zoologists

Dr. Mary Wicksten
Compiler

TAMU-SG-90-106
June 1990

Publication of this document partially supported by Institutional Grant No. NA89AA-D-SG139 to the Texas A&M University Sea Grant College Program by the National Sea Grant Program, National Oceanic and Atmospheric Administration, Department of Commerce.

Table of Contents

Introduction

MARY K. WICKSTEN

Color patterns in animals draw the attention of a wide span of viewers, including biologists, aquarists, pet fanciers, photographers, artists and teachers. Color patterns have been demonstrated to serve important functions in predator-prey relationships, communication, and regulation of heat and incoming radiation. Textbooks on evolutionary biology, animal behavior and ecology frequently cite such classic studies on animal coloration as industrial melanism (Kettlewell, 1955), mimicry between the viceroy and monarch butterflies (Brower *et al.*, 1968), and color patterns and courtship in the stickleback fish (Tinbergen, 1951).

Much of the literature on adaptive coloration has been particularly concerned with predator-prey relationships, especially among vertebrates and the Lepidoptera in terrestrial habitats. Many studies have been descriptive, or conducted strictly from an ecological or behavioral point of view. The literature is scattered, often appearing in specialized journals and systematic works. Recent symposia concerning adaptive coloration either have been restricted to one particular form of coloration or include coloration as part of a larger topic, such as predator-prey interactions in general.

Adaptive coloration related to predator-prey relationships and between- or within-species communication has received much attention. Extensive works on the subject include those by Cott (1957), Wickler (1968) and Edmunds (1974). Other recent reviews include those by Robinson (1969) on defenses against visually hunting predators by terrestrial arthropods, Burtt and others (1979) on the relationship between color and behavior, Owen (1980) on camouflage and mimicry in general, Hadeler *et al.* (1982) and **American Naturalist**, supplement to volume 131 (1988), on mimicry and Wicksten (1983) on camouflage among marine invertebrates. Hailman (1977) reviewed optical signals in animals. Adaptive coloration is considered in the book by Bagnara and Hadley (1973) on chromatophores and color change. Burtt (1981) provided an overview of adaptive coloration, particularly in birds.

Department of Biology, Texas A&M University, College Station, Texas 77843

Coloration can be due to physiological pigments that serve in processes such as heat absorption, uptake of oxygen, digestion, mechanical support and wound repair. Rossotti (1985) discussed the physics and chemistry of color in general. The works by Fox (1976) and Needham (1974) provide good reviews of biological pigmentation. A short but useful discussion of coloration with color photographs is given in Chapter 31 of **Living Invertebrates** by Pearse *et al.* (1987).

Among the invertebrates, insects, particularly the Lepidoptera, have received the most study of adaptive coloration. Increasingly detailed work is showing specializations for avoidance of predators, heat absorption and courtship. The first four papers in this symposium provide experimental work on the functions and evolution of insect coloration, including a reconsideration of industrial melanism.

The many papers on insects and their coloration may at times overshadow studies on aquatic and marine organisms, yet evolutionary processes likely are similar among them. The reader may note similarities between adaptations for object mimicry among insects such as mantids and thorn bugs, which blend in with terrestrial plants, and the marine pontoniine shrimps, which resemble their invertebrate hosts. Zmarzly's review provides a basis for comparison in her treatment of the coral reef shrimp. Pigmentation involved in optical signaling and absorption of radiation can be compared in the butterflies studied by Kingsolver and Ziff and the freshwater crustaceans described by Lueke. Thurman's paper on aspects of color change in fiddler crabs allows comparison of pigmentation in an animal with chromatophores with that in arthropods that cannot change color so quickly.

Marine invertebrates that are not arthropods may show conspicuous coloration, but have received relatively little study. Studying such animals and observing predator-prey relationships in their natural habitat may be difficult to impossible due to the reclusive habits, scarcity or small size of many species, or difficulties of conducting such research while scuba diving. Grober's paper presents an unusual form of aposematic coloration in a little-studied group, the Ophiuroidea. Gosliner and Behrens review the possible functions of coloration of opisthobranch mollusks, and provide observa-

tions suggesting that tropical species may be involved in mimetic complexes with turbellarian flatworms.

In preparing the symposium, an effort was made to provide a variety of aspects of adaptive coloration in diverse taxa. Biologists tend to specialize according to taxa (butterflies, cephalopods, echinoderms, etc.) or disciplines (ecology, behavior, etc.) Interdisciplinary comparisons can be difficult, yet rewarding — the methods used by a terrestrial behavioral biologist may give insight to the opisthobranch systematist; the physiologist interested in chromatophores of arthropods might find those in cephalopods worthy of consideration.

The papers presented here provide summaries for review as well as stimulation for further research. Many invertebrates are small, relatively inexpensive to obtain and keep for study, and not subject to regulations regarding maintenance and experimentation. Studies on adaptive coloration among invertebrates could show important differences in protective mechanisms between and within taxa as well as between land, freshwater and marine habitats.

This symposium was sponsored by the Divisions of Animal Behavior and Invertebrate Zoology of the American Society of Zoologists, and held at the 1987 meeting in New Orleans. A paper on adaptive coloration in cephalopods, presented at the meeting by Roger Hanlon of The University of Texas Medical Branch, will be published elsewhere. Publication of the proceedings was supported by the Sea Grant Program of Texas A&M University, whose enthusiastic assistance is gratefully acknowledged.

References

Bagnara, J.T. and M.E. Hadley. 1973. **Chromatophores and color change.** Prentice-Hall, Englewood Cliffs, New Jersey.

Brower, L.P., W.N. Ryerson, L.L. Coppinger and S.C. Glasier. 1968. Ecological chemistry and the palatability spectrum. **Science** 161: 1349-1351.

Burtt, E.H. Jr. (ed.) 1979. **The behavioral significance of color.** Garland Publishing, New York.

Burtt, E.H. Jr. 1981. The adaptiveness of animal colors. **Bioscience** 31: 723-729.

Cott, H.B. 1957. **Adaptive coloration in animals.** Methuen, London.

Edmunds, M. 1974. **Defence in animals.** Longman, Inc., New York.

Fox, D.L. 1976. **Animal biochromes and structural colors.** Univ. Calif. Press, Berkeley.

Hadeler, K.P., P. deMottoni and A. Tesei. 1982. Mimetic gain in Batesian and Mullerian mimicry. **Oecologia** 53: 84-92.

Hailman, J.P. 1977. **Optical signals: animal communication and light.** Univ. Indiana Press, Bloomington.

Kettlewell, H.B.D., 1955. Selection experiments on industrial melanism in the Lepidoptera. **Heredity** 10: 287-301.

Needham, A.E. 1974. **The significance of zoochromes.** Springer-Verlag, New York.

Owen, D. 1980. **Camouflage and mimicry.** Univ. Chicago Press, Chicago.

Pearse, V., J. Pearse, M. Buchsbaum and R. Buchsbaum. 1987. **Living invertebrates.** Blackwell Sci. Publ., Palo Alto, Calif.

Robinson, M.H. 1969.Defenses against visually hunting predator. **Evol. Biol.** 3: 225-259.

Rossoti, H. 1985. **Colour.** Princeton Univ. Press, Princeton.

Tinbergen, N. 1951. **The study of instinct.** Oxford Univ. Press, London.

Wickler, W. 1968. **Mimicry in plants and animals.** McGraw Hill, New York.

Wicksten, M.K. 1983. Camouflage in marine invertebrates. **Ann. Rev. Oceanogr. Mar. Biol.** 21: 177-193.

The Evolution of Animal Coloration — Adaptive Aspects from Bioenergetics to Demography

WARD B. WATT

Synopsis

Adaptive color variation in animals was at the center of early conceptual advances in evolutionary biology, but is not so central now. I propose ways in which modern biochemical and genetic approaches, and clarity as to the nature of the process of adaptation, can facilitate the use of varying coloration systems as subjects for study of general evolutionary problems. In particular, I focus on the current gap between mechanistic and evolutionary biology, arguing that this must be bridged, and that animal color systems are fine model systems in which such bridging studies can be carried out.

Although color patterns may appear inessential for living function in the laboratory, inappropriate coloration in the wild may be as damaging to an organism's Darwinian fitness as a breakdown in its genetic or physiological machinery. This is illustrated by natural variation in degree of crypsis, hence survivorship, on the part of butterfly larvae, in which failure of crypsis is effectively lethal. Such effects of camouflage only scratch the surface of color effects on fitness, however. Color patterns may have major fitness impacts via effects on photochemical processes or physical energy balance, as well as on biological interactions such as courtship signaling or predator-prey interactions of various sorts. Further, these effects may be expressed through differences in fecundity, not just in survivorship, as is shown by considering the effects of thermoregulatory color differences on egg output by female butterflies.

Color pattern effects on fitness may be evaluated in context of formal evolutionary bioenergetics. Simple theoretical analysis shows that the costs and benefits of color variation take on exactly the same form of bioenergetic expression as do processes more obviously central to metabolism. "Qualitative" color requirements may be brought into this formal scheme by considering them as examples of threshold and saturation processes in the relation of fitness-related output to metabolic resource allocation. The diverse possible fitness consequences of apparently simple changes in bioenergetic resource allocation to pigment synthesis are illustrated by reviewing the "alba" wing color polymorphism of *Colias* butterflies.

Two questions related to problems of preadaptation and constraint in evolutionary processes are examined using insect color examples: Do some evolutionary choices "predispose" toward others?, and, can evolutionary studies do without mechanistic (e.g. molecular or developmental) analyses? I answer "yes" to the first, and "no" to the second, using the examples to suggest how mechanistic analysis can illuminate evolutionary problems, uniquely suggest possible explanations, and uniquely offer ways of approaching the rigorous testing of such explanations.

Introduction

Adaptive color patterns of animals were prominent in early thinking about evolution. Darwin used many examples involving color in *The Origin of Species* (1859). Poulton (1908) devoted much attention to mimicry, countershading and defensive coloration in general, as a vehicle for consideration of major evolutionary issues. Yet, except for the study of coevolving mimicry systems (begun by J. Van Zandt Brower, 1958a,b,c,1960; see L.P. Brower, 1988 for the current state of the art), the study of general evolutionary issues has, of late, been less concerned with color. Interest in the adaptiveness of particular color-pattern systems continues, e.g. in camouflage or courtship-display patterns of many creatures (e.g. Endler, 1986, Parkin, 1980). However, major advances or conceptual debates have tended to use other sorts of evidence, such as enzyme polymorphisms or nucleic-acid sequence variation, for illustration or for hypothesis testing (e.g. Lewontin, 1974, Nei, 1987).

This volume demonstrates a great diversity of

Department of Biological Sciences, Stanford University, Stanford, Calif., and Rocky Mountain Biological Laboratory, Crested Butte, Colo.

ingenuity and insight among specific contemporary studies of animal coloration. The time seems right, if not indeed past due, for some new general perspective on the evolution of animal coloration, for its own sake and as a field in which to focus sharply on major evolutionary issues. Moreover, there is at least one such central issue, deserving much attention from evolutionary biologists, for which many color-pattern systems may offer ideal sources of evidence, or vehicles for the empirical testing of general questions.

That issue is the pertinence of molecular and physiological mechanisms, and their study, to the formulating and testing of important questions about evolution. As Mayr (e.g. 1980) and others have documented, there is a long-standing, even deepening, rift between what Mayr has called the biology of "proximate cause" (molecular, cellular or developmental biology) and the biology of "ultimate cause" (behavioral, ecological, evolutionary biology). Molecular biologists may speculate on broad-scale evolutionary patterns, or systematic biologists may use molecular data as characters for their analyses, and so forth, but rarely do such specialists truly interact with one another in joint consideration of conceptual problems. Individuals' work spans this rift from time to time, but often meets with puzzlement or skepticism from biologists firmly placed on one side or the other.

I believe that this rift must now be bridged, and that color patterns may provide ideal model systems for study of the mechanistic bases of adaptations, as a prime means of spanning the rift. I will begin by clarifying the actual meaning of the adaptation concept itself, since there has been much confusion in this area. I will next consider the central importance of color adaptations — why those who think of color variation as "inessential" are wrong, and what is the full variety of adaptive roles that color phenotypes can play, emphasizing the underlying mechanisms of color differences as important aspects of their adaptive natures. I will then focus on bioenergetic aspects, studying the costs of mechanisms of color adaptation in both formal and empirical terms. My last concern will be to show how this mechanistic approach to color-variation systems is crucial to understanding even evolutionary problems that can be initially defined without reference to mechanism — in this case, the potential importance of evolutionary predispositions and constraints.

Adaptation and Its Consequences for Fitness — A Clarification

Adaptation is an almost geometric concept: The relation of suitedness between creatures' phenotypes and the environments in which they find themselves. Its recognition as such begins in Darwin's introduction to the *Origin* (1859), although, of course, he did not use terms such as "phenotype." Indeed, it is clear that Darwin not only regarded the origin and improvement of adaptation as a problem coequal with the origin and increase of species diversity, but believed these problems to be intertwined in important ways.

We often believe we can rank alternative states of adaptation in particular cases, but no general quantitative measure of adaptation exists as yet. As Endler (1986) summarizes, numerous authors have suggested that r, the "intrinsic rate of natural increase" or "Malthusian parameter" of Fisher (1958), be used as an index of adaptation. But, this idea has little to recommend it. Most obviously, adaptations to environmental conditions prevailing near an organism's limiting carrying-capacity density could not be expressed well, if at all, in such terms. Perhaps an even more fundamental flaw is that such usage would collapse together quite different stages of the evolutionary process. r is a demographic measure, and adaptations are not the same thing as, but are clearly antecedent to, their demographic consequences.

Adaptations express themselves as what I have termed *fitness indexes*: Alternative character states that are visible to natural selection by means of their consequences for one or more *fitness components* (Watt, 1985). These basic components are survivorship and fecundity, the 1_x and m_x schedules of demographic ecology (these can be subdivided further at need: e.g. juvenile survivorship, female fecundity, etc.). In turn, the stage-specific 1_xm_x products are summed or integrated, depending on population structure, to give the net reproductive rate R_0 of an individual, a genotype, etc.:

$$R_0 = \Sigma\, 1_x m_x \text{ or } \int 1_x m_x\, dx$$

R_0 values, the replacement rates of individuals or genotypes in populations, are now often termed "absolute fitnesses" (Endler, 1986). When genotypic R_0 values in a population are all normalized to some standard genotype in that population, the results are the *net* or *relative* fitnesses of mathematical population genetics.

There are at least two benefits from a clear understanding of this causal progression from one stage of the evolutionary process to another:

1. It shows the error of the old notion that evolutionary theory is circular. Even in the recent literature (e.g. Peters, 1976), we find this claim, based on the assertion that "we only know the fittest by the fact that they survive," so that by this argument defining evolution as the survival of the fittest is tautological. This is simply nonsense in light of the above. We recognize the fittest genotypes by their demographic success,

which *results from* but is *not the same as* the functional relationships between their phenotypes and the surrounding environment. Or, we can start by observing putative adaptations, then ask empirically whether or not they have fitness consequences. In fact, if we study an evolutionary situation empirically, as for example in sickle-cell anemia, that is how we proceed: we first identify functional differences among genetically determined phenotypes, in relation to environmental pressures, then inquire whether the interactions of these phenotypic differences with those environmental pressures do indeed result in changes in fitness components, leading to differences in net fitness. Not even the (trivial) fact that this process repeats generation after generation makes it circular; rather, in that aspect it is *recursive*.

2. It emphasizes that no phenotypic difference can be said to be adaptive unless a successive chain of its effects can be traced to change in one or more fitness components, or ultimately to change in net fitness. Gould and Lewontin (1979) quite properly cried out against the uncritical assertion of universal phenotypic adaptiveness, emphasizing the possibility that some characters might be the passive result of constraints on developmental processes, or of previous evolutionary history, or of other selectively neutral processes. If one wishes to avoid this trap, it is essential to probe putatively adaptive characters with genetic variation (or some equally subtle and controllable external stimulus). If genetic perturbation of a character does not produce a fitness component difference, that character state is not specifically adaptive, at least in the environment in question and within the precision of the study and the magnitude of the perturbation imposed.

Color's Range of Adaptive Phenotypic Impacts

Fundamental adaptive importance of color variation

It will seem obvious to field biologists that animal or plant color patterns should be of deep and diverse importance to their bearers. However, experimental biologists commonly think of pigments as "non-essential" metabolites. Thus, it is important to emphasize the central adaptive importance of color before going any further.

It is often true that "null" mutants abolishing pigmentation (with the exception of such "targets" as respiratory or photosystem pigments) are not lethal, or even particularly harmful to organisms in lab culture. But, it is easily forgotten that lab culture is a protected environment, in which many forms of natural selection are relaxed. One genotype (and associated phenotype) may in the laboratory be equivalent to another in survivorship or fecundity, and yet in the field one of those genotypes may suffer lethality, or some other decisive degree of fitness difference.

Consider the "blue-green" variant of *Colias* butterfly larvae (Gerould, 1921, 1926; see Plate 1:1 for illustration of blue-green and normal larvae). Gerould, who isolated this autosomal Mendelian recessive from wild populations, found that sparrows, searching larval food plants, would find and consume all blue-green larvae, while failing to find normal-green sib larvae present in equal numbers on the same plants. We found later that the normal yellow hemolymph pigment, lutein bound to a carrier protein, is absent from the variant larvae, leaving only blue mesobiliverdin to color their hemolymph; this is the basis of their breakdown in camouflage (Hoffmann and Watt, 1974). In the presence of predators with normal color vision, this allele is as lethal when homozygous as any classical early-embryonic developmental lethal. Thus there is very powerful viability selection in the wild against the blue-green allele, even though in lab culture such larvae have normal viability. In fact, it is an open question what maintains this allele at high enough frequencies that Gerould could recover it from New England populations, and we could recover it independently *twice* from Colorado stocks!

Thus the laboratory-based classification of some phenotypic characters as "non-essential" is highly misleading in the evolutionary context. Proper physical energy balance, appropriate behavioral interactions, etc., are just as crucial to Darwinian fitness in the wild as effective protein synthesis, lipid metabolism, or gas exchange.

Diversity of color's fitness index/fitness component effects

We next survey quickly some of the numerous ways in which animal pigmentation can impact net fitness, through varying functional roles with diverse effects on fitness indexes and components related to many niche axes (Hutchinson, 1957, 1978). Note particularly that we are not treating (anywhere in the paper) *structural* color, produced by diffraction or interference effects at animal surfaces. This subject merits its own treatment at another time and place.

As *photochemical energy transducers*, pigments' absorption in specific wavebands is obviously the critical process in animal vision and timekeeping alike (and of course photosynthesis, in animals' algal symbionts as well as in free-living plants!). Likewise, although it is important to fewer animals, light *emission* as fluorescent bioluminescence oc-

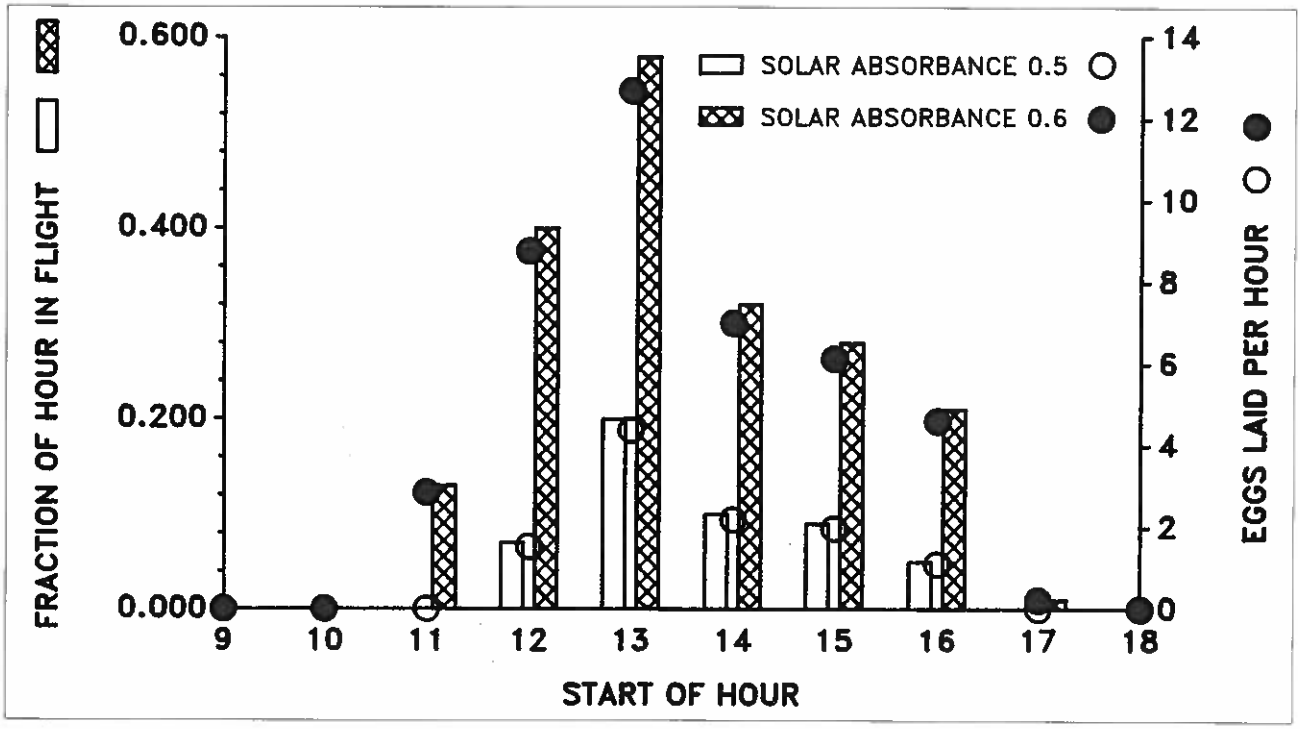


Figure 1. The impact of color on female *Colias* butterflies' fitness, through effects on body temperature. Output from a computer model revised from that of Kingsolver and Watt (1984), which computes the thermal balance, hence body temperature, of a *Colias*. It then predicts the resulting fraction of time in flight (bar graphs) and, using an observed (Kingsolver 1983b) value of eggs laid per time in flight, predicts the net resulting oviposition. For these simulations, all physical characteristics of the females were set identical except for their color, or absorbance for solar energy, as noted on the graph. Absorbance values of *Colias* wings can, physically, range from 0.4 to 0.7 (Kingsolver 1983a, Watt 1968). Meteorological data through the day are for the base of Crested Butte Mountain, Gunnison County, Colorado, on June 27, 1980, as reported by Kingsolver and Watt (1984). The total eggs laid through this day by the lighter-colored (absorbance 0.5) female would be 11.3, and the total laid by the darker (absorbance 0.6) female would be 42.3.

curs through emitting pigments. In both situations, the pigment that actually transduces light energy to or from chemical energy is typically formed by bonding a low-molecular-weight *chromophore* to a large *conjugate protein*. Whether in light absorption or in light emission, as well, both coarse and precise adjustments of photochemical pigment waveband are usually achieved not by change in the primary chromophore's structure, but by modification of the structure of the conjugate protein, hence the way this protein binds the primary chromophore and alters its resonance energy levels. This is true, for example, in mammalian vision (e.g. Nathans, *et al.* 1986, Piantanida, 1988), wherein one retinene derivative as chromophore is bound to different opsins to form the three primary-color rhodopsin complexes. Likewise, in insect bioluminescence (McElroy, *et al.* 1965), there is one luciferin, bound to a variety of luciferases, which thereby produces a variety of bioluminescent colors.

This common "strategy" of standard chromophore and variable conjugate, in energy-transducing pigment systems, arises from a number of sources:

1) all molecules of a photochemical pigment must have the same absorption/emission characteristics for optimal photochemical performance, and the waveband placement and breadth that are optimal may not be accessible by mutational varying of primary chromophore structure itself. However, both precision and broad range of such adjustments are typically achievable by change in the chromophore molecule's pi-electron orbital interactions with its conjugate protein, through mutational altering of that protein's structure;
2) photochemical pigments must show precise orientation to other compound(s) for energy transfer or related purposes, and this critical positioning is governed by the conjugate protein.

Pigment systems whose function is to absorb or reflect energy without photochemical consequences are typically constructed in a very different fashion. They are usually deposited *en masse*, without binding to any conjugate material, and are typically amorphous in microscopic organization. This means that to vary the color reflected, different chemical structures with distinct absorption spectra must be

produced. Often, to change the reflected wavelength distribution, a mixed deposit of such pigments is varied in composition. This is true, for example, of the pteridine wing pigments in pierid butterflies (Watt, 1964, Watt and Bowden, 1966).

Non-photochemical color patterns are most often thought of in terms of biological interactions — camouflage, signaling, and the like. However, reflected color patterns can be crucially important in physical as well as biotic contexts. As *thermoregulatory devices*, pigments may alter their carriers' thermal balance by absorbing or by reflecting energy. Absorption for heating is usually achieved with melanin or some other broad-spectrum-absorbing material (e.g. in *Colias* butterflies, Watt, 1968). Reflection, whether to shed excess energy by low absorption in warm climates (e.g. Watt, 1968), or to focus energy down insect wings onto the body in "solar furnace" fashion (Kingsolver, 1985a,b), is done with white or other pale, poorly-absorbing materials — pteridines, purines, etc.

Pigmentation's effects on the survivorship, 1_x, component of fitness may follow from photochemical benefits: the quality of vision, for example, has diverse effects on survivorship. Alternatively, color effects on physical energy balance may be important: *Colias* butterflies must fly to feed, to escape predators, or to escape bad weather by finding shelter, and since all these are critical to survivorship and flight depends on effective thermoregulation, evolutionary adjustment of thermoregulatory coloration affects survivorship (Watt, 1968). As for specifically biological color effects, we saw above a striking case of camouflage coloration changing survivorship, and many other such cases are known (e.g. Endler, 1984, 1986). Batesian and Mullerian mimicry obviously represent major ecologically mediated influences of color patterns on survivorship (e.g. J. Van Zandt Brower, 1958a,b,c, 1960; L. P. Brower, 1984, 1988; Poulton, 1908; Turner, 1987).

Moreover, net fitness may change as much or more through fecundity differences as through viability differences (e.g. Watt, *et al.* 1985, 1986; Feldman, *et al.* 1983, Liberman and Feldman, 1985; Carson, 1987), and fecundity may be drastically affected by changes of color, whether the ecological context in which the color difference acts is biophysical, behavioral, or some other aspect of niche structure. The intense pigmentation found in the testis sheaths of many small insects may protect germ-line cells or gametes against ultraviolet radiation damage, thus preserving fertility. Color differences, by drastically altering an animal's thermal balance and hence its body temperature (e.g. Watt, 1968, Kingsolver, 1983a,b), can have massive impacts on any temperature-dependent process. Overall gametogenesis rate, and many other aspects of reproductive physiology, certainly are such processes. Moreover, the ability of insects to fly, which depends crucially on their body temperature, is often essential to their mating success (Watt, *et al.* 1985, 1986) or their oviposition success (Kingsolver, 1983; Watt, Jacobs, and Donohue, unpubl.). If a female butterfly's absorptivity for sunlight is unsuited to its thermal environment, it may be much reduced in flight capacity, and thus in ability to lay eggs (Watt, 1968, Roland, 1982, Kingsolver, 1983a,b). This is illustrated in Figure 1, as its caption explains in detail: a relatively modest change in absorptivity for sunlight can change the net egg output of a female by several-fold.

Alternatively, in the behavioral context, if an insect displays an ineffective mating recognition pattern, it may have very poor mating success even though it is active in the right environment to find mates. Coloration as a visual courtship signal, whether in the ultraviolet (Silbergleid and Taylor, 1978) or the visible (Graham, *et al.* 1980), may have a massive effect on courtship effectiveness by male *Colias*, or male recognition of female *Colias* as potential mates (e.g. the *Colias* "alba" variant, see below and Graham, *et al.* 1980), respectively. Analogous effects have been analyzed in detail for the pierid genus *Pontia* by Rutowski (1981, 1984).

Clearly a diversity of mechanisms can be used to produce color differences, and these may have very diverse fitness-related effects. We need next to understand how cost-benefit analysis of such adaptive options can be carried out in an evolutionary context.

Color in Adaptive Bioenergetics

The formal bioenergetics of pigmentation

Pigments are made, deposited, or patterned only with some cost, large or small, to the organism, both in general energy currency and in specific elemental or functional-group constituents of the pigments in question. Some photochemical pigments, such as biological-clock photoreceptors, are effective in minute quantity, so that their cost may be negligible compared to their adaptive benefits. A pigment's color may not even be relevant to the adaptive function that determines the amount of the pigment made, and hence its cost. The observable color may be a "spandrel" — a phenotypic character that arises incidentally to another function, rather than having adaptive importance in its own right (Gould and Lewontin, 1979). For example, the deep colors of hemoglobin or myoglobin are incidental to their porphyrin rings' chelation of Fe^{++}, hence ability to bind and transport oxygen. On the other hand, the color of a slightly different porphyrin ring, chelating Mg^{++}, is critical to the photosynthetic function of chlorophyll (Figure 2). In either case, enough

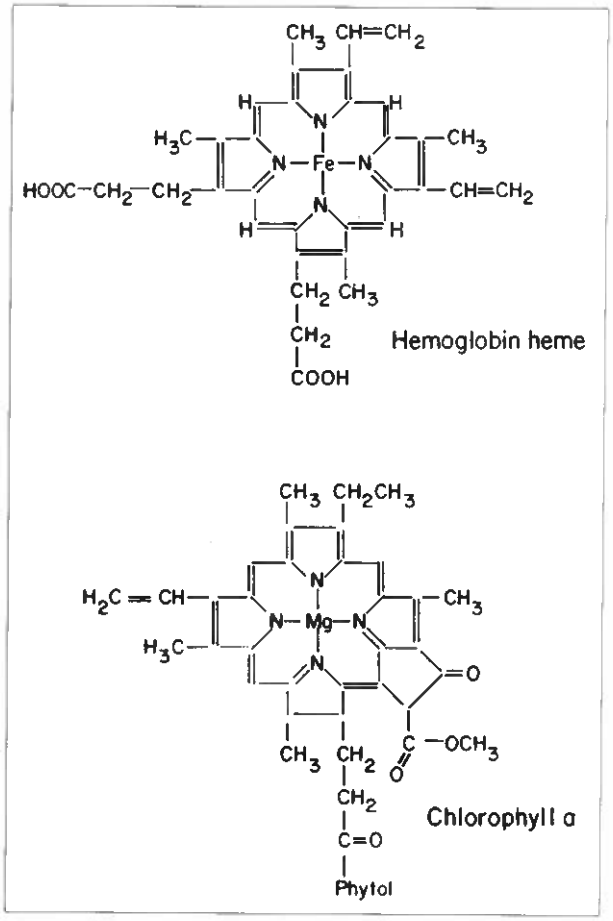


Figure 2. Color as adaptation and as happenstance. The colors of similar chemical structures, in this case two versions of the porphyrin tetrapyrrole ring, may be directly adaptive (chlorophyll a) or entirely incidental to the function of the molecule (hemoglobin heme).

pigment must be made to fulfill its functional role, and thus local solution concentrations will often fall in the micromolar to millimolar range, or local deposits will be on the order of micromoles per cm^2. In such cases, pigments may comprise up to several percent of their carriers' entire budget of energy or of some crucial element (e.g. Watt 1973). Thus, whatever their positive fitness consequences, pigments will often have major bioenergetic costs.

Recently, I have begun to analyze the evolutionary dynamics of metabolic energy allocation to fitness-related uses within organisms (Watt 1986; consult this also for others' approaches to the problem). Since the formal language of this analysis can be used to study the costs and benefits of pigmentation, I summarize it briefly here. Let the total of some energy currency (e.g. ATP) available at a particular time be M; let the fraction of that energy allocated to the "ith" fitness-related use be an "allocation coefficient", α_i; let the cost of a single offspring, within that fitness-related use of energy, be c_i; let whatever energy is allocated to that use, but wasted in practice, be W_i; and, let the total number of offspring ultimately produced be P. We then write in energy terms, for a single allocation:

$$P \cdot c_i = \alpha_i M - W_i \qquad (1)$$

Rearranging to emphasize the dependence of fitness, as indexed by offspring number, on allocation, and omitting waste hereafter to simplify the illustration (it is analyzed more thoroughly in Watt 1986), we have:

$$P = \frac{\alpha_i M}{c_i} \qquad (2)$$

Using such algebraic language, an allocation to pigment synthesis, or even uptake of pigments from the diet, and pigment deposition in relevant parts of the body takes the same form as any other fitness-related expenditure of metabolic currency.

Many classifications of α_is to fitness-related tasks are possible. Often an α_i assigned by a biologist might actually correspond to a single biological process: for example, the total input of metabolic currency to mature, functioning gonads would represent a *biologically* meaningful toward gametogenesis. On the other hand, some α_is may be intellectually useful but biologically artificial (as many previous energy-budget constructs have also been). For example, a summation of all α_i contributing to the survivorship, 1_x, component of fitness may be instructive to the biologist, but there is no unitary expenditure category in the organism's actual commitment of metabolic currency that corresponds to this. Sometimes reciprocal alterations of α_i values are separable in analysis (at least to a first approximation) between 1_x and m_x consequences, as in the work of Boggs on pupal resource allocation to adult somatic structure vs. reproductive effort in heliconiine butterflies (1981). In other circumstances, a particular commitment of currency resources may have intertwined effects on diverse fitness components.

Sometimes, an allocation to pigment production might be truly equivalent, in fitness-related outcome, to an allocation to a different biosynthetic or developmental process; but often, such allocations are uniquely effective. For example, investments of energy in the balance of lighter vs. darker sunlight-absorbing pigmentation, and in the thickness of insulating thoracic "fur," are both critical to the thermal balance of *Colias* butterflies in many grassland ecologies (Watt, 1968; Kingsolver, 1983a,b). However, these two investments of energy are not equivalent — their phenotypic states can compensate for one another's thermal effects to only a limited degree — because solar radiation load and convective cooling from wind do not have the same patterns of temporal variation in the wild (Kingsolver and Watt, 1983, 1984).

A major problem, which study of pigment sys-

tems share with study of other adaptive subsystems such as trace element metabolism, etc., is: how can a quantitative analysis of fitness-related energy (or other resource) allocation deal with apparently "qualitative" requirements — e.g. that a larva just "be green enough" to be camouflaged against visual predators? No matter how "qualitative" an allocation requirement may appear, it must be satisfied by chemical processes if it occurs in a live organism, so that it must be quantifiable by the counting up of chemical molecules used. Putting the problem in formal bioenergetic terms, this is the general issue of the geometry of the response of fitness, or one of its components, to changes in the values of α_i (M and c_i being held constant).

The simplest relationship possible between allocation values and fitness responses is the linear proportionality represented by equation 2 above — any non-zero allocation to a certain purpose returns some positive fitness-related consequence, and fitness gains increase linearly with added allocation "investment" (plot: Figure 3a). Such a simple pattern might occur in nature only rarely. For most fitness-related allocations, some minimum investment must be made before any fitness return is realized. Some "prerequisite-fixed-cost" allocation must be made beforehand — for example, there is the cost of building a gonad in which to make gametes, before any allocation of energy to gamete production can be effective. This can be described by building a threshold t_i into the allocation equation (plot: Figure 3b):

$$P = \frac{\alpha_i M - t_i}{c_i} \qquad (3)$$

Now, when we speak of a "qualitative" requirement, we mean in practice one for which the fitness-related return on resource investment, above whatever fixed-cost threshold t_i may exist, quickly approaches some maximum value, or "saturates." Let this maximum value $a = P_{max} \cdot c_i$, where P_{max} is the maximum number of offspring as set by other considerations, and let b equal the amount of energy investment (above the threshold t_i) which results in successful return of energy as offspring equal to half of a. (B might represent, for example, the expenditure on camouflage, above threshold, necessary to deceive a predator 50 percent of the time.) Increasing α_i farther and farther beyond b will then yield progressively diminishing returns in fitness, as a is approached asymptotically. (Eventually, such disproportionately large α_i values will contribute further to waste, although as above I omit this analysis here for simplicity.) We then have (plot: Figure 3c):

$$P = \frac{1}{c_i} \cdot \frac{a(\alpha_i M - t_i)}{b + (\alpha_i M - t_i)} \qquad (4)$$

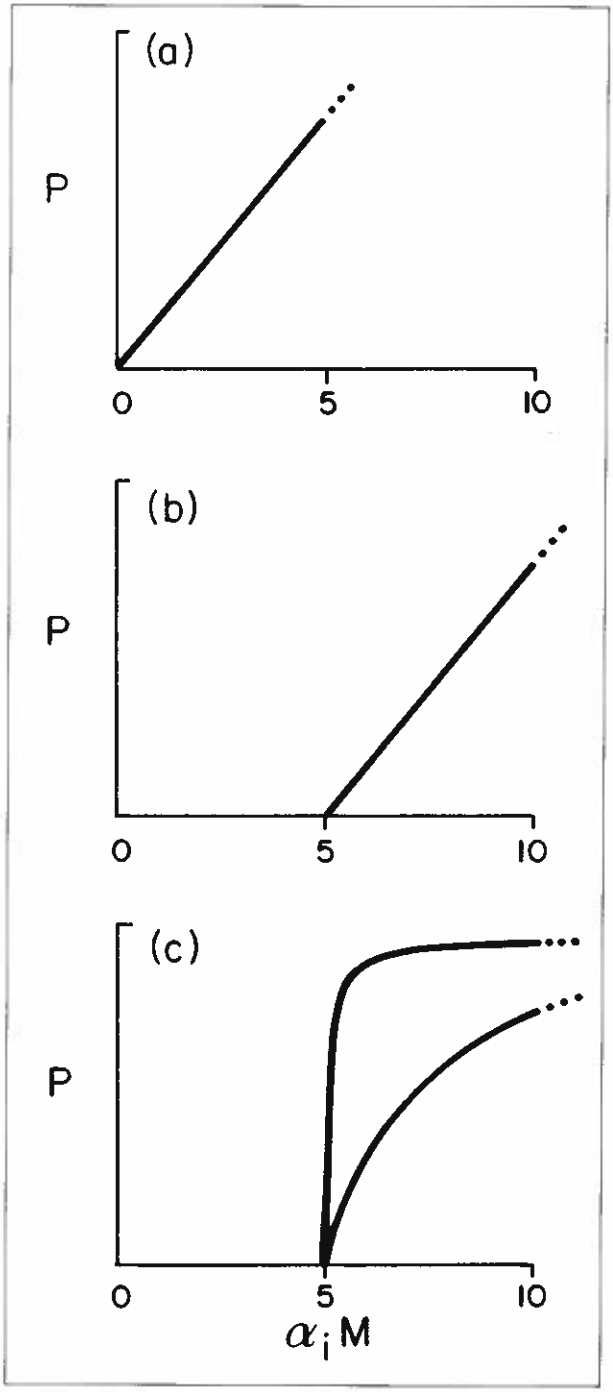


Figure 3. "Quantitative" vs. "qualitative" requirements for energy expenditure in relation to fitness. Three different possible patterns of the geometry of fitness response, as number of progeny P, to an organism's allocation of metabolic currency, $\alpha_i M$, to the ith category of fitness-related expenditure. a) linear relation of fitness to allocation; b) linear relation of fitness to allocation above a fixed threshold value of $\alpha_i M$ (=5.0); c) presence of moderate (lower curve) and steep (upper curve) saturation effects in the response curve, above the same threshold value as in b), illustrating quantitative chemical approximation to a "qualitative" requirement for a color pattern.

A "qualitative requirement," such as the adaptive need for a particular color pattern, thus becomes simply a limiting-case example of threshold and saturation effects that, in one way or another, may apply to most aspects of organisms' phenotypes in the real world. Whether the threshold and saturation occur in the fulfilling of some metabolic requirement, in the deception of a predator's visual system, or in the satisfaction of a behavioral release mechanism in a potential mate, the formal relationships and *kinds* of mechanisms to implement them will be essentially similar, as outlined here.

A single allocation shift with diverse, opposed effects on m_x

A particularly instructive example of such allocation effects is that of the "alba" polymorphism in *Colias* butterflies (Remington, 1954). In this case, a single alteration of allocation balance in a pigment pathway results in opposed consequences in different aspects of subsequent fecundity as a fitness component (Graham, *et al.* 1980). Transmitted autosomally by both sexes, the A allele is dominant in females, producing white wing color instead of the recessive (aa) yellow or orange (Plate 1:2), but is never expressed at all by male carriers. As Hovanitz (1944, 1950) showed, most North American *Colias* species display this polymorphism, and in these, the A allele is most common in the coldest parts of each species' range, although there may be little congruence in p_A among different sympatric species.

After ruling out direct effects on adult thermal balance as a cause of this variant's parallel biogeographic variation among species, I (Watt, 1973) confirmed and extended an earlier report of Descimon (1966) that "alba" lessens the quantity of colored pteridine pigments put into the wings, without major increase in the colorless ones. In fact, in the "alba" phenotype, roughly 3/4 micromole of guanosine triphosphate, the common precursor of all the pteridines, is diverted away from pupal wing pigment synthesis — this represents not only much diphosphoester bond energy, but also several percent of the pupa's total nitrogen budget. Further, data of Taylor (1970, 1972) suggested that male *Colias* might discriminate against "alba" females in courtship; if so, this could balance the polymorphism. Later, we (Graham, Watt and Gall, 1980) showed that the reallocated energy and nitrogen confer significant advantages on "alba," *at low temperatures,* in pupal developmental rate, gamete maturation rate, and holdover of fatbody storage reserves from the pupa for use by the adult. We also found that, indeed, male *Colias* do discriminate against "alba" females in the early, visual phase of courtship: in *C. alexandra*, which has a p_A of 5 to 8 percent, males discriminate against the "alba" phenotype by 5:1, but in the sympatric *C. scudderi*, with p_A ~85 to 90 percent, by only about 10:7.

At first glance, it appears maladaptive for males to discriminate against a female variant wthat allocates more of its resources to reproduction! However, in testing a quite different hypothesis about fitness effects of "alba," we have been able to clarify this situation as well. It had been suggested that "alba" might possibly be a mimic of putatively distasteful white butterflies, notably the genera *Pieris* and *Pontia*. We first tested this by studying the palatabilities to birds, *in the wild*, of these genera, together with all sexes and forms of *Colias*, as well as other butterflies from the same Colorado ecosystems. We found no significant differences in palatability among *Colias*, *Pontia*, and *Pieris*, and no evidence that any of these butterflies are "distasteful" (Ley and Watt, 1989). Moreover, we also found that *Colias* "alba" were most common where *Pieris* or *Pontia* were rarest — hardly the behavior expected from a mimic-model system (Watt, Kremen and Carter, 1989)! We suggest, in fact, that *Pieris* or *Pontia* may, via their visual resemblance to "alba" at a distance, confuse and distract male *Colias* seeking mates, and thus may be a proximate stimulus for the evolution of male *Colias'* discrimination against "alba." Based on initial observations, *Pieris* and *Pontia* may also impede "alba" female reproduction directly, by inappropriate "harassing" courtship of "alba" *Colias* as if these were pierine females, thus interrupting their oviposition.

While further testing of these ideas is needed and planned, as of now it appears that the balance of the "alba" polymorphism is due to tradeoff of physiological m_x advantage to this female phenotype, particularly at low temperature, against behavioral m_x disadvantage due to male discrimination. The situation may be even further complicated by density-dependent changes in the effects of male discrimination against "alba" (Gilchrist and Rutowski, 1986)! Thus, the effects of change in a single allocation coefficient, α_i, to a pigment system may act at many levels of organization, and stages of the life cycle, ultimately to affect one major fitness component, fecundity, in ways that are sufficiently different to maintain a color polymorphism.

Pre-adaptation and Constraint Versus "Optimal Adaptation" in Pigment Systems

The view held by many today, that evolution or evolutionary ecology is best pursued in terms of the "optimal strategies" of organisms, owes much to R. A. Fisher (1958). His advocacy of the power of natural selection to shape organisms' phenotypes, even to the claimed level of the selective modification of allelic dominance itself, has influenced many evolutionary biologists and ecologists (e.g. MacArthur,

1963, Pianka, 1976, Pyke *et al.*, 1977, Stearns, 1976; for a remarkable modern exegesis of Fisher's views and theoretical arguments, see Leigh, 1986, 1987).

An alternative view, that present-day phenotypes may reflect historically derived positive predispositions or negative constraints, as often as specific adaptations due to selection, has found favor from time to time, and is perhaps in the ascendancy now. The positive preadaptation of certain lineages to the subsequent evolution of particular character states was ably argued from palaeontological evidence by Bock (1959). His discussion complements the emphasis of Gould and Lewontin (1979) on the importance of overall animal body plans, evolved in "stem lineage" groups, in constraining evolutionary options among those groups' descendants.

Constraint on, or predisposition of, the course of adaptation may even arise from other selection pressures themselves. Adaptation to maximize body temperature-dependent daily flight time of *Colias* butterflies would maximize solar energy absorption and minimize convective heat loss (Watt, 1968, Kingsolver, 1983a,b). Kingsolver and Watt (1983, 1984) point out that this process is constrained in arctic or alpine habitats by the variance in environmental thermal variables such as wind speed: if these insects were optimally adapted to maximize flight time under average conditions, they would overheat to the point of self-cookery under uncommon but predictably recurrent periods of low air speed, hence low convective heat loss. Also, we have seen above the discrimination by *Colias* males against the "alba" female morph, apparently due to interference competition by pierine relatives, in spite of the fact that alba's greater resource commitment to reproduction would make it "optimal" for males to mate with this morph *preferentially*.

Animal color patterns, then, may provide fine model systems for studying these issues of adaptation, predisposition, and constraint — if the approach used explicitly incorporates the study of mechanism as an integral part of understanding evolutionary processes. I will now show how this necessity arises, with two further examples from pigment patterns in butterflies.

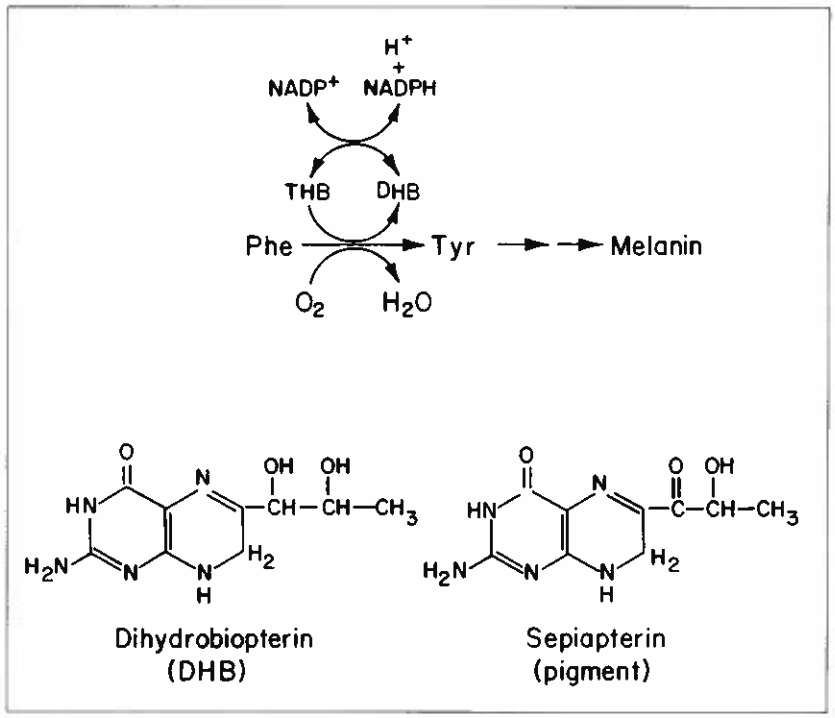


Figure 4. Possible preadaptation by the use of one pigment class to the use of another. Conversion of phenylalanine to tyrosine, the first step in melanogenesis, with the use of reduced biopterin derivatives (DHB, dihydrobiopterin; THB, tetrahydrobiopterin) which are closely similar in structure to the pigment sepiapterin, found (like other pteridines used as pigment in diverse animal groups but always in close association with the use of melanin. Reduced forms of biopterin are also used as cosubstrate in the second step of melanogenesis, the oxidation of tyrosine to dihydroxphenylalanine by tyrosine hydroxylase.

Mechanism and predisposition in the evolution of pteridine pigments

Some time ago, I observed that wherever in the animal kingdom pteridines are used as pigments (insects, fish, amphibia; Watt, 1964, Hama and Fukuda, 1964), they co-occur with melanin pigments, specifically eumelanin (Watt, 1967). It was known then that the first step in melanogenesis , the production of tyrosine from phenylalanine hydroxylase, uses the pteridine dihydrobiopterin as cosubstrate (Kaufman, 1963); it has since been found that tyrosine hydroxylase, the next enzyme on the pathway to eumelanin, also uses this compound as cosubstrate (e.g. Kaufman, 1979). Moreover, dihydrobiopterin is very close in structure to many pteridines used as pigment in diverse animals — indeed, only two hydrogens removed from one of them, sepiapterin (Watt, 1967; Figure 4). It seems likely that the use of eumelanin pigment predisposes to the evolution of pteridine pigmentation, via initial mutations for the overproduction of the phenylalanine and tyrosine hydroxylase pteridine cosubstrate. Further evolutionary modification of enzymic reaction sequences within and derived from the basic cosubstrate biosynthesis could then follow.

With the advent of modern recombinant and nucleic-acid sequencing technology, it may become possible to test these ideas further, by using a molecular-phylogenetic approach to study the DNA sequences now controlling the expression of pteridine metabolism. All members of the family Pieridae use pteridines as major wing pigments, but their close relatives the Papilionidae only use pteridines as pre-melanin cosubstrates (which pierids also do, Figure 4). One may thus, by using the Papilionidae as a closely related "outgroup," be able to construct a molecular phylogeny of the control sequences that have changed in the course of the evolutionary rise and refinement of pteridine pigmentation in Pieridae. It may thereby be possible to decide which changes in pteridine expression are the earliest, and thus to test explicitly the hypothesis of melanin pigment use as a preadaptation to pteridine pigment use.

Implied constraint on the evolution of mimicry, or why evolutionary studies cannot do without mechanistic analysis

Robert MacArthur (1963) used the example of color-pattern resemblance (due to Batesian mimicry) between the Viceroy butterfly (*Limenitis archippus* Cramer) and the Monarch butterfly (*Danaus plexippus* Linne') to argue for the primacy of strategic considerations over mechanistic details in evolutionary biology. The cell biologist, he said, explains the Viceroy's resemblance to its distasteful model (Plate 1:3) in terms of pigment structure and metabolic pathways, while the population biologist explains it in terms of escape from predation pressure by the evolution of resemblance to the model Monarch. Moreover, as he put it, "...the Viceroy does not care what orange pigment it contains...so long as it continues to resemble the monarch..." Now, this sort of argument has been common in evolutionary biology for decades. As noted earlier, Mayr (e.g. 1980) has documented it as the separation between "the biology of proximate cause" and "the biology of ultimate cause." Here, I want to stand the argument on its head, and suggest that, if this classical Batesian-mimetic system is considered in the right way, it argues strongly for the importance of mechanism in evolutionary studies, and for the necessity of bridging the gap between studies of "proximate" and "ultimate"causes in biology.

MacArthur's question was "why does the Viceroy resemble the Monarch?" But, perhaps an even more interesting approach arises from considering the monarch's open-meadow habitats in eastern and midwestern North America; these contain numerous other butterfly genera, at least as much "at home" there as *Limenitis*, which is mainly a forest-, brush-, or willow-swale-edge genus. Yet the Viceroy is the *only* mimic of the Monarch — why?

Numerous cases of Batesian mimicry systems are known in which one model is resembled by several mimics. It cannot be that the temperate-zone habitats of the Monarch are incapable of supporting as many mimics as might, for example, occur in the tropics — because the distasteful *Battus philenor* Linnè (Plate 1:4), occurring in most of the eastern north American range of the Monarch, is mimicked there by three other papilionids, and probably two nymphalids as well (J. Van Zandt Brower, 1958b).

Could it be that the ancestors of the Viceroy were, in some sense, predisposed to evolve Batesian mimicry of the Monarch? This suggestion gains credence when we realize that a second *Limenitis, L. astyanax* Fabricius, is one of the probable nymphalid mimics of a different distasteful model, *Battus philenor*, exhibiting a quite different departure form the "ancestral" generic *Limenitis* color pattern) and a third (*L. lorquini* Boisduval) is involved in an as yet unstudied, close resemblance with another nymphalid butterfly, *Adelpha bredowii* Geyer. Non-causal explanations (chance events, coincidence etc.) can always be advanced in the absence of evidence to the contrary, and certainly genetic drift can be a major force in evolutionary events (e.g. Nei, 1987). But, it is awfully "suspicious" that the one mimic of the Monarch comes from a genus of five species in North America, at least one and possibly two others of which are apparently also mimics of quite different putative models. It does suggest that, among the various butterfly genera "available" for ecological exposure to the monarch as potential model, only *Limenitis'* combination of pigment biochemistry and organization of pattern fields in the wings was such as to allow the requisite suite of mutations to arise and fix in its populations, "in response to" the evolutionary opportunity afforded by the distastefulness and distinctive warning pattern of the Monarch. A predisposition to sharp, dramatic changes in pigment distribution as a basic element of pattern is certainly apparent in the "ancestral" or "generic" *Limenitis* pattern of dramatic contrast between dark and light pigmentation (Plate 1:5).

In order to test this idea, one would be forced to recognize that the gap between mechanistic and population studies is an artificial one. One would deal not only with the comparative pigment biochemistries of a number of butterfly genera, but also with the complexities of developmental-field organization in insect wings (e.g. Nijhout, 1985). One would, in short, be forced to admit that organisms evolve as integrated wholes, *but assembled out of parts*, and that the nature of the parts may be critical to the results of the assembly. Not only may color-variation systems exemplify major evolutionary ideas, but they may force us to consider as essential the mechanistic underpinnings and constraints that allow, predispose to, or quite prevent the realization of certain kinds of adaptations.

Challenges for the Future

In this discussion, I have not tried to review exhaustively available case-study examples, hoping instead to sharpen my emphasis on present opportunities for re-orienting studies of animal coloration into a deeper conceptual context. The very obviousness and ease of measurement or color differences make it both possible and desirable that we not stop with mere documentation of apparent matchup between animal color patterns and some aspect of their wearers' environments, however dramatic.

It will be critical, whenever possible, to use *intraspecific*, indeed *intrapopulation genetic variation* as a *probe* of putative relationships between different color phenotypes and potential selective pressures in the wild. The power of this approach is evident in many of the foregoing examples (cf. also Endler, 1986). For example, the apparent inverse relationship between *frequency of "alba" morphs* in *Colias* populations and density of *Pieris* (or other white butterflies) sympatric with them is itself a more powerful evidence of the negative interactions of overlapping visual mate-recognition systems among these insects than would be a "simple" observation of inverse correlation of densities among species as a whole (Watt, Kremen and Carter, 1989). Sympatric presence of the *taxon*, but inverse behavior of the *morph frequency*, already rules out all sorts of generalized character-displacement or species-specific niche-requirement explanations, focusing more sharply on the color-pattern relationships themselves, The ability to compare, for example, the behavioral responses of male *Colias* to the two genetically determined color morphs, *all else remaining equal*, lends further power to discriminate a genuinely adaptive, evolutionarily relevant difference in pattern from possible mere coincidences (Graham *et al.*, 1980).

Combination of modern developmental analysis (e.g. Nijhout, 1985) with studies of the transmission genetics of major pattern elements in mimetic butterflies (e.g.. Remington, 1985) and the molecular biology of pigmentation will allow us to understand constraint and preadaptation in the evolution of mimicry systems in a thorough and mechanistic way. In systems that are already somewhat well understood, *de novo* induced mutations may be uniquely helpful probes — but often, particularly at the outset, it will be most practical as well as most informative to use naturally occurring variation as probes of putative adaptive state differences in coloration. This will also have the advantage of deepening our general understanding of natural selection in the wild, even as we probe the existence of adaptation in specific color-variation systems.

With tools of modern genetics and mechanistic biology put to the service of adaptive studies of color pattern variation, we can look forward to a new depth of results in this venerable aspect of evolutionary biology. Putative color adaptations may be analyzed in terms of bioenergetic costs. Their demographic, fitness-related benefits, if any, may be assessed by comparison of genetic alternatives within the same populations and environments. Such an integrated approach may confer a generality and importance to such studies that cannot be achieved in any other way.

Acknowledgements

I thank Carol Boggs, John Endler, and Tracy McLellan for stimulating discussion, and the U.S. National Science Foundation for research support (grants BSR 84-00299, BSR 87-05268).

References

Bock, W. 1959. Preadaptation and multiple evolutionary pathways. **Evolution** 13: 194-211.

Boggs, C.L. 1981. Nutritional and life history determinants of resource allocation in holometabolous insects. **Amer. Nat.** 117: 692-709.

Brower, L.P. 1984. Chemical defence in butterflies. pp. 109-134. *In* **The Biology of Butterflies,** ed. P. Ackery and R. Vane-Wright. Academic Press, London.

Brower, L.P., ed. 1988. Mimicry and the evolutionary process. **Amer. Nat.** 131: Supplement. pp. S1-S121.

Carson, H.L. 1987. High fitness of heterokaryotypic individuals segregating naturally within a long-standing laboratory population of *Drosophila silvestris*. **Genetics** 116: 415-411.

Darwin, C.R. 1859. **The Origin of Species.** Modern Library edition.

Descimon, H. 1966. Variations quantitatives de pterines de *Colias croceus* (Fourcroy) et de son mutant *helice* (Hbn.) (Lepidoptera Pieridae), et leur signification dans la biosynthèse des ptérines. **C.R. Acad. Sci.** Paris 262: 390-393.

Endler, J. A. 1984. Progressive background matching in moths, and a quantitative measure of crypsis. **Biol. J. Linnean Soc.** 22: 187-231.

Endler, J.A. 1986. **Natural selection in the wild.** Princeton Univ. Press, Princeton, NJ.

Feldman, M.W., F.B. Christiansen and U. Liberman. 1983. On some models of fertility selection. **Genetics** 105: 1003-1010.

Fisher, R.A. 1958. **The Genetical Theory of Natural Selection**, 2nd ed. Dover Press, NY.

Fox, H.M., and G. Vevers. 1960. **The Nature of Animal Colors.** Macmillan, N.Y.

Gerould, J.H. 1921. Blue-green caterpillars: the origin and ecology of a mutation in hemolymph color in *Colias (Eurymus) philodice*. **J. Exp. Zool.** 34: 385-415.

Gerould, J.H. 1926. Inheritance of olive-green and blue-green variations appearing in the life cycle of a butterfly, *Colias philodice*. **J. Exp. Zool.** 43: 413-427.

Gilchrist, G.W., and R.L. Rutowski. 1986. Adaptive and incidental consequences of the alba polymorphism in an agricultural population of *Colias* butterflies: female size, fecundity, and differential dispersal. **Oecologia** 68: 235-240.

Graham, S.M., W.B. Watt and L.F. Gall. 1980. Metabolic resource allocation vs. mating attractiveness: adaptive pressures on the "alba" polymorphism of *Colias* butterflies. **Proc. Nat'l. Acad. Sci.** USA 77: 3615-3619.

Gould, S.J., and R.C. Lewontin. 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. **Proc. Roy. Soc. London** B205: 581-596.

Hama, T., and S. Fukuda. 1964. The role of pteridines in pigmentation. pp. 495-505. *In* **Pteridine chemistry,** ed. W. Pfleiderer and E.C. Taylor, Macmillan, NY.

Hoffmann, R.J., and W.B. Watt. 1974. Naturally occurring variation in larval color of *Colias* butterflies: isolation from two Colorado populations. **Evolution** 28: 326-328.

Hovanitz, W. 1944. The distribution of gene frequencies in wild populations of *Colias*. **Genetics** 29: 31-60.

Hovanitz, W. 1950. The biology of *Colias* butterflies. II. Parallel geographical variation of dimorphic color phases in north American species. **Wasmann J. Biol.** 8: 197-219.

Hutchinson, G.E. 1957. Concluding remarks. **Cold Spring Harbor Symp. Quant. Biol.** 22: 415-427.

Hutchinson, G.E. 1978. **An Introduction to Population Ecology.** Yale Univ. Press, New Haven, CT.

Kaufman, S. 1963. The structure of the phenylalanine-hydroxylation cofactor. **Proc. Nat'l Acad. Sci.** USA 50: 1085-1093.

Kaufman, S. 1979. Biopterin and metabolic disease. pp. 117-124. *In* **Chemistry and Biology of Pteridines,** ed. R.L. Kisliuk and G.M. Brown. Elsevier/North Holland, NY.

Kingsolver, J.G. 1983a. Thermoregulation and flight in *Colias* butterflies: elevational patterns and mechanistic limitations. **Ecology** 64: 543-545.

Kingsolver, J.G. 1983b. Ecological significance of flight activity in Colias butterflies: implications for reproductive strategy and population structure. **Ecology** 64: 546-551.

Kingsolver, J.G. 1985a. Thermal ecology of *Pieris* butterflies: a new mechanism of behavioral thermoregulation. **Oecologia** 66: 540-545.

Kingsolver, J.G. 1985b. Thermoregulatory significance of wing melanization in *Pieris* butterflies: physics, posture, and pattern. **Oecologia** 66: 546-553.

Kingsolver, J.G., and W.B. Watt. 1983. Thermoregulatory strategies in *Colias* butterflies: Thermal stress and the limits to adaptation in temporally varying environments. **Amer. Nat.** 121: 32-55.

Kingsolver,J.G., and W.B. Watt. 1984. Optimality models and mechanistic constraints: thermoregulatory strategies in *Colias* butterflies. **Ecology** 65: 1835-1839.

Leigh, E. G., jr. 1986. Ronald Fisher and the development of evolutionary theory. I. The role of selection. **Oxford Surv. Evol. Biol.** 3: 187-223.

Leigh. E.G., jr. 1987. Ronald Fisher and the development of evolutionary theory. II. Influences of new variation on evolutionary process. **Oxford Surv. Evol. Biol.** 4: 212-263.

Ley, C., and W.B. Watt. 1989. Testing the "mimicry" explanation of the *Colias* "alba" polymorphism" palatability of *Colias* and other butterflies to wild bird predators. **Functional Ecology**: 3:183-192.

Liberman, U. and M.W. Feldman. 1985. A symmetric two locus model with viability and fertility selection. **J. Math. Biol.** 22: 31-60.

MacArthur, R.H. 1964. Ecological consequences of natural selection. pp. 388-397. *In* **Theoretical and Mathematical Biology,** ed. T.H. Waterman and H.J. Morowitz. Blaisdell, NY.

Mayr, E. 1980. Prologue: some thoughts on the history of the evolutionary synthesis. pp. 1-48. *In* **The Evolutionary Synthesis,** ed. E. Mayr and W. B. Provine. Harvard Press, Cambridge, MA.

McElroy, W.D., H.H. Seliger, and M. DeLuca. 1965. Enzyme catalysis and color of light in bioluminescent reactions. pp. 319-340. *In* **Evolving Genes and Proteins,** ed. V. Bryson and h.J. Vogel, Academic Press, NY.

Nathans, J., D. Thomas, and D. Hogness. 1986. Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. **Science** 232: 193-202.

Nei, M. 1987. **Molecular Evolutionary Genetics.** Columbia Univ. Press, NY.

Nijhout, H.F. 1985. The developmental physiology of colour patterns in Lepidoptera. **Adv. Insect Physiol.** 18: 181-247.

Parkin, D.T. 1980. The ecological genetics of plumage polymorphism in birds. **Linn. Soc. Symp.** 9: 219-234.

Peters, R.H. 1976. Tautology in ecology and evolution. **Amer. Nat.** 110: 1-12.

Pianka, E.R. 1976. Natural selection of optimal reproductive tactics. **Am. Zool.** 16: 775-784.

Piantanida, T. 1988. The molecular genetics of color vision and color blindness. **Trends in Genet.** 4: 3219-323.

Poulton, E.B. 1908. **Essays on Evolution, 1889-1907.** Clarendon Press, Oxford.

Pyke, G.H., H.R. Pulliam, and E.L. Charnov. 1977. Optimal foraging: a selective review of theory and tests. **Quart. Rev. Biol.** 52: 137-154.

Remington, C.L. 1954. The genetics of *Colias* (Lepidoptera). **Adv. in Genet.** 6: 403-450.

Remington, C.L. 1985. Genetical differences in solutions to the crises of hybridization and competition in early sympatry. **Boll. Zool.** 52: 21-43.

Roland, J. 1982. Melanism and diel activity of alpine *Colias* (Lepidoptera: Pieridae). **Oecologia** 53: 214-221.

Rutowski, R.L. 1981. Sexual discrimination using visual cues in the checkered white butterfly (*Pieris protodice*). **Zeits. Tierpsych.** 55: 325-334.

Rutowski, R.L. 1984. Sexual selection and the evolution of butterfly mating behavior. **J. Res. Lepid.** 23: 125-142.

Silbergleid, R.E., and O.R. Taylor, jr. 1978. Ultraviolet reflection and its role in the courtship of the sulphur butterflies Colias eurytheme and Colias philodice (Lepidoptera: Pieridae). **Behav. Ecol. Sociobiol.** 3: 203-243.

Stearns, S.C. 1976. Life-history tactics: a review of the ideas. **Quart. Rev. Biol.** 521: 3-47.

Taylor, O.R., jr. 1970. Reproductive isolation in *Colias eurytheme* and *Colias philodice* (Lepidoptera: Pieridae). Ph.D. thesis, University of Connecticut, Storrs. 121 pp.

Taylor, O.R., jr. 1972. Random vs. non-random mating in the sulfur butteflies *Colias eurytheme* and *Colias philodice* (Lepidoptera: Pieridae). **Evolution** 26: 344-356.

Turner, J.R.G. 1987. The evolutionary dynamics of batesian and mullerian mimicry: similarities and differences. **Ecol. Entomol.** 12: 81-95.

Van Zandt Brower, J. 1958a. Experimental studies of mimicry in some North American butterflies. I. The Monarch, *Danaus plexippus*, and Viceroy, *Limenitis archippus archippus*. **Evolution** 12: 32-47.

Van Zandt Brower, J. 1958b. Experimental studies of mimicry in some North American butterflies. II. *Battus philenor* and *Papilio troilus*, *P. polyxenes* and *P. glaucus*. **Evolution** 12: 123-136.

Van Zandt Brower, J. 1958c. Experimental studies of mimicry in some North American butterflies. III. *Danaus gilippus berenice* and *Limenitis archippus floridensis*. **Evolution** 12: 273-285.

Van Zandt Brower, J. 1960. Experimental studies of mimicry, IV. The reactions of starlings to different proportions of models and mimics. **Amer. Nat.** 94: 271-282.

Watt, W.B. 1964. Peridine components of wing pigmentation in the butterfly *Colias eurytheme*. **Nature** 201: 1326-1327.

Watt. W.B. 1967. Pteridine biosynthesis in the butterfly *Colias eurytheme*. **J. Biol. Chem.** 242: 565-572.

Watt, W.B. 1968. Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation of melanin pigment in relation to thermoregulation. **Evolution** 22: 437-458.

Watt, W.B. 1985. Bioenergetics and evolutionary genetics — opportunities for new synthesis. **Amer. Naturalist** 125: 118-143.

Watt, W.B. 1986. Power and efficiency as indexes of fitness in metabolic organization. **Amer. Naturalist** 127: 629-653.

Watt, W.B., and S.R. Bowden. 1966. Chemical phenotypes of pteridine colour forms in *Pieris* butterflies. **Nature** 210: 304-306.

Watt, W.B., P.A. Carter, and S.M. Blower. 1985. Adaptation at specific loci. IV. Differential mating success among glycolytic allozyme genotypes of *Colias* butterflies. **Genetics** 109: 157-175.

Watt, W.B., P.A. Carter, and K. Donohue. 1986. Females' choice of "good genotypes" as mates is promoted by an insect mating system. **Science** 233: 1187-1190.

Watt, W.B., C. Kremen and P.A. Carter. 1989. Testing the "mimicry" explanation for the *Colias* "alba" polymorphism: patters of co-occurrence of *Colias* and Pierine butterflies. **Functional Ecology**. 3:193-199.

Industrial Melanism in Moths: A Review and Reassessment

THEODORE D. SARGENT

Synopsis

Increases in the incidence of melanism in moths in industrialized parts of the world over the last century have been generally interpreted as an evolutionary consequence of the superior crypsis of black moths on tree trunks that have been darkened by industrial pollutants. This classical interpretation assumes (1) that melanism is based on relatively simple genetic changes; (2) that melanic moths do rest on darkened substrates; and (3) that birds are significant predators on the moth species involved. These assumptions are examined here, and I conclude that the evidence on these matters is equivocal. In particular, I question the close association of melanism in moths with industrially darkened substrates, and the adequacy of the experimental evidence purporting to demonstrate selection based on crypsis in the field. An examination of the literature, and some new experiments presented here, also indicate that induction cannot yet be ruled out as a factor in the overall melanism phenomenon. Finally, I present a revised hypothesis that, although still assuming that crypsis is the critical factor in promoting increased melanism, emphasizes various effects of human activities on forest succession as the basis for the substrate changes to which cryptic moths are responding.

Introduction

Industrial melanism in moths has become the standard textbook example of microevolution in nature. The so-called "classical" explanation of this phenomenon was developed by Kettlewell and his associates working in England with the peppered moth, *Biston betularia* (L.) (Geometridae) (for summary, see Kettlewell 1973). In this paper I will examine the observations and assumptions upon which this classical case has been built, and assess the extent to which they are supported by existing data. I will also present some new data bearing on certain previously held assumptions regarding the inheritance of melanism, and I will conclude with a revised hypothesis that I believe incorporates more of what is now known about the phenomenon than does the classical hypothesis.

The classical explanation of industrial melanism is based on a series of observations that seem to create a straightforward adaptive story. Among the critical observations in this scenario are (1) recent increases in the frequencies of melanic moths, (2) a simple genetic basis for the melanic condition, (3) an environmental change (darkening of tree trunks) that correlates both spatially and temporally with the change in appearance of the moths, and (4) a selective agent (birds preying on resting moths) that directs the entire process. If one assumes that the moths exhibiting melanism do rest on tree trunks, and that birds are significant predators on the moth species involved, then the conclusion that natural selection based on cryptic advantage has created the industrial melanism phenomenon seems inescapable. This "inescapable" conclusion, however, has not fared well in several recent papers (Hailman, 1982; Jones, 1982; Sermonti and Catastini, 1984; Lambert *et al*, 1986; Brakefield, 1987), and it seems worthwhile, in this symposium on protective coloration in invertebrates, to review the entire matter.

The Melanism Phenomenon

The problem with which we are concerned may be stated simply: melanic morphs of many species of moths have increased in abundance in recent times. Why?

The increased incidence of melanism in moths seems clearly established. Numerous data documenting these increases in more than 100 species in England and many parts of Europe have been tabulated by Kettlewell (1973), and Owen (1961, 1962) has documented similar increases in melanism in several North American species. With respect to North America, it is interesting to note that Holland's **Moth Book** (1903) provided no illustrations of

Department of Zoology, University of Massachusetts, Amherst, Mass. 01003.

melanics among the hundreds of specimens depicted in his plates, yet melanism is now a common occurrence in North America (e.g., Sargent, 1974). In the genus *Catocala* (Noctuidae) alone, there are now 14 described melanic morphs (Sargent, 1976), none of which were noted by Holland.

It seems safe to conclude that starting in about 1850 in England and Europe, and some 50 years later in this country, something favored the appearance and spread of melanism in many species of moths. Whether or not melanics were ever previously abundant, cannot be known — but there can be little dispute regarding the overall trend since 1850.

There is, of course, some evidence for a recent reversal of this trend in *B. betularia* in certain smoke-free zones in England (Bishop and Cook, 1975; Cook *et al*, 1986), but this is a very localized phenomenon, and one whose relationship to crypsis is somewhat obscure (Clarke *et al*, 1985; Howlett and Majerus, 1987; Liebert and Brakefield, 1987). At any rate, the major evolutionary problem remains: what factor or factors can account for the rise and spread of melanism in so many species of moths, worldwide, over the past 100 to 150 years?

Genetics and induction

Reviews of the genetic bases of melanism in moths are provided by Ford (1937), Robinson (1971), and Kettlewell (1973). Kettlewell (1973) concluded that, "More than 90 percent of industrial melanics are controlled by a single major gene, and the melanic character is inherited as a Mendelian dominant..." This picture of specific alleles, usually dominant, controlling melanism is supported by extensive breeding studies on a wide array of species.

There is, however, another line of evidence suggesting that some form of induction may play a role in the production of melanism in moths. A number of very early workers maintained that melanism was an induced trait (e.g. Cooke, 1877; Chapman, 1988; Merrifield, 1890, 1891, 1892), and some anomalous breeding results were interpreted in that fashion as well (e.g., Hamling, 1905; Bowater, 1914; Onsolow, 1920a, b; 1921). The first extensive experimental evidence for induction, however, was reported by Harrison (1926, 1928, 1935; Harrison and Garrett, 1926). Harrison's remarkable results, achieved primarily with *Selenia bilunaria* and *Tephrosia bistortata* (Geometridae), revealed an apparent induction of adult melanism by various salts (lead nitrate, manganese sulfate, or manganese chloride) that were added to plant leaves upon which his larvae were fed. Moreover, once induced, the melanic condition was inherited like a Mendelian trait (usually as a recessive, but as a dominant in *Tephrosia crepuscularia* [Geometridae]), even in the absence of the inducing salts.

These results generated a considerable controversy (e.g., Fisher, 1933; Haldane, 1935; Ford, 1937; Harrison, 1956) that continues to the present day (e.g., Lambert *et al*, 1986). Harrison's experiments were repeated by Hughes (1932) and Thomsen and Lemcke (1933), and these workers were not able to produce melanics in their treatment groups. However, Lambert *et al* (1986), in a critical review of these studies, conclude that, "...this contradictory experimental evidence is, at best, questionable." Other critics of Harrison (e.g., Ford, 1936) have suggested that melanism was introduced into his treatment groups via rare recessive alleles from the wild. This criticism, however, fails to consider why melanics never appeared in Harrison's control groups, and it cannot explain the one case of dominant melanism mentioned above.

It is ironic that Harrison's contention that his salts exerted effects on the "soma," which, in turn, affected the "germ plasma," has been dismissed as "pure Buffonism" on the one hand (Kettlewell, 1973), yet been discussed with reference to such modern concepts as transposons and promiscuous DNA on the other (Lambert *et al*, 1986). Certainly, recent evidence for environmentally induced and heritable DNA sequence rearrangements in flax (Cullis, 1977, 1983, 1985) should prevent us from dismissing Harrison's results out-of-hand, although there seem to be no precedents for such a phenomenon in animals. At any rate, the induction controversy with respect to melanism seems far from over.

Later in this paper I will discuss some recent experiments of my own that provide additional evidence for environmental induction of melanism in the noctuid, *Panthea pallescens*. In this case, as in Harrison's, induction occurred via larval ingestion, although here the foodplant variable was differences in the age of the vegetation on which the larvae fed, rather than the presence or absence of added chemicals. Here again, though, there is strong evidence for an induction phenomenon and a suggestion that the effect is genotypic, rather than phenotypic only.

It seems clear that melanism in moths often exhibits a Mendelian mode of inheritance. What is less clear, however, is whether or not the condition always arises as a mutation in the first place, thence to increase or decrease in frequency in a population as natural selection dictates. It also seems plausible at the moment to suggest that melanism may arise via some form of induction that is triggered by an environmental change. This induction response may itself represent a long-term adaptation, assuming that the environmental change in question has recurred from time to time in the evolutionary history of the species involved.

The speed of the process would be very different in the two cases. In the first case, an appropriate

mutation would have to occur, and the resulting trait then be selected — a relatively slow process. In the second case, a considerable proportion of a population might immediately exhibit the new trait, and any subsequent selection for that trait would have a broader base of individuals from which to commence. In this regard, it is interesting to note that the speed with which melanics have replaced typicals is regarded as one of the most striking features of the industrial melanism phenomenon (Kettlewell, 1973). Populations of *B. betularia* around Manchester, England, for example, changed from having virtually no melanics to having more than 95 percent melanics in no more than 47 years (1848 to 1895) (Haldane, 1956). Such a change, if due to a change in the frequency of a dominant allele, requires a 50 percent selective advantage of the melanic over the typical morph (Ford, 1975). Even faster changes have been suggested in other cases (e.g., Harrison and Garrett, 1926; Clarke and Sheppard, 1966; Owen, 1961). Such rapid changes would not seem so striking, however, if an induction process affecting many individuals, rather than a single mutation in a single individual, was the original source of the melanic moths.

It is important to note that an induced trait may seem to be inherited in a Mendelian fashion. This would be true if the induction affected only the phenotype, provided that each new generation was also exposed to the inducing agent. If the induction affected the genotype (as suggested by Harrison), then the trait involved might persist for some time even after the inducing agent was removed. In any event, the observation that melanism in moths is usually inherited as a Mendelian dominant says little about the possibility of induction as a basis of the melanic condition.

Crypsis and predation

A presumed cryptic advantage of melanic moths on industrially darkened tree trunks is the primary proposition in the classical interpretation of the industrial melanism phenomenon. To examine this matter, I will address the following questions:

1. Are industrialization, darkening of tree trunks, and increased melanism always associated?
2. Do melanic moths actually rest on darkened tree trunks?
3. If so, do melanics have a cryptic advantage over typicals on these trees?

There is no question that industrialization, darkening of tree trunks, and increased melanism in moths are often associated. This statement, in fact, could serve as a general summary of the findings of Kettlewell and his associates on *B. betularia* in England (1965, 1973). There are, however, numerous exceptions to this generalization. Thus, Creed *et al* (1973) reported high frequencies of melanic *B. betularia* from East Anglia where the trees are generally pale, and where selective predation experiments suggest strong visual selection against melanics (Lees and Creed, 1975). In North America, Klots (1964, 1966, 1968a, b), Jones (1977) and Sargent (1971, 1974, 1983, 1985) have reported high melanic frequencies in several species of moths from areas in New England where the trees are not devoid of lichens nor noticeably darkened by soot. Similarly, West (1977) and Manley (1981) have reported recent increases in the frequencies of melanics in populations of *B. betularia cognataria* in rural areas of Virginia and Pennsylvania. Other examples of so-called "rural melanism" are discussed by Owen (1962), Bishop and Harper (1970), Lees (1971), Bishop *et al* (1978) and Steward (1977a, b).

Various attempts to incorporate these cases into the general explanation of melanism have been made. These generally involve positing heterozygous advantage (e.g. Ford, 1937, 1975; Kettlewell, 1973), non-visual selection (e.g., Bishop, 1972; Creed *et al*, 1973; Lees and Creed, 1975; Steward, 1977a, b, d; Whittle *et al*, 1976; Lees, 1981), or migration phenomena (e.g., Bishop and Cook, 1975; Bishop *et al*, 1978; Cook and Mani, 1980; Mani, 1980, 1982; Cook *et al*, 1986) to account for the observed disparities between the incidence of melanics and assessments of their cryptic status.

It is clear that these disparate cases pose problems for the classical explanation of melanism. The question is whether this classical explanation can be modified to fit all cases, or whether another explanation with wider applicability can be found. I will return to this question in the final section of this paper.

It is also important to ascertain whether melanic moths actually rest on the trunks of darkened trees as the classical explanation requires. Kettlewell's famous mark-release-recapture experiments (1955a, 1956) involved placing live *B. betularia* onto tree trunks, and many subsequent predation studies have utilized dead moths affixed to trees (e.g., Clarke and Sheppard, 1966; Bishop, 1972).

The dearth of field observations of naturally resting *B. betularia* had always been puzzling, and it was Mikkola (1979) who obtained the first experimental evidence that these moths tend to rest high in trees, mostly on the undersides of small horizontal branches. This finding has since been confirmed by Howlett and Majerus (1987) and Liebert and Brakefield (1987), and it certainly confounds any interpretation of Kettlewell's mark-release-recaptive experiments.

Another problems is posed by some early observations of melanic moths resting on very light backgrounds in the field (e.g., Barrett, 1897; Harrison, 1920), and more recent experimental findings that melanic morphs of several species prefer light (white)

over dark (black) backgrounds when given a choice between the two (Sargent, 1968, 1969; Lees, 1975; Steward, 1976, 1977c). The case of *B. betularia*, where melanics prefer dark over light backgrounds (Kettlewell, 1955b; Boardman *et al*, 1974; Kettlewell and Conn, 1977), is an exception, although Steward (1985) has recently shown that this preference is true of the "carbonaria," but not the "insularia" morph (Steward, 1985).

I have addressed this general problem in a recent paper (Sargent, 1985), suggesting that some melanics may prefer white backgrounds because they are most cryptic on white birches; which, although prevailingly white-barked, do have black patches and may thereby provide opportunities for a disruptive kind of crypsis to black moths. At any rate, the demonstrated preference of many melanics for light (white) backgrounds is difficult to accommodate within the classical framework of the melanism problem.

Finally, these questions about the resting habits of melanic moths suggest that the primary evidence for the classical explanation of industrial melanism, namely the previously mentioned mark-release-recapture experiments of Kettlewell in Birmingham (polluted woods) and Dorset (rural woods) in England, should be re-examined. These oft-cited experiments are usually seen as compelling evidence for the classical view, but Sermonti and Catastini (1984), in a recent critical review, conclude that, "...Kettlewell's experiments do not prove in any acceptable way, according to the current scientific standard, the process he maintains to have experimentally demonstrated."

There are problems with these experiments and we have already suggested some of them. For example, *Biston* may not rest on the trunks of trees as they were forced to do in these experiments. Also, it seems certain that *B. betularia* never reaches the densities in nature that Kettlewell used. In some ways, then, the procedures used created a "feeding tray" situation that may have attracted far more predation than the moths normally encounter. In these circumstances it is perhaps not surprising that birds tended to take the more conspicuous moths before the less conspicuous ones. However, whether this result would also have been obtained had the moths been resting in their normal fashion and at their typical densities can only be surmised.

It might also be noted that the selective advantages that would be required to yield Kettlewell's results are much higher than those previously calculated to exist at the sites that he used (Sermonti and Catastini, 1984). And, of course, mark-release-recapture experiments do not permit distinction between predation and other reasons for failure to recapture (e.g., emigration). This last problem was mitigated to some extent by Kettlewell's observations and filming of bird predation on released *B. betularia*, although again, the questions of normal resting sites and densities apply here as well.

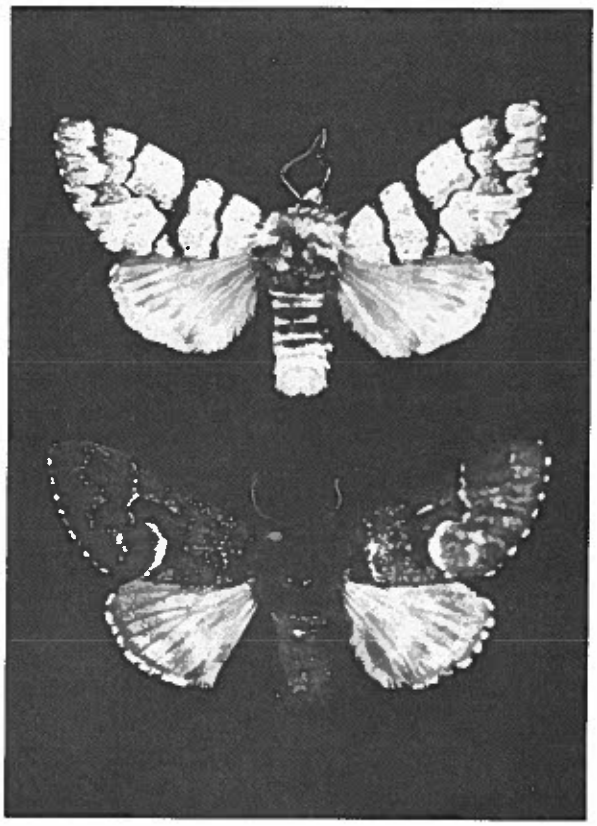


Figure 1. The typical (above) and melanic (below) morphs of *Panthea pallescens* McDunnough. Specimens from Leverett, Mass., 1981. Approximately life-size.

In summary, it appears that Kettlewell's experiments, as well as subsequent efforts to repeat them (e.g., Liebert and Brakefield, 1987), are suggestive, but that we have yet to see compelling experimental evidence that increased melanism in moths is based primarily on the cryptic advantage of black moths on industrially darkened trees.

New Experiments

In the summer of 1986, I was able to conduct some rearing experiments with *Panthea pallescens* McDunnough (Noctuidae) in Amherst, Mass., that provided evidence for an induction phenomenon with respect to the expression of melanism in that species. These experiments differed from prior induction studies in that the variable affecting adult melanism was not the presence or absence of a foreign substance, but rather some constituent(s) of the normal hostplant that varied with the age of the foliage upon which the larvae were fed.

Panthea pallescens is bivoltine in Massachusetts, with adults flying primarily in June and August.

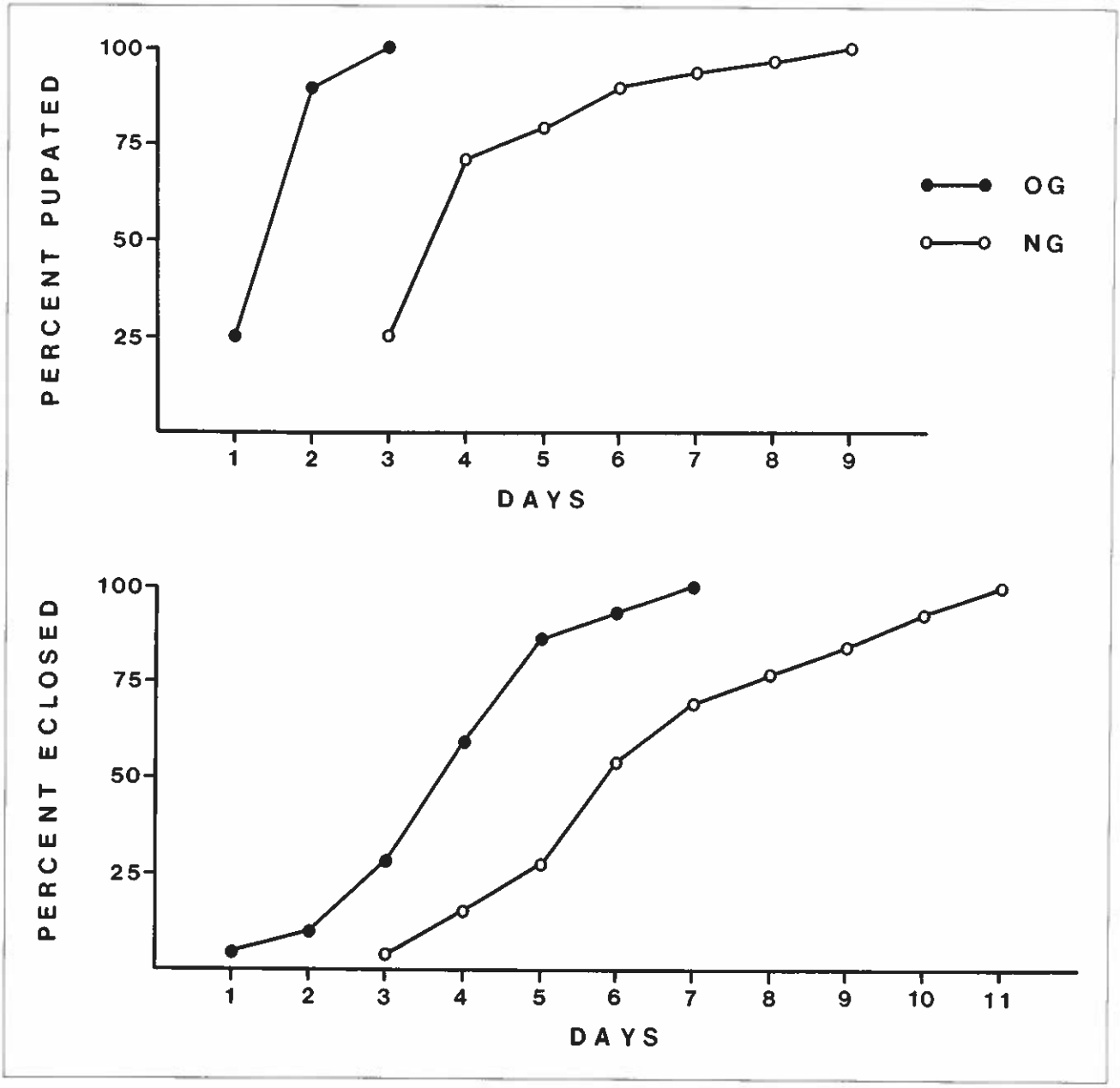


Figure 2. The cumulative rates of pupation (above) and adult eclosion (below) of *P. pallescens* in Experiment 1 on old-growth (OG) and new-growth (NG) white pine.

The melanic morph, "atrescens" McDunnough (1942), has increased in abundance in recent years (Klots, 1964, 1966; Ginevan, 1971; Sargent, 1974; Jones, 1977), and presently makes up about 60 percent of the local population. The two morphs of this species are easily distinguished (Fig. 1), and there is substantial evidence that melanism in this case is controlled by a sex-linked, dominant allele (Ginevan, 1971).

In the first of the present two experiments, an adult melanic female was taken at lights at my home in Leverett, Mass., on May 23, 1986. This female was placed in a plastic jar with a small sprig of the larval foodplant, white pine (*Pinus strobus* L.), including needles of the current year ("new growth") and older needles of previous years ("old growth"). Egg-laying commenced immediately, and the eggs hatched on May 30. Fresh pine was then added, again including both new growth and old growth. (All of the pine used in these 1986 experiments was taken from a single tree growing near my home in Leverett.)

On June 1, three days after hatching, the larvae were divided into two groups in pint-sized, plastic ice cream containers. One group was provided with only new growth pine (NG group), and the other

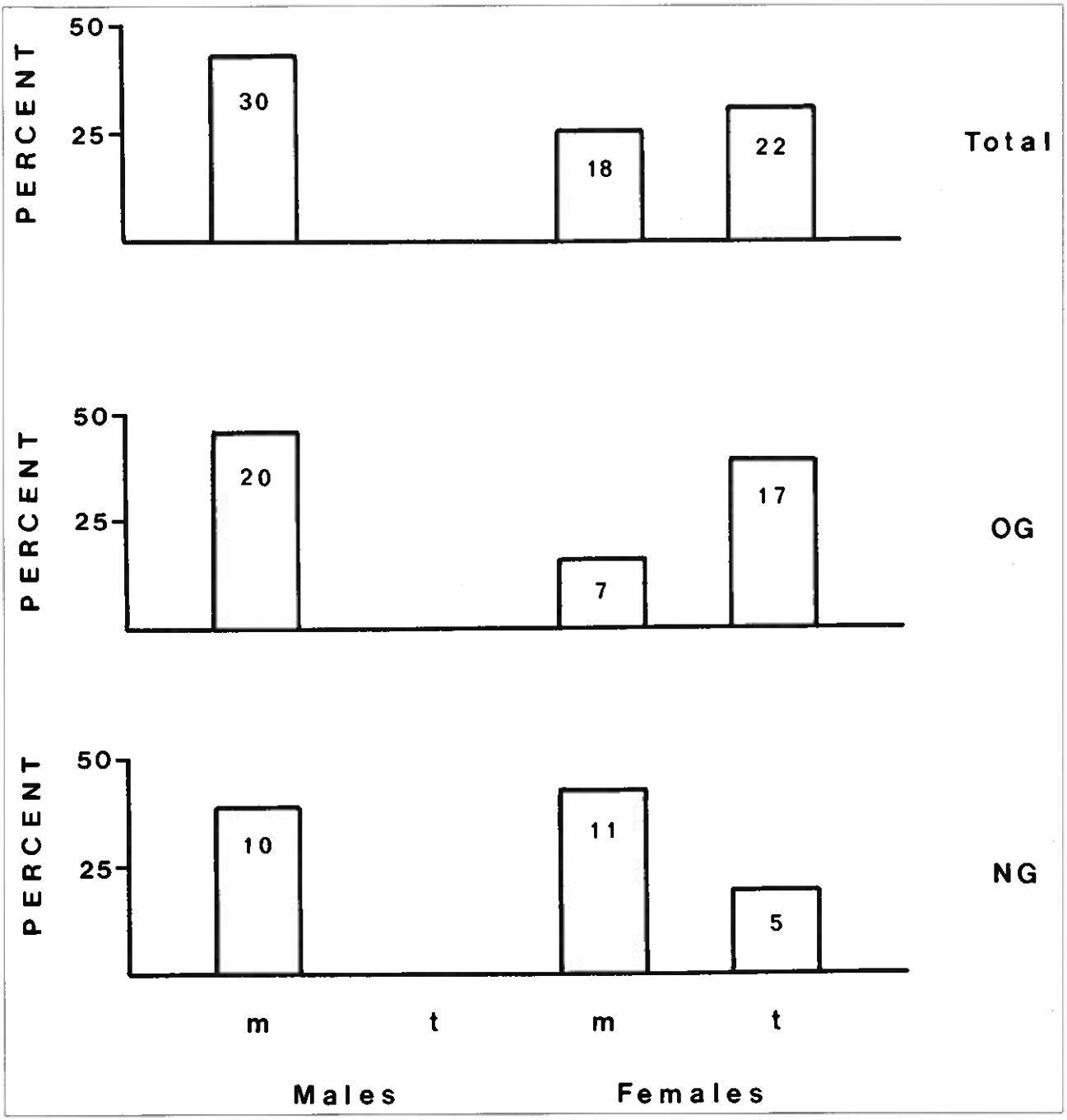


Figure 3. The phenotype ratios of the offspring of *P. pallescens* in Experiment 1; overall (above), and separated according to rearing conditions (below). m = melanic adults; t = typical adults; OG = old growth white pine; NG - new growth white pine. The numbers within the bars represent the numbers of individuals in each case.

group was provided with only old growth pine (OG group). Then, on a daily basis, the larvae in each group were counted, and provided with fresh pine needles of the appropriate kind. Periodically, as the larvae grew, they were separated into smaller groups in more pint-sized containers until there were only one or two larvae per container (June 12).

Larval growth was considerably slower on the new growth pine than on the old growth pine, and both pupation and adult eclosion were delayed by an average of two to three days in the NG, as compared with the OG, group (Fig. 2).

All of the males that emerged were melanics, and the females were 50:50, typicals and melanics. Assuming that melanism in *P. pallescens* is controlled by a sex-linked dominant allele (Ginevan, 1971), this overall phenotype ratio indicated that the melanic female collected had mated with a

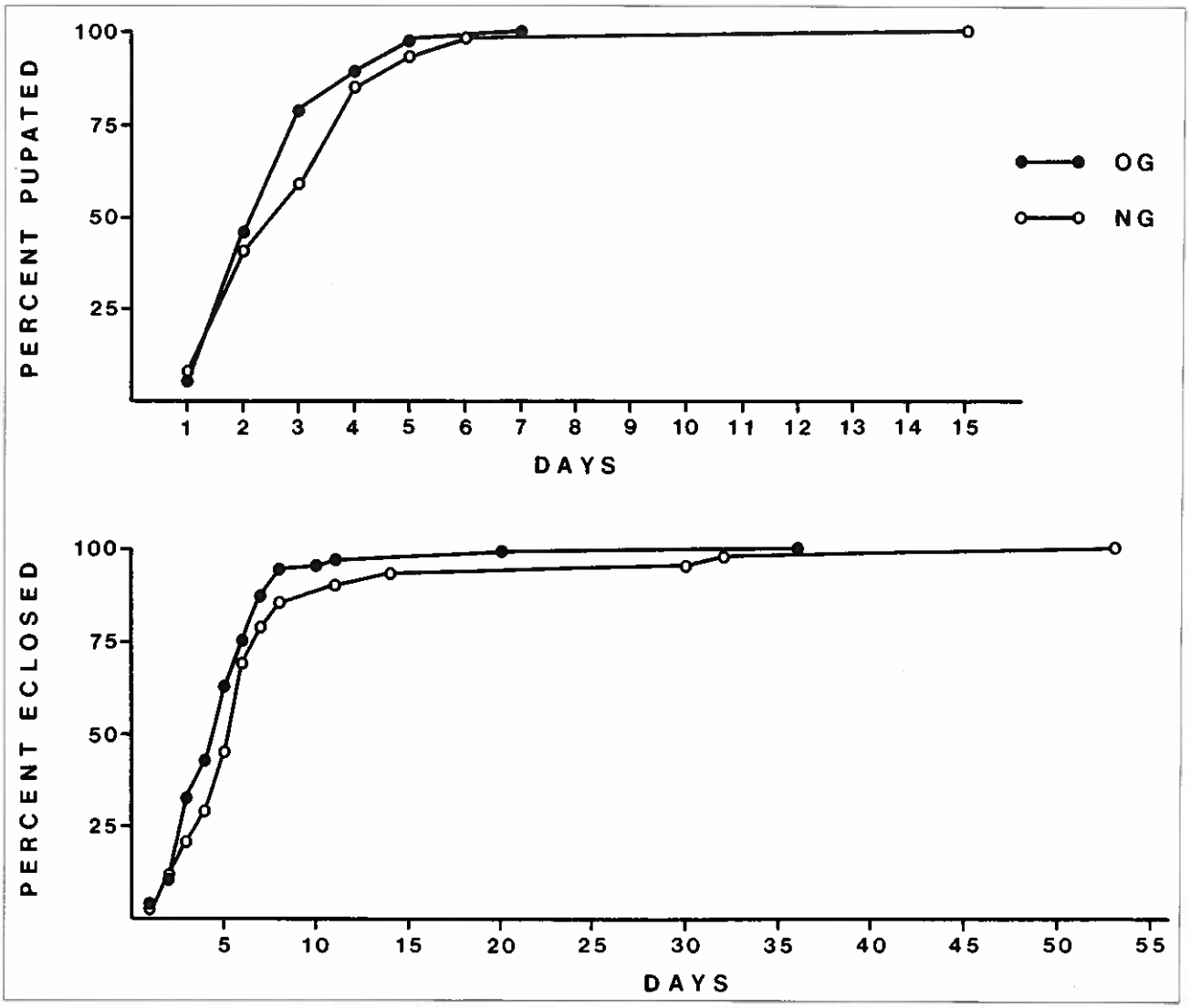


Figure 4. The cumulative rates of pupation (above) and adult eclosion (below) of *P. pallescens* in Experiment 2 on old-growth (OG) and new-growth (NG) white pine.

heterozygous melanic male (recalling that the female is the heterogametic sex in moths). The total numbers of moths in the three phenotypic categories (melanic males, melanic females, typical females) did not differ from those expected in an MY x Mm cross (chi-square goodness-of-fit, $\chi^2 = 1.88$, $p > 0.40$) (Fig. 3, top).

However, the picture was very different when the ratios of typical to melanic females were examined separately in the OG and NG groups (Fig. 3, bottom). More than two-thirds of the OG females were typicals, and more than two-thirds of the NG females were melanics — a difference that was significant (G-test of association, G-6.2, $p<0.05$).

An opportunity to repeat this experiment was presented when a second melanic female was taken at lights at my home on July 8, 1986. The procedures that had been followed with the first female were followed again. This time, the lag in larval growth that had been noted previously on NG was barely discernible (Fig. 4). However, the adult phenotype ratios were similarly biased toward typical females in the OG group and toward melanic females in the NG group (G-test of association, $G=4.5$, $p<0.05$), despite the fact that the overall phenotype ratio did not differ from that expected in an MY x Mm cross (chi-square goodness-of-fit, $\chi^2 = 0.43$, $p>0.80$) (Fig. 5).

When the adults of the two groups are combined, the difference in phenotype ratios between the OG and NG groups is very highly significant (G-test of association, $G=10.4$, $p<0.001$) (Fig. 6).

Thus, among females, something associated with larval feeding on new growth pine favored the expression of adult melanism; and conversely, something associated with larval feeding on old growth pine favored typical adults. There would

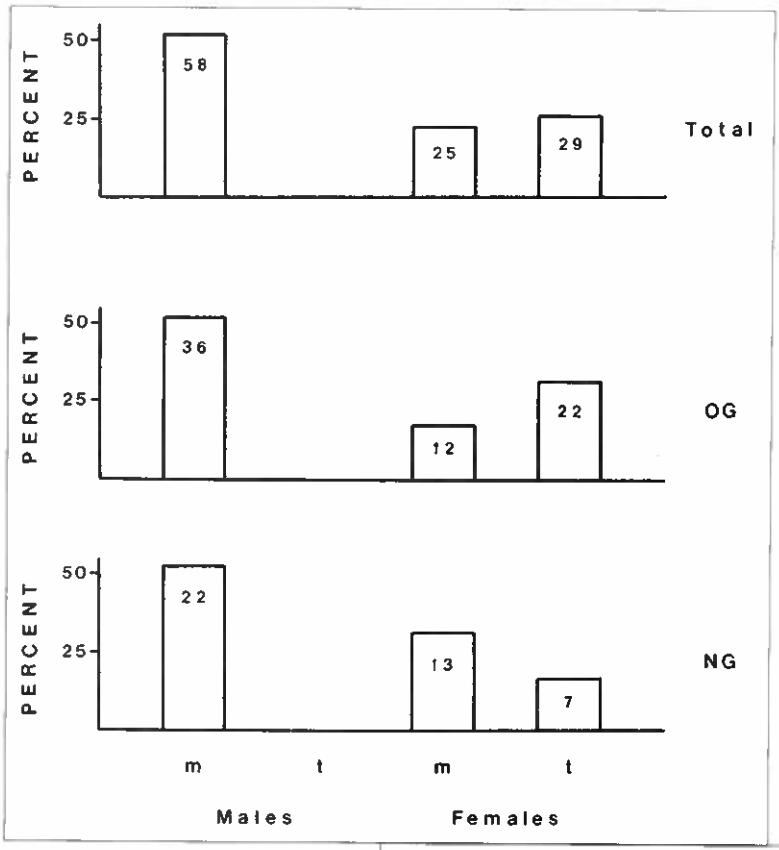


Figure 5. The phenotype ratios of the offspring of *P. pallescens* in Experiment 2; overall (above), and separated according to rearing conditions (below). m = melanic adults; t = typical adults; OG = old-growth white pine; NG = new growth white pine. The numbers within the bars represent the numbers of individuals in each case.

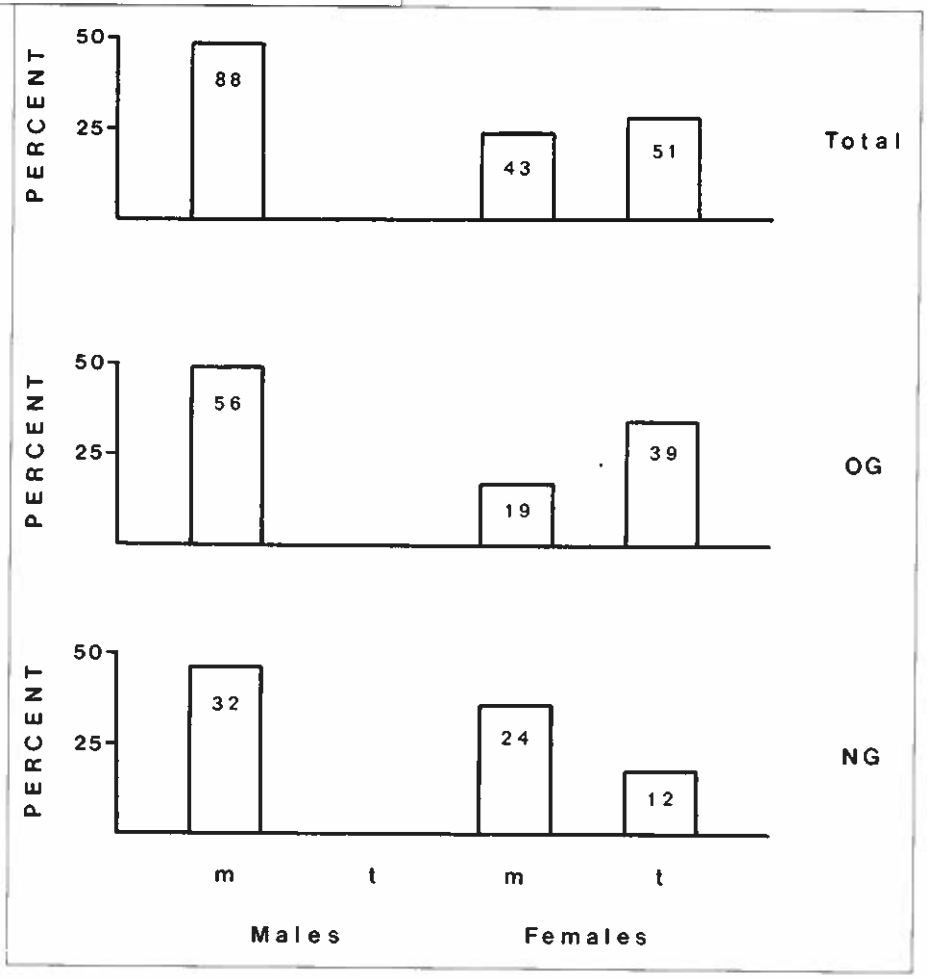


Figure 6. The phenotype ratios of the offspring of *P. pallescens* in Experiments 1 and 2; overall (above), and separated according to rearing conditions (below). m = melanic adults; t = typical adults; OG = old growth white pine; NG = new growth white pine. The numbers within the bars represent the numbers of individuals in each case.

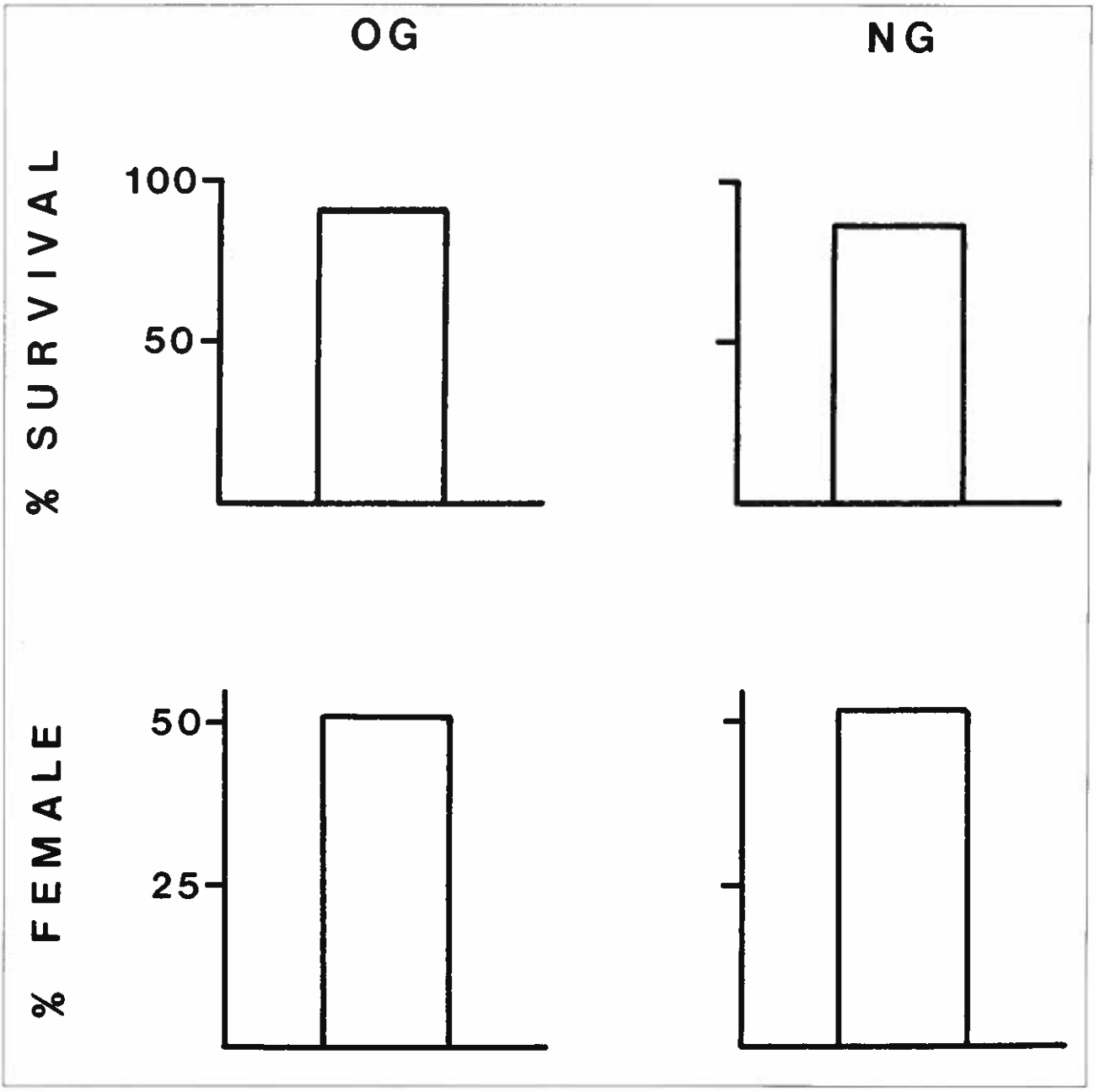


Figure 7. The percent survival of larvae to adult *P. pallescens* in Experiments 1 and 2 on old-growth (OG) and new-growth (NG) white pine (above); and the percent of females among the adults reared on old-growth (OG) and new-growth (NG) white pine in Experiments 1 and 2 (below).

seem to be two possible explanations of this result: (1) differential mortality favoring one or the other type of individual on each of the two larval diets, or (2) environmental induction favoring either typical or melanic morphs on old growth and new growth pine, respectively. The first possibility seems ruled out by the very high survivorship of larvae under both conditions in these experiments (Fig. 7, top). Furthermore, there was no deficit of females in the NG condition (Fig. 7, bottom), as would have been expected if typicals had experienced higher mortality on this diet (recalling that no typical males were expected).

This seems to leave induction as the most likely explanation of the results. It is known that environmental variables do influence adult appearance in the Lepidoptera, as witnessed, for example, by the widespread occurrence of seasonal polyphenism in this group (e.g., Shapiro, 1976). Most of the polyphenisms are controlled by variations in either temperature or photoperiod, and I know of no reports of adult polyphenisms based on variations

in larval diet. However, the possibility of such an occurrence in adults certainly seems plausible.

In the present experiments, it appears that new growth and old growth white pine needles differ in some way that exerts an effect on the expression of melanism. Analyses of the leaf oil terpene composition of eastern white pine have been carried out (e.g., von Rudloff, 1985), although differences in the chemical composition of new and old needles have apparently not been examined in this species (von Rudloff, pers. comm.). There are, however, substantial chemical differences between new and old needles in Douglas fir (Maarse and Kepner, 1970) and white spruce (von Rudloff, 1972), and such differences are to be expected in white pine as well. Whether such differences will prove to be the basis of the induction effect seen here, and, if so, how they exert that effect, must be topics for future research.

Whatever the basis, the changes in adult appearance seen in *P. pallescens* may be adaptive, since young white pine trees (with relatively high percentages of new foliage) have very dark bark, while old white pine trees (with relatively low percentages of new foliage) have very pale bark. Assuming that adult moths rest on the trunks of the trees on which they feed, then dark (melanic) moths would be favored on younger trees, and light (typical) moths on older trees. However, further studies of the resting habits of these moths, and of the precise match of typical and melanic morphs to the bark of younger and older pines, must be carried out before this suggestion can be evaluated.

There remains the question of the nature of this apparent induction effect — is it phenotypic only, or is it genotypic as well (as claimed, for example, by Harrison in the induction experiments previously described)? No definitive answer can be provided, as I have not reared progeny from the adults obtained in these experiments. It is noteworthy, however, that my students and I have reared *P. pallescens* on dozens of other occasions, including many cases where the phenotypes of both parents were known, and that we have never reared a brood where the ratio of typicals to melanics in the F_1 was inconsistent with the assumption that melanism is controlled by a sex-linked, dominant allele (Given, 1971; Hendrickson, 1973). This suggests that phenotype and genotype are normally linked in this species, and raises the intriguing possibility that the induction seen here may involve a genetic change. This suggestion may not be as heretical as it once was, given the previously mentioned recent evidence for such a phenomenon in flax (Cullis, 1985).

A Revised Hypothesis

We have seen that there are problems with the so-called "classical" interpretation of the industrial melanism phenomenon. It is not clear, for example, whether all cases of industrial melanism currently seen in moths arose and spread via mutations and selection, or whether some form of environmental induction has contributed to the situation. A more serious problem, from the point of view of a functional explanation, is the question of whether a close association between melanism in moths and an industrial darkening of resting substrates exists. We have discussed cases where melanism occurs and the trees are not darkened by pollutants (so-called "rural melanism"). We also know that a slow, progressive darkening of moth populations via directional selection may occur (as occurred around Pittsburgh, Penn., after the turn of the century — see Sargent, 1976) and that this process alone could result in a match of moths to their substrates.

These problems might lead one to suggest that the sudden appearance of black morphs in moths is a case of indeterminate evolution, and that chance alone may account for many of the melanic frequencies that presently exist. There are two reasons, however, to reject this view. First, the concurrence of increased melanism in so many species throughout the world over the last 100 to 150 years is highly improbable on the basis of chance alone. Second, the relatively constant frequencies of melanics that are being maintained in many species (e.g., more than 20 years in *Phigalia titea* in Massachusetts, Fig. 8) imply that melanism is under some sort of control, and not merely a drifting, essentially neutral, trait.

If melanism is not a response to a general darkening of trees caused by air-borne pollutants, but does represent an adaptive response to some environmental change that has occurred in relatively recent times, what could that change be? My own view is that the change in question, at least in New England and other parts of North America, is another outcome of human activity — the reversion of forests to earlier seral stages. Many human activities associated with settlement, urbanization and industrialization encouraged the cutting back of forests, whether simply to clear the land for grazing and agricultural purposes, or to provide wood for building purposes and fuel. In New England, for example, virtually all of the area has been cut at least once since 1750 (Bormann and Likens, 1979). As agricultural lands have been abandoned, much of the New England countryside has returned to woodland, although logging and other human activities have prevented a return to the original, pre-settlement conditions. Thus, the hallmark of the European presence in North America has been forest disturbance, and the result is forests whose species composition today is very different from that of the "virgin" forests that the first settlers encountered (Bromley, 1935; Siccama, 1971; Lorimer, 1977).

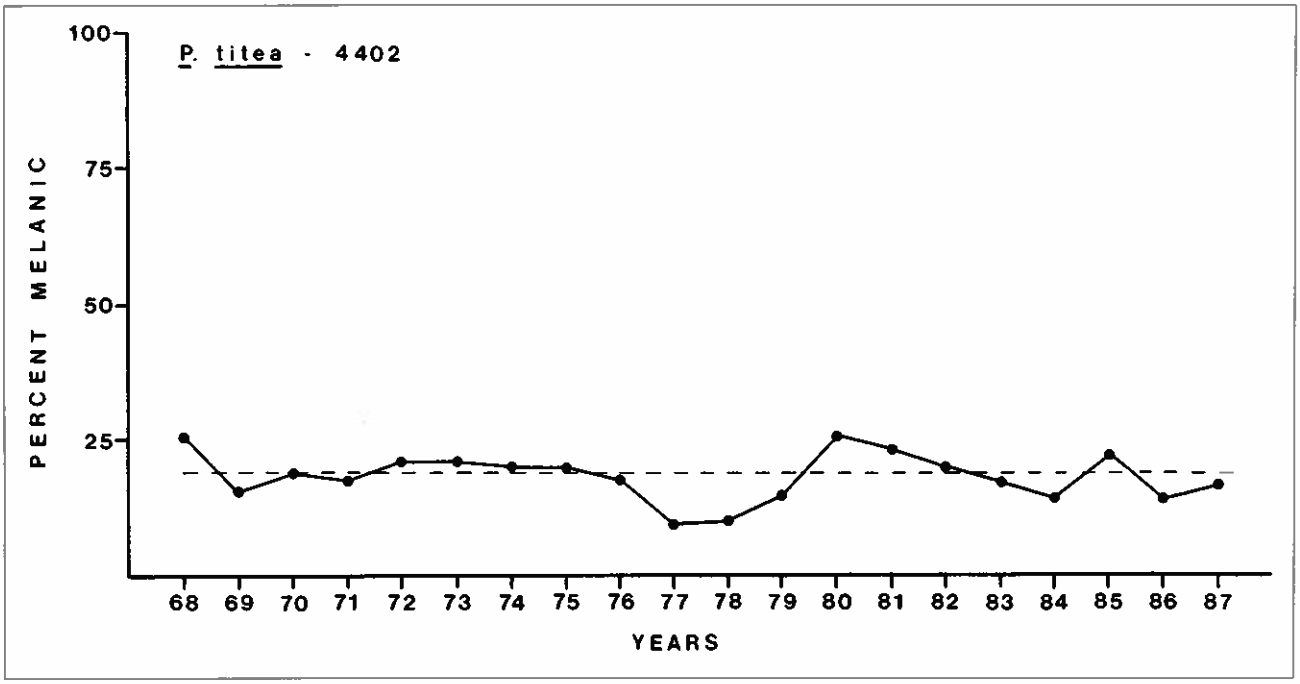


Figure 8. The percent of melanics in yearly samples of adult *Phigalia titea* (Cramer) taken at light sources at a single location in Leverett, Mass., 1968-1987. Total sample = 4402 individuals.

Because of these changes in forest trees, the substrates available to bark-like cryptic moths have also changed, and some of these changes seem to favor melanics. Many of the climax trees in New England have relatively light bark (e.g., beech, maples, oaks, hickories), whereas some of the earlier successional trees have relatively dark bark (e.g., cherries) or variegated black-and-white bark (e.g., white birches, poplars), on which melanic moths may be quite cryptic (Sargent, 1985). Finally, there are some trees whose bark characteristics change with age, such as the previously mentioned white pine, where younger trees have very dark bark and older trees have very light bark. In this case, logging may maintain conditions that favor melanic moths.

The suggestion here is that many cases of melanism may represent a response of moth species to cryptic opportunities provided by changes in the composition of forests that have been disturbed by human activities. Generally, the moths would be adapting to earlier successional stages than those occurring prior to human disturbance, although historically such disturbances might have recurred from time to time through natural processes (e.g., hurricanes, fire, etc.) (Bormann and Likens, 1979; Davis, 1981). In this scenario, melanism might be an ancient trait that was originally associated with forests that no longer flourish, but that do recur from time to time in any geographic area.

The recurrence of earlier conditions, if frequent enough, might provide opportunities for the evolution of inducible melanism. Certainly, any mechanism that would enable moth populations to change in appearance in an appropriate direction as the environment changed would be favored. Ordinarily, one would postulate fortuitous mutations and subsequent selection to arrive at this end, but an inducible change would be a faster and more certain mechanism.

The explanation of melanism advanced here does not differ from the classical explanation in terms of the ultimate cause of the phenomenon. In both cases, a cryptic advantage of the melanic condition is assumed. The two views do differ, however, in that the classical explanation suggests that a single consequence of industrialization — darkening of tree trunks — fits all cases; whereas the present explanation suggests that the changes in forests that follow human disturbance provide a variety of cryptic opportunities for melanic moths. Melanism in this latter case would often be associated with industrialization, but would also occur in response to many other kinds of human activity (clearing, burning, logging, etc.), and so might better be described as *forest disturbance*, rather than industrial, melanism.

The other difference between the classical and the present interpretations involves differing viewpoints on the possibility of induction as a factor in the overall picture. Kettlewell tended to reject the idea of induction (1973), whereas this paper has encouraged further study of the phenomenon. In a sense, the possibility of induction is more compat-

ible with the present ecological interpretation than it is with the classical view, in that a recurring event (succession) might provide opportunities for the evolution of an adaptive induction mechanism, whereas induced melanism would be entirely fortuitous in response to industrialization alone.

References

Barrett, C.G. 1897. **The Lepidoptera of the British Isle.** Reeve and Co., London.

Bishop, J.A. 1972. An experimental study of the cline of industrial melanism in *Biston betularia* (L.) (Lepidoptera) between urban Liverpool and rural North Wales. **J. Anim. Ecol.** 41:209-243.

Bishop, J.A. and L.M. Cook. 1975. Moths, melanism and clean air. **Sci. Amer.** 232:90-99.

Bishop, J.A. and P. Harper. 1970. Melanism in the moth *Gonodontis bidentata*: a cline within the Merseyside conurbation. **Heredity** 5:449-456.

Bishop, J.A., L.M. Cook and J. Muggleton. 1978. The response of two species of moths to industrialization in northeast England. **Phil. Trans. Roy. Soc. London** B 281:489-542.

Boardman, M., R.R. Askew and L.M. Cook. 1974. Experiments on resting site selection by nocturnal moths. **J. Zool.**, London 172:343-355.

Bormann, F.H. and G.E. Likens. 1979. **Pattern and process in a forested ecosystem.** Springer-Verlag, New York.

Bowater, W. 1914. Heredity of melanism in Lepidoptera. **J. Genet.** 3: 299-315.

Brakefield, P.M. 1987. Industrial melanism: do we have the answers? **Trends in Ecol. and Evol.** 2: 117-122.

Bromley, S. W. 1935. The original forest types of southern New England. **Ecol. Monogr.** 5: 61-89.

Chapman, J.W. 1888. On melanism in Lepidoptera. **Entomol. Monthly Mag.** 25:40.

Clarke, C.A. and P.M. Sheppard. 1966. A local survey of the distribution of the industrial melanic forms in the moth *Biston betularia* and estimates of the selective values of these forms in an industrial environment. **Proc. Roy. Soc. London** B 165: 424-439.

Clarke, C.A., G.S. Mani and G. Wayne. 1985. Evolution in reverse: clean air and the peppered moth. **Biol. J. Linn. Soc.** 26: 189-199.

Cook, L.M. and G.S. Manni. 1980. A migration-selection model for the morph frequency variation in the peppered moth over England and Wales. **Biol. J. Linn. Soc.** 13: 179-198.

Cook, L.M., G.S. Mani and M.E. Varley. 1986. Postindustrial melanism in the peppered moth. **Science** 231: 611-613.

Cooke, N. 1877. On melanism in Lepidoptera. **Entomologist** (London) 10: 92-96, 151-153.

Creed, E.R., D.R. Lees and J.G. Duckett. 1973. Biological method of estimating smoke and sulphur dioxide pollution. **Nature** (London) 244: 278-280.

Cullis, C.A. 1977. Molecular aspects of the environmental induction of heritable changes in flax. **Heredity** 38: 129-154.

Cullis, C.A. 1983. Environmentally induced DNA changes in plants. **CRC Critical Reviews in Plant Sciences** 1: 117-131.

Cullis, C.A. 1985. Sequence variation and stress. *In* B. Hohn and E.S. Dennis (eds.), **Genetic flux in plants,** pp. 158-167. Springer-Verlag, New York.

Davis, M. B. 1981. Quaternary history and the stability of forest communities. *In* D.C. West, H.H. Shugart and D.B. Botkin (eds.), **Forest succession: concepts and applications,** pp. 132-153. Springer-Verlag, New York.

Fisher, R.A. 1933. On the evidence against the chemical induction of melanism in Lepidoptera. **Proc. Roy. Soc. London** B. 112: 407-416.

Ford, E.B. 1937. Problems of heredity in the Lepidoptera. **Biol. Rev.** (Cambridge) 12: 461-503.

Ford, E.B. 1975. **Ecological genetics.** 4th ed. Chapman and Hall, London.

Franclemont, J.g. 1938. Description of new melanic forms (Lepidoptera: Geometridae, Noctuidae and Arctiidae). **Entomol. News** 49: 108-114.

Ginevan, M.E. 1971. Genetic control of melanism in *Panthea furcilla* (Packard) (Lepidoptera: Noctuidae). **J. New York Entomol. Soc.** 79: 195-200.

Hailman, J.P. 1982. Evolution and behavior: an iconoclastic view. In H.C. Plotkin (ed.), **Learning, development, and culture,** pp. 205-254. John Wiley and Sons, London.

Haldane, J.B.S. 1935. The rate of spontaneous mutation of a human gene. **J. Genet.** 31: 317-326.

Haldane, J.B.S. 1956. The theory of selection for melanism in Lepidoptera. **Proc. Roy. Soc. London** B. 145: 303-306.

Hamling, T.H. 1905. *Hemerophila abruptaria* - heredity statistics. **Trans. City London Entomol. Soc.** (1905): 5.

Harrison, J.W.H. 1920. Genetical studies in the moths of the geometrid genus *Oporabia (Oporinia)* with a special consideration of melanism in the Lepidoptera. **J. Genet.** 9: 195-280.

Harrison, J.W.H. 1926. The inheritance of wing colour and pattern in the lepidopterous genus *Tephrosia (Ectropis).* II. Experiments involving melanic *Tephrosia bistortata* and typical *T. crepuscularia.* J. **Genet.** 17: 1-19.

Harrison, J.W.H. 1928. A further induction of melanism in the lepidopterous insect, *Selenia bilu-*

naria Esp., and its inheritance. **Proc. Roy. Soc. London** B. 102: 338-347.

Harrison, J.W.H. 1935. The experimental induction of melanism and other effects, in the geometrid moth *Selenia bilunaria* Esp. **Proc. Roy. Soc. London** B 117: 78-92.

Harrison, J.W.H. 1956. Melanism in the Lepidoptera. **Entomol. Record and J. Var.** 68: 172-181.

Harrison, J.W.H. and F.C. Garrett. 1926. The induction of melanism in the Lepidoptera and its subsequent inheritance. **Proc. Roy. Soc. London** B 99: 241-263.

Hendrickson, D. 1977. An investigation into selective agents affecting melanism in the Lepidoptera, with special reference to *Panthea furcilla* (Packard) (Noctuidae). Unpublished Master's project, Department of Zoology, Univ. of Massachusetts, Amherst, Massachusetts.

Holland, W.J. 1903. **The moth book.** Doubleday, New York.

Howlett, R.J. and M.E.N.Majerus. 1987. The understanding of industrial melanism in the peppered moth (*Biston betularia*) (Lepidoptera: Geometridae). **Biol. J.Linn. Soc.** 30: 31-44.

Hughes, A.W.M. 1932. Induced melanism in Lepidoptera. **Proc. Roy. Soc. London** B 110:378-402.

Jones, J.S. 1982. More to melanism than meets the eye. **Nature** (London) 300: 109-110.

Jones, T.K. 1977. Melanism in *Panthea furcilla* (Packard) (Lepidoptera: Noctuidae): field studies in central Massachusetts. **J. New York Entomol. Soc.** 85: 102-114.

Kettlewell, H.B.D. 1955a. Selection experiments in industrial melanism in the Lepidoptera. **Heredity** 9: 323-342.

Kettlewell, H.B.D. 1955b. Recognition of appropriate backgrounds by the pale and black phases of Lepidoptera. **Nature** (London) 175: 943-944

Kettlewell, H.B.D. 1956. Further selection experiments on industrial melanism in the Lepidoptera. **Heredity** 10: 287-301.

Kettlewell, H.B.D. 1965. A 12-year survey of the frequencies of *Biston betularia* L. and its melanic forms in Great Britain. **Entomol. Record and J. Var.** 77: 195-218.

Kettlewell, H.B.D. 1973. **The evolution of melanism.** Clarendon Press, Oxford.

Kettlewell, H.B.D. and D.L.T. Conn. 1977. Further background-choice experiments on cryptic Lepidoptera. **J. Zool.**, London 181: 371-376.

Klots, A.B. 1964. notes on melanism in some Connecticut moths. **J. New York Entomol. Soc.** 72: 142-144.

Klots, A.B. 1966. Melanism in Connecticut *Panthea furcilla* (Packard) (Lepidoptera: Noctuidae). **J. New York Entomol. Soc.** 74: 95-100.

Klots, A.B. 1968a. Melanism in Connecticut *Charadra deridens* (Guenee) (Lepidoptera: Noctuidae). **J. New York Entomol. Soc.** 76: 58-59.

Klots, A.B. 1968b. Further notes on melanism in Connecticut *Panthea furcilla* (Packard) (Lepidoptera: Noctuidae). **J. New York Entomol. Soc.** 76: 92-95.

Lambert, D.M., C.D. Miller and T.J. Hughes. 1986. On the classic case of natural selection. **Riv. di Biol.** 79: 11-49.

Lees, D.R. 1971. The distribution of melanism in the pale brindled beauty moth, *Phigalia pedaria*, in Great Britain. *In* E.R. Creed (ed.), **Ecological genetics and evolution,** pp, 152-174. Blackwell Scientific Publications, Oxford.

Lees, D.R. 1975. Resting site selection in the geometrid moth *Phigalia pilosaria* (Lepidoptera: Geometridae). **J. Zool.**, London 176: 341-352.

Lees, D.R. 1981. Industrial melanism: genetic adaptation of animals to air pollution. *In* J.A. Bishop and L.M. Cook (eds.), **Genetic consequences of man-made change,** pp. 129-176. Academic Press, New York.

Lees, D.R. and E.R. Creed. 1975. Industrial melanism in *Biston betularia*: the role of selective predation. **J. Anim. Ecol.** 44: 67-83.

Liebert, T.G. and P.M. Brakefield. 1987. Behavioral studies on the peppered moth *Biston betularia* and a discussion of the role of pollution and lichens in industrial melanism. **Biol. J. Linn. Soc.** 31: 129-150.

Lorimer, C.G. 1977. The presettlement forest and natural disturbance cycle of northeastern Maine. **Ecology** 58: 139-148.

Maarse, H. and R.E. Kepner. 1970. Changes in composition of volatile terpenes in Douglas fir needles during maturation. **J. Agr. Food Chem.** 18: 1095-1101.

Mani, G.S. 1980. A theoretical study of morph ratio clines with special reference to melanism in moths. **Proc. Roy. Soc. London** B 210: 299-316.

Mani, G.S. 1982. A theoretical analysis of the morph frequency variation in the peppered moth over England and Wales. **Biol. J. Linn. Soc.** 17: 259-267.

Manley, T.R. 1981. Frequencies of the melanic morph of *Biston cognataria* (Geometridae) in a low-pollution area of Pennsylvania from 1971 to 1978. **J. Lepid. Soc.** 35: 257-265.

McDunnough, J. 1942. notes on *Pantheinae* (Lepidoptera, Phalaenidae). **Canadian Entomol.** 74: 93-95.

Merrifield, F. 1890. systematic temperature experiments on some Lepidoptera, in all their stages. **Trans. Entomol. Soc.** London (1890): 131-159.

Merrifield, F. 1891. Conspicuous effects on the markings and colouring of Lepidoptera caused by

exposure of the pupae to different temperature conditions. **Trans. Entomol. Soc.** London (1891): 155-167.

Merrifield, F. 1892. The effects of artificial temperature on the colouring of several species of Lepidoptera. **Trans. Entomol. Soc.** London (1892): 33-44.

Mikkola, K. 1979. Resting site selection by *Oligia* and *Biston* moths (Lepidoptera: Noctuidae and Geometridae). **Ann. Entomol. Fenn.** 45: 81-87.

Onslow, H. 1920a. The inheritance of wing-colour in Lepidoptera. III. Melanism in *Boarmia consortaria* (var. consobrinaria Bkh.). **J. Genet.** 9: 339-346.

Onslow, H. 1920b. Inheritance of wing-colour in Lepidoptera. IV. Melanism in *Boarmia abietaria*. **J. Genet.** 10: 135-140.

Onslow, H. 1921. The inheritance of wing-colour in Lepidoptera. VII. Melanism in *Hemerophila abruptaria* (var. *fuscata* Tutt). **J. Genet.** 11: 293-298.

Owen, D.G. 1961. Industrial melanism in North American moths. **Amer. Nat.** 95: 227-233.

Owen, D.F. 1962. The evolution of melanism in six species of North American geometrid moths. **Ann. Entomol. Soc. Amer.** 55: 695-703.

Robinson, R. 1971. **Lepidoptera genetics.** Pergamon Press, Oxford.

Sargent, T.D. 1966. Background selections of geometrid and noctuid moths. **Science** 154: 1674-1675.

Sargent, T.D. 1968. Cryptic moths: effects on background selections of painting the circumocular scales. **Science** 159: 100-101.

Sargent, T.D. 1969. Background selections of the pale and melanic forms of the cryptic moth, *Phigalia titea* (Cramer). **Nature** (London) 222: 585-586.

Sargent, T.D. 1971. Melanism in *Phigalia titea* (Cramer) (Lepidoptera: Geometridae). **J. New York Entomol. Soc.** 79: 122-129.

Sargent, T.D. 1973. Behavioral adaptations of cryptic moths. VI. Further experimental studies on bark-like species. **J. Lepid. Soc.** 27: 8-12.

Sargent, T.D. 1974. Melanism in moths of central Massachusetts (Noctuidae, Geometridae). **J. Lepid. Soc.** 28: 145-152.

Sargent, T.D. 1976. **Legion of night: the underwing moths.** Univ. of Massachusetts Press, Amherst, Massachusetts.

Sargent, T.D. 1983. Melanism in *Phigalia titea* (Cramer) (Lepidoptera: Geometridae): a fourteen-year record from central Massachusetts. **J. New York Entomol. Soc.** 91: 75-82.

Sargent, T.D. 1985. Melanism in *Phigalia titea* (Cramer) (Lepidoptera: Geometridae) in southern New England: a response to forest disturbance? **J. New York Entomol. Soc.** 93: 1113-1120.

Sargent, T.D. 1987. On the relative acceptability of the typical and melanic morphs of *Panthea pallescens* McDunnough (Lepidoptera: Noctuidae) to birds. **J. New York Entomol. Soc.** 95: 495-503.

Sermonti, G. and P. Catastini. 1984. On industrial melanism: Kettlewell's missing evidence. **Riv. di Biol.** 77: 35-52.

Shapiro, A.M. 1976. Seasonal polyphenism. **Evol. Biol.** 9: 259-333.

Siccama, T.G. 1971. Presettlement and present forest vegetation in northern Vermont with special reference to Chittenden County. **Amer. Midl. Nat.** 85: 153-172.

Steward, R.C. 1976. Experiments on resting site selection by the typical and melanic forms of the moth *Allophyes ocyacanthae* (Caradrinidae). **J. Zool.,** London 178: 107-111.

Steward, R.C. 1977a. Industrial melanism in the moths *Diurnea fagella* (Oecophoridae) and *Allophyes oxyacanthae* (Caradrinidae). **J. Zool.,** London 183: 47-62.

Steward, R.C. 1977b. Industrial and non-industrial melanism in the peppered moth, *Biston betularia* (L.). **Ecol. Entomol.** 2: 231-243.

Steward, R.C. 1977c. Further experiments on resting site selection by the typical and melanic forms of the moth, *Allophyes oxyacanthae* (Caradrinidae). **J. Zool.,** London 181: 395-406.

Steward, R.C. 1977d. Melanism and selective predation in three species of moths. **J. Anim. Ecol.** 46: 483-496.

Steward, R.C. 1985. Evolution of resting behaviour in polymorphic industrial melanic moth species. **Biol. J. Linn. Soc.** 24: 285-293.

Thomsen, M. and H. Lemcke. 1933. Experimente zur Erzielung eines erblichen melanismus bei dem Spanner *Selenia bilunaria* Esp. **Biol. Zentralbl.** 53: 541-560.

von Rudloff, E. 1972. Seasonal variation in the composition of the volatile oil of the leaves, buds, and twigs of white spruce (*Picea glauca*). **Canad. J. Botany** 50: 1595-1603.

von Rudloff, E. 1985. The leaf oil terpene composition of eastern white pine, *Pinus strobus* L. **Flavour and Fragrance J.** 1: 33-35.

West, D.A. 1977. Melanism in *Biston* (Lepidoptera: Geometridae) in the rural central Appalachians. **Heredity** 39: 75-81.

Whittle, P.D.J., C. Clarke, P.M. Sheppard and J.A. Bishop. 1976. Further studies on the industrial melanic moth *Biston betularia* (L.) in the northwest of the British Isles. **Proc. Roy. Soc. London** B 194: 467-480.

Relationships Between Visual Characteristics of Rainforest Butterflies and Responses of a Specialized Insectivorous Bird

PENG CHAI

Synopsis

The responses of five rufous-tailed jacamars (*Galbula ruficauda*, two adults and three hand-reared young) to local butterflies (1,679 individuals of 140 morphs) were investigated in a series of feeding experiments. In each experiment, a single bird was presented with approximately 15 individual butterflies, each a different morph. Its responses were then observed for four hours. The birds showed considerable discrimination in their attacks and consumption of butterflies. Based on their responses, the butterfly morphs could be clearly divided into an unacceptable (either sight-rejected or taste-rejected) group and an acceptable group, with relatively fewer morphs of intermediate acceptability in between.

Jacamars responses were closely associated with the color patterns, flight behaviors, and morphologies of the butterflies. Unacceptable butterflies typically have conspicuous and mimetic color patterns, fly regularly, and have long, slender bodies. The majority were ignored by the birds; however, if attacked, they could be more easily captured. Most acceptable butterflies are non-mimetic, have cryptic color patterns on the resting side (usually the underside) of their wings, fly erratically, and have short, stout bodies. They were attacked readily but more often escaped the birds' attacks. The five birds behaved very similarly in feeding experiments, and individual differences, though statistically significant, contribute little to a description of their responses.

The present study indicates that, to the jacamars, the local butterfly community is organized according to several categories such that the acceptability of butterflies is closely reflected by their color pattern, flight and morphological characteristics. Young, naive jacamars can learn these categories and behave like adults in selecting prey butterflies in a relatively short period of time. The close relationship between butterfly phenotypic characteristics and their palatabilities is presumably molded by selective pressures from a complement of local predators in which some, like the jacamars, are relatively specialized on butterflies, whereas others are opportunistic butterfly predators.

Introduction

Our knowledge of prey selection behavior of visual predators comes largely from studies about prey color pattern and mimicry (see reviews in Rettenmeyer, 1970; Ford, 1975; Turner, 1977; Brower, 1984; Huheey, 1984), taste aversion learning (Domjan, 1980; Brower and Fink, 1985), and optimal foraging theory (Pyke et al., 1977; Pyke, 1984). Most experimental studies in this field were conducted in simplified, often unnatural, and tightly controlled settings (Schluter, 1981).

In nature, however, predators such as omnivorous and insectivorous vertebrates, the predator type most frequently used in the above studies, face a large variety of potential prey with different appearance, profitability and antipredator defenses. Under these conditions, a diverse suite of unprofitable or harmful prey needs to be discriminated and avoided. It is difficult to infer from simplified experiments how predators will select prey in a more complicated situation (Zach and Smith, 1981; Sutherland and Anderson, 1987). The present study uses a naturalistic approach in which local free-flying butterflies were offered to rufous-tailed jacamars (*Galbula ruficauda*) in feeding experiments designed to investigate the response patterns of a specialized predator to a variety of potential prey they attack in the field.

Many problems faced by jacamars and other predators are due to the antipredator defenses of their potential prey. In general, a complete predation sequence has five distinct stages (Fig. 1 modified from Endler, 1986). Antipredator defenses of

Texas Parks and Wildlife Department, 4200 Smith School Road, Austin, Texas 78744

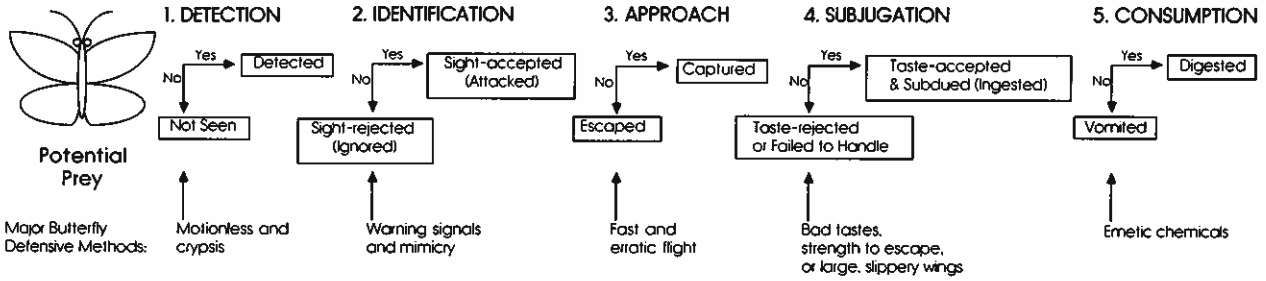


Figure 1. Five stages of a butterfly predation sequence by a visually foraging predator and major defensive methods of butterflies.

prey serve to block the progression of this sequence. Prey, through morphological, behavioral, and physiological adaptations, can impose limitations on the predator's abilities to detect, capture and consume them, and predator foraging tactics and prey defensive tactics are closely interrelated. Butterflies are known to possess highly diverse protective methods functioning in all five stages of the predation sequence (Fig. 1). The present study seeks to identify the butterfly characteristics that are closely related to the jacamars' responses.

In previous feeding experiments, two adult jacamars exhibited considerable discriminatory, attacking, and handling capabilities on local butterflies (Chai, 1986). Several color pattern and flight pattern characteristics of the 114 butterfly morphs tested in that study were shown to be closely associated with the responses of the two jacamars. The present study further examines the nature and development of these associations using two adult and three naive young jacamars in more controlled feeding experiments.

Methods

Study site and study organisms

This study was conducted at Sirena station (8°28'N, 83°35'W) in the Corcovado National Park on the Osa Peninsula of southwestern Costa Rica. The vegetation is lowland tropical rainforest (described by Hartshorn 1983). At the study site, extensive second growth of different stages, mostly created by past agricultural activities before the establishment of the park in 1975, is interspersed among patches of forest that have also been disturbed to different extents in the past. These habitats support a large resident population of the rufous-tailed jacamar and hundreds of species of butterflies (Papilionoidea, DeVries, 1987 described Costa Rican butterflies except for Lycaenidae and Riodinidae).

The black-chinned race of the rufous-tailed jacamar (*G. ruficauda melanogenia*) in the neotropical family Galbulidae is common in the lowland forests of Central America. They catch insects by sallying, and often further pursue the escaping prey. The long, slender bill of these agile, aerial insectivores facilitates handling prey with large bodies and/or wings. Consequently, their diet includes a high proportion of large winged insects (Sherry 1983). At Corcovado, wild rufous-tailed jacamars were observed only to eat flying insects, and frequently attack and consume a variety of butterflies (Chai 1986).

Data for the present study were collected over a two-year period: from April to October 1984 and from February to August 1985. I studied a total of five birds: one Adult Female (at least four years old, based on its leg band) and one Young Female (Young Female 1) in 1984; one Adult Male (at least three years old, based on bird band), one Young Female (Young Female 2), and one Young Male in 1985. The three juvenile birds were collected from their nest tunnels shortly before fledging. The two young studied in 1985 came from the same brood (two males and one female having been observed feeding the four nestlings of this brood).

The young were hand-fed using a pair of tweezers until they could catch insects independently, at which point feeding experiments commenced. Initially, their attack behavior looked awkward, and unsuccessful attacks were frequent. The first feeding experiment with Young Female 1 started 73 days after it was caught, 22 days for the Young Female 2, and 23 days for the Young Male. At this age the young were characterized by having short bills, about two-thirds of the adult length (their bills grew from about 32 mm to 40 mm during the experimental period). Wild juvenile jacamars were also observed foraging by themselves about 20 days after fledging, although they still received food from the parents from time to time. A juvenile in the field was seen to be able to capture and consume a butterfly successfully 45 days after fledging.

All birds were fed a diet of live insects and did not show any signs of illness throughout the experi-

mental period. Their diet was comprised of locally caught palatable insects, mostly dragonflies, cicadas, and moths, but also grasshoppers, katydids, wasps, and flies. Butterflies were used as prey only during the feeding experiments. The birds were fed about five times each day: early morning, mid-morning, noon, early afternoon and before dark. Each bird received 7 to 8 g of insects daily and was weighed weekly so that it could be maintained at similar weights throughout its experimental period. The body weights of study birds measured during the experimental period are lighter than the weights when they were first caught so they were probably hungrier than in the wild. Indeed, my young birds more frequently emitted begging calls than the wild fledglings that I had followed and observed. The average body weight measured during the experimental period of Jacamar Adult Female was 25 ± 1.2 g (±1 SD, 86 percent of the weight when it was first caught in the field), 24 ± 0.8 g (85 percent) for Jacamar Adult Male, 23 ± 1.1 g (85 percent) for Jacamar Young Female 1, 23 ± 0.8 g (83 percent) for Jacamar Young Female 2, and 25 ± 1.1 g (83 percent) for Jacamar Young Male.

Local butterflies (Papilionoidea) netted or trapped in the park were used for feeding experiments. Those with wing lengths less than 3 cm were generally excluded because they were too small to measure and handle; thus, most lycaenids and riodinids were excluded (Appendix 1). Only healthy and active butterflies were used. They were kept either in an insectary or separately in butterfly envelopes. If butterflies needed to be maintained for several days, I fed them sugar water.

Morphological traits of butterflies

Prior to the feeding experiment, each butterfly was identified and sexed. Four easily obtainable morphological traits of most tested butterflies were measured: body weight (to the nearest 0.01 g), body length (mm, from tip of head to tip of abdomen), wing length (mm, from base of forewing to the forewing apex), and thoracic width (to the nearest 0.1 mm, measured using a pair of calipers). Several individuals of each species were collected as vouchers for comparison of color patterns.

Wing areas of butterflies were measured from wings that were knocked off by jacamars during handling or wings of collected specimens. The fore- and hindwing of one pair of the wings of an individual butterfly were first arranged and overlapped in a normal manner. Next, they were photocopied, and wing area was measured by tracing the outline of its photocopy using a planimeter, or cutting out the picture, weighing it, and then comparing it with the weight of a unit area (only a small proportion of wings were measured using the latter method).

Four morphological variables were derived by standardizing for body weight. They are relative thoracic width, relative body length, relative wing length and relative wing area, which are measured as deviations (standardized residuals) from a best-fit line relating the respective morphological trait to body weight (detailed in Chai, 1987). These relative values can show the relative size of the traits with respect to that of other butterflies in the same community without the confounding effects of body size differences (see Harvey and Mace, 1982). These four derived morphological variables were used in the correlation analyses with jacamar responses.

Butterfly flight pattern

After capturing a butterfly in the field, it was taken back to the insectary as quickly as possible, taking care that it was active and undamaged. The butterfly was released in the insectary (7.2 x 3.6 m wide, 1.8 m high, Chicopee saran shade cloth covered cage) and its flight pattern was recorded with a video camera (GE, model 1CVC5036E; recorder: RCA, VKP900), which makes 30 complete images per second. From these recordings, the flight paths and postures of the butterflies were traced. This tracing allowed me, in a general way, to compare the butterflies for speed and degree of irregularity (fluctuations) in their flight paths (see Chai, 1987 and Chai & Srygley, in press for details).

Feeding experiments

Single birds were housed in a cylindrical wire mesh cage lined with nylon net (Fig. 2). It was hung under a tree. (This kind of cage was also used to keep the young birds before they were available for feeding experiments.) The cage was designed to allow the bird enough room to chase and catch insects, and to see all the surrounding butterflies easily. This cage size also enabled the observer to maintain an ample visual field to record the behavior of the bird and offered butterflies. The bird usually perched at the middle of the upper branch, and sallied out for the prey. In the previous study with two captive jacamars, one was tested in the same-sized cage; the other in a 12 m^3 aviary. Their responses to butterflies were similar to each other and also to the responses of jacamars observed in the field. Thus, I concluded that this cage size would not significantly alter jacamar behavior during the experiments (cf., Neumann & Klopfer, 1969).

Fourteen to 20 individual butterflies (average 15.8 ± 1.8 SD individuals per feeding experiment, Fig. 2), each representing a distinct butterfly morph, were used in each experiment. Each butterfly morph usually means one species. However, I refer to butterfly morphs instead of species for two reasons: first, some species contain two distinct color morphs and were split accordingly; secondly, some congeners are so similar that they can be distinguished

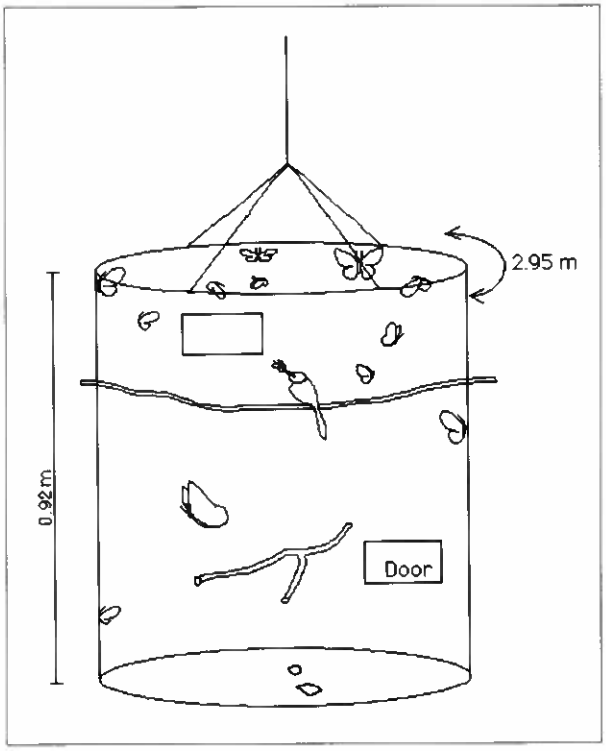


Figure 2. A diagram of the feeding experiments.

only by close examination and are combined and treated here as a single morph (butterfly species combined as one morph for analysis do not show any acceptability difference to jacamars, see Appendix 1). Butterflies were presented together rather than one at a time so that the bird had to select from a variety of potential prey. The ratio of unacceptable to acceptable butterflies presented in each experiment was about 2:1 (relative palatability was based on the responses of the two jacamars in Chai, 1986). In jacamar's habitat, the natural ratio of unacceptable to acceptable butterflies may be closer to 1:1 (Chai, pers. obs.). Using this proportion of unacceptable to acceptable butterflies made it harder for the experimental bird to select prey, and kept the bird hungry and responsive. With a selection of butterflies in its cage, the bird had to discriminate constantly between prey items. I usually avoided using the same butterfly morph in two consecutive feeding experiments so the bird would not be able to memorize a single morph easily. Just before the beginning of each feeding experiment, I offered the bird two to four dragonflies one after another (jacamars generally prefer dragonflies to butterflies, especially when the released butterflies contained many distasteful ones). This engaged its attention while I released all the experimental butterflies as soon as possible. Thus, when the bird finished handling and consuming the dragonflies, it was surrounded by a variety of butterflies engaged in different behaviors: flying, flapping wings while climbing the cage wall, or perching motionlessly, etc. In most cases, the bird would immediately begin attacking the offered butterflies. The total mass of the consumed dragonflies was about 0.5 g, but the repletion mass for a jacamar is about 2.0 g, so the bird was still hungry and responsive (Chai, unpubl. data). During the feeding experiment, I sat next to the cage to record my observations, taking care not to disturb the bird or the butterflies. However, I would occasionally attempt to activate a motionless butterfly by moving a long stick in the area where it was perched. All unkilled butterflies as well as any wings of the killed butterflies knocked off during handling were removed after a feeding experiment, and the bird was subsequently fed with local insects other than butterflies.

Each feeding experiment lasted four hours, with one experiment per bird on a particular day. Since jacamars recognize butterflies and other winged insects as prey only through their movement, the

Table 1. Total number of morphs and individuals of butterflies tested with each of the five rufous-tailed jacamars. The degree of overlap between two sets of butterflies is expressed by Horn's (1966) R_o which varies from 0 when these two sets are completely distinct (containing no morph in common), to 1 when the sets are identical with respect to proportional morph composition.

	Adult Female	**Adult Male**	**Young Female 1**	**Young Female 2**	**Young Male**
Number of butterfly morphs	86	111	107	101	99
Number of individual butterflies	293	315	469	296	306
Overlap measures:					
Adult Female	1.000	0.799	0.853	0.810	0.807
Adult Male		1.000	0.814	0.854	0.812
Young Female 1			1.000	0.852	0.848
Young Female 2				1.000	0.938
Young Male					1.000

long experimental period provided the bird ample time to view all the experimental butterflies. Also as the time passed, the bird presumably became hungrier, thus the long period allowed me to observe its behavior under increasing hunger state. However, the long period of viewing caged butterflies in close distances probably made it easier for the birds to identify specific butterflies, whereas, in nature, the bird probably detected most butterflies as they flew by and had very little time to view and make its decision to attack. The feeding experiment was generally conducted every other day. This enabled me to collect butterflies on intervening days. Most of the feeding experiments were started between 12:30 and 13:30 hours (wild jacamars were observed to forage intermittently throughout the day).

The combination of specific butterfly morphs used in a feeding experiment varied and mostly depended on what could be collected in the previous day(s). Consequently, both morphs and individuals of tested butterflies as well as their presenting sequence varied from bird to bird. However, because each bird was tested with a large number of local butterflies, as a whole the five birds were tested with similar sets of butterflies in terms of proportional morph composition. Table 1 expresses the degree of overlap of butterfly sets tested between two birds based on Horn's (1966) overlap index (R_o) derived from information measures.

In each experiment, I closely observed not only the bird's response but also the butterflies' behavior in the cage. I recorded which butterfly was attacked, the time of the attack, whether the butterfly was successfully captured or not, and the bird's response to the butterfly after it was captured. I paid special attention to the behavior of the butterfly immediately before the bird initiated its attack (the bird often stared at the prey for a brief moment before sallying out). When the butterfly was captured, I recorded the bird's handling behavior, *i.e.*, how many times the bird beat the butterfly against the branch and how long the bird handled the butterfly. During feeding experiments, many butterflies were observed to escape, even repeatedly, from the jacamar's attacks under the test conditions where the butterfly could not escape from the experimental cage. These unsuccessful attacks were recorded.

Individuals of a given butterfly morph were continuously used until the bird evinced a consistent response to this morph, that is, until individuals of a particular butterfly morph were consistently eaten or ignored in at least three consecutive feeding experiments. When these responses were observed, I concluded the bird had learned the palatability of this particular butterfly morph and would

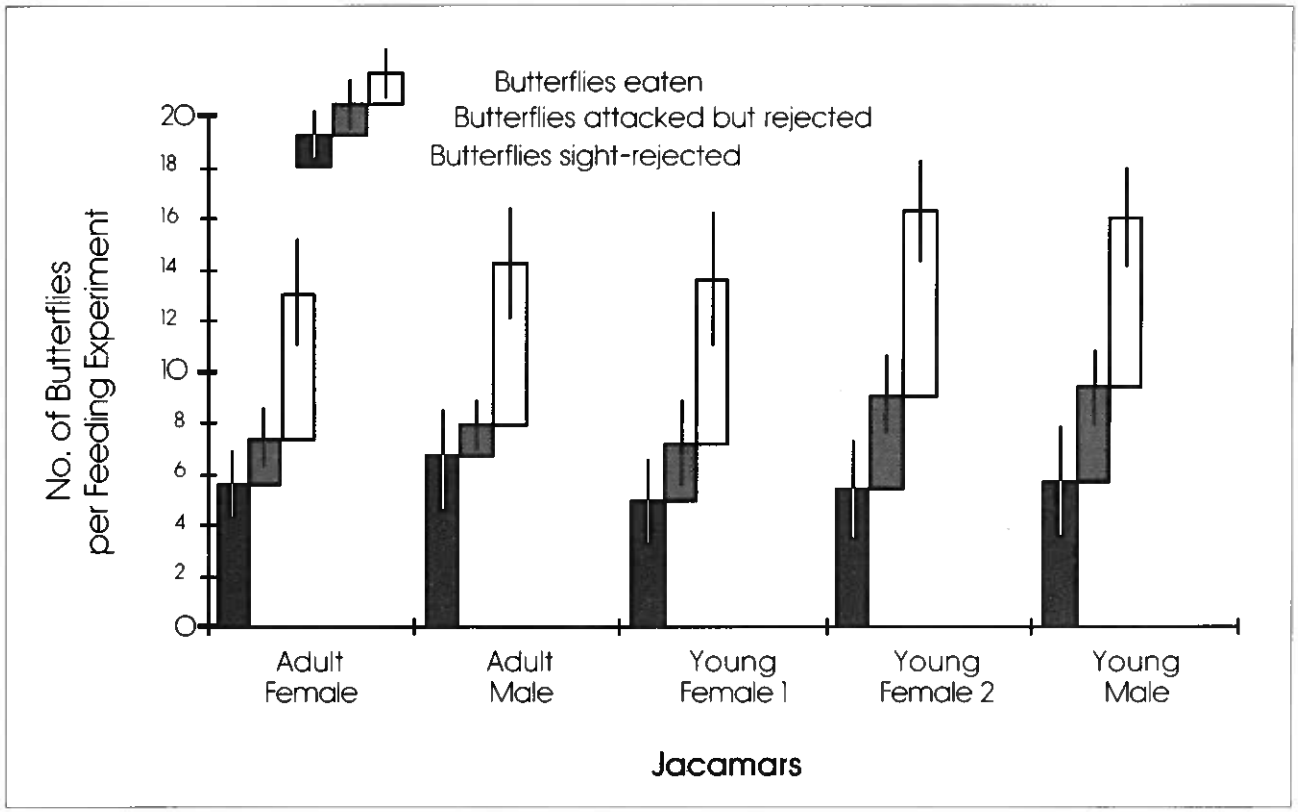


Figure 3. Summary of the responses of five rufous-tailed jacamars in feeding experiments. The mean and ±1 S.D. of butterflies in feeding experiments under each response category were shown.

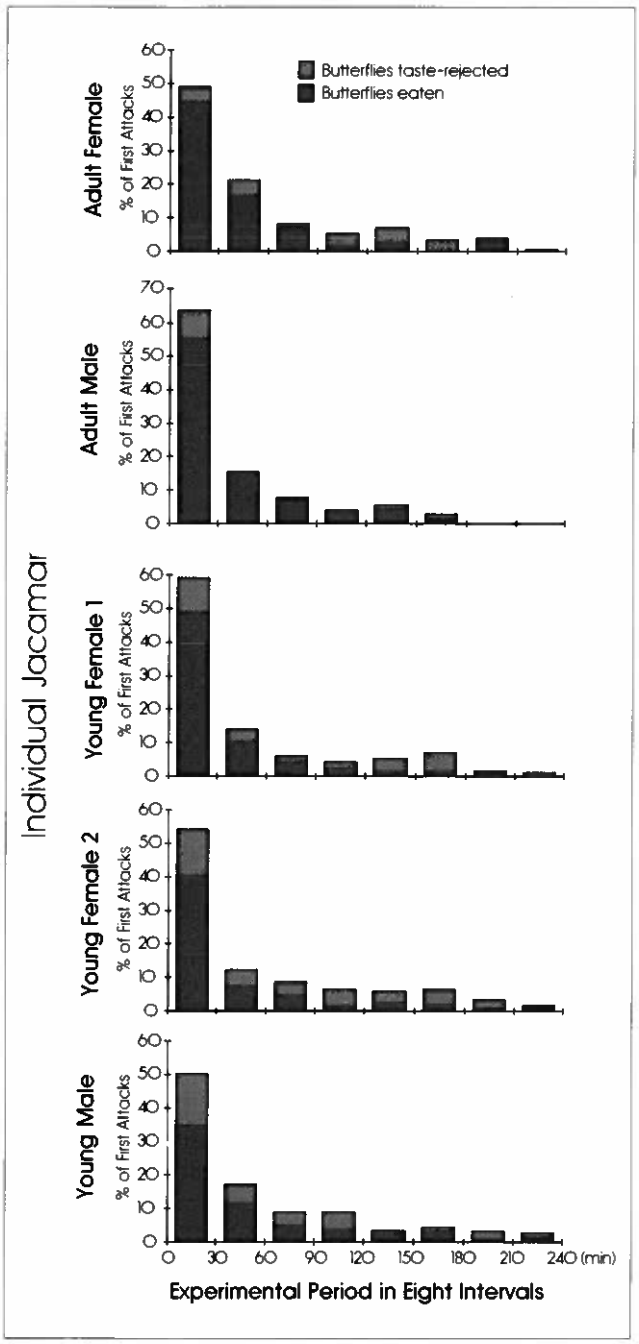


Figure 4. Percentage distributions of records of the time of first attack on a given individual butterfly during the experimental period by each jacamar. Attacks within each time interval were further divided into those in which the attacked butterflies were taste-rejected, and those eaten.

often stop using individuals of this morph for feeding experiments. In this way, more butterfly morphs could be tested. However, many species of butterflies were just too rare or too hard to capture in large enough numbers during the experimental periods for adequate testing.

Results

General response pattern of jacamars

Fig. 3 summarizes the responses of the five jacamars to a total of 1,839 Lepidoptera in 115 feeding experiments. For most butterflies, the response of a jacamar to an individual butterfly in a feeding experiment can be summarized and assigned to one of the three categories (cf., Fig. 1): (1) **Sight-rejected**, ignored, and not attacked during the entire four-hour period, (2) **Taste-rejected**, attacked but rejected after tasting once or several times, (3) **Eaten**, attacked and accepted. Thus, all rejections observed were either on sight or by taste. The jacamars were never observed vomiting or showing signs of illness after consuming butterflies (however, another jacamar was observed vomiting in other feeding experiments, see Chai, 1988 and Swynnerton, 1915b; Brower, 1969).

(A group of 24 day-flying moths and 86 artificially painted *Heliconius* butterflies were used in feeding experiments; however, they are excluded from the present analyses because of their different taxonomy or treatment. Fifty butterflies were attacked but failed to capture, subdue or consume [Chai, 1987]. This leaves a total of 1,679 individual butterflies of 140 morphs with determined acceptability [see Appendix 1]. Table 1 shows the total number of morphs and individuals of butterflies tested according to each of the five birds.)

The three response categories — sight-rejected, taste-rejected and eaten — are separated by two major decision points made by the bird (Fig. 1). The information for the first decision, to attack or not, presumably only comes from distant visual inspection of the butterfly (most birds have very poor sense of smell, Kare and Mason, 1986). After a successful attack, the second decision, to eat or not, is made on the captured butterflies. Now the bird can closely view and taste this butterfly as well as, presumably, smell the odor and feel the body texture of this butterfly (many unacceptable butterflies have a tough and flexible thorax). Thus, several types of information become available, and so-called taste-rejection may be due to reasons other than bad taste.

From the point of view of butterfly survival, taste-rejected butterflies can further be divided into those that survived, and those that were killed in the process of being taste-rejected (butterflies that could not fly after having been rejected by the bird were considered killed).

The five birds showed very similar response patterns (Fig. 3). The majority of the butterflies tested fell into the "sight-rejected" and the "eaten" categories. The three young birds sampled the butterflies more frequently and, on the average, taste-rejected a higher proportion of butterflies (17 percent, 23 percent and 23 percent, respectively) than the two adult birds (14 percent and 9 percent). All the birds tended to attack butterflies at the beginning of the feeding experiment with most of the attacked morphs (individuals) sampled during the first half hour (Fig. 4). As time passed, the number of jacamar attacks dropped quickly. Those butterflies attacked by the bird for the first time later (say, after an hour) in a feeding experiment tended to be those taste-rejected. Thus, the bird seemed to expect certain butterflies to be unacceptable and was reluctant to attack; however, as the time passed and the bird presumably became hungrier, it became more willing to sample butterflies previously ignored. Since a higher proportion were taste-rejected, this indicates that the bird was initially right, *i.e.*, not to attack the butterfly that was unacceptable.

The considerable discrimination in the jacamars' attack and consumption associates with many phenotypic traits of local butterflies. These relationships are examined in the following sections.

Butterfly color patterns and jacamar responses

The color pattern of a butterfly morph was assessed in terms of its resemblance with the general background. Except for the sky, plant materials essentially compose the entire visual background in a rainforest. Since all butterflies are diurnal and fly in different light and microhabitat conditions, their color patterns cannot be compared against the specific background on which they rest as in Endler's (1984) work on moths. In the present study, the degree of crypsis was assessed by comparing the resemblance of a butterfly's color pattern to plant parts, *e.g.*, leaves, bark, epiphytes or forest floor litter. Some contrastingly patterned butterflies that are seemingly cryptic from a distance but are aposematic at close range are not classified as cryptic here (cf., Papageorgis, 1975; Endler, 1981; Rothschild, 1981).

The color patterns on the two wing sides of many butterflies are very different. Most butterflies close their wings when resting, and their resting color pattern is that on the wing underside. A few butterflies (*Hamadryas* species and most riodinids), however, keep their wings open when resting, and their resting color pattern is that on the wing upperside. The crypsis of the color patterns on the two sides of the wings is evaluated separately.

I identified four attributes that characterize the appearance of plant parts, and a cryptic color pattern should also express these. First, the color should be green, brown or gray; these are the colors commonly shown by leaves, bark or epiphytes. Second, patterns should show details, *i.e.*, fine, irregular lines resembling veins or fissures, and dots and blotches resembling lenticels, fungal infection or herbivore damaged areas. Third, the surface of plant parts is usually not as smooth as that of a butterfly's wings. This is especially true for dead and crumpled leaves, the objects resembled by most cryptic butterflies. Hence, the color hue, brightness and saturation on a butterfly's wings, like these plant parts, should not be uniformly distributed. Fourth, colors should not be boldly, orderly and contrastingly patterned with abrupt boundaries.

A categorizing system based on these four plant characteristics was used to evaluate the degree of crypsis of local butterfly color patterns (see Chai, 1987 for details). Under each trait, three scores were assigned to the color pattern of the considered butterfly morph with "-1" representing the cryptic state, "0" intermediate and "+1" conspicuous. Each characteristic was evaluated independently, and a sum "conspicuous-crypsis score" was finally determined with nine possibilites ranging from "-4," the most cryptic (showing all four attributes characterizing the appearance of plant parts), to "+4," the most conspicuous (with no resemblance to plant parts), and was assigned to the color pattern considered.

Using pooled data, Fig. 5 shows that the birds tend to attack and consume more cryptic butterflies, and reject more conspicuous butterflies. This trend is more clearly shown in the resting color pattern than in that of the opposite color pattern. The resting color pattern is usually more cryptic than that of the opposite side. The opposite wing patterns of most butterflies do not show cryptic details and hue variation; thus, they lack detailed resemblance to plant parts. Many acceptable butterflies, such as charaxines and morphines, have highly cryptic undersides (the resting sides) as opposed to very conspicuous uppersides. For discussion purposes, based on the resting color pattern, the experimental butterflies can be further categorized into three groups: cryptic (score: -4 to -1), intermediate (0 to +2), and conspicuous (+3 to +4).

Flight patterns of butterflies

After comparing the flight patterns of approximately 120 species (1 to 3 individuals per species) of Corcovado butterflies from video recordings, I found clear variations among species in several flight pattern parameters: speed, acceleration, flight path, and wingbeat frequency and amplitude. I could distinguish two unambiguous types of flight, and species could be classified as erratic fliers or regular fliers. This typification is based on the way that butterflies flap their wings and the path of their

flight. Although erratic fliers usually fly faster than regular fliers, flight speed is not used for distinction here.

Erratic fliers beat their wings more deeply (wingstroke amplitude > 120°, Appendix 1), and each wingbeat causes the body to jerk upwards to create a bouncing and fluctuating flight path. They then glide horizontally or downward before initiating another wingbeat. There exist noticeable variations between different subfamilies. Morphine, brassoline, and satyrine butterflies typically fly more slowly and with greater fluctuations by making steeper downward glides after each wingbeat and waiting a relatively longer time before initiating another stroke. On the other hand, charaxine and nymphaline butterflies tend to glide horizontally, flying faster but with less fluctuation. Indeed, some members in these two subfamilies fly so quickly and erratically that only blurred images were shown in my recordings.

The bodies of regular fliers do not suddenly lift upwards after each downstroke but maintain a smooth and relatively straight flight path. Unless escaping, they do not beat their wings deeply (wingstroke amplitude < 120°, Appendix 1), and they show a generally slow, fluttering flight. *Parides*, acraeines, *Heliconius*, and ithomiines are typical fluttering fliers. Less typical ones such as *Battus*, non-*Heliconius* heliconiines, and some distasteful lycaenids and riodinids fly relatively faster but still maintain a straight flight path.

In the cage, those butterflies that were observed in the field flying slowly and regularly would maintain this behavior and repeatedly circle the cage. When not flying, they tended to flap their wings while climbing the cage wall. They were also calmer and less disturbed by the bird's activities. Those butterflies that flew fast and erratically in the field would also display this flight behavior. Because of enclosure by the cage, especially when disturbed by the bird, they often repeatedly bounce against the cage wall. When not flying, they tended to perch motionlessly on the wall. Jacamars are active birds. During the feeding experiment, the jacamar's movements in the cage would often disturb the experimental butterflies. All butterflies, when disturbed, would exhibit faster and more erratic escape flight than their normal flight. This behavior would catch the bird's attention,

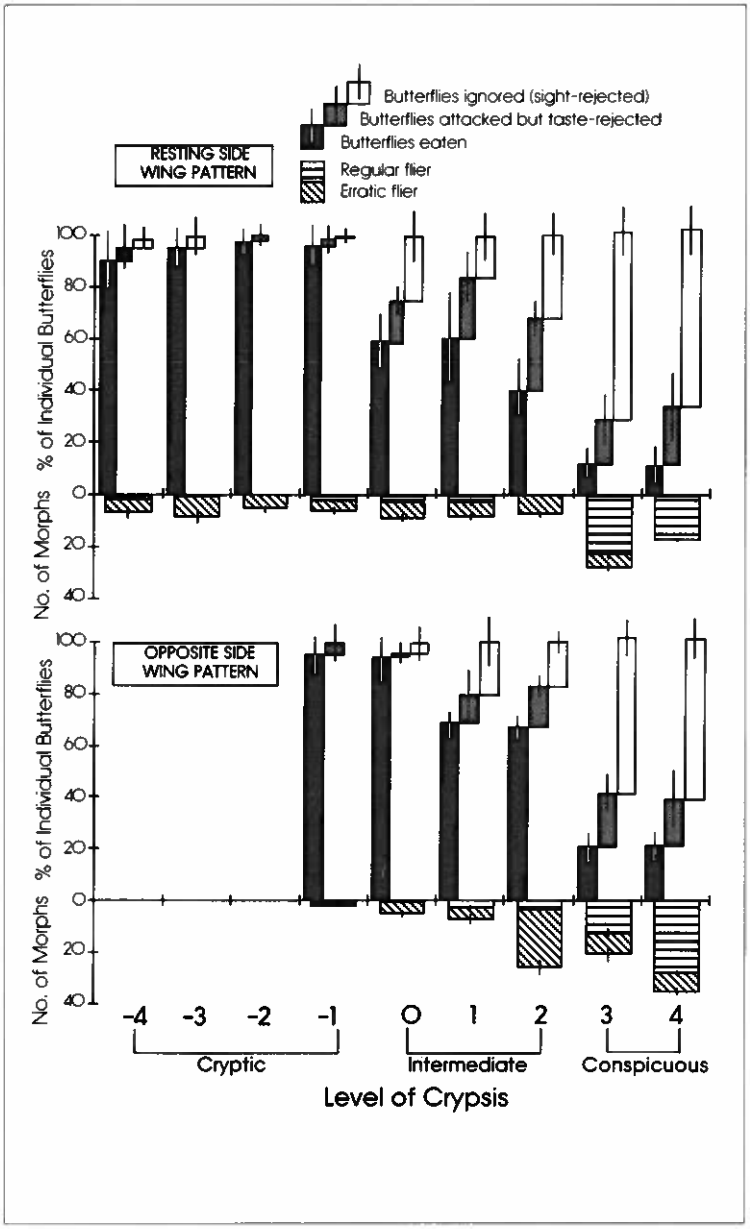


Figure 5. Relationships between jacamar responses and butterfly color patterns. The percentage mean and ±1 S.D. of butterflies in each response category for the five birds were shown.

and attacks were frequently initiated by just such movements.

Butterfly morphological characteristics and jacamar responses

The four morphological traits (thoracic width, body length, wing length and wing area) were chosen because they might correlate with flight pattern traits (the aerodynamic and thermoregulatory effects of diffierent butterfly morphologies are discussed in Chai and Srygley, in press; Srygley and Chai, in press). The first three measurements were collected from a total of 1,613 butterflies just before they were presented to the birds in feeding experiments. Wing area measurements came from 506 individual butterflies.

Relative thoracic width — In Neotropical butterflies, thoracic width positively correlates with thoracic mass (R. Dudley, unpub. data). A relatively wider thorax can contain more flight muscles which probably increases flight speed and potential acceleration. The larger thoracic mass should make this type of prey more profitable (Pyke, 1984). Erratic fliers generally have wider thoraxes than regular fliers of the same weight. Fig. 6 shows that butterflies with relatively wider thoraxes tended to be attacked and consumed, whereas butterflies with narrower thoraxes tended to be sight- or taste-rejected.

Relative body length — Shorter bodies may enhance the escape ability by making it easier for a butterfly to manouver and harder for a predator to seize the body. Butterfly wings are slippery and pieces can be broken off when pecked at by the predator, and the butterfly escape. A butterfly grabbed by the body is unlikely to escape. Even if it does, organ damage may reduce its time of survival. Fig. 6 shows that the birds tended to attack and

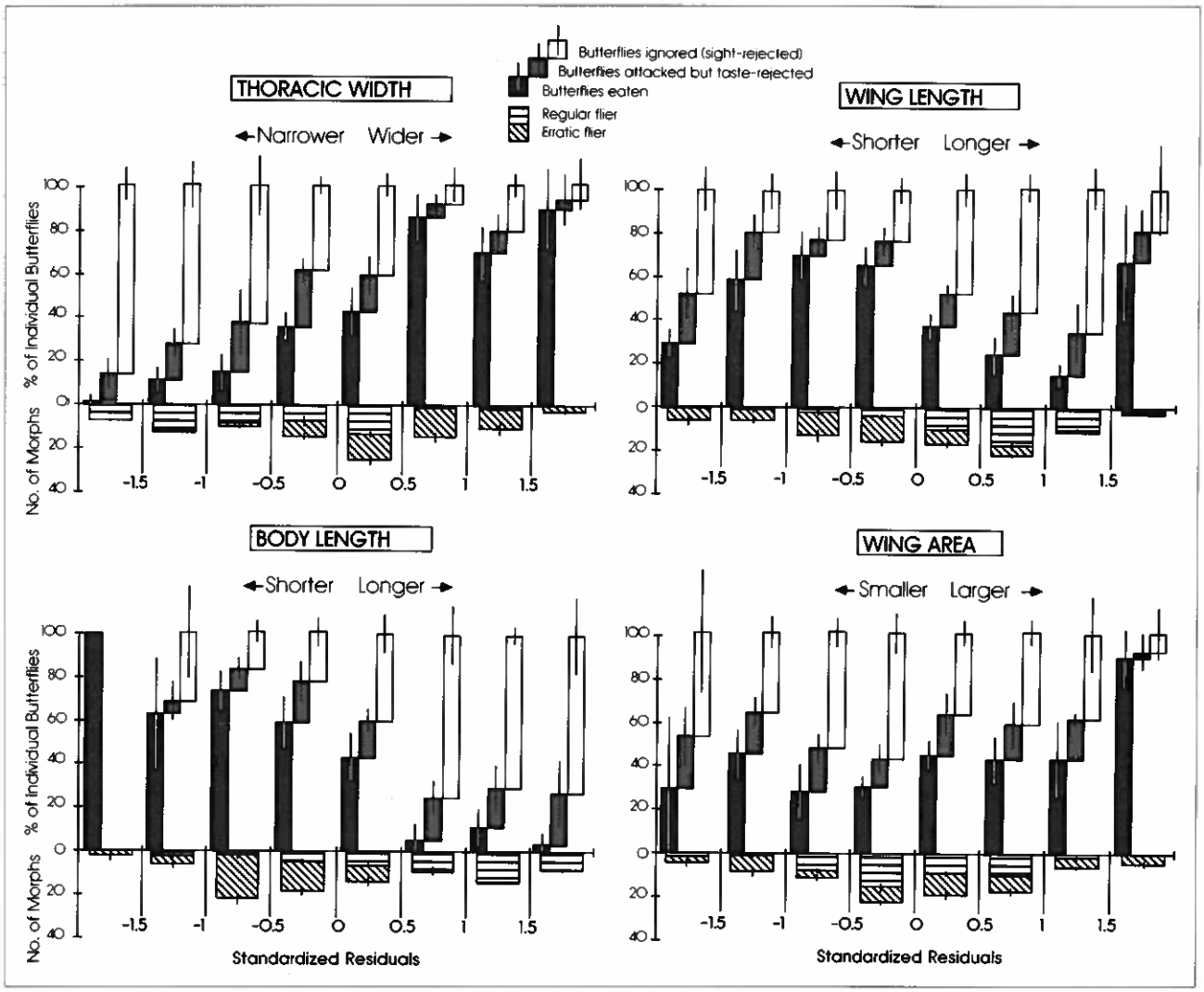


Figure 6. Relationships between jacamar responses and relative thoracic width, body length, wing length, and wing area of butterflies. The percentage mean and ±1 S.D. of butterflies in each response category for the five birds were shown.

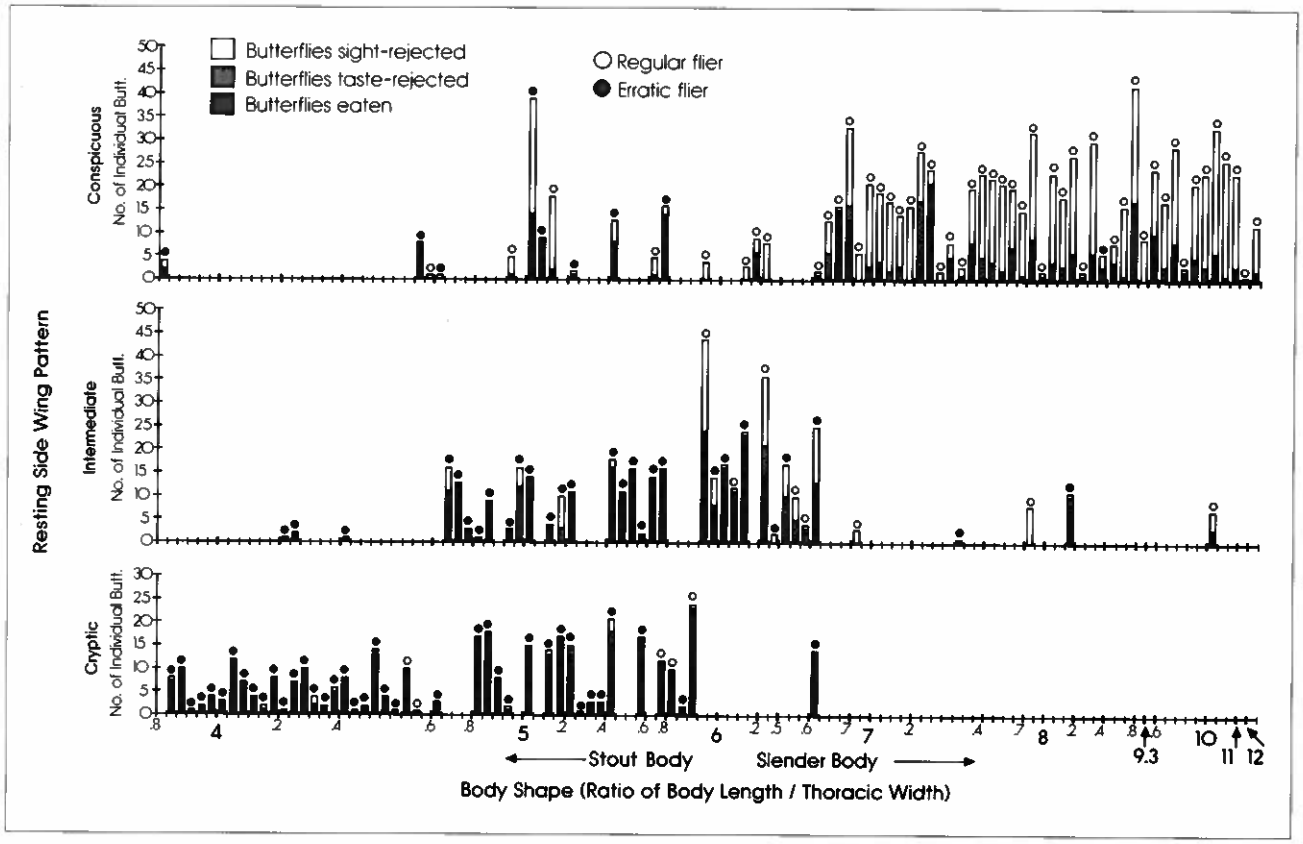


Figure 7. Summary of jacamar responses to butterfly morphs in color pattern (based on the resting side wing pattern), flight pattern, and body shape categories. Each histogram represents the combined jacamar responses to one butterfly morph.

consume butterflies with relatively shorter bodies but tended to ignore or taste-reject butterflies with longer bodies.

Relative wing length — Smaller wings probably make it easier for a butterfly to manouver and avoid predation, in addition to the greater difficulty of random intersection of a smaller object. Fig. 6 shows that jacamars are inclined to sight- or taste-reject butterflies with relatively longer wings than those with shorter wings, though this tendency is less strong compared to the patterns shown by the relative thoracic width and body length. Several common species are exceptions to the above trend, *e.g.*, *Morpho* are palatable but with very long forewings, whereas *Diaethria* are unpalatable but with very short forewings.

Relative wing area — Wing area is the only morphogical characteristic that does not show a relationship to butterfly flight pattern and jacamar responses (Fig. 6). Although the wing areas of regular and erratic fliers are generally similar, their wing shapes are quite different. The regular fliers tend to have relatively longer forewings but shorter wing chords; in contrast, the erratic fliers tend to have shorter forewings but longer wing chords (thus, the resulting wing areas are similar). The effect of butterfly wing shape on flight pattern is discussed in Chai and Srygley (in press).

Statistical analyses of the relationships between butterfly visual characteristics and jacamar responses

I have shown, through separate graphic presentations, that the five jacamars' responses are closely related to the color pattern, flight pattern, and several morphological characteristics of local butterflies. Fig. 7 summarizes these relationships by presenting the pooled jacamar responses to each of 140 butterfly morphs that are categorized by color and flight patterns and are arranged according to their body shapes from stout- to slender-bodied. The body shape of each morph is represented by the ratio of its body length/thoracic width.

Because both the color and flight patterns of butterflies as well as the responses of jacamars are categorical variables, I used the generalized linear model package GLIM (Baker and Nelder, 1978) to fit, by maximum likelihood, the following binary linear logistic regression model (Cox, 1970) to the data presented previously:

$$\text{Log}[P(\text{RESPOND})/P(\text{NOT RESPOND})] = \text{BIRD}_i + \text{COLOR}_j + \text{FLIGHT}_k + \text{SHAPE}_l \quad (1)$$

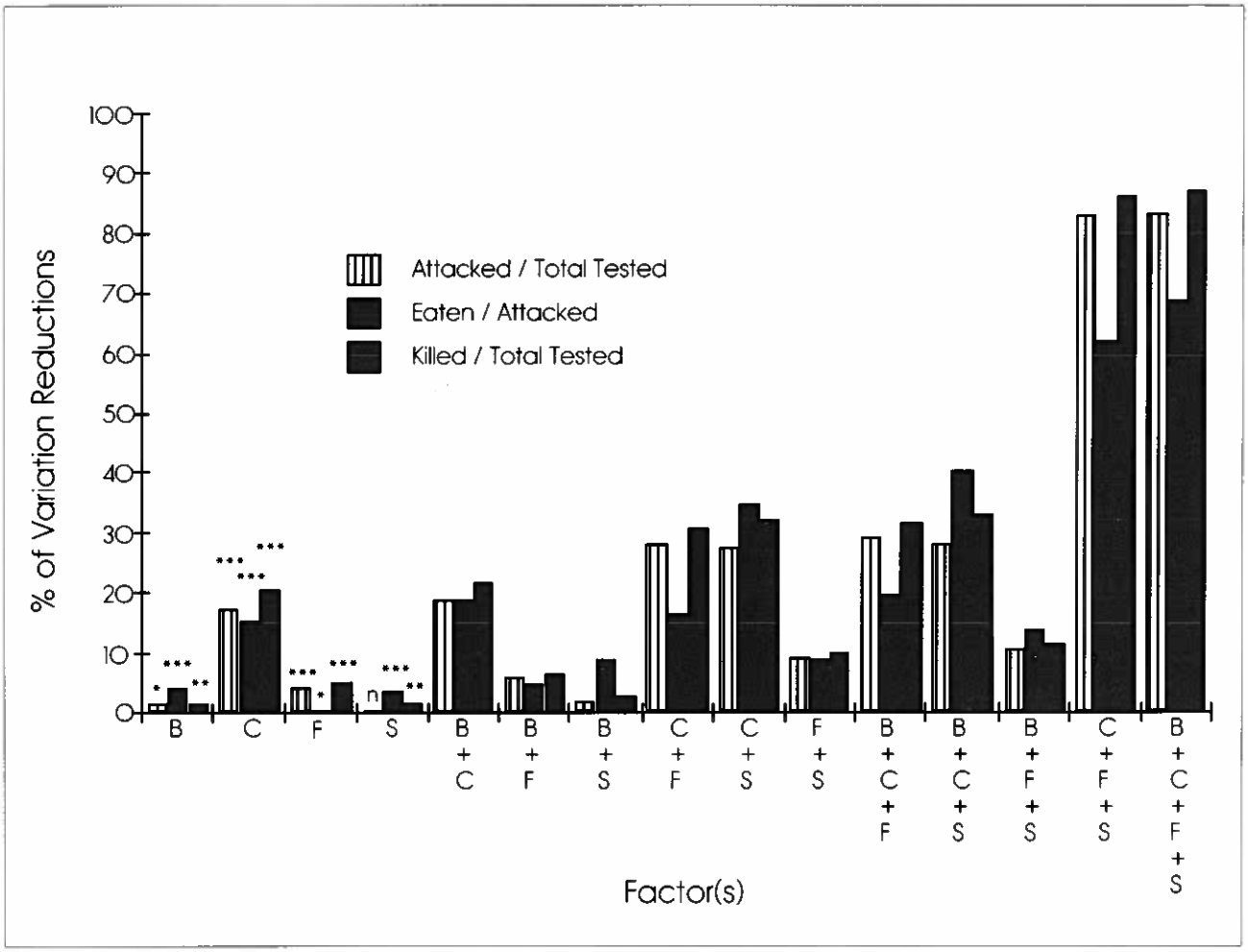


Figure 8. Unique contributions in variation reductions by a single factor or subsets of factors used in fitting the binary linear logistic regression model which estimates that the probability of a particular bird response to butterflies will occur rather than not occur. The same model was used to fit three response probabilities (i.e., attack, eat after being captured, and kill) respectively (see text). B: the effect of individual bird (five jacamars); C: the effect of butterfly color pattern (three categories); F: the effect of butterfly flight pattern (two categories); S: the effect of butterfly body shape (ratio of body length/thoracic width, four categories). Significance level of these four factors: n = not significant; * = P < .05; ** = p < .01; *** = p < .001.

where P(RESPOND) is the probability of responding; thus, P(NOT RESPOND) = 1 - P(RESPOND), BIRD is the effect due to one of the five individual jacamars (representing individual differences among birds), COLOR is the effect due to one of the three color pattern categories (based on the resting color pattern), FLIGHT is the effect due to one of the two flight pattern categories, and SHAPE is the effect due to one of the four body shape categories: <5, 5 - 6, 6 - 7, or >7 (body length/thoracic width ratio).

Because there are very few interactions among the dependent variables in earlier model fitting trials, this model does not include any interactive terms. The data correspond to jacamar responses categorized by 120 BIRD x COLOR x FLIGHT x SHAPE combinations (5 x 3 x 2 x 4). (Just as in chi-square tests, it is necessary to avoid having many cells in the contingency table with zero or very small counts. Thus, the number of categories in the factors is limited by the sample size, though more categories may make the model more realistic.) This model describes the probability that a particular response by a bird will occur rather than not occur for each group (combination). The same technique was used to fit three possible jacamar responses, *i.e.*, (1) attacked or not to the 1,679 experimental butterflies of 88 groups (32 groups with no butterflies, *i.e.*, zero cells, were excluded and not weighed after fitting the model), (2) eaten or not to the 934 attacked and captured butterflies of 80 groups, and (3) killed or not to the 1,679 butterflies of 88 groups.

Appendix 2 presents the observed and fitted proportions of the jacamars' three possible responses for each group of butterflies. Model (1), which fits the probability of being attacked rather than not

attacked (sight-rejected), can reduce 83.2 percent (=.832, which is analogous to R^2 in parametric regression model) of the total variation (analogous to total sum of squares in parametric regression model), Model (2), which fits the probability of being eaten if captured by the bird rather than not eaten (taste-rejected), can reduce 68.6 percent of the total variation, and Model (3), which fits the probability of being killed rather than not killed (survived in a feeding experiment), can reduce 87.0 percent of the total variation.

In order to examine the effects of the four factors (BIRD, COLOR, FLIGHT and SHAPE) of butterflies independently as well as in subsets, I calculated the unique contributions of each factor and subset (two to three factors considered together) in variation reductions for each of the three models (Fig. 8). The amount of variation uniquely reduced by the factor or subset in question is the residual variation of the reduced model in which the factor or subset in question is not included **minus** the residual variation of the original, complete model in which all four factors are included. This, divided by the total variation, is then the percentage of variation reduction uniquely accounted for by the factor or subset in question to the complete model.

Fig. 8 shows the unique effects in variation reduction accounted for by the four independent factors (variables) as well as all possible subsets. The results of chi-square tests on each factor in each model are also shown. When considered individually, COLOR uniquely contributes the greatest amounts of variation reductions for all the three models. The other three factors taken separately account for little in variation reduction, though their effects are still statistically significant (except for SHAPE in Model [1]). In Model (1), a jacamar's response to a butterfly, i.e., attack or not, is based on distant, visual signals emitted by this butterfly. In Model (2), a jacamar's response to a captured butterfly, i.e., eat or not, is based on signals that can only be perceived through close contact, *e.g.*, chemical or tactile signals. Thus, it is interesting to see that SHAPE alone is insignificant and contributes almost nothing in variation reduction in Model (1), whereas FLIGHT alone is almost insignificant and contributes next to nothing in variation reduction in Model (2). COLOR, FLIGHT and SHAPE of butterflies are highly correlated with one another. Thus, when considered together, these three factors can account for almost all the variation reductions made by the three models.

Because there is little individual difference in responses among the five birds, the unique effect of BIRD is very small, suggesting that the underlying decision-making processes are probably very similar among the jacamars. The unique effect of BIRD is highest in Model (2), and primarily reflects the different proportions of taste-rejections on captured butterflies between the adult and young birds. Probably because of their inexperience, the three young birds sampled butterflies more frequently and, as a result, made more mistakes (*i.e.*, attacking butterflies that were subsequently taste-rejected) than the adults. Thus, the three young ate smaller proportions of captured butterflies (69 percent, 59 percent and 61 percent, respectively) than the two adults (75 percent and 85 percent). The proportions of attacked butterflies out of total tested butterflies are similar among the five birds. The three young birds attacked 53 percent, 56 percent and 59 percent of the butterflies respectively compared to the 57 percent and 56 percent attacked by the two adults. Models (1) and (2) should be in close agreement. This means that a specialized predator should visually reject (ignore) an unacceptable butterfly that would be taste-rejected anyway, and should visually accept (attack) an acceptable butterfly that would be taste-accepted (eaten). The results (variation reductions of Models [1] and [2]: 83 percent and 69 percent) show this to be the case.

Model (3) is from the perspective of the prey. It describes the probability of a butterfly with known color pattern, flight pattern and body shape being killed by a jacamar in a feeding experiment. However, because the butterflies were confined in the cage and basically denied the chance to hide and/or escape, this is highly biased toward unacceptable butterflies when compared to what might occur under more natural conditions. Under the experimental conditions, hide and longterm escape were not viable alternatives for avoiding predation.

Butterfly morphological and behavioral characteristics and unsuccessful attacks by the jacamars

During the feeding experiments, many butterflies were observed to escape, even repeatedly, from the jacamars' attacks. However, in most cases, because of the confinement, the bird would eventually succeed in capturing the intended prey. Only species of *Archaeoprepona*, *Historis*, *Morpho*, and *Caligo*, with their large size (forewing length > 50 mm and body mass > 0.6 g) and powerful struggle, sometimes survived the feeding experiments with young birds even after being repeatedly attacked but never survived with the adults. The two adult jacamars showed much more skill in terms of capturing and subduing butterflies than did the three young. On the average, the two adults showed much lower percentages of unsuccessful attacks, 25 percent and 37 percent respectively, than the three young, 56 percent, 45 percent and 53 percent.

The butterflies' own phenotypic traits clearly affect their chances of being captured. Large wing size makes it difficult for the bird to pass the wings and grasp the body. Large body size provides more

Table 2. The relationship between the upperside wing pattern categorized by its mimetic effect and the underside wing pattern categorized by its cryptic effect among 140 butterfly morphs.

	Color pattern (underside wing)	Mimicry (upperside wing)	
	No. of morphs with common mimetic pattern	With imperfect or uncommon mimetic pattern	Not mimetic
Conspicuously patterned	37	15	8
Intermediately patterned	9	11	16
Cryptically patterned	3	12	29

struggling power. Short abdomen, especially when hidden between hind wings, makes it hard for the bird to grasp. Combining these factors, I generated another binary linear logistic regression model to estimate the probability that a jacamar attack would be unsuccessful rather than successful:

$$\text{Log}[P(\text{UNSUCCESS})/P(\text{SUCCESS})] = \text{BIRD}_i + \text{FLIGHT}_j + \text{WEIGHT}_k + \text{SHAPE}_l \quad (2)$$

where P(UNSUCCESS) is the probability of an unsuccessful attack (P(SUCCESS)=1 - P(UNSUCCESS)), BIRD, FLIGHT, and SHAPE are as in equation (1), and WEIGHT represents the effect due to one of the three body weight categories: <0.2, 0.2 - 0.6, or >0.6 g.

The data correspond to a total of 2,221 jacamar attacks categorized by 120 BIRD x FLIGHT x WEIGHT x SHAPE combinations (5 x 2 x 3 x 4). Because 48 groups with no butterflies were not weighted after fitting the model, only 72 groups were actually used. (Data of the observed and fitted proportions of the unsuccessful attacks for each group are available upon request.) This model gives a close fit and can reduce 84.3 percent of the total variation. Chi-square tests on each individual factor show that they are all statistically significant, and BIRD and WEIGHT uniquely account for significant amounts of variation reductions (10.6 percent and 24.8 percent, respectively). Again FLIGHT and SHAPE are correlated with each other, and only when considered together do they show a large unique effect (21.6 percent).

Butterfly mimicry and jacamar responses

Mimicry among Neotropical rainforest butterflies is extensive and eye-catching. Several mimicry complexes can coexist in an area (e.g., Brown and Benson, 1974; Papageorgis, 1975; Gilbert, 1983; DeVries, 1987). However, because the mimetic relationships among butterflies are dynamic, the actual role of a given species is relative and can only be determined with thorough research (VaneWright, 1980; Endler, 1981). Hence, mimicry is treated here in a general, commonly used manner (Remington, 1963). The species called mimetic here are (1) those that resemble one another in color pattern but not in background pattern such as plant parts (thus are not cryptic), and (2) those, within their respective color pattern group, where some members, such as the Müllerian models, are unacceptable to the jacamars. Thus, several acceptable species are not considered as mimetic, even though they are all characterized by conspicuous, iridescent blue patches on the upperside of their wings (e.g., *Memphis* and *Morpho*).

In the previous section, I categorized wing patterns of butterflies according to the degree of resemblance to plant parts. The color pattern on the opposite, usually dorsal, and more brightly patterned side is shown only when a butterfly is active, *e.g.*, flying or flapping its wings. Based on the color pattern opposite to the resting side, I can broadly separate the mimetic butterflies from non-mimetic ones (Chai, 1987 further discussed the color patterns of local butterflies).

In the mimetic group, there exist three broad types of butterflies. The first type shows the common mimetic patterns, i.e., color patterns shown by the common mimicry complexes of local butterflies. Butterfly morphs with common mimetic patterns not only resemble other members in color pattern but also in movement. They can be considered as the core species of this mimicry complexes. As a general phenomenon, the second type contains many butterfly morphs whose color patterns only imperfectly and partially resemble common mimetic patterns (i.e., the imperfect mimics; Ford, 1963). The mimetic function of the color patterns of this second type of butterflies is only speculative. The third type of butterflies possess uncommon mimetic patterns that were not frequently encountered in the field (*i.e.*, the uncommon mimics; Chai, unpubl. data).

Based on the opposite color patterns, the local butterfly morphs can also be assigned to one of three groups — the group with common mimetic patterns, the group with imperfect and/or uncommon mimetic patterns, and the group that is not mimetic. Although the cryptic and mimetic effects of the color patterns are determined according to different wing sides, they are associated (Table 2).

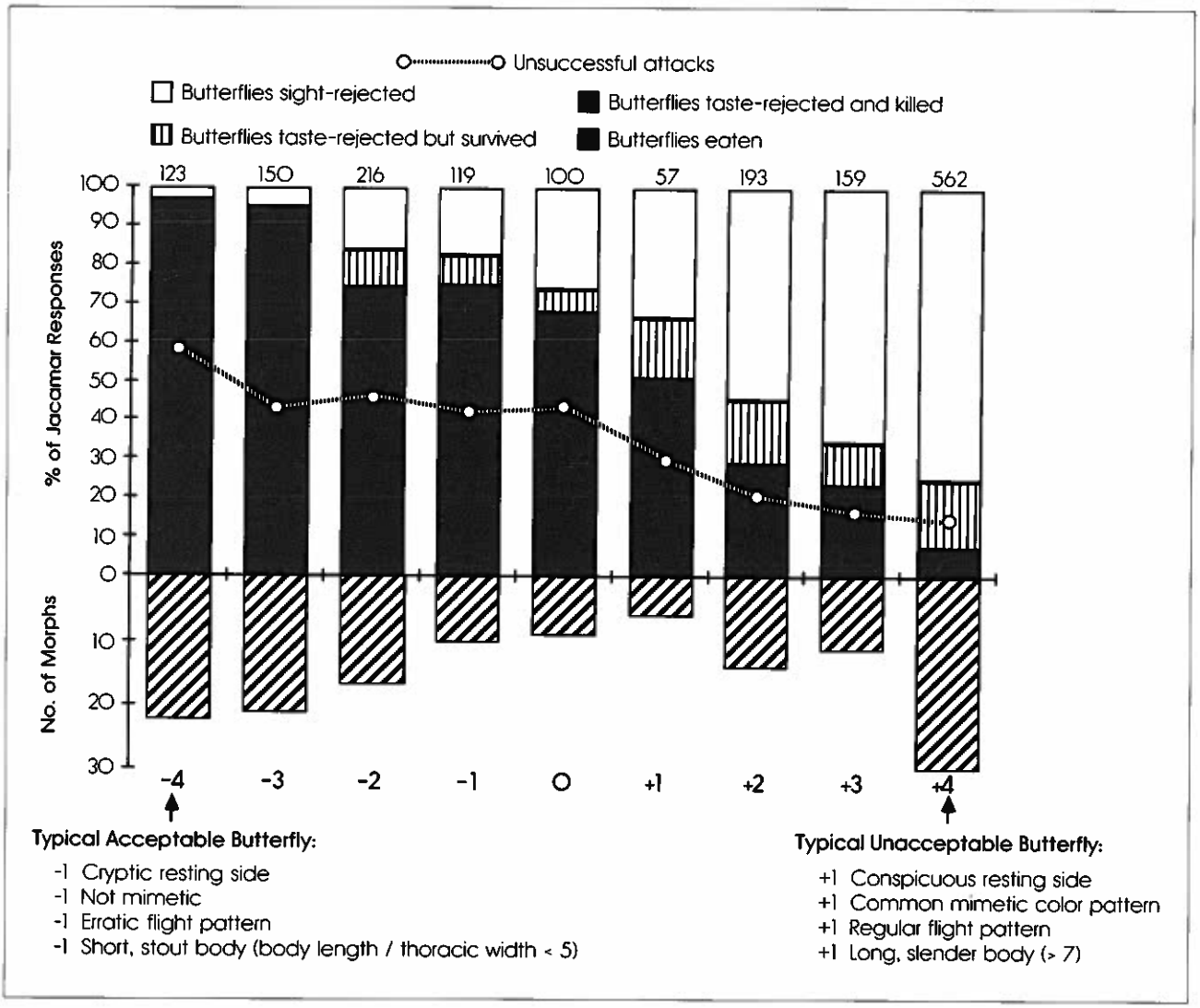


Figure 9. 1,679 butterflies of 140 morphs categorized into nine groups according to their color pattern, flight behavior, and morphological characteristics. The assignment of a butterfly morph to a group is based on its total score evaluated according to the four criteria shown in the figure. Intermediately cryptic resting side, imperfect or uncommon mimetic color pattern, and body length/thoracic width ratio between 5 and 7 are intermediate states and are represented by "0". For example, *Heliconius charitonius* belongs to the group with a +3 sum score because it is conspicuously patterned on the resting side (+1), an imperfect mimic (0), a regular flier (+1), and has a long, slender body (+1). The number of individuals in each group is indicated on the top of each column. The combined responses of five jacamars to each butterfly group are presented in percentages. In order to reduce the body size effect, percentages of unsuccessful attacks do not include jacamar attacks on butterflies larger than 0.6 g (this is because there were no unacceptable butterfly morphs with body masses heavier than this weight, and because butterflies weighing more than 0.6 g are very difficult for jacamars to subdue and require repeated attacks). The other percentages of jacamar responses are based on total number of individual butterflies tested.

Thus, butterflies with common mimetic patterns tend to have conspicuous resting sides, whereas butterflies that are not mimetic tend to have cryptic resting sides. Most mimetic butterflies have similar upperside and underside wing patterns, they are thus both conspicuously and mimetically patterned. Butterflies that are not mimetic generally have cryptic undersides, even though many have conspicuous uppersides such as many nymphalines (Chai, 1987).

Summary of the relationships between the visual characteristics of individual butterflies and jacamar responses

Fig. 9 summarizes the regularity of jacamar responses in relation to the visual characteristics of the Corcovado butterfly community. The visual characteristics of a butterfly that indicate its acceptability are represented by the sum of the number of "unacceptable" characteristics (each is represented by +1) that it possesses plus the number of

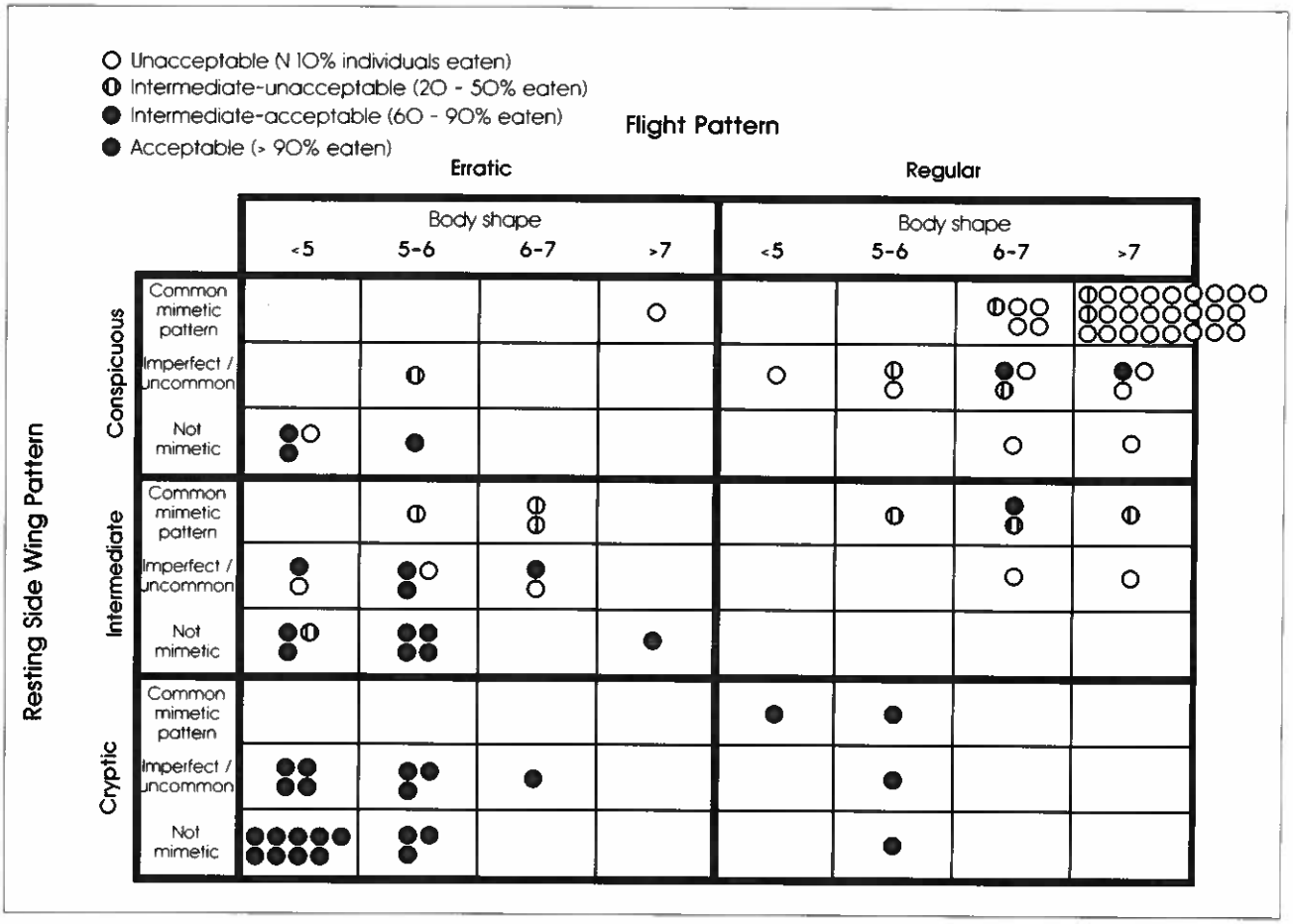


Figure 10. 95 butterfly morphs categorized by color pattern, flight behavior, and morphological characteristics as well as their accepatability to five jacamars.

"acceptable" characteristics (each is represented by -1) that it possesses. Thus, in feeding experiments, jacamars were reluctant to pursue and consume butterflies that possess unacceptable characteristics. However, compared to butterflies with acceptable characterisitcs, these butterflies are easier for jacamars to catch as shown by the lower rate of unsuccessful attacks. It is likely that in nature, with more space to escape than in the cage, butterflies with more acceptable characteristics will become far harder for the jacamars to catch than butterflies with more unacceptable characteristics.

Visual characteristics of butterfly morphs and jacamar responses

To understand the pattern of jacamar responses to a specific morph, several individuals per morph should be tested. Here, I selected the 95 morphs of which at least five individuals per morph were tested with at least two jacamars. Fig. 10 clearly shows the dichotomized response pattern by jacamars to butterfly morphs. Fifty-five (57 percent) morphs were either eaten or never eaten by the jacamars, and relatively few morphs show intermediate acceptability. This suggests that conspecific individuals have consistent palatability to predators such as jacamars. Of course, this pattern is also generated by the highly selective and stable response pattern of jacamars, which, in turn, is probably primarily maintained by the simple generalities in the visual signals exhibited by the local butterfly community. Thus, butterflies with the greatest number of unacceptable characteristics were least attacked, and best survived if captured (Fig. 9). These results clearly indicate the advantages of possessing visual characteristics that reflect unacceptability.

As shown in Fig. 11, although only five species were never attacked, most unacceptable morphs were not sampled often, *i.e.*, the birds learned to ignore them. Because there exists consistent acceptability within a butterfly morph and clear associations of visual characteristics to indicate it, jacamars can categorize and learn these "rules of thumb." Thus, in Fig. 11, for the respective 95 morphs, there exists a relationship between the proportion of individuals attacked and the proportion of individuals eaten. Morphs that were unacceptable were attacked infrequently; hence, for most unacceptable morphs, less than 20 percent of the individuals were

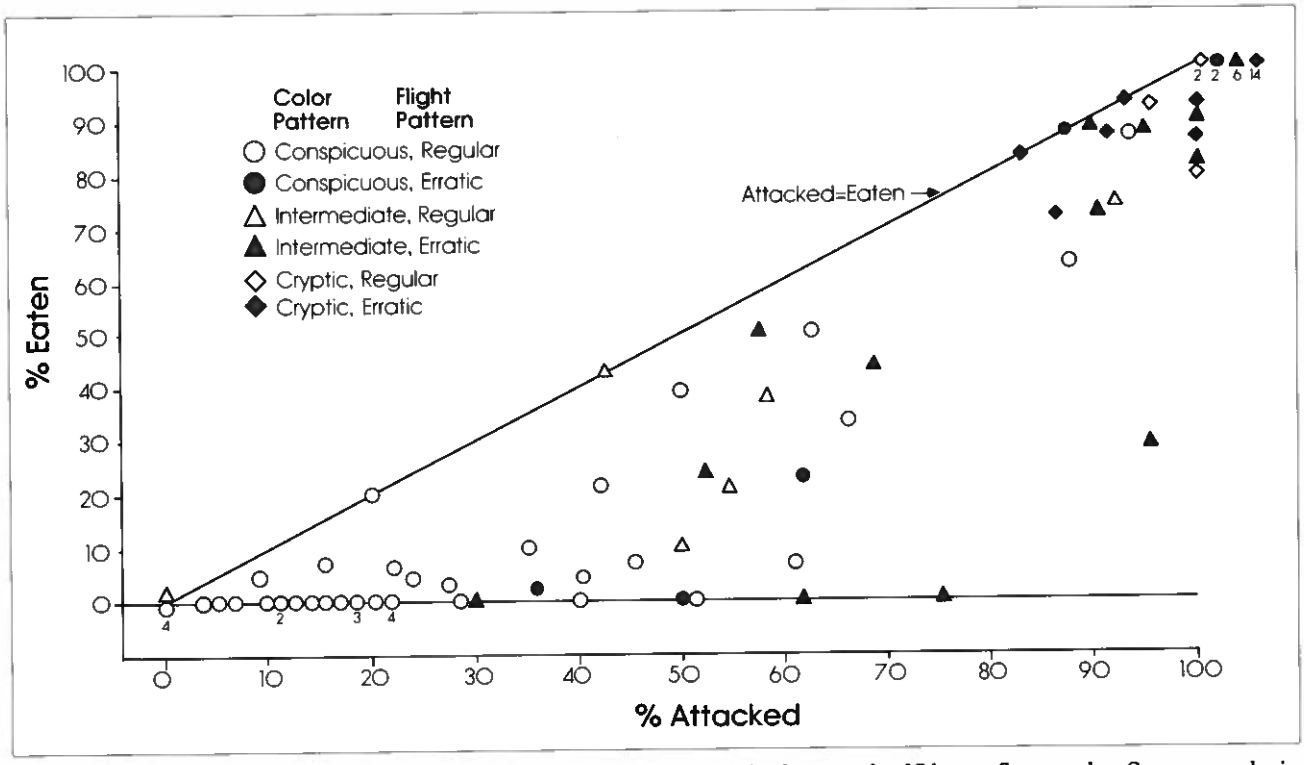


Figure 11. Percentage of individuals attacked vs. percentage of individuals eaten for 95 butterfly morphs. Some morphs in the same category and with very close distributions were only shown by a single symbol with the number of morphs indicated right below it.

sampled. If the birds' learning ability is poor, we would expect a much higher proprotion of unacceptable morphs to be attacked frequently.

Phylogenetic relationships of butterflies and jacamar responses

In the above discussion, I have not considered phylogenetic relationships among the butterflies. In fact, very high correlations exist between the visual characteristics of butterflies and their taxonomic position. In general, member species in a subfamily have similar phenotypic traits. Some subfamilies are especially homogeneous. Charaxines (except for *Consul fabius*), morphines, brassolines, and satyrines (except for *Cithaerias menander*) always have a cryptic underside wing pattern, fly erratically, have short, stout bodies, and are all acceptable to jacamars; in contrast, danaines and ithomiines always have conspicuous, mimetic patterns which are similar on both sides of their wings, fly regularly, have long, slender bodies, and are unacceptable. Butterflies of the Papilioninae, Pierinae, Nymphalinae, Heliconiinae, Lycaenidae, and Riodinidae, however, show relatively heterogeneous phenotypic traits and acceptability (Appendix 1). In general, the associations between butterfly visual characteristics and jacamar responses within these phenotypically less uniform subfamilies are consistent with the overall trends (see Chai, 1987 for further discussions).

Discussion

Divergent phenotypical characteristics of butterflies

The correlative evidence presented shows that the jacamars did not attack butterflies with more complete suites of unacceptable characteristics. When the birds did attack them, the majority were quickly taste-rejected (the tasting process by jacamars is usually very brief, see Chai, 1986). On the other hand, butterflies acceptable to jacamars tended to possess a different suite of characteristics. Those butterflies with more complete "acceptable" characteristics were usually quickly attacked and consumed.

Because the visual characteristics of local butterflies reflect their palatability, these generalities helped the jacamars to learn and to develop their response patterns. In a relatively short period of time, the three, naive young learned to behave like the two adults in selecting prey butterflies. There existed only small individual differences among them. The feeding behavioral pattern of jacamars appears to be very different from that of the Darwin's Finch of Cocos Island, Costa Rica, which exhibited extreme intraspecific variability in feeding (Werner & Sherry, 1987).

Several past studies on bird feeding behavior have demonstrated initial inhibition toward attacking or handling novel, conspicuously colored prey

by naive, hand-reared young of at least some species (Coppinger, 1970; Smith, 1975, 1977; Schuler, 1982; Greenberg, 1984; but see Smith, 1980). In the beginning of my experiments, the young did not exhibit any indication of fear of novel, conspicuously patterned butterflies, and there was no initial preference shown by any of the three young birds toward attacking butterflies with particular visual characteristics (Chai, 1987). It appears that the three young jacamars were able to develop their response patterns through rapid learning. After three or four feeding experiments, the response pattern of the young was generally similar to that of the adults.

Because being recognized as unpalatable is equivalent to avoiding confusion with palatable species, unpalatable butterflies should differ in as many traits as possible from their palatable counterparts (Fisher, 1958). These differences can facilitate predators in learning the association between the unpalatable prey and their visual characteristics (Gittleman and Harvey, 1980; Sillén-Tullberg, 1985; Roper and Redston, 1987), and can also reduce recognition errors by ensuring that predators do not make hasty attacks on unpalatable prey that they have already experienced (Guilford, 1986).

Since palatable butterflies (except for Batesian mimics) survive by avoiding detection and capture by predators, their color patterns, morphologies and behaviors, as reflected in their visual characteristics, are primarily designed for concealment, distraction and escape. In order to reduce the possibility of confusion, unpalatable butterflies, on the other hand, evolve away from palatable butterflies in visual characteristics at the expense of easier detection and capture by the predators.

By advertising their distastefulness through being easily detected, identified and captured, unpalatable butterflies are protected from more specialized and experienced predators such as jacamars. However, they also face greater risk of being captured by generalized and opportunistic predators less specialized in hunting flying insects such as foliage-gleaning birds. The consequence of this is that the set of potential predators is increased. In order to survive the enlarged set of predators, unpalatable butterflies have to become better adapted to higher frequency of predator sampling. They can achieve this through a combination of chemical defenses (e.g., bad and warning taste, Brower, 1984; Guilford *et al.*, 1987) and structural protection (*e.g.*, tough and flexible body, Rothschild, 1971). (Notice that in Fig. 9, butterflies with more unacceptable characteristics not only tended to be sight-rejected, but if attacked, they tended to survive the sampling episode because the jacamars quickly taste-rejected them, see Chai, 1986.)

As Fig. 9 also shows, butterflies with more unacceptable visual characteristics were less likely to be consumed when captured. In other words, to the jacamars, the proportion of acceptable to unacceptable butterflies with more unacceptable characteristics is very low. Butterflies that are palatable but that possess unacceptable visual characteristics, such as Batesian mimics or automimics (Brower *et al.*, 1967), are cheating because they mimic the visual signals but do not possess chemical deterrents to cause taste-rejection. The low frequency of cheaters is probably due to the fact that unacceptable visual characteristics facilitate detection and capture; however, being palatable, the cheaters cannot afford to be captured and tasted. Thus, sampling by a broader complement of local predators toward prey with more unacceptable visual characteristics presumably functions as a finer filter to reduce the existence of palatable members. This then results in highly selective response patterns of jacamars such that a total of 44 percent (745/1,679) of tested butterflies were completely ignored in four-hour-long feeding experiments.

Implications regarding warning coloration and mimicry

Whether a predator will attack a model:mimic complex depends on its ability to discriminate and its likelihood of attacking once it has made such a discrimination (Brower *et al.*, 1971). Experimental evidence has shown that when the penalty for an attack on a model is very severe, the mimics of this model tend to be well protected (Duncan and Sheppard, 1965; Alcock, 1970; O'Donald and Pilecki, 1970). However, it is to the advantage of both predators and noxious prey (*i.e.*, the models) to establish efficient communications so that predators can identify noxious prey and stop their interactions as early as possible. Consequently, natural selection should favor rejection occurring as early as possible. In the feeding experiments reported here, most rejections observed were by sight, the rest by taste and none by vomiting. A variety of other bird species also rejected distasteful butterflies on sight or by taste (discussed in Chai, 1986). Beak-marked specimens are more common in taxa known to be distasteful or toxic (Carpenter, 1941). Hence, the ability of many predators to taste-reject noxious prey reduces the penalty of sampling butterflies, and this relatively low-risk learning process should encourage a predator to sample (Huheey, 1980). Indeed, field observations and feeding experiments have shown that a great many wild birds were ready to attack butterflies (Marshall, 1909; Swynnerton, 1915a; Jones, 1932, 1934; Swynnerton in Carpenter, 1942; Wourms and Wasserman, 1985). Thus, when unpalatable butterflies evolve toward further increasing the effectiveness of warning signals to more specialized and/or experienced predators at the expense of enlarging the set of potential preda-

tors, this process must be especially detrimental to a Batesian mimic. Although experimental studies and theoretical models suggest that a high proportion of Batesian mimics is possible in a community (Brower, 1960; O'Donald and Pilecki, 1970; Ford, 1971; Charlesworth and Charlesworth, 1975), my feeding experiments with jacamars showed that most mimics were Müllerian mimics, and there were few Batesian mimics in the Corcovado rainforest habitat.

Pattern discrimination and generalization by predators is essential for the functioning and evolution of both Batesian and Müllerian mimicry. Previous feeding experiments have demonstrated these two abilities in a variety of predators using small sets of artificial prey (Schmidt, 1958, 1960; Morrell and Turner, 1970; Ford, 1971; Ikin and Turner, 1972; Terhune, 1977) or dead butterflies (Brower, 1958 a, b, c; Brower *et al.*, 1963; Brower *et al.*, 1971; Platt *et al.*, 1971). In the present study, the general response patterns of jacamars, on the basis of several visual characteristics from a large set of free-flying butterflies, clearly indicate pattern generalization by these birds. The jacamars' ability to learn these general characteristics of prey categories allows them to assess the likely palatability of a given butterfly, even of one they have not encountered before.

The experimental jacamars were also highly discriminating. This is examplified by several cases in which the birds were able to consistently capture and consume particular species of Batesian mimics while signt-rejecting other more distasteful members in the same mimicry complexes (Chai, 1986, 1987 described these cases).

In the present study, the free-flying butterflies provided additional behavioral cues to the jacamars. Indeed, the jacamars were very sensitive to the flight behaviors of caged butterflies, and they might discriminate specific butterfly species based on their behavioral pattern in addition to color pattern and/or morphology. Cheaters such as Batesian mimics cannot afford to be sampled in contrast to Müllerian mimics. When a predator initiates attack, they should be more likely to behave differently and try to escape (Rothschild, 1971). The sensitivity of jacamars to the movement patterns of butterflies helped them to discriminate Batesian mimics.

Because of their diurnal habit, butterflies constantly face a suite of visual predators. The optimal traits for protection exhibited by unpalatable butterflies probably represent a balance between the need to signal unambiguously to specialized and/or experienced predators and the need to reduce capture by generalized and/or opportunistic predators. With greater selective pressures from specialized predators, the color pattern and morphological and behavioral traits of unpalatable and palatable butterflies may diverge to a greater extent. In the tropical rain forest, because of the need for prey to escape specialized predators, the divergence in the visual characteristics of butterflies would conceivably be most pronounced. At Corcovado, the divergent butterfly characteristics and the corresponding jacamar responses suggest reciprocal evolutionary interactions.

Acknowledgments

I am indebted to the members of my doctoral committee: L. E. Gilbert (chairman), R. H. Barth, M. Domjan, J. C. Lang and M. C. Singer, for their comments, support and inspiration. I also thank J. A. Endler, M. Rothschild, C. D. Thomas and two anonymous reviewers for constructive suggestions and comments on the manuscript. The assistance of D. V. Hinkley and D. Ng with statistical analyses is gratefully acknowledged. R. Dudley kindly provided his unpublished data on butterfly thoracic mass and width. Servicio Parques Nacionales de Costa Rica granted permission to conduct the research in Corcovado. I thank them and the staff of Corcovado National Park. My research in Costa Rica was supported by the Chapman Foundation of the American Museum of Natural History, a fellowship from the Graduate School of The University of Texas at Austin, and NSF grant BSR-8315399 to L. E. Gilbert. This paper is part of a dissertation submitted in partial satisfaction of the requirements for a doctorate in zoology.

References

Alcock, J. 1970. Punishment levels and the response of white-throated sparrows (*Zonotrichia albicollis*) to three kinds of artificial models and mimics. **Anim. Behav.** 18: 733-739.

Baker, R. J. and J. A. Nelder. 1978. **The GLIM system, Release 3, Generalized linear interactive modeling.** Numerical Algorithms Group, Oxford.

Brower, J. V. Z. 1958 a. Experimental studies of mimicry in some North American butterflies. Part I. The monarch, *Danaus plexippus*, and the viceroy, *Limenitis archippus archippus*. **Evolution** 12: 32-47.

Brower, J. V. Z. 1958 b. Experimental studies of mimicry in some North American butterflies. Part II. *Battus philenor* and *Papilio troilus*, *P. polyxenes* and *P. glaucus*. **Evolution** 12: 123-136.

Brower, J. V. Z. 1958 c. Experimental studies of mimicry in some North American butterflies. Part III. *Danaus gilippus berenice* and *Limenitis archippus floridensis*. **Evolution** 12: 273-285.

Brower, J. V. Z. 1960. Experimental studies of mimicry. IV. The reactions of starlings to different proportions of models and mimics. **Am. Nat.** 94: 271-282.

Brower, L. P. 1969. Ecological chemistry. **Scient. Am.** 220: 22-29.

Brower, L. P. 1984. Chemical defense in butterflies. In R. I. Vane-Wright and P. R. Ackery (eds.), **The Biology of Butterflies,** pp. 109-134. Academic Press, New York.

Brower, L. P. and L. S. Fink. 1985. A natural toxic defense system: cardenolides in butterflies versus birds. **Ann. N. Y. Acad. Sci.** 443: 171-188.

Brower, L. P., J. Alcock, and J. V. Z. Brower. 1971. Avian feeding behavior and the selective advantage of incipient mimicry. In E. R. Creed (ed.), **Ecological Genetics and Evolution,** pp. 261-274. Blackwell Scientific, Oxford.

Brower, L. P., J. V. Z. Brower, and C. T. Collins. 1963. Experimental studies of mimicry. 7. Relative palatability and Müllerian mimicry among neotropical butterflies of the subfamily Heliconiinae. **Zoologica** (New York) 48: 65-84.

Brower, L. P., J. V. Z. Brower, and J. M. Corvino. 1967. Plant poisons in a terrestrial food chain. **Proc. Nat. Acad. Sci.** 57: 893-898.

Brown, K. S., Jr. and W. W. Benson. 1974. Adaptive polymorphism associated with multiple Müllerian mimicry in *Heliconius numata* (Lepid. Nymph.). **Biotropica** 6: 205-228.

Carpenter, G. D. H. 1941. The relative frequency of beak-marks on butterflies of different edibility to birds. **Proc. Zool. Soc. Lond.,** (A) 111: 223-230.

Chai, P. 1986. Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. **Biol. J. Linn. Soc.** 29: 161-189.

Chai, P. 1987. Patterns of prey selection by an insectivorous bird on butterflies in a tropical rainforest. Ph.D. Diss., Univ. of Texas, Austin.

Chai, P. 1988. Wing coloration of free-flying Neotropical butterflies as a signal learned by a specialized avian predator. **Biotropica** 20: 20-30.

Chai, P. and R. B. Srygley. 1990. Predation and the flight, morphology, and temperature of Neotropical rainforest butterflies. **Am. Nat.,** in press.

Charlesworth, D. and B. Charlesworth. 1975. Theoretical genetics of Batesian mimicry. I. Single-locus models. **J. Theoret. Biol.** 55: 283-303.

Coppinger, R. P.,1970. The effect of experience and novelty on avian feeding behaviour with reference to the evolution of warning coloration in butterflies. Part II. Reactions of naive birds to novel insects. **Am. Nat.** 104: 323-335.

Cox, D. R. 1970. **The analysis of binary data.** Chapman and Hall, London.

DeVries, P. J. 1987. **Butterflies of Costa Rica and Their Natural History.** Princeton Univ. Press, Princeton.

Domjan, M. 1980. Ingestional aversion learning: unique and general processes. In J. S. Rosenblatt, R. A. Hinde, C. Beer, and M. C. Busnel (eds.), **Advances in the Study of Behavior** 11: 275-336. Academic Press, New York.

Duncan, C. J. and P. M. Sheppard. 1965. Sensory discrimination and its role in the evolution of Batesian mimicry. **Behaviour** 24: 269-282.

Endler, J. A. 1981. An overview of the relationships between mimicry and crypsis. **Biol. J. Linn. Soc.** 16: 25-31.

Endler, J. A. 1984. Progressive background in moths, and a quantitative measure of crypsis. **Biol. J. Linn. Soc.** 22: 187-231.

Endler, J. A. 1986. Defense against predators. In M. E. Feder and G. V. Lauder (eds.), **Predator-Prey Relationships,** pp. 109-134. Univ. of Chicago Press, Chicago.

Fisher, R. A. 1958. **The Genetical Theory of Natural Selection,** 2nd ed. Dover, New York.

Ford, E. B. 1963. Mimicry. **Proc. XVI Int. Congr. Zool.** Washington 4: 184-186.

Ford, E. B. 1975. **Ecological Genetics,** 4th ed. Oxford Univ. Press, Oxford.

Ford, H. A. 1971. The degree of mimetic protection gained by new partial mimics. **Heredity** 27: 227-236.

Gilbert, L. E. 1983. Coevolution and mimicry. In D. J. Futuyma and M. Slatkin (eds.), **Coevolution,** pp. 263-281. Sinauer, Sunderland, Massachusetts.

Gittleman, J. L. and P. H. Harvey. 1980. Why are distasteful prey not cryptic? **Nature** 286: 149-150.

Greenberg, R. 1984. Differences in neophobia in the tropical migrant wood warblers *Dendroica pensylvanica* and *Dendroica castanea*. **J. Comp. Psychol.** 98: 131-136.

Guilford, T. 1986. How do 'warning colours' work? conspicuousness may reduce recognition errors in experienced predators. **Anim. Behav.** 34: 286-288.

Guilford, T., C. Nicol, M. Rothschild, and B. P. Moore. 1987. The biological roles of pyrazines: evidence for a warning odour function. **Biol. J. Linn. Soc.** 31: 113-128.

Hartshorn, G. S. 1983. Plants, introduction. In D. H. Janzen (ed.), **Costa Rican Natural History,** pp. 118-157. Univ. of Chicago Press, Chicago.

Harvey, P. H. and G. M. Mace. 1982. Comparisons between taxa and adaptive trends: problems of methodology. In King's College Sociobiology Group (eds.), **Current Problems in Sociobiology,** pp. 343-361. Cambridge Univ. Press, Cambridge.

Horn, H. S. 1966. Measurement of "overlap" in comparative ecological studies. **Am. Nat.** 100: 419-424.

Huheey, J. E. 1980. Studies in warning coloration

and mimicry. VIII. Further evidence for a frequency-dependent model of predation. **J. Herpet.** 14: 223-230.

Huheey, J. E. 1984. Warning coloration and mimicry. In W. J. Bell and R. T. Cardé (eds.), **Chemical Ecology of Insects,** pp. 257-297. Chapman and Hall, London.

Ikin, M. and J. R. G. Turner. 1972. Experiments on mimicry: Gestalt perception and the evolution of genetic linkage. **Nature** 239: 525-527.

Jones, F. M. 1932. Insect colouration and relative acceptability of insects to birds. **Trans. Ent. Soc. Lond.** 80: 345-385.

Jones, F. M. 1934. Further experiments on colouration and relative acceptability of insects to birds. **Trans. Ent. Soc. Lond.** 82: 443-453.

Kare, M. R. and J. R. Mason. 1986. The chemical senses in birds. In P. D. Sturkie (ed.), **Avian Physiology,** 4th ed., pp. 59-73. Springer-Verlag, New York.

Marshall, G. A. K. 1909. Birds as a factor in the production of mimetic resemblances among butterflies. **Trans. Ent. Soc. Lond.** 1909: 329-383.

Morrell, G. M. and J. R. G. Turner. 1970. Experiments on mimicry: I. The response of wild birds to artificial prey. **Behaviour** 36: 116-130.

Neumann, C. P. and P. H. Klopfer. 1969. Cage size and discrimination tests in birds: a methodological caution. **Behaviour** 34: 132-137.

O'Donald, P. and C. Pilecki. 1970. Polymorphic mimicry and natural selection. **Evolution** 24: 395-401.

Papageorgis, C. 1975. Mimicry in neotropical butterflies. **Am. Scient.** 63: 522-532.

Platt, A. P., R. P. Coppinger, and L. P. Brower. 1971. Demonstration of the selective advantage of mimetic *Limenitis* butterflies presented to caged avian predators. **Evolution** 25: 692-701.

Pyke, G. H. 1984. Optimal foraging theory: a critical review. **Ann. Rev. Ecol. Syst.** 15: 523-575.

Pyke, G. H., H. R. Pulliam, and E. L. Charnov. 1977. Optimal foraging: A selective review of theory and tests. **Quart. Rev. Biol.** 52: 137-154.

Remington, C. L. 1963. Historical backgrounds of mimicry. **Proc. XVI Int. Congr. Zool.** Washington 4: 145-149.

Rettenmeyer, C. W. 1970. Insect mimicry. **Ann. Rev. Ent.** 15: 43-74.

Roper, T. J. and S. Redston. 1987. Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. **Anim. Behav.** 35: 739-747.

Rothschild, M. 1971. Speculations about mimicry with Henry Ford. In E. R. Creed (ed.), **Ecological Genetics and Evolution,** pp. 202-223. Blackwell Scientific, Oxford.

Rothschild, M. 1981. The mimicrats must move with the times. **Biol. J. Linn. Soc.** 16: 21-23.

Schluter, D. 1981. Does the theory of optimal diets apply in complex environments? **Am. Nat.** 118: 139-147.

Schmidt, R. S. 1958. Behavioral evidence on the evolution of Batesian mimicry. **Anim. Behav.** 6: 129-138.

Schmidt, R. S. 1960. **Predator behaviour and the perfection of incipient mimetic resemblances.** Behaviour 16: 149-158.

Schuler, W. 1982. Zur Function von Warnfarben: Die Reaktion junger stare auf wespenähnlich schwarz-gelbe Attrappen. **Z. Tierpsychol.** 58: 66-78.

Sherry, T. W. 1983. *Galbula ruficauda.* In D. H. Janzen (ed.), **Costa Rican Natural History,** pp. 579-581. Univ. of Chicago Press, Chicago.

Sillén-Tullberg, B. 1985. The significance of coloration per se, independent of background, for predator avoidance of aposematic prey. **Anim. Behav.** 33: 1382-1384.

Smith, S. M. 1975. Innate recognition of coral snake pattern by a possible avian predator. **Science** 187: 759-760.

Smith, S. M. 1977. Coral-snake pattern recognition and stimulus generalization by naive great kiskadees (Aves: Tyrannidae). **Nature** 265: 535-536.

Smith, S. M. 1980. Responses of naive temperate birds to warning coloration. **Am. Midl. Nat.** 103: 346-352.

Srygley, R. B. and P. Chai. 1990. Predation and the elevation of thoracic temperature in brightly-colored, Neotropical butterflies. **Am. Nat.**, in press.

Sutherland, W. J. and C. W. Anderson. 1987. Six ways in which a foraging predator may encounter options with different variances. **Biol. J. Linn. Soc.** 30: 99-114.

Swynnerton, C. F. M. 1915 a. A brief preliminary statement of a few of the results of five years' special testing of the theories of mimicry. **Proc. Ent. Soc. Lond.**, I: xxxii-xliv.

Swynnerton, C. F. M. 1915 b. Birds in relation to their prey: experiments on wood-hoopoes, small hornbills and a babbler. **J. S. Afr. Ornit. Un.** 111: 32-109.

Swynnerton, C. F. M. (in G. D. H. Carpenter). 1942. Observations and experiments in Africa by the late C. F. M. Swynnerton on wild birds eating butterflies and the preference shown. **Proc. Linn. Soc. Lond.** 154: 10-46.

Terhune, E. C. 1977. Components of a visual stimulus used by scrub jays to discriminate a Batesian model. **Am. Nat.** 111: 435-451.

Turner, J. R. G. 1977. Butterfly mimicry, the

genetical evolution of an adaptation. In M. K. Hecht, W. C. Steere, and B. Wallace (eds.), **Evolutionary Biology** 10: 163-206. Plenum Press, New York.

Vane-Wright, R. I. 1980. On the definition of mimicry. **Biol. J. Linn. Soc.** 13: 1-6.

Werner, T. K. and T. W. Sherry. 1987. Behavioral feeding specialization in *Pinaroloxias inornata*, the "Darwin's Finch" of Cocos Island, Costa Rica. **Proc. Natl. Acad. Sci.** 84: 5506-5510.

Wourms, M. K. and F. E. Wasserman. 1985. Bird predation on Lepidoptera and the reliability of beak-marks in determining predation pressure. **J. Lepid. Soc.** 39: 239-261.

Zack, R. and J. N. M. Smith. 1981. Optimal foraging in wild birds? In A. C. Kamil and T. D. Sargent (eds.), **Foraging Behavior: Ecological, Ethological and Psychological Approaches,** pp. 95-109. Garland, New York.

Appendix 1. Summary of the visual characteristics of 140 butterfly morphs and the combined responses of five jacamars to individuals of each butterfly morph.

*Sp. N	*CFMB	Butterfly Morph	Color Pattern	Mimicry	Flight Pattern	Body Shape	Total No. of Butterflies	Sight-Rejected	Taste-Rejected	Eaten	No. of Att. Observed	Unsuccess. Attacks
0.1		**Papilionidae**										
0.2		Papilioninae										
1	3	*Parides childrenae (m)*	Conspic.	Mimetic	Regular	6.98	17	15	2	0	5	0
2	4	*Parides childrenae (f)*	Conspic.	Mimetic	Regular	7.13	16	16	0	0	0	0
3	4	*Parides lycimenes,*	Conspic.	Mimetic	Regular	7.41	21	19	1	1	2	0
3.1		*P. erithalion, P. iphidamas (m)*										
4	4	*Parides lycimenes,*	Conspic.	Mimetic	Regular	7.32	20	12	8	0	10	1
4.1		*P. erithalion, P. iphidamas (f)*										
5	4	*Parides arcas (m)*	Conspic.	Mimetic	Regular	8.03	18	15	3	0	4	0
6	4	*Parides arcas (f)*	Conspic.	Mimetic	Regular	7.58	15	14	1	0	1	0
7	2	*Battus polydamas*	Conspic.	Imperfect	Regular	6.14	9	3	3	3	8	1
8	2	*Battus crassus*	Conspic.	Imperfect	Regular	6.56	2	1	1	0	1	0
9	-1	*Papilio thoas*	Conspic.	Not mime.	Erratic	5.66	16	2	0	14	22	8
10	2	*Papilio androgeus (f)*	Conspic.	Imperfect	Regular	5.61	5	4	0	1	1	0
11	3	*Papilio anchisiades*	Conspic.	Mimetic	Regular	5.90	4	4	0	0	0	0
12	-1	*Eurytides orabilis*	Conspic.	Not mime.	Erratic	5.17	2	1	1	0	1	0
12.1		**Pieridae**										
12.2		Dismorphiinae										
13	0	*Enantia licinia*	Interm.	Imperfect	Erratic	7.31	1	0	1	0	3	1
14	4	*Dismorphia theucharila*	Conspic.	Mimetic	Regular	10.91	1	0	0	1	1	0
15	3	*Dismorphia amphiona*	Interm.	Mimetic	Regular	10.30	7	4	0	3	5	2
15.1		Pierinae										
16	-1	*Melete isandra*	Interm.	Imperfect	Erratic	6.50	17	7	10	0	25	4
17	0	*Appias drusilla (m)*	Interm.	Mimetic	Erratic	5.93	14	6	1	7	18	10
18	-1	*Appias drusilla (f)*	Interm.	Imperfect	Erratic	5.60	14	0	1	13	29	14
19	2	*Perrhybris pyrrha (m)*	Conspic.	Mimetic	Erratic	8.33	6	3	3	0	4	0
20	4	*Perrhybris pyrrha (f)*	Conspic.	Mimetic	Regular	8.35	8	4	4	0	5	0
21	0	*Ascia monuste (m)*	Interm.	Mimetic	Erratic	6.57	25	12	7	6	33	20
22	0	*Ascia monuste (f)*	Interm.	Mimetic	Erratic	6.14	24	1	16	7	43	15
23	0	*Ascia limona (f)*	Interm.	Mimetic	Erratic	6.45	2	2	0	0	0	0
23.1		Coliadinae										
24	-2	*Phoebis philea*	Interm.	Not mime.	Erratic	5.10	4	0	0	4	14	10

Appendix 1. (continued)

*Sp. N	*CFMB	Butterfly Morph	Color Pattern	Mimicry	Flight Pattern	Body Shape	Total No. of Butterflies	Sight-Rejected	Taste-Rejected	Eaten	No. of Att. Observed	Unsuccess. Attacks
25	-2	*Phoebis argante*	Interm.	Not mime.	Erratic	5.39	11	0	2	9	22	9
26	-2	*Phoebis sennae*	Interm.	Not mime.	Erratic	5.16	11	0	0	11	43	32
27	-2	*Phoebis trite*	Interm.	Not mime.	Erratic	5.46	2	0	0	2	2	0
28	-1	*Aphrissa boisduvalii*	Interm.	Imperfect	Erratic	5.68	16	0	0	16	55	39
29	-1	*Eurema proterpia*	Interm.	Not mime.	Erratic	8.14	11	1	2	8	19	9
29.1		**Nymphalidae**										
29.2		Charaxinae										
30	-4	*Archaeoprepona demophon*	Cryptic	Not mime.	Erratic	3.90	10	0	0	10	83	73
31	-4	*Archaeoprepona meander*	Cryptic	Not mime.	Erratic	3.91	1	0	0	1	1	0
32	-3	*Zaretis ellops*	Cryptic	Imperfect	Erratic	4.33	6	1	0	5	7	2
33	0	*Consul fabius*	Cryptic	Mimetic	Regular	4.45	10	0	0	10	17	7
34	-3	*Memphis eurypyle,*	Cryptic	Imperfect	Erratic	4.00	12	0	0	12	33	19
34.1		*M. chrysophana, M. glycerium*										
35	-4	*Memphis artacaena*	Cryptic	Not mime.	Erratic	4.37	2	0	0	2	3	1
36	-4	*Memphis pithyusa*	Cryptic	Not mime.	Erratic	4.03	7	0	0	7	12	4
37	-4	*Memphis forreri*	Cryptic	Not mime.	Erratic	4.13	8	0	0	8	17	9
38	-4	*Memphis sp. 1*	Cryptic	Not mime.	Erratic	3.92	2	0	0	2	2	0
39	-4	*Memphis sp. 2*	Cryptic	Not mime.	Erratic	4.44	1	0	0	1	1	0
40	-4	*Memphis sp. 3*	Cryptic	Not mime.	Erratic	3.92	4	0	0	4	6	2
40.1		Nymphalinae										
41	-2	*Colobura dirce*	Interm.	Not mime.	Erratic	5.37	18	2	0	16	26	8
42	-2	*Tigridia acesta*	Cryptic	Imperfect	Erratic	6.57	14	0	1	13	24	9
43	-4	*Historis odius*	Cryptic	Not mime.	Erratic	4.20	7	0	0	7	94	87
44	-4	*Historis acheronta*	Cryptic	Not mime.	Erratic	4.43	4	0	0	4	25	21
45	-4	*Hamadryas feronia*	Cryptic	Not mime.	Erratic	4.40	14	0	0	14	42	28
46	-4	*Hamadryas ipthime*	Cryptic	Not mime.	Erratic	4.35	8	0	0	8	12	4
47	-3	*Hamadryas amphinome*	Interm.	Not mime.	Erratic	4.68	16	5	4	7	45	15
48	-1	*Hamadryas laodamia (m)*	Interm.	Imperfect	Erratic	5.14	10	7	3	0	17	4
49	-2	*Hamadryas laodamia (f)*	Interm.	Imperfect	Erratic	4.95	16	4	12	0	53	14
50	0	*Marpesia petreus*	Cryptic	Mimetic	Regular	4.55	1	0	0	1	1	0
51	-3	*Marpesia chiron*	Interm.	Not mime.	Erratic	4.90	3	0	0	3	8	5
52	-3	*Marpesia iole*	Cryptic	Imperfect	Erratic	4.36	1	0	0	1	1	0
53	-2	*Marpesia berania*	Interm.	Imperfect	Erratic	4.69	13	0	0	13	27	14
54	-3	*Temenis laothoe*	Cryptic	Imperfect	Erratic	4.76	17	0	0	17	31	14

Appendix 1. (continued)

*Sp. N	*CFMB	Butterfly Morph	Color Pattern	Mimicry	Flight Pattern	Body Shape	Total No. of Butterflies	Sight-Rejected	Taste-Rejected	Eaten	No. of Att. Observed	Unsuccess. Attacks
55	-3	*Nica flavilla*	Cryptic	Imperfect	Erratic	4.83	8	0	0	8	14	5
56	-2	*Pyrrhogyra neaerea*	Conspic.	Not mime.	Erratic	4.52	8	0	0	8	25	17
57	-2	*Pyrrhogyra crameri*	Conspic.	Not mime.	Erratic	4.98	9	0	0	9	17	8
58	-2	*Catonephele mexicana (m)*	Cryptic	Imperfect	Erratic	5.37	21	3	3	15	38	19
59	-2	*Catonephele mexicana (f)*	Cryptic	Imperfect	Erratic	5.15	15	0	2	13	23	7
60	-3	*Catonephele numilia (f)*	Cryptic	Imperfect	Erratic	4.90	2	1	0	1	2	1
61	-4	*Nessaea aglaura*	Cryptic	Not mime.	Erratic	4.60	3	0	0	3	6	3
62	-2	*Diaethria marchalii*	Conspic.	Not mime.	Erratic	4.96	39	25	13	1	40	11
63	0	*Callicore atacama*	Conspic.	Imperfect	Erratic	5.43	13	5	5	3	14	1
64	-2	*Callicore texa*	Conspic.	Not mime.	Erratic	4.59	1	1	0	0	0	0
65	-3	*Adelpha cytherea*	Interm.	Not mime.	Erratic	4.97	14	0	0	14	22	8
66	-3	*Adelpha cocala*	Interm.	Not mime.	Erratic	4.24	2	0	0	2	2	0
67	-3	*Adelpha heraclea*	Interm.	Not mime.	Erratic	4.75	1	0	0	1	1	0
68	-3	*Adelpha lerna*	Interm.	Not mime.	Erratic	4.21	1	0	0	1	1	0
69	-3	*Adelpha celerio*	Interm.	Not mime.	Erratic	4.84	9	0	0	9	17	8
70	-4	*Siproeta stelenes*	Cryptic	Not mime.	Erratic	4.81	18	0	0	18	45	27
71	-2	*Anartia fatima*	Interm.	Not mime.	Erratic	5.42	16	0	0	16	37	21
72	-2	*Anartia jatrophae*	Cryptic	Imperfect	Erratic	5.13	17	0	0	17	37	20
73	-3	*Junonia evarete*	Cryptic	Not mime.	Erratic	5.05	14	1	0	13	23	9
74	0	*Euptoieta hegesia*	Cryptic	Imperfect	Regular	5.75	10	0	2	8	23	12
74.1		Acraeinae										
75	2	*Actinote anteas*	Interm.	Mimetic	Regular	6.67	3	3	0	0	0	0
76	2	*Actinote lapitha*	Interm.	Imperfect	Regular	7.70	8	8	0	0	0	0
76.1		Heliconiinae										
77	-1	*Philaethria dido*	Cryptic	Not mime.	Regular	5.65	12	0	0	12	18	6
78	2	*Dryadula phaetusa*	Conspic.	Imperfect	Regular	6.61	16	1	1	14	31	13
79	2	*Dione juno*	Interm.	Mimetic	Regular	5.89	44	20	15	9	41	3
80	2	*Agraulis vanillae*	Interm.	Mimetic	Regular	6.01	12	1	2	9	17	5
81	1	*Dryas iulia*	Cryptic	Mimetic	Regular	5.83	24	1	1	22	39	16
82	2	*Eueides aliphera*	Interm.	Mimetic	Regular	6.18	36	15	7	14	39	8
83	3	*Eueides lybia libioides*	Conspic.	Mimetic	Regular	6.64	33	17	3	13	27	4
84	4	*Eueides isabella*	Conspic.	Mimetic	Regular	7.24	8	3	1	4	7	2
85	3	*Heliconius doris (green morph)*	Conspic.	Mimetic	Regular	6.82	21	18	3	0	5	0
86	3	*Heliconius doris (red morph)*	Conspic.	Imperfect	Regular	7.11	14	11	3	0	4	1

Appendix 1. (continued)

*Sp. N	*CFMB	Butterfly Morph	Color Pattern	Mimicry	Flight Pattern	Body Shape	Total No. of Butterflies	Sight-Rejected	Taste-Rejected	Eaten	No. of Att. Observed	Unsuccess. Attacks
87	3	*Heliconius charitonius*	Conspic.	Imperfect	Regular	7.17	24	3	6	15	37	6
88	4	*Heliconius melpomene*	Conspic.	Mimetic	Regular	7.47	20	13	5	2	9	1
89	3	*Heliconius cydno*	Conspic.	Imperfect	Regular	7.84	2	1	1	0	2	1
90	4	*Heliconius pachinus*	Conspic.	Mimetic	Regular	7.40	22	18	4	0	12	5
91	4	*Heliconius erato*	Conspic.	Mimetic	Regular	7.73	32	23	9	0	15	2
92	4	*Heliconius hecalesia*	Conspic.	Mimetic	Regular	7.29	3	2	1	0	6	4
93	4	*Heliconius hecale zuleika*	Conspic.	Mimetic	Regular	8.23	30	24	6	0	18	7
94	4	*Heliconius ismenius clarescens*	Conspic.	Mimetic	Regular	8.52	42	25	15	2	27	4
95	3	*Heliconius sara theudela*	Conspic.	Mimetic	Regular	6.74	6	6	0	0	0	0
96	3	*Heliconius hewitsoni*	Conspic.	Mimetic	Regular	6.96	19	15	4	0	8	1
96.1		Melitaeinae										
97	2	*Chlosyne janais*	Conspic.	Imperfect	Regular	6.19	8	8	0	0	0	0
98	1	*Thessalia ezra*	Conspic.	Not mime.	Regular	6.57	13	7	5	1	7	0
99	4	*Eresia eutropia*	Conspic.	Mimetic	Regular	7.21	2	2	0	0	0	0
99.1		Danainae										
100	1	*Danaus gilippus*	Interm.	Imperfect	Regular	6.54	4	1	3	0	13	3
101	1	*Danaus eresimus*	Interm.	Imperfect	Regular	6.53	10	5	4	1	12	3
102	4	*Lycorea cleobaea*	Conspic.	Mimetic	Regular	7.17	28	11	15	2	39	1
102.1		Ithomiinae										
103	4	*Tithorea tarricina*	Conspic.	Mimetic	Regular	7.32	23	18	5	0	11	2
104	4	*Melinaea scylax*	Conspic.	Mimetic	Regular	9.58	29	21	7	1	12	1
105	4	*Thyridia psidii*	Conspic.	Mimetic	Regular	8.37	16	15	1	0	2	1
106	4	*Mechanitis lysimnia*	Conspic.	Mimetic	Regular	9.99	33	27	6	0	6	0
107	4	*Mechanitis polymnia*	Conspic.	Mimetic	Regular	10.26	26	25	1	0	1	0
108	4	*Ithomia patilla*	Conspic.	Mimetic	Regular	9.71	21	16	4	1	5	0
109	4	*Ithomia celemia*	Conspic.	Mimetic	Regular	9.58	3	1	2	0	4	0
110	3	*Aeria eurimedia*	Conspic.	Imperfect	Regular	12.20	12	10	1	1	3	1
111	4	*Hyposcada virginiana*	Conspic.	Mimetic	Regular	10.42	23	20	3	0	3	0
112	4	*Oleria paula*	Conspic.	Mimetic	Regular	9.72	23	20	3	0	4	1
113	4	*Ceratinia tutia*	Conspic.	Mimetic	Regular	9.26	24	14	5	5	13	0
114	4	*Callithomia hezia*	Conspic.	Mimetic	Regular	8.14	27	21	4	2	8	0
115	2	*Godyris zygia (m)*	Conspic.	Not mime.	Regular	7.95	23	19	4	0	5	1
116	4	*Godyris zygia (f)*	Conspic.	Mimetic	Regular	8.21	2	1	1	0	1	0
117	4	*Hypoleria cassotis*	Conspic.	Mimetic	Regular	9.55	17	14	3	0	3	0

Appendix 1. (continued)

*Sp. N	*CFMB	Butterfly Morph	Color Pattern	Mimicry	Flight Pattern	Body Shape	Total No. of Butterflies	Sight-Rejected	Taste-Rejected	Eaten	No. of Att. Observed	Unsuccess. Attacks
118	4	*Pteronymia agalla*	Conspic.	Mimetic	Regular	8.83	9	9	0	0	0	0
118.1		Morphinae										
119	-4	*Morpho peleides marinita*	Cryptic	Not mime.	Erratic	4.09	4	0	0	4	14	9
120	-4	*Morpho amathonte (m)*	Cryptic	Not mime.	Erratic	4.20	10	0	0	10	44	33
121	-4	*Morpho amathonte (f)*	Cryptic	Not mime.	Erratic	4.24	4	2	0	2	22	18
121.1		Brassolinae										
122	-4	*Opsiphanes tamarindi*	Cryptic	Not mime.	Erratic	4.00	3	0	0	3	6	3
123	-4	*Eryphanis polyxena*	Cryptic	Not mime.	Erratic	4.19	1	0	0	1	1	0
124	-4	*Caligo memnon*	Cryptic	Not mime.	Erratic	3.89	8	1	0	7	62	53
125	-4	*Caligo eurilochus*	Cryptic	Not mime.	Erratic	4.12	2	1	0	1	9	8
126	-4	*Caligo atreus*	Cryptic	Not mime.	Erratic	4.26	2	0	0	2	27	24
126.1		Satyrinae										
127	-1	*Cithaerias menander*	Interm.	Imperfect	Erratic	6.01	17	1	1	15	22	5
128	-2	*Pierella helvetia*	Cryptic	Imperfect	Erratic	5.21	1	0	0	1	1	0
129	-3	*Pierella luna*	Cryptic	Not mime.	Erratic	5.04	15	0	0	15	20	5
130	-3	*Taygetis andromeda*	Cryptic	Not mime.	Erratic	5.50	17	0	0	17	35	17
131	-3	*Megeuptychia antonoe*	Cryptic	Not mime.	Erratic	5.23	3	0	0	3	15	12
132	-3	*Cissia libye*	Cryptic	Not mime.	Erratic	5.27	3	0	1	2	5	2
132.1		**Lycaenidae**										
133	2	*Eumaeus minyas*	Conspic.	Imperfect	Regular	5.14	18	16	2	0	2	0
134	1	*Theorema eumenia*	Conspic.	Imperfect	Regular	4.87	5	4	1	0	1	0
135	-3	*Pseudolycaena damo*	Interm.	Not mime.	Erratic	4.75	3	0	0	3	6	3
135.1		**Riodinidae**										
136	2	*Hades noctula*	Conspic.	Imperfect	Regular	6.09	3	3	0	0	0	0
137	-3	*Erybia patrona*	Cryptic	Not mime.	Erratic	5.81	2	0	0	2	2	0
138	-1	*Ancyluris inca*	Conspic.	Imperfect	Erratic	3.84	4	2	2	0	3	0
139	1	*Esthemopis sp.*	Conspic.	Imperfect	Regular	4.55	1	1	0	0	0	0
140	-3	*Juditha sp.*	Interm.	Not mime.	Erratic	4.40	1	0	0	1	2	1

Appendix 2. The observed and fitted numbers of butterflies attacked, eaten when attacked and captured, and killed by each jacamar for each group of butterflies The 120 groups of butterflies are categorized by five birds and three color pattern, two flight pattern, and four body shape categories of butterflies. See text for explanation of the statistical model used. Bird: AF = adult female; AM = adult male; YF1 = young female 1; YF2 = young female; YM - young male.

Butt. Group	No. of Butt. Morphs	Bird	Butt. Color Pattern	Butt. Flight Pattern	Butt. Body Shape	No. of Butt. Test.	Obs. (Att./No. Test.)	Fit.	No. of Butt. Capt	Obs. (Eat./No. Capt.)	Fit.	No. of Butt. Test.	Obs. (Kill./No. Test.)	Fit.
1	31	AF	Consp.	Reg.	>7	120	29	35	29	9	10	120	14	16
2	8	AF	Consp.	Reg.	6-7	26	16	10	16	13	8	26	13	6
3	1	AF	Consp.	Reg.	5-6	5	1	2	1	1	1	5	1	1
4	0	AF	Consp.	Reg.	<5	0	0	0	0	0	0	0	0	0
5	1	AF	Consp.	Err.	>7	3	0	2	0	0	0	3	0	1
6	0	AF	Consp.	Err.	6-7	0	0	0	0	0	0	0	0	0
7	2	AF	Consp.	Err.	5-6	6	6	4	6	5	5	6	5	4
8	2	AF	Consp.	Err.	<5	13	7	8	7	3	5	13	3	7
9	0	AF	Interm.	Reg.	>7	0	0	0	0	0	0	0	0	0
10	3	AF	Interm.	Reg.	6-7	10	8	6	8	6	5	10	7	5
11	1	AF	Interm.	Reg.	5-6	7	4	5	4	2	3	7	2	4
12	0	AF	Interm.	Reg.	<5	0	0	0	0	0	0	0	0	0
13	0	AF	Interm.	Err.	>7	0	0	0	0	0	0	0	0	0
14	4	AF	Interm.	Err.	6-7	12	8	10	8	3	6	12	5	10
15	8	AF	Interm.	Err.	5-6	23	20	20	20	20	17	23	20	19
16	5	AF	Interm.	Err.	<5	14	13	12	13	9	11	14	12	11
17	0	AF	Cryptic	Reg.	>7	0	0	0	0	0	0	0	0	0
18	0	AF	Cryptic	Reg.	6-7	0	0	0	0	0	0	0	0	0
19	2	AF	Cryptic	Reg.	5-6	7	7	7	7	7	7	7	7	7
20	1	AF	Cryptic	Reg.	<5	2	2	2	2	2	2	2	2	2
21	0	AF	Cryptic	Err.	>7	0	0	0	0	0	0	0	0	0
22	1	AF	Cryptic	Err.	6-7	2	2	2	2	2	2	2	2	2
23	6	AF	Cryptic	Err.	5-6	20	20	20	20	20	20	20	20	20
24	10	AF	Cryptic	Err.	<5	23	23	23	23	23	23	23	23	23
25	29	AM	Consp.	Reg.	>7	92	7	19	7	5	3	92	6	9
26	11	AM	Consp.	Reg.	6-7	27	7	7	7	6	4	27	6	5
27	2	AM	Consp.	Reg.	5-6	5	0	1	0	0	0	5	0	1

Appendix 2 (continued)

Butt. Group	No. of Butt. Morphs	Bird	Butt. Color Pattern	Butt. Flight Pattern	Butt. Body Shape	No. of Butt. Test.	Obs. (Att./No. Test.)	Fit.	No. of Butt Capt	Obs. (Eat./No. Capt.)	Fit.	No. of Butt. Test.	Obs. (Kill./No. Test.)	Fit.
28	2	AM	Consp.	Reg.	<5	2	0	0	0	0	0	2	0	0
29	0	AM	Consp.	Err.	>7	0	0	0	0	0	0	0	0	0
30	0	AM	Consp.	Err.	6-7	0	0	0	0	0	0	0	0	0
31	2	AM	Consp.	Err.	5-6	8	4	5	4	3	3	8	3	4
32	3	AM	Consp.	Err.	<5	8	5	4	5	5	4	8	5	3
33	1	AM	Interm.	Reg.	>7	3	0	1	0	0	0	3	0	1
34	4	AM	Interm.	Reg.	6-7	17	10	9	10	5	6	17	5	7
35	1	AM	Interm.	Reg.	5-6	14	10	7	10	7	8	14	7	6
36	0	AM	Interm.	Reg.	<5	0	0	0	0	0	0	0	0	0
37	1	AM	Interm.	Err.	>7	2	2	1	2	2	1	2	2	1
38	3	AM	Interm.	Err.	6-7	20	18	16	18	7	14	20	17	16
39	10	AM	Interm.	Err.	5-6	29	26	23	26	25	23	29	25	23
40	7	AM	Interm.	Err.	<5	14	12	11	12	9	10	14	10	10
41	0	AM	Cryptic	Reg.	>7	0	0	0	0	0	0	0	0	0
42	0	AM	Cryptic	Reg.	6-7	0	0	0	0	0	0	0	0	0
43	3	AM	Cryptic	Reg.	5-6	9	9	8	9	9	9	9	9	8
44	1	AM	Cryptic	Reg.	<5	3	3	3	3	3	3	3	3	3
45	0	AM	Cryptic	Err.	>7	0	0	0	0	0	0	0	0	0
46	1	AM	Cryptic	Err.	6-7	2	2	2	2	2	2	2	2	2
47	9	AM	Cryptic	Err.	5-6	20	20	19	20	20	20	20	20	20
48	21	AM	Cryptic	Err.	<5	40	40	39	40	40	39	40	40	39
49	30	YF1	Consp.	Reg.	>7	192	50	46	50	7	12	192	14	16
50	8	YF1	Consp.	Reg.	6-7	39	13	12	13	10	5	39	11	6
51	2	YF1	Consp.	Reg.	5-6	7	1	2	1	0	1	7	0	1
52	0	YF1	Consp.	Reg.	<5	0	0	0	0	0	0	0	0	0
53	1	YF1	Consp.	Err.	>7	3	3	2	3	0	1	3	0	1
54	0	YF1	Consp.	Err.	6-7	0	0	0	0	0	0	0	0	0
55	3	YF1	Consp.	Err.	5-6	12	8	7	8	6	6	12	7	6
56	4	YF1	Consp.	Err.	<5	20	11	12	11	5	7	20	8	7
57	1	YF1	Interm.	Reg.	>7	1	0	0	0	0	0	1	0	0
58	2	YF1	Interm.	Reg.	6-7	14	7	8	7	7	4	14	7	5

Appendix 2 (continued)

Butt. Group	No. of Butt. Morphs	Bird	Butt. Color Pattern	Butt. Flight Pattern	Butt. Body Shape	No. of Butt. Test.	Obs. (Att./No. Test.)	Fit.	No. of Butt. Capt	Obs. (Eat./No. Capt.)	Fit.	No. of Butt. Test.	Obs. (Kill./No. Test.)	Fit.
59	1	YF1	Interm.	Reg.	5-6	12	4	7	4	0	3	12	1	5
60	0	YF1	Interm.	Reg.	<5	0	0	0	0	0	0	0	0	0
61	1	YF1	Interm.	Err.	>7	2	2	2	2	0	1	2	2	1
62	5	YF1	Interm.	Err.	6-7	20	14	16	14	7	9	20	11	15
63	9	YF1	Interm.	Err.	5-6	28	23	23	23	21	18	28	22	21
64	7	YF1	Interm.	Err.	<5	21	20	17	20	17	16	21	17	14
65	0	YF1	Cryptic	Reg.	>7	0	0	0	0	0	0	0	0	0
66	0	YF1	Cryptic	Reg.	6-7	0	0	0	0	0	0	0	0	0
67	3	YF1	Cryptic	Reg.	5-6	12	12	11	12	12	12	12	12	11
68	2	YF1	Cryptic	Reg.	<5	3	3	3	3	3	3	3	3	3
69	0	YF1	Cryptic	Err.	>7	0	0	0	0	0	0	0	0	0
70	1	YF1	Cryptic	Err.	6-7	2	2	2	2	2	2	2	2	2
71	7	YF1	Cryptic	Err.	5-6	27	26	26	26	25	25	27	26	27
72	20	YF1	Cryptic	Err.	<5	54	49	53	49	49	48	54	49	52
73	28	YF2	Consp.	Reg.	>7	106	30	26	30	0	4	106	1	6
74	9	YF2	Consp.	Reg.	6-7	29	11	9	11	2	3	29	3	3
75	2	YF2	Consp.	Reg.	5-6	5	1	2	1	0	0	5	0	1
76	1	YF2	Consp.	Reg.	<5	2	1	1	1	0	0	2	0	0
77	0	YF2	Consp.	Err.	>7	0	0	0	0	0	0	0	0	0
78	0	YF2	Consp.	Err.	6-7	0	0	0	0	0	0	0	0	0
79	2	YF2	Consp.	Err.	5-6	3	3	2	3	2	2	3	3	1
80	4	YF2	Consp.	Err.	<5	9	5	5	5	3	3	9	3	3
81	2	YF2	Interm.	Reg.	>7	6	0	3	0	0	0	6	0	1
82	3	YF2	Interm.	Reg.	6-7	12	6	7	6	3	2	12	5	4
83	1	YF2	Interm.	Reg.	5-6	5	3	3	3	0	2	5	0	2
84	0	YF2	Interm.	Reg.	<5	0	0	0	0	0	0	0	0	0
85	1	YF2	Interm.	Err.	>7	4	3	3	3	3	1	4	3	2
86	4	YF2	Interm.	Err.	6-7	16	12	13	12	5	6	16	12	11
87	8	YF2	Interm.	Err.	5-6	19	17	16	17	15	11	19	16	13
88	8	YF2	Interm.	Err.	<5	16	13	13	13	10	8	16	11	9
89	0	YF2	Cryptic	Reg.	>7	0	0	0	0	0	0	0	0	0

Appendix 2 (continued)

Butt. Group	No. of Butt. Morphs	Bird	Butt. Color Pattern	Butt. Flight Pattern	Butt. Body Shape	No. of Butt. Test.	Obs. (Att./No. Test.)	Fit.	No. of Butt. Capt	Obs. (Eat./No. Capt.)	Fit.	No. of Butt. Test.	Obs. (Kill./No. Test.)	Fit.
90	0	YF2	Cryptic	Reg.	6-7	0	0	0	0	0	0	0	0	0
91	3	YF2	Cryptic	Reg.	5-6	10	9	9	9	7	8	10	9	9
92	1	YF2	Cryptic	Reg.	<5	2	2	2	2	2	2	2	2	2
93	0	YF2	Cryptic	Err.	>7	0	0	0	0	0	0	0	0	0
94	1	YF2	Cryptic	Err.	6-7	3	3	3	3	3	3	3	3	3
95	8	YF2	Cryptic	Err.	5-6	22	20	22	20	17	19	22	20	21
96	15	YF2	Cryptic	Err.	<5	27	26	26	26	26	25	27	26	26
97	29	YM	Consp.	Reg.	>7	127	54	41	54	16	12	127	26	15
98	8	YM	Consp.	Reg.	6-7	26	6	10	6	0	2	26	1	6
99	2	YM	Consp.	Reg.	5-6	5	0	2	0	0	0	5	0	1
100	1	YM	Consp.	Reg.	<5	2	0	1	0	0	0	2	0	0
101	0	YM	Consp.	Err.	>7	0	0	0	0	0	0	0	0	0
102	0	YM	Consp.	Err.	6-7	0	0	0	0	0	0	0	0	0
103	2	YM	Consp.	Err.	5-6	2	2	1	2	1	1	2	2	1
104	4	YM	Consp.	Err.	<5	11	5	7	5	2	3	11	3	5
105	2	YM	Interm.	Reg.	>7	5	3	3	3	3	1	5	3	2
106	4	YM	Interm.	Reg.	6-7	12	9	8	9	3	4	12	6	6
107	1	YM	Interm.	Reg.	5-6	6	3	4	3	0	2	6	1	3
108	0	YM	Interm.	Reg.	<5	0	0	0	0	0	0	0	0	0
109	2	YM	Interm.	Err.	>7	4	4	3	4	3	2	4	4	3
110	4	YM	Interm.	Err.	6-7	17	10	15	10	6	6	17	10	14
111	9	YM	Interm.	Err.	5-6	17	15	15	15	13	12	17	14	14
112	7	YM	Interm.	Err.	<5	14	12	12	12	9	9	14	9	10
113	0	YM	Cryptic	Reg.	>7	0	0	0	0	0	0	0	0	0
114	0	YM	Cryptic	Reg.	6-7	0	0	0	0	0	0	0	0	0
115	3	YM	Cryptic	Reg.	5-6	8	8	8	8	7	8	8	8	8
116	1	YM	Cryptic	Reg.	<5	1	1	1	1	1	1	1	1	1
117	0	YM	Cryptic	Err.	>7	0	0	0	0	0	0	0	0	0
118	1	YM	Cryptic	Err.	6-7	5	5	5	5	4	5	5	5	5
119	7	YM	Cryptic	Err.	5-6	19	18	19	18	16	18	19	18	19
120	12	YM	Cryptic	Err.	<5	25	25	25	25	25	24	25	25	25

A Kaleidoscope of Cryptic Colors: Polymorphic Caterpillars and Camouflaged Adults on a Multi-Colored Host Plant

JUSTIN O. SCHMIDT

Synopsis

The larvae of *Eumorpha typhon* feed mainly on the leaves of grape, a host plant often concurrently eaten by two other *Eumorpha* species. Grape plants generate a variety of background colors and textures: leaves are green, leaf veins and petioles and new vine can be either green or pink to red, the bark on the main vines is brown, dying tendrils are reddish to brown or black, and dying leaves are yellowish.

In larval *E. typhon* each time a caterpillar molts to another instar, an individual that is green either can remain green or change to a different color pattern. Non-green morphs do not molt to a green morph. A total of eleven color and size morphs exist within the population with various morphs being green, pink, yellow, or a disrupted burgundy-gray. Adult *E. typhon* are monomorphic in color pattern but well camouflaged by their color pattern of variously shaded and shaped patches of brown, gray, and burgundy. Birds and social wasps are major predators of *E. typhon* caterpillars. Both have well developed visual acuity, can see color, and hunt by sight. Additional predators of *E. typhon* also have color vision and most hunt, at least partly, visually. Birds are particularly serious predators of *E. typhon* because they probably can form search images for particular prey, or can learn to vary their searching rates to optimize prey discovery, plus they have potential for learning where to hunt for caterpillars based on microhabitat, leaf damage, and plant species. The crypsis plus color and size polymorphism of *E. typhon* caterpillars appear to be evolved strategies to reduce the numbers of caterpillars of similar appearance in the microhabitat. By so doing, individuals in the population appear to gain protection from predators by reducing the formation of density dependent-search behaviors by birds and by benefiting from the rare morph phenomenon of apostatic selection.

Southwestern Biological Institute, 1961 W. West Brichta Drive, Tucson, Ariz. 85745.

Introduction

> I think all will admit that the larvae upon a tree stand a better chance against their various enemies if they belong to two differently colored species (both well protected) than if they are all the same. So dimorphism is an advantage when divergence in color is quite complete.
>
> E. B Poulton, 1884

For foliage feeding caterpillars, color is an essential fact of life. Simply viewed, caterpillars are non-reproductive eating machines whose goals usually are to eat and grow as quickly as possible and not to be eaten. Survival in an environment filled with predators is no trivial part of the life of a caterpillar. Predators are numerous and exact an immense toll on caterpillar populations: many passerine birds feed their young almost exclusively with caterpillars (Holmes *et al.*, 1979; Heinrich, 1979) and as a group, forest bird species were responsible for a 37 percent weekly caterpillar loss in a New England forest (Holmes *et al.*, 1979) and 78 percent of the variation in larval loss of Douglas-fir tussock moth caterpillars (Torgersen *et al.*, 1984). Social vespid wasps also severely reduce caterpillar populations (Morris, 1972; Mason and Torgersen, 1983; Steward *et al.*, 1988). In Costa Rica the pattern of habitat use by leaf-eating caterpillars is apparently determined more by the seasonal abundance of predators and parasitic insects than by the mere presence of leaves (Janzen, 1987).

One of the major means available for both caterpillars and moths to avoid predation is the use of color. Sometimes color is used in an aposematic fashion to warn predators that the caterpillar or moth is toxic or unpalatable (Blum, 1981; Bowers, 1990) or to mimic an unpalatable species (Wickler,

1968), but in the majority of lepidopterous species color is used primarily to render the animal inconspicuous or unrecognizable to the predator. Various categories of color usage for defense have been designated and defined, e.g. crypsis (or camouflage), masquerade, mimicry, and others (see Endler, 1981 for overview), but by far the most common usage by both caterpillars and moths is crypsis. Crypsis can be defined as the use of color and pattern by an individual so that it resembles the background perceived by visually hunting predators in the microhabitat and at the time in which the individual is most vulnerable to predation (Endler, 1978). Crypsis is a relative term. An organism is most cryptic on specific backgrounds, often when specifically oriented relative to the background, and at specific times under specific lighting conditions. An animal that is cryptic in one circumstance can be conspicuous in others. Overriding factors in crypsis are the predators and their visual and perception of potential prey.

The value of cryptic coloration in prey organisms has been most thoroughly studied with birds as potential predators. Birds are natural subjects because they are major predators of insects and other invertebrates, search for food mainly visually (Tinbergen, 1960), generally have color vision, are intelligent and capable of learning, and are easy to experimentally manipulate. Birds also effectively discover and prey upon cryptic prey. They appear to accomplish this either through formation of specific search images (Tinbergen, 1960) or by altering rates of searching (Gendron, 1986; Guilford and Dawkins 1987, 1989).

Density dependent selection by predators on cryptic prey is an important factor in prey color and defensive strategies. Apostatic selection, or selection that favors survival of rare morphs relative to common morphs, was understood by Poulton (1884) and formalized by Clarke (1962). In essence, for apostatic selection to favor the rare morph, the prey should be cryptic and match the background (Cooper, 1984; reviewed by Allen, 1988). Unlike apostatic selection, selective predation on "odd" (or rare) individuals, individuals that are conspicuously different from the bulk of the population, occurs only in situations where the prey are conspicuous, fast moving, and/or are in high density. In the case of flocks, schools, or herds, the "odd" individual of the group is probably selected for attempted capture by the predator in an effort to reduce the "confusion effect" generated by the escape movements of the similar appearing members of the group (Landeau and Terborgh, 1986). In the case of cryptic prey, selection for predation of rare individuals is unexpected except, perhaps, in situations of extreme prey abundance where both common and "rare" types occur. In lower density situations where rare types are truly rare, apostatic selection favoring decreased predation on the rare morphs should be the overriding factor.

A common observation within populations of cryptic prey species is the occurrence of color polymorphism. In cryptic polymorphism the prey exhibit two or more distinctly different color morphs which sometimes are found in the same, and sometimes in different microhabitats. Differential rates of predation on various color morphs has been amply demonstrated (e.g. Cesnola, 1904; Gerould, 1921; Kettlewell, 1956; Den Boer, 1971; Sims and Shapiro, 1983) especially when the backgrounds upon which the individuals are resting render one form more cryptic than the other (Cesnola, 1904). Beyond this, the intriguing observation that predation upon the population as a whole is reduced when polymorphism exists was noted by Poulton (1890; see Endler, 1988 for a review of predator factors and prey coloration).

Evidence is suggestive that individuals of color morphs of polymorphic prey sometimes behave differently from one another. Fifth instar brown-gray caterpillars, the more common morph, of the sphinx moth *Sphecodina abbotti* feed on the host grapevine (*Vitis vulpina*) almost exclusively at night and after feeding travel as much as 2 m from the feeding site to rest on the brown bark of the grapevine. The rarer green fifth instar larvae feed both during the day and night and rest primarily on the leaves and green vines (Heinrich, 1979). Similarly, pink *Amphion floridensis* (Sphingidae) rest primarily on stems (but do not discriminate among stems of different colors), whereas green larvae rest primarily under leaves (Fink, 1987). Leaf or catkin mimicking morphs of *Nemoria arizonaria* (Geometridae), when removed from the cryptic part of the plant and placed on a less cryptic part, returned preferentially to that part of the plant on which they were most cryptic (Greene, 1989).

Observational evidence indicates that the different behaviors of the different color morphs of *Erinnis ello*, a sphinx moth feeding on *Poinsettia pulcherrima* (Euphorbiaceae), is a result of predation pressure. The green, blue, or green-gray morphs all rested on the leaves of the host plant where they were heavily preyed upon by the wasp *Polistes crinitus*. The brown morph rested primarily on the branches and trunk where it was infrequently captured by *Polistes*. The lizards *Anolis lineatopus* and *A. grahami* forage primarily on the stems of the plant and more frequently attack non-brown larvae than brown ones. Consequently, the various greenish morphs are forced to rest on the leaves where they are more vulnerable to *Polistes* (Curio, 1970).

Eumorpha typhon and Its Predators

Eumorpha typhon is an enormous hawk moth

Table 1. Developmental rates and body sizes of *Eumorpha typhon* reared in the laboratory on domestic grape cuttings and at ambient temperatures of 25° to 30°C.

Day	Stage	Head capsule width (mm)	Approximate body length (mm)
0-4	Egg	-	1.8 x 2.0
5-6	1st instar	1.05	9
7-8	2nd instar	1.64	17
9-11	3rd instar	2.42	30
12-16	4th instar	3.63	54
17-22	5th instar	5.51	100-125
23	Wanderer	-	-
24	Prepupa (in pupal cell underground)	-	-

whose larvae feed on the leaves of at least grape (*Vitis arizonica* and *V. vinifera*) and Virginia creeper (*Parthenocissus quinquefolia*). It ranges from the mountains of southern Arizona and New Mexico to Honduras. In the U.S., *E. typhon* is sympatric with three other *Eumorpha* species, *E. fasciata*, *E. achemon* and *E. vitis* (Hodges, 1971). Two of these, *E. achemon* and *E. vitis*, also feed on grape, often on the same individual plant (personal observations). Until this investigation, the larvae and host plant of *E. typhon* were so "cryptic" as to remain unknown.

Caterpillars of *E. typhon* were discovered and investigated at two sites: at an elevation of 1635 m on the grounds of the Southwestern Research Station of the American Museum of Natural History, located in the Chiricahua Mountains at Portal, Arizona; and located in Patagonia, Arizona, in the Santa Rita Mountains at an elevation of 1235 m. Both sites were mountain riparian habitats having permanent streams and an abundance of large deciduous trees including cottonwoods, (*Populus fremontii*) and sycamores (*Platanus wrightii*) plus a dense understory vegetation including abundant wild grape (*V. arizonica*) and ornamentally planted Virginia-creeper. The surrounding non-riparian rocky hillsides were dominated by oaks (*Quercus* spp.), mesquite (*Prosopis velutina*), juniper (*Juniperus* spp.) and grasses. Both sites are well known for their diversities and abundant populations of birds, insects and other animals. *E. typhon* caterpillars were investigated both at their sites of discovery and reared and propagated in the lab.

The caterpillars of *E. typhon* are among the very largest found in the U.S. with maximal larval sizes of 12.5 cm and weights of 20 g. Mature larvae are often larger than sparrows and warblers that typically feed on caterpillars. Developmental rates and size data for the species are listed in Table 1.

Potential predators of *E. typhon* and the defenses against these predators are listed in Table 2. The major predators of the smallest larvae (first and second instars) are most likely ants, spiders, predacious bugs, and polistine wasps (Itô and Miyashita 1968; Den Boer, 1971; McNeil *et al.*, 1978; Mason and Torgersen, 1983; Mason and Paul, 1988) with birds being relatively unimportant. Birds are likely to be less important predators of small caterpillars because they appear to selectively prey upon caterpillars that are large (>18 mm) (Tinbergen, 1960; Morris, 1972; Torgersen *et al.*, 1984). Mid-sized larvae (late second to early fourth instars) are too large to fall easy prey for spiders, ants and most other occasional arthropod predators of the caterpillars (I have observed crab spiders [Thomisidae] preying on first and second instar *E. typhon*, but not on larger instars; Mason and Torgersen, 1983). At this size *Polistes* and the yellowjacket wasps (primarily *Vespula pensylvanica*) and various parasitic and hunting wasps are probably important predators, with birds becoming a factor for the largest caterpillars (Morris, 1972; Mason and Torgersen, 1983; Steward *et al.*, 1988).

Large (fourth and fifth instar) larvae are probably preyed upon primarily by birds (Eliot and Soule, 1902; Den Boer, 1971; Morris, 1972; Torgersen *et al.*, 1984) with polistine wasps and other predators being less important. *Polistes arizonensis* have been observed actively searching grape leaves, concentrating on areas freshly chewed by *E. typhon*, yet when the large fifth instars caterpillars themselves were discovered, the wasps flew off (author's personal observations). Adult *E. typhon* are likely preyed upon primarily by birds and bats.

Many local environmental factors plus properties inherent to a potentially wide diversity of predators determine the net severity of predation pres-

Table 2. Potential predators of *Eumorpha typhon* and its defenses against these predators.

Predator	Predator's means of detection	Prey defense
	Larvae	
Birds	Vision	Cryptic polymorphism
Social wasps (*Polistes*, *Mischocyttarus*, etc.)	Vision, odor	Cryptic polymorphism, others?
Tachinid flies	Vision, odor	Cryptic polymorphism, others?
Sphecid and eumenid wasps	Vision, odor	Cryptic polymorphism, others?
Parasitic wasps	Odor, vision	?
Spiders and predaceous insects	Close range vision, movement detection	?
Ants	Close range vision, odor	Cling to substrate?
	Adults	
Birds	Vision	Crypsis, tibial spurs, scales, flight
Bats	Ultrasonics	Tibial spurs, scales, protean escape?

sure to prey such as caterpillars and moths. Birds hunt primarily visually (Tinbergen, 1960) and have excellent color vision (Endler, 1978; Lythgoe, 1979; Jacobs, 1981). Hunger can also be a major factor in the behavior of birds and other predators (Holling, 1965; Beukema, 1968; Rechten *et al.*, 1983; Houston and McNamara, 1985; Janzen, 1988) and can greatly modify the effectiveness of various anti-predator strategies of prey. Birds find prey by using search images, by reducing foraging speeds, by selecting certain habitats for searching, and are known to search selectively on certain parts of plants, e.g. under leaves (Greenberg and Gradwohl, 1980), near areas of leaf damage (Greenberg and Gradwohl, 1980; Heinrich and Collins, 1983), and on certain species of trees where cryptic caterpillars occur (Heinrich and Collins, 1983).

Social wasps (*Polistes, Mischocyttarus, Vespula, Dolichovespula*, etc.), probably the major non-avian predators of caterpillars, search for prey mainly through visual search of foliage (Dowell and Johnson, 1986; Raveret Richter, 1990) with odor playing a role at close range (Den Boer, 1971; Morris, 1972; Steward *et al.*, 1988; S. B. Vinson, personal communication). Social wasps, like most insects (Gruber, 1979), possess excellent color vision, which helps to distinguish prey from the background (Weiss, 1953; Mazokhin-Porshniakov, 1969; Menzel, 1971; Beier and Menzel, 1972).

The senses used in prey detection by the less important potential predators of *E. typhon* (Table 2) are not as well studied as those of birds. Many of the parasitic wasps and flies rely in part on chemical cues for host location (Sugimoto *et al.*, 1988; Hérard *et al.*, 1988; Auger *et al.*, 1989; Ding *et al.*, 1989; Strand *et al.*, 1989; Roland *et al.*, 1989). Nevertheless, they, as well as the spiders, predatory wasps and ants, all possess color vision (Gruber, 1979) and most, with the possible exception of crab spiders (Den Boer, 1971), use vision, at least in part, in their search behavior.

Crypsis and color polymorphism are the major *E. typhon* defenses against caterpillar predators. *E. typhon* caterpillars are palatable, slow moving, and possess no highly effective "fight back" defenses against birds and wasps. When grasped they do not vigorously thrash and writhe, nor do they regurgitate gut contents as do some other sphingid larvae; rather, they tend to retract their head into the thorax and very firmly hold onto the substrate with their legs. Their attachment to the substrate is so tight that it is often difficult to remove them from vines without tearing or injuring one or more prolegs (personal observations). In *Eumorpha*, behaviors such as head flicking or squirming in response to sound (Minnich, 1936; Myers and Smith, 1978; Tautz and Markl, 1978) and selective feeding and pruning of leaves to reduce leaf damage conspicuousness (Heinrich and Collins, 1983) are not known.

Cryptic Color Polymorphism in *Eumorpha typhon*

E. typhon feeds exclusively on species in the Vitaceae, particularly on grape. The grape plant is

an ideal background substrate for a polymorphic species. Grape plants come with numerous shades of colors and textures of surfaces. The leaves are greenish, but the leaf petioles can be either green or pinkish to red. Likewise, the growing vine stems can be green or pinkish to red. The older woody vines are covered with a brown bark, dying leaves turn yellowish-tan, and dying tendrils range in color from reddish to black. An alternate host plant, Virginia-creeper, is green with some areas being pink. The coloration between and within individual grape plants is highly variable. The red color in grape vegetation is apparently caused primarily by anthocyanins which are variably produced in grapes as a result of light (Slabecka-Szweykowska, 1955) and likely by other factors such as temperature and nutrition (Noggle and Fritz, 1976). The various environmentally-induced grape plant colors and variable incidences of pink-red color make conditions ideal for larval color polymorphism to evolve and for apostatic selection to occur. Evidence of crypsis in all instars and for all color morphs of *E. typhon* is shown in Figs. 1 through 15 taken of animals naturally found on plants.

The eggs of *E. typhon* are a light green and are laid singly on grape leaves (Plate 2:1). All first instar larvae are pale green with long black tails (Plate 2:2) that resemble dried tendrils of the host grape (Plate 2:3). Second instar larvae are either pink (Plate 2:4) or green (Plate 2:5), both with pink tails that resemble withering grape tendrils (Plate 2:6). These caterpillars are approximately the same diameter as grape leaf petioles (Plate 2:4). Third instar caterpillars are either pink or green with pink tails. Both body and tail color closely match those observed in grape (Plates 3:7 and 3:8). Fourth instar larvae can be pink, green, or, rarely, yellow, all with short matchingly colored curved tails. All three color morphs can be cryptic when resting on matching parts of the grape plant (Plate 3: 9, 3:10 and 3:11). The fifth, and last instar, caterpillars can be either a disrupted burgundy-gray color pattern (Plate 4:12) or green (Plate 4:13). Both lack tails and readily match the appropriate color background of the grape plant (Plate 4:14). Both sexes of *E. typhon* are monomorphic and colored indentically; but they, like the larvae, have diurnal predators that hunt visually, are cryptic when resting on the host plant (Plate 4:15).

As pointed out by Clarke (1962), when two or more species share the same predators, habitat, and conditions favorable for apostatic selection, the species will tend to diverge in appearance. In the case of the grape feeding *Eumorpha* in Southern Arizona, the most abundant color morphs of the last two instar larvae of the grape feeding congeneric *E. achemon* are predominantly brown (Plates 4:16 and 4:17), a color different from any *E. typhon* caterpillars. The fourth and fifth instar larvae of *E. vitis*, also a congeneric grape feeder, are rather different from *E. typhon* in possessing a pair of dorsolateral bands running from the fourth to eleventh segments (Moss, 1912). The larvae of these three species, if considered from a visually searching predator's point of view, represent a melange of some approximately 20 color morphs all potentially living on the same plant.

Acknowledgments

I thank Wade Sherbrooke of the Southwest Research Station in Portal, Arizona who made facilities available for use and provided information on the presence of caterpillars on nearby vines; Abbey Seltzer who kindly informed me of the locations of caterpillars in Patagonia, Arizona; John Endler for many helpful suggestions and comments on the manuscript; and, especially, Pat Schmidt who performed much of the field and laboratory work and for many helpful suggestions throughout the research and manuscript writing.

References

Allen, J. A. 1988. Frequency-dependent selection by predators. **Phil. Trans. R. Soc.** Lond. B 319:485-503.

Auger, J., C. Lecomte, J. Paris and E. Thibout, 1989. Identification of leek-moth and diamondback-moth frass volatiles that stimulate parasitoid, *Diadromus pulchellus*. **J. Chem. Ecol.** 15:1391-1398.

Beier, W. and R. Menzel. 1972. Untersuchungen über den Farbensinn der deutschen Wespa (*Paravespula germanica* F., Hymenoptera, Vespidae): Verhaltensphysiologischer Nachweis des Farbensehens. **Zool. Jahr. Allg. Zool. Physiol. Tiere** 76: 441-454.

Beukema, J. J. 1968. Predation by the three-spined stickleback (*Gasterosteus aculeatus* L.): the influence of hunger and experience. **Behaviour** 31:1-126.

Blum, M. S. 1981. **Chemical Defenses of Arthropids.** Academic, New York.

Bowers, M. D. 1990. Recycling plant natural products for insect defense. *In* D. L. Evans and J. O. Schmidt (eds.), **Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators**, pp. 353-386. SUNY Press, Albany, NY.

Cesnola, A. P. 1904. Preliminary note on the protective value of colour in *Mantis religiosa*. **Biometrika** 3:58-59.

Clarke, B. 1962. Balanced polymorphism and the diversity of sympatric species. *In* D. Nichols, (ed.) **Taxonomy and Geography**, pp. 47-70. The Systematics Association, London.

Cooper, J. M. 1984. Apostatic selection on prey

that match the background. **Biol. J. Linn. Soc.** 23:221-228.

Curio, E. 1970. Die Selektion dreier Raupenformen eines Schwarmers (Lepidopt., Sphingidae) durch einen *Anolis* (Rept., Iguanidae). **Z. Tierpsychol.** 27:899-914.

Den Boer, M. H. 1971. A colour-polymorphism in caterpillars of *Bupalus piniarius* (L.) (Lepidoptera: Geometridae). **Neth. J. Zool.** 21:61-116.

Ding, D., P. D. Swedenborg and R. L. Jones. 1989. Plant odor preferences and learning in *Macrocentrus grandii* (Hymenoptera: Braconidae), a larval parasitoid of the European corn borer, *Ostrina nubilalis* (Lepidoptera: Pyralidae). **J. Kansas Ent. Soc.** 62:164-176.

Dowell, R. V. and M. Johnson. 1986. *Polistes major* (Hymenoptera: Vespidae) predation of the treehopper, *Umbonia crassicornis* (Homoptera: Membracidae). **Pan-Pac. Ent.** 62:150-152.

Eliot, I. M. and C. G. Soule. 1902. **Caterpillars and Their Moths**. Century, New York.

Endler, J. A. 1978. A predator's view of animal color patterns. *In* M. K. Hecht, W. L. Steere, and V. Wallace, (eds.), **Evolutionary Biology**, Vol. 2, pp. 319-364. Plenum, New York.

Endler, J. A. 1981. An overview of the relationships between mimicry and crypsis. **Biol. J. Linn. Soc.** 16:25-31.

Endler, J. A. 1988. Frequency-dependent predation, crypsis and aposematic coloration. **Phil. Trans. R. Soc. Lond.** B 319:505-523.

Fink, L. S. 1987. Behavioral differences among color morphs of sphingid caterpillars. **Amer. Zool.** 27:102A.

Gendron, R. P. 1986. Searching for cryptic prey: evidence for optimal search rates and the formation of search images in quail. **Anim. Behav.** 34:898-912.

Gerould, J. H. 1921. Blue-green caterpillars: the origin and ecology of a mutation in hemolymph color in *Colias* (*Eurymus*) *philodice*. **J. Exp. Zool.** 34:385-415.

Greenberg, R. and J. Gradwohl. 1980. Leaf surface specializations of birds and arthropods in a Panamanian forest. **Oecologia** 46:115-124.

Greene, E. 1989. A diet-induced developmental polymorphism in a caterpillar. **Science** 243:643-646.

Gruber, S. H. 1979. Mechanisms of color vision: an ethologist's primer. *In* E. H. Burtt, Jr. (ed.), **The Behavioral Significance of Color**, pp. 183-236. Garland STPM Press, New York.

Guilford, T. and M. S. Dawkins. 1987. Search images not proven: a reappraisal of recent evidence. **Anim. Behav.** 35:1838-1845.

Guilford, T. and M. S. Dawkins. 1989. Search image versus search rate: a reply to Lawrence. **Anim. Behav.** 37:160-162.

Heinrich, B. 1979. Foraging strategies of caterpillars: leaf damage and possible predator avoidance strategies. **Oecologia** 42:325-327.

Heinrich, B. and S. L. Collins. 1983. Caterpillar leaf damage, and the game of hide-and-seek with birds. **Ecology** 64:592-602.

Hérard, F., M. A. Keller, W. J. Lewis and J. H. Tumlinson. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals. III influence of age and experience on flight chamber responses of *Microplitis demolitor* Wilkinson. **J. Chem. Ecol.** 14:1583-1596.

Hodges, R. W. 1971. **The Moths of America North of Mexico, Fascicle 21 Sphingoidea**. E. W. Classey, London.

Holling, C. S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. **Mem. Ent. Soc. Canada.** 45:1-60.

Holmes, R. T., J. C. Schultz, and P. Nothnagle. 1979. Bird predation on forest insects: an exclosure experiment. **Science** 206:462-463.

Houston, A. and J. McNamara. 1985. The choice of two prey types that minimizes the probability of starvation. **Behav. Ecol. Sociobiol.** 17:135-141.

Itô, Y. and K. Miyashita. 1968. Biology of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) in Japan. V. Preliminary life tables and mortality data in urban areas. **Res. Popul. Ecol.** 10:177-209.

Jacobs, G. H. 1981. **Comparative Color Vision**. Academic Press, New York.

Janzen, D.H. 1987. How moths pass the dry season in a Costa Rican dry forest. **Insect Sci. Applic.** 8:489-500.

Janzen, D.H. 1988. Ecological characterization of a Costa Rican dry forest caterpillar fauna. **Biotropica** 20:120-135.

Kettlewell, H. B. D. 1956. Further selection experiments on industrial melanism in the Lepidoptera. **Heredity**10:287-301.

Landeau, L. and J. Terborgh. 1986. Oddity and the "confusion effect" in predation. **Anim. Behav.** 34:1372-1380.

Lythgoe, J. N. 1979. **The Ecology of Vision**. Oxford Univ. Press, Oxford.

Mason, R.R. and H.G. Paul. 1988. Predation on larvae of Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae), by *Metaphidippus aeneolus* (Araneae: Salticidae). **Pan-Pac. Ent.** 64:258-260.

Mason, R. R. and T. R. Torgersen. 1983. Mortality of larvae in stocked cohorts of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae). **Can. Entomol.** 115:1119-1127.

Mazokhin-Porshniakov, G. A. 1969. **Insect Vision**. Plenum Press, New York.

McNeil, J. N., J. Delisle and R. J. Finnegan. 1978. Seasonal predatory activity of the introduced red wood ant, *Formica lugubris* (Hymenoptera: Formicidae) at Valcartier, Quebec in 1976. **Can. Entomol.** 110:85-90.

Menzel, R. 1971. Uber den Farbensinn von *Paravespula germanica* F. (Hymenoptera): ERG und selektive Adaptation. **Z. Vergl. Physiol.** 75:86-104.

Minnich, D. E. 1936. The responses of caterpillars to sounds. **J. Exp. Zool.** 72:439-453.

Morris, R. F. 1972. Predation by wasps, birds, and mammals on *Hyphantria cunea*. **Can. Entomol.** 104:1581-1591.

Moss, A. M. 1912. On the Sphingidae of Peru. **Trans. Zool. Soc. Lond.** 20:73-135.

Myers, J. H. and J. N. M. Smith. 1978. Head flicking by tent caterpillars: a defensive response to parasite sounds. **Can. J. Zool.** 56:1628-1631.

Noggle, G. R. and G. J. Fritz. 1976. **Introductory Plant Physiology**. Prentice-Hall, Englewood Cliffs, New Jersey.

Poulton, E. B. 1884. Notes upon, or suggested by, the colours, markings, and protective attitudes of certain lepidopterous larvae and pupae, and of a phytophagous hymenopterous larva. **Trans. Ent. Soc. Lond.** 1884:27-60.

Poulton, E. B. 1890. **The Colours of Animals.** D. Appleton, New York.

Raveret Richter, M. 1990. Hunting social wasp interactions: influence of prey size, arrival order, and wasp species. **Ecology** 71: (in press).

Rechten, C., M. Avery, and A. Stevens. 1983. Optimal prey selection; why do great tits show partial preferences? **Anim. Behav.** 31:576-84.

Roland, J., W. G. Evans and J. H. Myers. 1989. Manipulation of oviposition patterns of the parasitoid *Cyzenis albicans* (Tachinidae) in the field using pland extracts. **J. Insect Behav.** 2:487-503.

Sims, S. R. and A. M. Shapiro. 1983. Pupal color dimorphism in California *Battus philenor* (L.) (Papilionidae): mortality factors and selective advantage. **J. Lepidop. Soc.** 37:236-243.

Slabecka-Szweykowska, A. 1955. Wplyw dlugosci fali swiatla na biogeneze barwnika antocyjanowego w tkance winorosli hodowanej in vitro [The influence of wave length of light on the biogenesis of anthocyanin pigment in *Vitis vinifera* tissue in vitro]. **Acta Soc. Bot. Polon** 24:3-11.

Steward, V. B., K. G. Smith and F. M. Stephen. 1988. Predation by wasps on lepidopteran larvae in an Ozark forest canopy. **Ecol. Entomol.** 13:81-86.

Strand, M. R., H. J. Williams, S. B. Vinson and A. Mudd. 1989. Kairomonal activities of 2-acylcyclohexane-1, 3-diones produced by *Ephestia kuehniella* Zeller in eliciting searching behavior by the parasitoid *Bracon hebetor* (Say). **J. Chem. Ecol.** 15:1491-1500.

Sugimoto, T., Y. Shimono, Y. Hata, A. Nakai and M. Yahara. 1988. Foraging for patchily-distributed leaf-miners by the parasitoid, *Dapsilarthra rufiventris* (Hymenoptera: Braconidae) III. visual and acoustic cues to a close range patch-location. **Appl. Ent. Zool.** 23:113-121.

Tautz, J. and H. Markl. 1978. Caterpillars detect flying wasps by hairs sensitive to airborne vibration. **Behav. Ecol. Sociobiol.** 4:101-110.

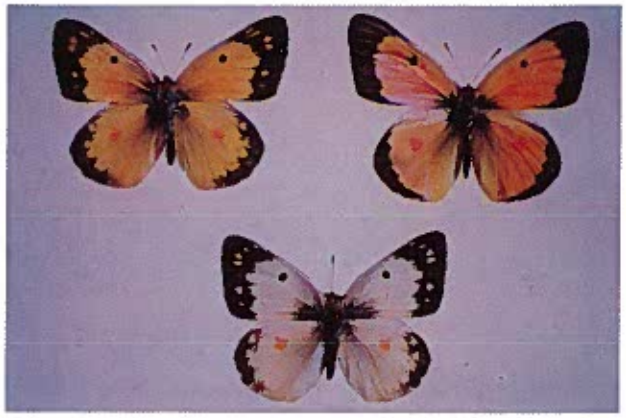
Tinbergen, L. 1960. The natural control of insects in pinewoods. I. factors influencing the intensity of predation by songbirds. **Arch. Neerl. Zool.** 13:265-343.

Torgersen, T. R., J. W. Thomas, R. R. Mason and D. van Horn. 1984. Avian predators of Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough), (Lepidoptera: Lymantriidae) in southwestern Oregon. **Environ. Entomol.** 13:1018-1022.

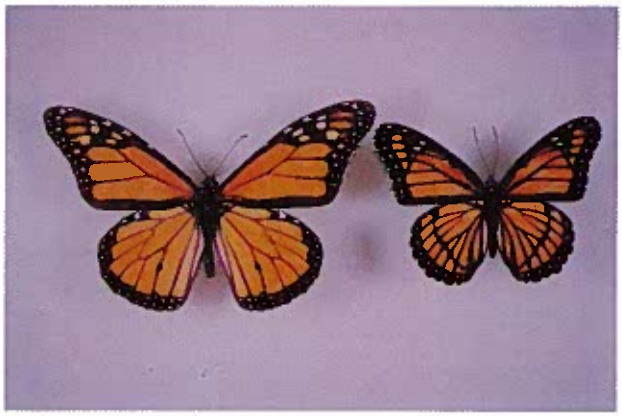
Weiss, K. 1953. Versuche mit Bienen und Wespen in farbigen Labyrinthen. **Z. Tierpsychol.** 10:29-44.

Wickler, W. 1968. **Mimicry in Plants and Animals**. McGraw-Hill, New York.

1. Inappropriate color as a lethal condition in the wild. Blue-green recessive mutant, and wild-type, larvae of *Colias eurytheme*, as studied by Gerould (1921, 1926) and Hoffmann and Watt (1984), on domestic vetch (*Vicia*) in the laboratory. Gerould found that the blue-green variant is easily found and eaten by predatory birds, which fail to find larvae of the "wild-type" green color.



2. A polymorphism in resource allocation to pigment patterns. Male (solid black borders) and female (light spots in black borders) "alba" (white) and "normal" (orange) *Colias eurytheme* butterflies.



3. Mimicry in North American butterflies. Left, the Monarch, *Danaus plexippus*, distasteful model; right, *Limenitis archippus*, edible mimic (J. Van Zandt Brower 1958a).

4. Mimicry in North American butterflies. Left, the Pipevine Swallowtail, *Battus philenor*, distasteful model; right, *Limenitis astyanax*, probable edible mimic (J. Van Zandt Brower 1958b).

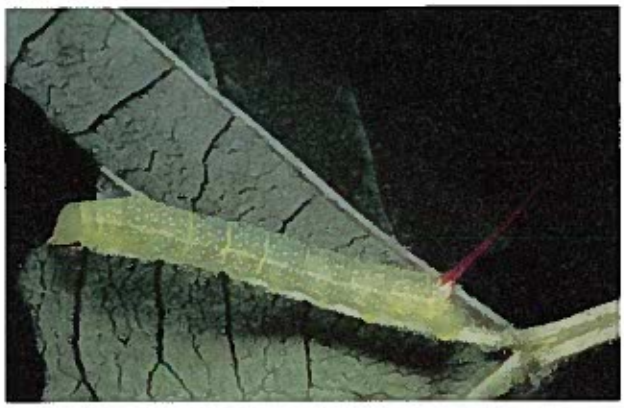
5. Evidence of possible predisposition to mimetic evolution. Different departures of *Limenitis* mimetic patterns from the apparently ancestral pattern, shown by *L. weidemeyerii* below *L. archippus* and *L. astyanax*.

1. Egg on the underside of a grape leaf.

2. First instar caterpillar.

3. Dead tendril remnant that resembles the tail of first instar caterpillar.

4. Second instar pink morph caterpillars on grape.



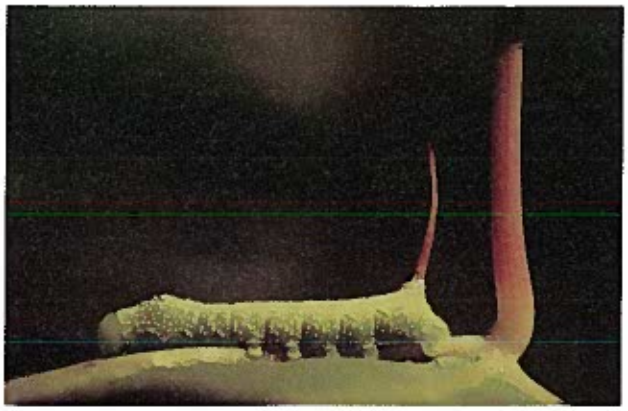
5. Second instar caterpillar, green morph.

6. Grapevine tendril that resembles the tails of second instar caterpillars.

Crypsis of the egg and small caterpillars of *Eumorpha typhon*.

Plate 2 — Schmidt

7. Third instar caterpillar, pink morph.



8. Third instar caterpillar, green morph.

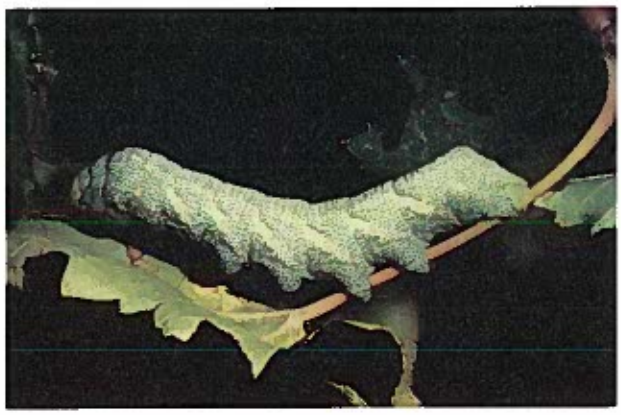
9. Fourth instar caterpillar, pink morph.

10. Fourth instar caterpillar, green morph.

11. Fourth instar caterpillar, yellow morph.

Cryptic color polymorphism of the third and fourth instar caterpillars of *E. typhon*.

12. Disrupted burgundy-gray color pattern of fifth instar caterpillar of *E. typhon*.



13. Green morph of fifth instar caterpillar of *E. typhon*.

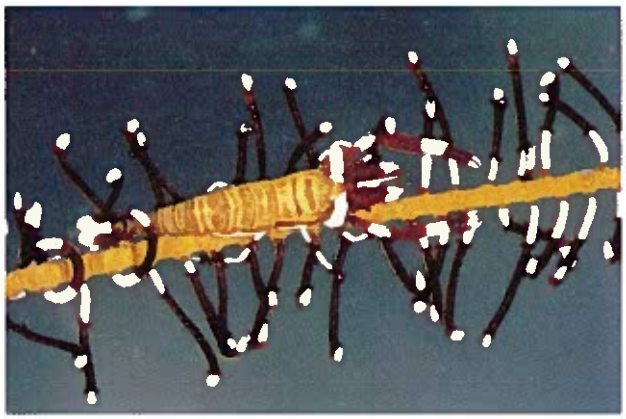
14. Both color morphs on grape (fifth instar caterpillar of *E. typhon*).

15. Adult *E. typhon* resting on the host grape plant.

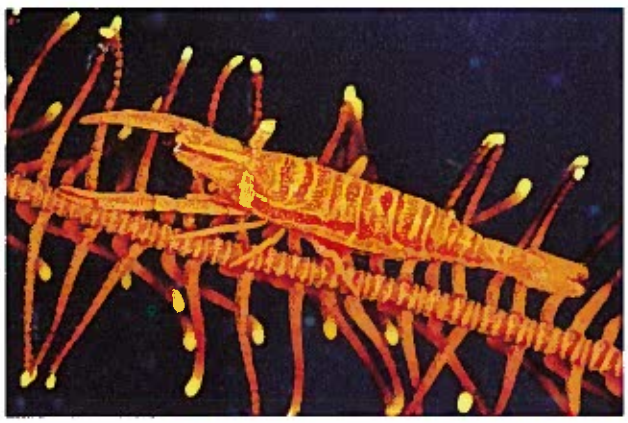
16 and 17. Brown color morphs of fourth and fifth instars of *E. achemon*.

1. An example of disruptive coloration: *Periclimenes brevicarpalis* on a cnidarian host at Enewetak Atoll, Marshall Islands. (Photograph by A. Charles Arneson.)

2. *Periclimenes tenuis* mimicking a pinnule on the arm of its crinoid host, *Comanthus bennetti*, at Enewetak Atoll, Marshall Islands. In this example of homochromy, the body of the shrimp is largely transparent, with longitudinal stripes of pigment that match the host. (Photograph by the author.)



3. An example of homochromy achieved through complex pigmentation: *Periclimenes amboinensis* on the crinoid, *Comantheria briareus* at Kwajalein Atoll, Marshall Islands. (Photography by the author.)

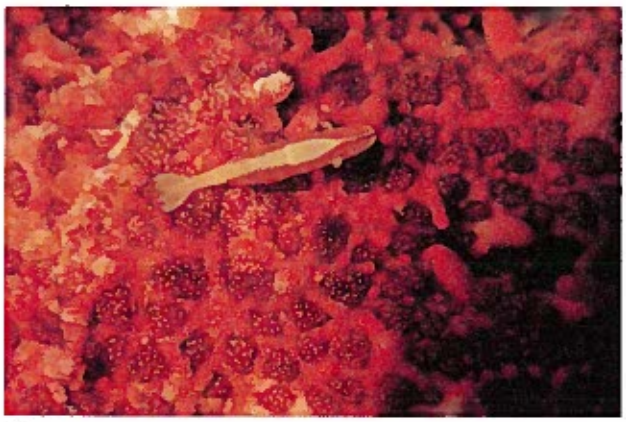


4. (A and B) Two specimens of *Periclimenes amboinensis*, collected from two different color varieties of the crinoid, *Comanthus bennetti*, Enewetak Atoll, Marshall Islands. (Photographs by the author.)

5. Two Caribbean cleaner shrimps with contrasting coloration: A. *Periclimenes yucatanicus*. B. *Periclimenes pedersoni*. (Photographs by A. Charles Arneson.)

A

6. (A, B and C) Three color patterns displayed by *Periclimenes soror*, an associate of Indo-Pacific asteroids. (Photographs by A. Kerstitch.)

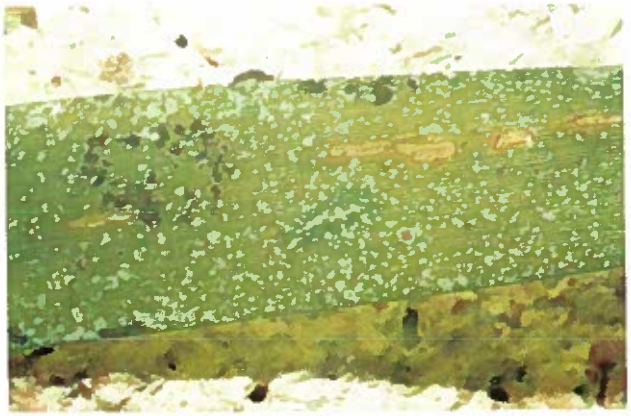


B

C

Common fiddler crabs (*Uca*) of Texas. A. *Uca subcylindrica* (Stimpson). B. *Uca panacea* Novak and Salmon(courtesy of F.H. Barnwell). C. *Uca spinicarpa* Rathbun. D. *Uca longisignalis* Salmon and Atsaides. E. *Uca rapax* (Smith).

Plate 7 — Thurman



A. *Phyllaplysia engeli* Marcus, 1955, on turtle grass *Thalassia testudinum* Banks ex Konig, Punta Allen, Quintana Roo Mexico, T.M.G.

B. *Aldisa sanguinea* (Cooper, 1863) on sponge prey, Pt. Lobos, California, Marc Chamberlin.

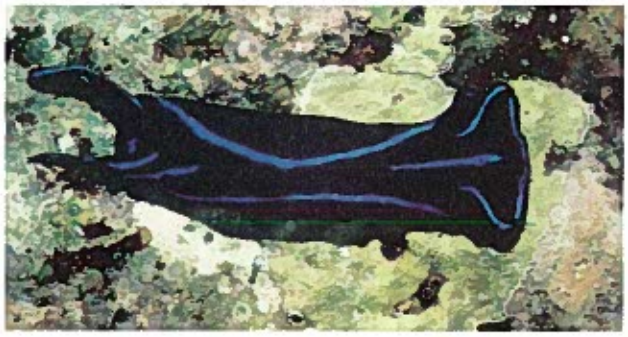
C. *Lomanotus vermiformis* O'Donoghue, 1929, Loreto, Baja California Sur, Mexico, T.M.G.

D. *Doto* sp. on plumularid hydroid, Madang, Papua New Guinea, T.M.G.

Special resemblance in opisthobranch gastropods.

Plate 8 — Gosliner and Behrens

A. *Chelidonura pallida* Risbec, 1951.



B. *Chelidonura varians* Eliot, 1903.

C. *Elysia bayeri* Marcus, 1965.

D. *Elysia* sp.

E. *Chromodoris kuniei* (Pruvot-Fol, 1930).

F. *Chromodoris magnifica* (Quoy and Gaimard, 1832).

G. *Phyllidia* sp.

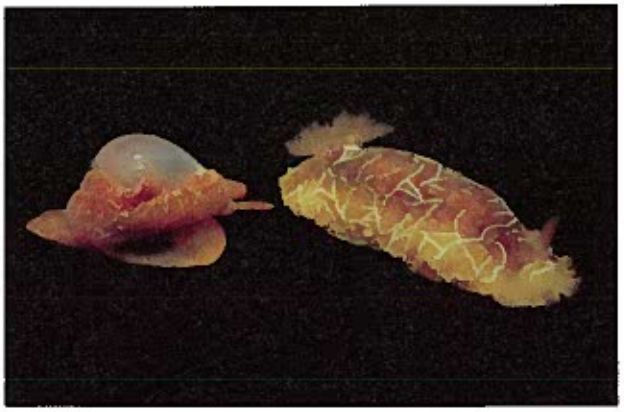
H. *Phyllidia* sp.

Aposematic coloration in opisthobranchs. (All specimens from Madang, Papua New Guinea, photographed by T.M.G.).

A. Nudibranch Batesian model, *Flabellina iodinea* (Cooper, 1862), Morris Point, California, D.W.B.

B. Amphipod Batesian mimic, *Podocerus cristatus* (Thompson, 1879).

C. Nudibranch model, *Doriopsilla* sp. and prosobranch Batesian mimic, *Trivia ovulata* (Lamarck, 1810), Danger Point, South Africa, W.R. Liltved.



D. Nudibranch mimic, *Doriopsilla miniata* (Alder and Hancock, 1864) and prosobranch Batesian mimic, *Trivia millardi* (Cate, 1979), Llandudno, South Africa, W.R. Liltved.

E-G. Mullerian mimics from Madang, Papua New Guinea, T.M.G.
E. *Chelidonura* sp.

F. Undescribed haminoeid.

G. *Gastropteron bicornutum* Baba and Tokioka, 1965.

Mimicry involving opisthobranch models.

Plate 10 — Gosliner and Behrens

A. Top: undescribed cryptobranch dorid; bottom: *Phyllidia nobilis* Bergh, 1869.

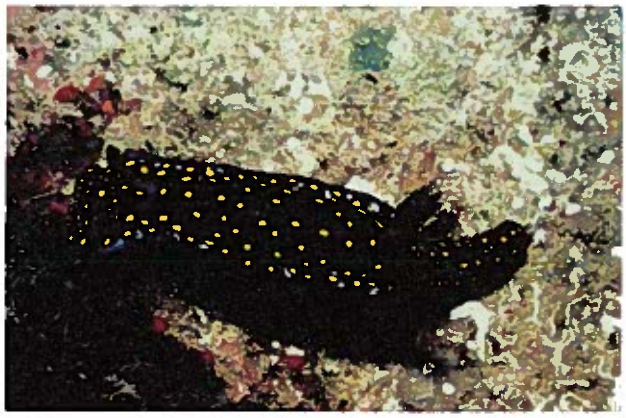
B. Phyllidiid and holothurian mimics. Left: *Phyllidia coelestis* Bergh, 1905; top: *Phyllidia varicosa* Lamarck, 1801; lower right: *Fryeria ruppelli* Bergh, 1869; center: the sea cucumber *Bohadschia graeffei* (Semper, 1868).

C. *Chromodoris geometrica* Risbec, 1928.

D. Unidentified polyclad flatworm.

E. *Pseudoceros bajae* Hyman, 1953.

F. *Navanox inermis* (Cooper, 1862).



G. *Hypselodoris ghiselini* Bertsch, 1973.

Mullerian mimics from Madang, Papua New Guinea.

Plate 11 — Gosliner and Behrens

Adaptive Coloration of Pontoniine Shrimps (Crustacea: Decapoda: Caridea)

DEBORAH L. ZMARZLY

Synopsis

Pontoniine shrimps, which occur predominantly as symbionts of other macroinvertebrates, exhibit two trends in coloration. One trend is toward increasing crypsis; the other is toward increasing conspicuousness. Crypsis is achieved by transparency, disruptive coloration, or homochromy (coloration which matches that of the host). In some cases, shrimp coloration mimics a particular region or microhabitat on the host. Host mimicry may be further enhanced by morphological or behavioral adaptations of shrimps. Coloration is often uniform across vast geographic ranges, thus indicating a degree of genetic control over the development and expression of color patterns. There are also species capable of matching a variety of host color patterns, which indicates a degree of environmental influence and raises the question of how host coloration is perceived and translated by newly settled juveniles.

Introduction

Many species in the caridean subfamily Pontoniinae occur as obligate symbionts of other macroinvertebrates (Bruce, 1976, 1982, 1983) and exhibit remarkable adaptations in coloration related to their highly specialized lifestyle. Prior to extended observation of living material and careful hand collection that preserved the association between shrimps and their hosts, the adaptive significance or functional nature of coloration in this group was not fully appreciated.

In his study of the organisms associated with crinoids at Torres Strait, Potts (1915) was one of the first to comment on the color resemblance between symbiotic shrimps and their hosts. However, many studies of pontoniines have been based on preserved material, which typically lacks any trace of color. Much of the available information on color patterns of these shrimps is fragmentary, often isolated in single species descriptions. General reviews of coloration in various caridean groups may be found in Bruce (1975a, 1976) and Patton (1967). This paper provides an overview of coloration in the Pontoniinae, in relation to aspects of the biology, ecology, and behavior of the shrimps, and is based on personal observations in the field as well as the published observations of other workers.

The subfamily Pontoniinae Kingsley, 1878 is contained within the large family Palaemonidae, along with a second major subfamily, the Palaemoninae, and several smaller, highly specialized subfamilies (Holthuis, 1955). Bruce (1986) recently proposed that members of the small family Gnathophyllidae Dana, 1852 should be merged with the Pontoniinae, based primarily on similarities in larval morphology. In this case, the subfamilial designation Gnathophyllinae would take taxonomic precedence. However, since the proposed revision has not yet been widely adopted (Bruce, pers. comm.), the scope of this review is restricted to the genera traditionally contained in the Pontoniinae.

Comparison of ecological strategies between the Pontoniinae and the closely related subfamily Palaemoninae provides valuable insight into the evolution of coloration and other features in the Pontoniinae. The major points of divergence between the two subfamilies are summarized in Fig. 1. The Palaemoninae is comprised almost entirely of free-living, relatively unspecialized species that occur predominantly in tropical freshwater and temperate marine habitats. In contrast, members of the Pontoniinae are most abundant in tropical and subtropical marine habitats (Bruce, 1983). Relatively few species are free-living; a large proportion live in permanent, obligatory associations with other invertebrates, including sponges, a variety of cnidarians, bivalve mollusks, all classes of echinoderms, and ascidians (Table 1) (see Bruce, 1983 and extensive bibliography contained within; Castro, 1971; Criales, 1984).

Many pontoniine genera are monotypic, representing highly derived forms, and many species exhibit a high degree of host specificity (Bruce, 1983). Evolution in the Pontoniinae thus appears to

Ocean Monitoring Program, MS-45A, City of San Diego, 4077 N. Harbor Drive, San Diego, Calif. 92101

Table 1. Associations of pontoniine shrimps by major host taxa, expanded from the Indo-Pacific records of Bruce (1983) to include reports from all oceans.

Host Taxa	Associated Genera (number of species)
Porifera	*Anchistioides* (3); *Apopontonia* (2); *Onycocaridella* (3); *Onycocaris* (12); *Periclimenaeus* (14); *Periclimenes* (2); *Thaumastocaris* (1); *Typton* (8)
Cnidaria	
Hydroida	*Hamodactyloides* (1); *Periclimenes* (7)
Antipatharia	*Dasycaris* (1); *Periclimenes* (1); *Pontonides* (2); *Pseudocoutierea* (2)
Alcyonacea	*Hamodactylus* (1); *Periclimenes* (1); *Propontonia* (1)
Telestacea	*Periclimenes* (1)
Gorgonacea	*Hamodactylus* (2); *Mesopontonia* (1); *Neopontonides* (1); *Periclimenes* (2); *Pontonides* (1); *Pseudocoutierea* (3)
Pennatulacea	*Dasycaris* (1)
Corallimorpharia	*Pliopontonia* (1)
Actiniaria	*Periclimenes* (11)
Scleractinia	*Anapontonia* (1); *Coralliocaris* (8); *Fennera* (1); *Hamopontonia* (2); *Harpiliopsis* (3); *Ischnopontonia* (1); *Jocaste* (2); *Paratypton* (1); *Periclimenes* (11); *Philarius* (3); *Platycaris* (1)
Rhizostomeae	*Periclimenes* (1)
Mollusca	
Gastropoda	*Periclimenes* (1); *Pontonia* (1)
Bivalvia	*Anchistus* (7); *Chernocaris* (1); *Conchodytes* (5); *Neoanchistus* (1); *Paranchistus* (2); *Platypontonia* (2); *Pontonia* (6)
Annelida	
Polychaeta	?*Pontonia* (1)
Echinodermata	
Asteroidea	*Periclimenes* (1); *Zenopontonia* (1)
Echinoidea	*Allopontonia* (1); *Periclimenes* (7); *Stegopontonia* (1); *Tuleariocaris* (3)
Ophiuroidea	*Periclimenes* (3)
Holothuroidea	*Periclimenes* (4)
Crinoidea	*Araiopontonia* (1); *Lipkebe* (1); *Palaemonella* (1); *Parapontonia* (1); *Periclimenes* (15); *Pontoniopsis* (1)
Chordata	
Ascidiacea	*Dasella* (2); *Periclimenaeus* (7); *Pontonia* (4)
Host unknown	*Periclimenes* (3)
Free-living	*Palaemonella* (7); *Periclimenes* (15)

Decapoda
Caridea
Palaemonidae

Palaemoninae
Habitats: tropical freshwater and temperate marine
Lifestyle: majority of species free-living

Pontoniinae
Habitats: tropical and subtropical marine, reaching greatest diversity on coral reefs in Indo-Pacific
Lifestyle: many species obligate symbionts of other invertebrates

Figure 1. Ecological dichotomy between the caridean subfamilies Palaemoninae and Pontoniinae.

be driven by selective pressures arising from the symbiotic lifestyle. The more generalized, free-living palaemonine species, as well as free-living representatives of the Pontoniinae, exemplify the plesiomorphic condition against which the characteristics of the more specialized symbiotic pontoniines may be compared (Hipeau-Jacquotte, 1973). Such a comparison provides insight into the evolution of coloration within the Pontoniinae.

Trends in Coloration in the Pontoniinae

Palaemonine shrimps are typically semitransparent with brown or green mottling, reminiscent of army camouflage gear. Free-living pontoniines are also largely transparent or drab colored (Bruce, 1975a, 1976). Individuals with this type of coloration can blend with a variety of natural substrates.

Coloration among symbiotic pontoniines varies widely, although some general trends are apparent. Endo-symbiotic species, found inside of bivalves, ascidians, or sponges, are either transparent (*Chernocaris* in the bivalve *Placuna*; *Anchistioides* in sponges), or they have monochromatic chromatophores dispersed over the body. In the latter case, the pigmentation is often inconspicuous (for example, *Paranchistus ornatus* in the fan shell *Atrina*; *Anchistus custos* in the fan shell *Pinna*; *Conchodytes meleagrinae* in the pearl oyster *Pinctada*), but some species in the genera *Anchistus* and *Conchodytes* are quite conspicuously colored, with pigments which do not necessarily relate to host coloration (Bruce, 1972). Since the biology of most pontoniines is poorly known, further study may elucidate the significance of such coloration.

Species which live on external surfaces of their host show two distinct trends in coloration. They are either extremely cryptic, achieving concealment by one of several strategies, or they are rendered highly conspicuous by virtue of coloration which contrasts sharply with their background. Both trends are intimately related to the symbiotic lifestyle.

Although experimental evidence for the adaptive significance of coloration among marine invertebrates is available for relatively few taxa (Cott, 1957; Wicksten, 1983), adaptiveness may be inferred from several lines of evidence (Robinson, 1969; Wicksten, 1983), including (1) the presence of behavioral or morphological adaptations that interact with the color pattern (a point eloquently discussed by Huxley (in Cott, 1957: p. viii)), and (2) convergence on similar coloration strategies by different taxa. Both types of evidence are found in the Pontoniinae.

Strategies for concealment

Symbiotic pontoniines achieve concealment by one of three strategies: complete transparency, disruptive coloration, or homochromy (coloration which mimics that of the host). These strategies involve increasingly complex adaptations in coloration, morphology, and behavior.

Transparency, involving complete loss of color from the cuticle and underlying dermis, is most often observed among pontoniines associated with cnidarian hosts, many of which are relatively colorless. Examples include several species in the genus *Periclimenes* (*diversipes, kempi, inornatus, madreporae*) and *Propontonia pellucida*, all of which occur on cnidarians (Bruce, 1975a, 1976, 1979).

Disruptively colored individuals are largely transparent, with opaque white or dark patches scattered over the body. This pattern makes it difficult to focus on the true outline of an animal, particularly when it is motionless, and thereby prevents or delays discrimination of the animal from its background (Cott, 1957). Examples include *Periclimenes brevicarpalis*, associated with sea anemones (Plate 5:1), and species in the genera *Harpiliopsis* and *Jocaste*, which are found on the peripheral branches of corals (Bruce, 1976).

Various degrees of homochromy have evolved in the Pontoniinae. In some species, the body is mostly transparent except for dorsal or dorso-lateral stripes of pigment which match host coloration. *Periclimenes tenuis* and *P. commensalis*, for example,

inhabit the arms of crinoids and have longitudinal stripes of pigment that match the host's pinnules (Plate 5:2). Similarly, two shrimps that occur on the wire coral *Cirripathes* (*Dasycaris zanzibarica* and a species of *Pontonides* that shows affinities to *P. unciger*) both have largely transparent bodies with transverse yellow stripes that mimic the color of the coral's polyps (Bruce, 1975a, 1976).

Some species achieve concealment through more complex homochromatic patterns. Two symbionts of crinoids, *Periclimenes amboinensis* and *Araiopontonia odontorhyncha*, often display patterns, involving two or more colors, that mimic the complex coloration of their hosts (Plate 5:3). *Periclimenes imperator* matches the mottled red and white pattern of its nudibranch host, *Hexabranchus* (Bruce, 1972).

In addition to resembling general host coloration, the pigment patterns of some shrimps create three-dimensional effects that mimic host texture. For example, *Stegopontonia commensalis*, which perfectly matches the color of the echinoid spines on which it is found, also has thin white longitudinal lines that mimic the etching of the spines (Bruce, 1976; Castro, 1971).

The color patterns of some species specifically match particular regions or microhabitats on the host. Zmarzly (1984) reported the microhabitat specificities of shrimps occurring on tropical shallow-water crinoids. The coloration of *Periclimenes amboinensis* mimics the pattern on the proximal parts of the crinoid's arms (Plate 5:4A), while the coloration of *P. commensalis* and *P. tenuis* mimics the pinnules on the distal parts of the arms (Plate 5:2).

Morphological adaptations, described in greater detail in a later section, and behavioral adaptations often enhance the mimetic value of a shrimp's coloration. For example, the bodies of both *Stegopontonia* and *Tuleariocaris* are thin and elongate like the echinoid spines on which they occur. Crypsis is further enhanced by a special posture, in which the body is oriented longitudinally on a spine, with the head towards the test and the uropods folded in (Castro, 1971). When removed from their host, these species tend to maintain their longitudinal orientation while swimming rather than swimming in the typical horizontal position (Bruce, pers. comm.).

Periclimenes tenuis orients itself on the arm of its crinoid host such that it is perpendicular to the rib of the arm, in the same way the pinnules are held, with the head toward the rib. In this orientation, the shrimp's longitudinal pigment stripes mimic projecting pinnules (Plate 5:2). In many cases, the tips of the uropods and telson are contrastingly colored like the distal tips of the pinnules.

Contrasting coloration

Species displaying contrasting coloration typically have large opaque white patches on the body, with accents of bright orange, pink, red, or violet. The caudal fan in particular is conspicuously colored and may possess well developed "eyespots". The antennae are extremely long and bright white (Bruce, 1975a, 1979; Limbaugh *et al.*, 1961).

Most pontoniine species with this type of coloration have been observed to engage in cleaning symbioses with fishes, and they usually occur in association with large, solitary anemones. Known cleaners include the Caribbean species *Periclimenes pedersoni* and *P. yucatanicus* (Limbaugh *et al.*, 1961) (Plate 6:5). Although cleaning behavior has not yet been observed in *Periclimenes magnificus*, it is associated with sea anemones and shares many specific elements of the morphology and coloration of known cleaners (Bruce, 1979).

In cleaning symbioses, shrimps maintain a cleaning station to which they must attract client fishes. Thus, a color pattern which renders shrimps visually conspicuous is highly adaptive for this function. Convergence on a similar pattern by several species greatly reinforces the "signal" value of the pattern. Specialized "advertisement" behaviors, such as body swaying and waving of the antennae, have also been observed. Cleaner shrimps in two other families show convergences with respect to antennal morphology and advertisement behaviors (Limbaugh *et al.*, 1961).

Morphological Adaptations

The typical palaemonid body form is exemplified by free-living species such as *Palaemonella rotumana* and *Periclimenes elegans*. The body form of symbiotic species that live on exposed surfaces of their host deviates little from the general palaemonid plan. Among pontoniines inhabiting more confined spaces on their host, four general types of morphological adaptations have been observed:

1. **Dorso-ventral flattening of the body**
   Dorso-ventral flattening results in a thinner body form. This condition has been documented for genera such as *Chernocaris*, which inhabits bivalves, and *Platycaris*, which lives in the narrow spaces between corallites in the scleractinian coral *Galaxea* (Bruce, 1972, 1976).
2. **Lateral compression of the body**
   Lateral compression also produces a thinner body form and is seen in the genus *Ischnopontonia*, which lives among the densely packed corallites of *Galaxea* (Bruce, 1976).
3. **Inflation of the body**
   A more robust, inflated body is typical of genera such as *Onycocaris* and *Typton*, which occupy the tubular channels of sponges (Bruce, 1976).
4. **Elongation and slenderization of the body**
   An elongate body is characteristic of several

species that occupy long, narrow spaces on their host. For example, species of *Tulеariocaris* and *Stegopontonia*, which cling to the spines of echinoids, have more slender, elongate bodies than species found on the tests of echinoids (Bruce, 1975b). *Periclimenes attenuatus* and *P. tenuis*, which occur on the arms of crinoids, have slender, elongate bodies, while species normally found near the bases of the arms or on the oral disk of a crinoid show little modification of the general body form.

Intra- and Interspecific Variation in Color Patterns

The color pattern or patterns of pontoniine species are often consistent enough to be useful in taxonomic diagnoses (Bruce, 1975a; Bruce and Zmarzly, 1983). Such constancy implies a level of genetic control over the expression of the chromatophore system.

Some species exhibit a single color pattern throughout their geographic and bathymetric range. This is usually the case for conspicuously and disruptively colored species. For species that mimic host coloration, intraspecific variation in color pattern is a function of the number of host species utilized and the number of color varieties which exist for the host. Color patterns are typically uniform among conspecific shrimps occurring on the same color variety of the same host species, whereas conspecifics from different host species or different host color varieties usually display different pigment patterns, each corresponding to the particular coloration of their host. Thus, a degree of environmental influence in the development of chromatic patterns is also indicated.

Neopontonides beaufortensis exhibits several color patterns that correspond to the color varieties of its gorgonian host, *Leptogorgia virgulata* (Patton, 1972). Three distinct but constant color patterns are known for *Periclimenes soror*, which occurs throughout the Indo-Pacific Ocean in association with a variety of asteroids (Bruce, 1982; Hayashi, 1973; pers. obs.) (Plate 6:6). Bruce and Zmarzly (1983: Fig. 6) described four different color patterns for *Periclimenes pilipes*, collected from four color varieties of the crinoid *Comanthina schlegeli*. The color patterns of shrimps collected from crinoids of the same color variety were constant to a fine level of detail.

Many other pontoniines associated with crinoids exhibit an impressive array of color patterns which match their polychromatic hosts. The extent of intraspecific variation is illustrated in Plates 5:3 and 5:4. Plate 5:3 shows the color pattern developed by *Periclimenes amboinensis* when it utilized the crinoid host *Comantheria briareus*; Plates 5:4A and B show specimens of *P. amboinensis* collected from two different color varieties of the crinoid *Comanthus bennetti*;

Caridean Chromatophores and Color Change

The color patterns of caridean shrimps are formed by particular arrangements of pigment-containing cells, called chromatophores, which are typically found in the hypodermis underlying the cuticle (Bauer, 1981; Fingerman, 1963). Each chromatophore usually contains a single pigment. Complex pigmentation may be achieved through the aggregation of different colored chromatophores into color units. These multicellular aggregations are referred to as chromatosomes (Bauer, 1981; Elofsson and Kauri, 1971).

Caridean chromatophores are stellate cells, consisting of a cell body with radiating processes which remain fixed in outline during pigment migrations. When pigment granules are concentrated in the cell body, color is minimized; when they are dispersed into the radiating processes, color is maximally expressed (Rao, 1985). The physiological mechanisms by which pigment migration is controlled, thus effecting color change, are reviewed by Fingerman (1963) and Rao (1985).

There are two basic types of color change. The first, morphological color change, is usually effected by a change in background coloration. It involves an increase or decrease in the quantity of pigment and/or the number of chromatophores. As a result, morphological color change is slow, occurring gradually over periods of weeks. In contrast, physiological color change, which involves a change in the dispersion of pigment granules within the chromatophores, occurs more rapidly. It is usually prompted by physical, chemical, or tactile stimuli (Fingerman, 1963).

Studies of chromatophore systems and the capacity for color change in caridean shrimps have focused primarily on hippolytids and crangonids (Bauer, 1981; Chassard-Bouchaud, 1965; Gamble and Keeble, 1900; Keeble and Gamble, 1900; Kuris and Carlton, 1977). Brown (1934, 1935) described color change in the palaemonine shrimp, *Palaemonetes*, but for pontoniines, our knowledge is limited to a few observations.

Nocturnal paling, a form of physiological color change, has been documented in some pontoniines (Bruce, 1975a). In my studies of the species associated with crinoids, only *Periclimenes commensalis* was observed to change from its normal coloration to a translucent red after dark.

The capacity for morphological color change in the Pontoniinae has yet to be investigated experimentally. Cases are known of shrimps which fail to settle or to remain on their normal host yet still

display the color pattern appropriate for the normal host. The color pattern may be distinctly non-adaptive on alternate hosts or backgrounds, rendering the individual quite conspicuous.

Bruce (1982) described such a situation for *Periclimenes imperator*. The characteristic red and white coloration of this shrimp provides camouflage on its usual host, the red and white nudibranch *Hexabranchus*. However, Bruce observed individuals of *P. imperator*, with the characteristic red and white coloration, on brown holothurians. It is not know whether these individuals settled on their normal host and failed to remain there, or if they originally settled on the abberant host. Such situations may indicate that the capacity for morphological color change is limited.

Newly released zoea are mostly transparent, with chromatophores concentrated in a few areas, especially around the bases of the eyes. I observed the zoea of several crinoid-associated species in the genus *Periclimenes* to possess yellow and red chromatophores at time of release, similar to the description given by Gore *et al.* (1981) for the first zoea of *Periclimenes pandionis*. Pigmentation changed relatively little in the course of larval development. Larval chromatophores may undergo diurnal changes in the dispersion of pigment, but the capacity for background adaptation appears to be lacking (Pautsch, 1967). Further work is needed on ontogenetic development of color patterns in pontoniine shrimps to distinguish between the components of genetic control and environmental inducement of coloration.

Acknowledgements

Thanks are due to Mary Wicksten for conceiving and organizing the adaptive coloration symposium at which this paper was presented. This work resulted from field observations made in the course of my doctoral dissertation research on the biology and ecology of crinoid symbionts. I am grateful to A.J. Bruce, Division of Natural Sciences, The Darwin Museum, for the loan of color slides for reference and to A.C. Arneson and A. Kerstitch for the loan of color slides for publication. The manuscript was improved by the comments of A.J. Bruce, who has authored a vast amount of literature on pontoniine shrimps, and those of an anonymous reviewer.

References

Bauer, R.T. 1981. Color patterns of the shrimps *Heptacarpus pictus* and *H. paludicola* (Caridea: Hippolytidae). **Mar. Biol.** 64:141-152.

Brown, F.A., Jr. 1934. The chemical nature of the pigments and the transformations responsible for color changes in *Palaemonetes*. **Biol. Bull. Mar. Biol. Lab.**, Woods Hole 67:365-380.

Brown, F.A., Jr. 1935. Color changes in *Palaemonetes*. **J. Morph.** 57:317-334.

Bruce, A.J. 1972. Shrimps that live with molluscs. **Sea Frontiers** 18:218-227.

Bruce, A.J. 1975a. Coral reef shrimps and their colour patterns. **Endeavour** 34:23-27.

Bruce, A.J. 1975b. Shrimps that live with echinoderms. **Sea Frontiers** 21:44-53.

Bruce, A.J. 1976. Shrimps and prawns of coral reefs, with special reference to commensalism. Pp. 37-94 *In*: O.A. Jones and R. Endean (eds), **Biology and Geology of Coral Reefs,** Vol. III: Biology 2. Academic Press, New York.

Bruce, A.J. 1979. Notes on some Indo-Pacific Pontoniinae. XXXI. *Periclimenes magnificus* sp. nov., a coelenterate associate from the Capricorn Islands (Decapoda, Palaemonidae). **Crustaceana**, Suppl. 5:195-208.

Bruce, A.J. 1982. The shrimps associated with Indo-West Pacific echinoderms, with the description of a new species in the genus *Periclimenes* Costa, 1844 (Crustacea: Pontoniinae). **Aust. Mus. Mem.** No. 16:191-216.

Bruce, A.J. 1983. The pontoniine shrimp fauna of Australia. **Aust. Mus. Mem.** No. 18:195-218.

Bruce, A.J. 1986. Observations on the family Gnathophyllidae Dana, 1852 (Crustacea: Decapoda) **J. Crust. Biol.** 6:463-470.

Bruce, A.J. and D.L. Zmarzly. 1983. *Periclimenes pilipes*, new species, a crinoid associate from Enewetak Atoll, Marshall Islands (Crustacea: Decapoda: Pontoniinae). **J. Crust. Biol.** 3:644-654.

Castro, P. 1971. The natantian shrimps (Crustacea, Decapoda) associated with invertebrates in Hawaii. **Pac. Sci.** 25:395-403.

Chassard-Bouchaud, C. 1965. L'adaptation chromatique chez la Natantia (crustaces decapodes). **Cah. Biol. Mar.** 6:469-576.

Cott, H.B. 1957. **Adaptive Coloration in Animals.** Methuen and Company, Ltd., London; 508pp.

Criales, M. 1984. Shrimps associated with coelenterates, echinoderms, and molluscs in the Santa Marta region, Columbia. **J. Crust. Biol.** 4:307-317.

Elofsson, R. and T. Kauri. 1971. The ultrastructure of the chromatophores of *Crangon* and *Pandalus* (Crustacea). **J. Ultrastruct. Res.** 36:263-270.

Fingerman, M. 1963. **The Control of Chromatophores.** The Macmillan Company, New York; 184pp.

Gamble, F.W. and F.K. Keeble. 1900. *Hippolyte varians*: a study in color change. **J. Microsc. Sci.** 43:589-698.

Gore, R.H., C.L. Van Dover and J.R. Factor. 1981. Studies on decapod Crustacea from the Indian River

region of Florida. XVII. Rediscovery of *Periclimenes (Periclimenes) pandionis* Holthuis, 1951 (Caridea, Palaemonidae) with notes on the males and zoeal stages. **Crustaceana** 40:253-265.

Hayashi, K.-I. 1973. *Periclimenes soror* Nobili associated with the Crown of Thorns starfish from Japan (Decapoda, Natantia, Palaemonidae). **Proc. Jap. Soc. Syst. Zool.** 9:29-35.

Hipeau-Jacquotte, R. 1973. Étude des crevettes Pontoniinae (Palaemonidae) associees aux mollusques Pinnidae a Tuléar (Madagascar). 3. Morphologie externe et morphologie des pièces buccales. **Tethys** Suppl. 5:95-116.

Holthuis, L.B. 1955. The recent genera of the caridean and stenopodidean shrimps (Class Crustacea, Order Decapoda, Supersection Natantia) with keys for their determination. **Zool. Verhand.**, Leiden 26:1-157.

Keeble, F.K. and F.W. Gamble. 1900. The color physiology of *Hippolyte varians*. **Proc. R. Soc.**, Ser. B, 65:461-468.

Kuris, A. and J.T. Carlton. 1977. Description of a new species, *Crangon handi*, and new genus, *Lissocrangon*, of crangonid shrimps (Crustacea: Caridea) from the California coast, with notes on adaptation in body shape and coloration. **Biol. Bull. Mar. Biol. Lab.**, Woods Hole 153:540-559.

Limbaugh, C., H. Pederson, and F.A. Chace, Jr. 1961. Shrimps that clean fishes. **Bull. Mar. Sci. Gulf Caribb.** 11:237-257.

Patton, W.K. 1967. Commensal Crustacea. Pp. 1228-1243 *In*: **Proc. Symp. Crust., Ernakulam, 1965**; Mar. Biol. Assoc. India, Pt. 3.

Patton, W.K. 1972. Studies on the animal symbionts of the gorgonian coral, *Leptogorgia virgulata* (Lamarck). **Bull. Mar. Sci.** 22:419-431.

Pautsch, F. 1967. Pigmentation and color change in decapod larvae. Pp. 1108-1123. *In*: **Proc. Symp. Crust., Ernakulam, 1965**; Mar. Biol. Assoc. India, Pt. 3.

Potts, F.A. 1915. The fauna associated with the crinoids of a tropical coral reef: With especial reference to its color variations. **Pap. Dept. Mar. Biol. Carnegie Inst.**, Wash. 8:73-96.

Rao, K.R. 1985. Pigmentary effectors. Pp. 395-462 *In*: D.E. Bliss and L.H. Mantel, eds., **The Biology of Crustacea**, Vol. 9. Academic Press, Inc., New York.

Robinson, M.H. 1969. Defenses against visually hunting predators. **Evol. Biol.** 3:225-259.

Wicksten, M. 1983. Camouflage in marine invertebrates. **Oceanogr. Mar. Biol. Ann. Rev.** 21:177-193.

Zmarzly, D.L. 1984. Distribution and ecology of shallow-water crinoids at Enewetak Atoll, Marshall Islands, with an annotated checklist of their symbionts. **Pac. Sci.** 38:105-122.

Aposematism and Bioluminescence in Coastal Marine Communities

MATTHEW S. GROBER

Synopsis

The co-occurrence of conspicuous coloration and noxious characteristics in many coastal marine species suggests that aposematism is a common predator deterrent in the marine environment. However, few studies have rigorously tested this suggestion, and those studies that do exist have concentrated on diurnally active species. The possibility that bioluminescence functions as an aposematic signal has been suggested, but no empirical studies have directly addressed this point. In this paper, I will briefly review past research regarding aposematism, specifically with respect to coastal marine habitats. I then present the common characteristics and possible functions of a common class of luminescent signals produced by coastal marine organisms. As a means of integrating these two areas of knowledge, I describe a system whereby bioluminescence in the brittle-star, *Ophiopsila riisei* (Echinodermata: Ophiuroidea), functions as a facultative aposematic signal to deter crab (Brachyura: Portunidae) predators. Finally, the ability of *Ophiopsila riisei to* survive predator attacks suggests that, although selection acting at the level of groups of individuals may occur, selection acting at the level of the individual provides a sufficient mechanism for the evolution of bioluminescent aposematism.

Introduction

The process of natural selection acting on both predators and prey has produced a variety of remarkable mechanisms whereby prey species can deter predators (for review see Edmunds, 1974; Endler, 1986). One such mechanism is aposematism, or warning coloration, whereby prey advertize their toxic, noxious, or unpalatable properties to predators by being conspicuously colored. Although the specific characteristics of unprofitability may vary, the most commonly studied is unpalatability or bad taste. The observation that unpalatability can be associated with bright coloration was recognized over a century ago and was clearly presented by Charles Darwin:

> Under these circumstances it would be highly advantageous to a caterpillar to be instantaneously and certainly recognised as unpalatable by all birds and other animals. Thus the most gaudy colors would be serviceable, and might have been gained by variation and the survival of the most easily-recognised individuals (Darwin, 1871).

Thus, the effectiveness of aposematic signals is dependent on the predator being able to associate the conspicuous signal with prey unprofitability, enabling the predator to avoid the costs of pursuing and attacking the prey. A number of excellent recent studies have shown that predators quickly learn to avoid conspicuously colored distasteful prey (Gittleman and Harvey, 1980; Gittleman *et al.*, 1980; Sillen-Tullberg, 1985; also see Guilford, 1988 for additional citations). However, as was pointed out by Guilford (1988), predators can also learn to avoid cryptic unpalatable prey, illustrating that simple avoidance of conspicuous unpalatable prey does not establish the presence of aposematic coloration. Additional studies establishing that survival is higher for brightly colored versus cryptic individuals of the same species (Jarvi *et al.*, 1981; Wiklund and Jarvi, 1982; Wiklund and Sillen-Tullberg, 1985) provide a more convincing demonstration of the aposematic function of conspicuous coloration in unpalatable prey. One aspect that these and most other studies concerning aposematism have in common is the use of birds as predators, and insects, or insect-like models, as prey. Few studies have addressed the use of aposematic signals against non-avian vertebrate predators (see references in Guilford, 1988), and only one previous study has investigated aposematic deterrents against invertebrate predators (Berenbaum and Miliczky, 1984). Moreover, the study of aposematism in marine species remains relatively untouched in comparison to our knowledge of terrestrial systems.

Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853. Formerly with Department of Biology, University of California, Los Angeles, Calif.

Many studies have suggested that conspicuous coloration functions aposematically in a variety of marine species including sea snakes (Rubinoff and Kropach, 1970; Caldwell and Rubinoff, 1983), fish (Cameron, 1976), tunicate larvae (Young and Bingham, 1987), brittle-stars (Basch, 1988; Grober, 1988 and 1989), opisthobranch gastropods (see Faulkner and Ghiselin, 1983 and Edmunds, 1987 for reviews), and zooanthid cnidarians (West, 1976). However, only the studies by Young and Bingham (1987) and Grober (1988, 1989) have provided the experimental manipulations necessary to eliminate alternative mechanisms of predator deterrence. Moreover, the study by Young and Bingham (1987) shows only a learned avoidance of orange tunicate larvae, not an increase in deterrence based on coupling color with unpalatability. The possibility that nocturnal marine species might use bioluminescence as an aposematic signal has been suggested by several previous authors (see Morin, 1983 for review), although definitive studies on any luminescent species are lacking. A brief introduction to the predominant type of luminescent signal produced by coastal organisms, and the possible functions of this distinct signal type will provide a basis for addressing the use of aposematic luminescent deterrents by coastal marine species.

The vast majority of bioluminescent coastal marine species produce either brief flashes of light (in the range of 10's to 100's of msec), slow glows (greater than 2 sec in duration), or both (Morin, 1983). Although certain "glowing" species can be relatively common, it is clear that short flashes of light are the most common signal in coastal habitats. In addition to their short duration, these signals, and the species that produce them, have several other features in common. Bioluminescent flashes are only produced when the signaler has been mechanically stimulated and the intensity of the flash is proportional to the intensity of the mechanical stimulus. Additionally, the species that produce these signals have poor photosensory abilities and are either sessile or sedentary. These characteristics can be used to assess the possible functions for brief light flashes. Morin (1983) suggested that luminescence, in general, can serve the following functions: intraspecific communication, advertisement, prey acquisition, or predator evasion. Intraspecific communication signals normally function to either bring individuals closer together (e.g. mate attraction) or to keep individuals at a given distance (e.g. territorial disputes). Regardless of which of these two broad categories is considered, the fact that most coastal light emitters have poor or no visual abilities and very limited mobility strongly suggests that luminescence is not used to signal between members of the same species. As is evidenced by our own media, the effectiveness of an advertisement is often direclty proportional to the frequency of its occurrence. Thus, the most effective advertisements are those that are regularly repeated, or are on continuously. This is certainly not the case for coastal luminescent signals, which are only produced for very brief durations when the signaler is disturbed. It is certainly possible that light flashes can be used to facilitate prey capture by temporarily stunning light sensitive prey. However, very bright luminescent flashes are produced when nocturnal foragers, which are much too large to be considered prey, mechanically stimulate emitter species. These relatively large and bright flashes probably function as a deterrent to nocturnally active predators. Morin (1983) suggests a variety of mechanisms whereby luminescent flashes may function as predator deterrents. These mechanisms range from simple startle signals to more complex decoy or camouflage effects. My research focuses on aposematic luminescent deterrents, although this by no means suggests that other mechanisms of deterrence are not also involved.

This paper will consider an aposematic system in which a Caribbean reef invertebrate produces light flashes as a deterrent to nocturnally active crabs (Brachyura: Portunidae). The prey species studied was the nocturnally active brittle-star *Ophiopsila riisei* (Echinodermata: Ophiuroidea). Brittle-stars generally have five long thin arms that insert on a small central disk. As the name suggests, these animals are very sensitive to mechanical disturbance and when attacked (or handled), they will autotomize or throw off their arms in an attempt to escape from the predator. Thus, they are well adapted to survive predation events. The signal in this system is bioluminescence that is only produced upon contact with a predator, and for this reason, can be considered a facultative signal.

Although the individual components of this system are quite different from those previously studied, all aposematic systems should be under similar selective constraints and show similar characteristics. A review of recent theoretical models concerning the evolution of aposematic signals provides the following characteristics of both prey and predators that should favor the evolution of these signals (Harvey *et al.*, 1982; Sillen-Tullberg and Bryant, 1983; Turner *et al.*, 1984; Leimar *et al.*, 1986):

1. Species whose normal behavioral activities exposes them to predators should be more likely to evolve aposematic signals.
2. By definition, only prey species that are unprofitable in some way should evolve aposematic signals.
3. Predators show an initial reluctance to attack aposematic prey.
4. Predators show more rapid avoidance learning toward aposematic prey.
5. The evolution of aposematic signals is favored

Table 1. Palatability of ophiuroids, as percentage of trials, offered to normal (N) and blind (B) individuals of three species of portunid crabs.

Crab species Treatment (N)*	Percentage Palatable *Ophiopsila*	*Ophioderma*	Crabmeat
P. sebae			
Sighted (30)	0	100	100
Blind (25)	0	96	100
P. spinimanus			
Sighted (25)	0	88	100
Blind (20)	0	100	100
P. ordwayi			
Sighted (25)	0	0	100
Blind (20)	0	0	100

*Total number of trials given in parentheses (divide by five to get the number of individual crabs used). From Grober, 1988a.

if the prey species is able to survive predator attacks.

Therefore, individual selection would be sufficient to account for the evolution of the signal, allowing it to arise in species that do not form groups of related individuals.

Methods and Results

In this section, the selective factors that favor the evolution of aposematic coloration will be used as a framework to consider the results of my research. Two additional experiments will then be considered because of their relation to the effects of luminescence on predator behavior. The research described in this paper was conducted at a Smithsonian Tropical Research Institute field station, in the San Blas Islands, Panama. Detailed descriptions of the study site and methods can be found in Grober (1988a,b,1989).

Regular exposure to predators should favor the evolution of aposematic coloration

The brittle-star, *Ophiopsila riisei*, stays hidden in shallow Caribbean reefs during the day, emerges at dusk, and spends the entire night feeding on suspended matter. The disk remains hidden in the reef while the arms (mean length of 10cm) extend into the water column. Dense aggregations can be found that persist for long periods of time. Densities of greater than 1000 individuals per m^2 can be found in rocks and dead areas of coral heads, with average densities, within patches, of 150 per m^2.

The predators studied were three species of common crabs from the genus *Portunus (P. spinimanus, P. ordwayi,* and *P. sebae).* All three are similar in their biology and have a carapace width of approximately 27 mm. I chose to study these crabs for two main reasons. First, they were common and could be easily observed at the study site, and active only at night near dense *O. riisei* aggregations. Second, qualitative gut content analyses showed that these crabs do eat ophiuroids, and previous work has demonstrated that portunids are generalist predators that will capitalize on almost any abundant food resource (Williams, 1982). Thus, *O. riisei* is exposed to predation by virtue of its suspension feeding habit, and the high densities of this brittle-star could provide a substantial food resource for opportunistic portunid crabs.

Species that are unprofitable to predators are more likely to become aposematic

This second factor addresses the question of whether or not crabs will eat *Ophiopsila riisei* if given the opportunity. I offered three different food items to crabs that were isolated in aquaria. The crabs were offered *O. riisei, Ophioderma cinereum* (another common reef ophiuroid), and crab meat, which was used as a control for satiation, since it was palatable to all of the crabs. These tests were done during the day so that *O. riisei* luminescence did not affect crab behavior. An item was scored as palatable if the crab kept it within its grinding mouthparts for at least three minutes. The results of these tests show that *O. riisei* is extremely unpalatable to all three crab species (Table 1). *Ophiopsila* was never accepted by any of the crabs, while *Ophioderma* was palatable to two of the crab species and unpalatable to one. Because the crabmeat control was accepted by all crabs, satiation did not affect the results.

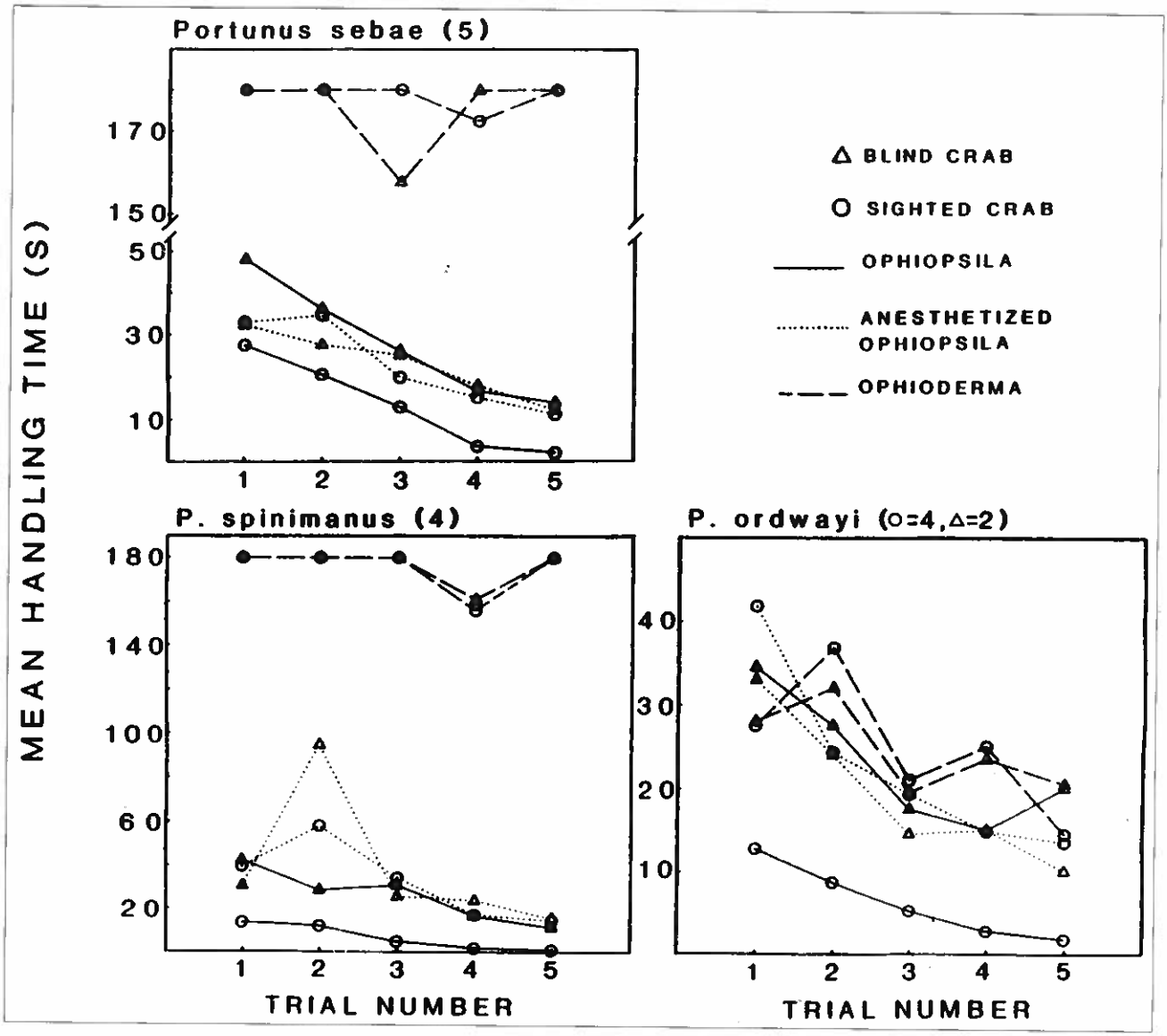


Figure 1. Mean time that sighted and blind crabs spent handling ophiuroids over the course of five consecutive trials separated by 30-minute intervals. Non-luminescent preparations were *Ophioderma cinereum* and anesthetized *Ophiopsila riisei*. Normal *O. riisei* is the single luminescent preparation. Number of individuals tested is in parentheses. From Grober, 1988a.

Predators show an initial reluctance to attack aposematic prey

Since *O. riisei* is both exposed to predators and is unpalatable, I next assessed whether predators show an initial reluctance to attack this potential prey. To do this, I needed a control ophiuroid that was identical to *O. riisei* in all ways except for the ability to produce luminescence. Unfortunately, ophiuroids of this kind were not available, so two alternative controls were employed. First, I visually deprived some crabs by clipping off the distal part of their compound eyes. Marked changes in behavior in response to visual ablation has been demonstrated for a number of decapod crustaceans (Hazlett, 1971 and references therein), however, Hazlett showed that portunid crabs exhibit little or no change in behavior after eye removal. To determine whether visual deprivation had significant effects on the behavior of the crabs used in this study, I repeated the palatability experiments with blind crabs. The results of this experiment suggest that visual deprivation had little effect on the behavior of the crabs. (Table 1). There were no substantial differences in the palatability results between normal and blind crabs, and there were no obvious differences in the behavior of the crabs during the tests.

The second control was to offer crabs *O. riisei* that had been anesthetized in a magnesium chloride solution. Anesthetization inhibited light production by the ophiuroids for the duration of the experi-

ment, but also rendered them immobile. Therefore, as an additional control, *Ophioderma* was used as a non-luminescent and mobile ophiuroid. These experiments were conducted in the same way as the palatability experiments except they were done at night so that the luminescent signal was detectable, and the crabs were given the same prey item five times in succession with 30 min between trials. The order of presentation of the different ophiuroids was randomized. Observations were conducted under moonlight conditions or with far red lighting, to which crabs are very insensitive (Goldsmith and Fernandez, 1968). For all trials, the duration of time that a crab manipulated the ophiuroid before releasing it was recorded and is presented as "handling time".

The results of a three-way ANOVA of handling times for *P. sebae* and *P. spinimanus* were similar and yielded significant interaction effects between trial number, predator treatment, and prey types. The results for *P. ordwayi* were not significant. However, this species followed the same general trends exhibited by the other two crab species, when the differences in the palatability to *Ophioderma* are taken into consideration (Fig. 1). To determine whether crabs show initial reluctance towards luminescent ophiuroids, we need only consider the data for the first trial. For all three crab species, handling times for luminescence-mediated interactions are less at the outset, than handling times for interactions that do not involve sighted crabs and luminescent ophiuroids (Fig. 1). Although these differences are not statistically significant, they suggest an initial reluctance to handle luminescent unpalatable prey as compared to non-luminescent unpalatable prey. In addition, the handling times for *Ophioderma* are in accordance with the results of the earlier palatability studies, suggesting that visual ablation had little effect on the outcome of the experiment. Thus, all three species of crab appear to show a greater initial reluctance to handle luminescent prey.

Predators show more rapid avoidance learning towards aposematic prey

Assessment of whether predators show more rapid avoidance learning in response to luminescent prey can be determined from the results of the previously described handling time experiment (Fig. 1), but with special reference to the slope of the lines (this slope represents how quickly the crab learns to decrease handling time for unprofitable prey). If luminescence triggers more rapid avoidance learning, then the slope of the line representing the interaction between *O. riisei* and normal crabs should be steeper than the slope of all other lines, which represent any interactions that were unaffected by luminescence. For all three crab species, there was no difference in the speed at which crabs learn to ignore the unpalatable food items. However, all sighted crabs spent significantly less time handling luminescent ophiuroids than did blind crabs (across all five trials). Also, by the end of the final trial, normal crabs paired with luminescent ophiuroids showed five times faster rejection than any of the interactions where luminescence was not involved.

One problem with the previous experiment is that handling time may not be the best behavior to use for addressing the question of how quickly crabs learn to avoid distasteful prey. With respect to the prey species, the critical measure is how much damage or mortality occurs before the association between coloration and unpalatability is formed. The data from Table 2 can be re-analyzed to provide a measure of the damage caused by portunid crabs. Combining the data for all crabs within treatments and across all five trials allows for a comparison of the differences in damage between prey types and crab treatments. The data are plotted on a per trial basis (Fig. 2), but since each data point in the figure comprise all crabs within a trial they represent a sample size of one and thus are not suitable for statistical analysis. Nonetheless, it is clear that for two of the crab species the proportion of ophiuroids damaged decreases much more rapidly for interactions where luminescence and unpalatability are coupled versus interactions involving prey that are only unpalatable (Fig. 2). These data are consistent with the requirement for more rapid learning of aposematic signals where conspicuousness (luminescence) and unpalatability are coupled.

The ability of prey to survive predator attacks facilitates the evolution of aposematic signals

To assess whether *Ophiopsila riisei* can survive predator attacks, I determined the percentage of prey that were damaged during the previously described handling time experiments (Fig. 1, Table 2). There was a significant relationship between the proportion of individuals damaged and the different ophiuroid prey. During interactions with normal individuals of all three species of crab, a lower proportion of luminescent ophiuroids suffered damage versus non-luminescent controls. In addition, even after five trials 50 to 80 percent of the non-luminescent ophiuroids were damaged before being rejected by the crabs, while only 0 to 40 percent of the luminescent ophiuroids that were offered to normal crabs were damaged over all five trials, and no appreciable damage occurred after the first or second trial.

Behavioral responses of crabs in spontaneous interactions

The results of the previous experiments support the hypothesis that luminescence functions as an aposematic deterrent to crab predators. However,

Table 2. Percentage of ophiuroids damaged after being handled by sighted and blind crabs.

Crab species Treatment (N)*	Percentage of Prey Items Damaged	Anesthetized	
	Ophiopsila	*Ophiopsila*	*Ophioderma*
P. sebae			
Sighted (25)	40	75	100
Blind (25)	95	90	100
P. spinimanus			
Sighted (20)	24	84	100
Blind (20)	96	88	100
P. ordwayi			
Sighted (20)	0	56	75
Blind (10)	70	60	80

*Total number of trials given in parentheses (divide by five to get the number of individual crabs used). From Grober, 1988a.

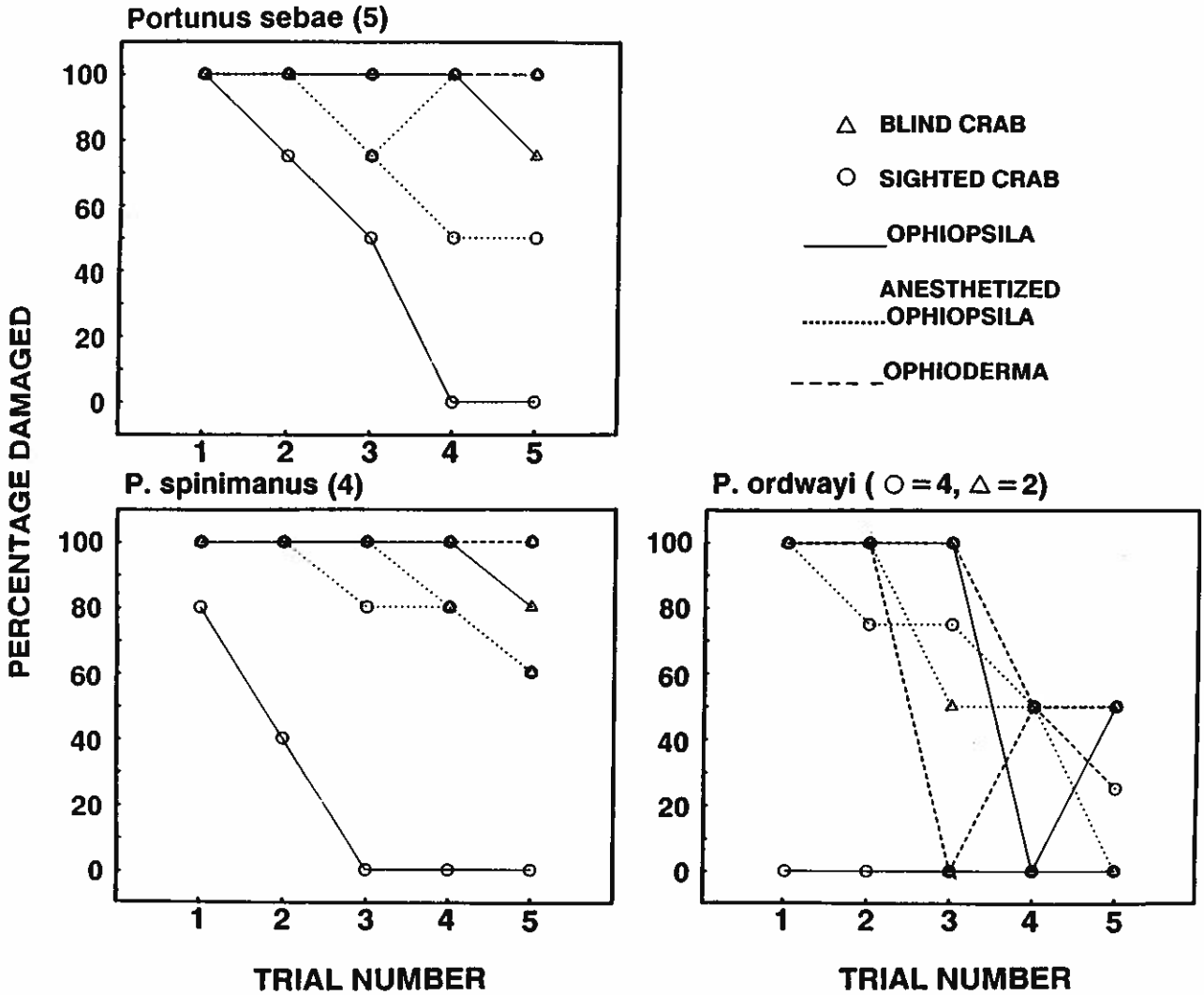


Figure 2. The percentage of ophiuroids damaged over the course of five consecutive trials separated by 30-minute intervals. Normal *Ophiopsila* is the only prey that is both unpalatable and luminescent (i.e. aposematic). Number of individuals tested is in parentheses. From Grober, 1989.

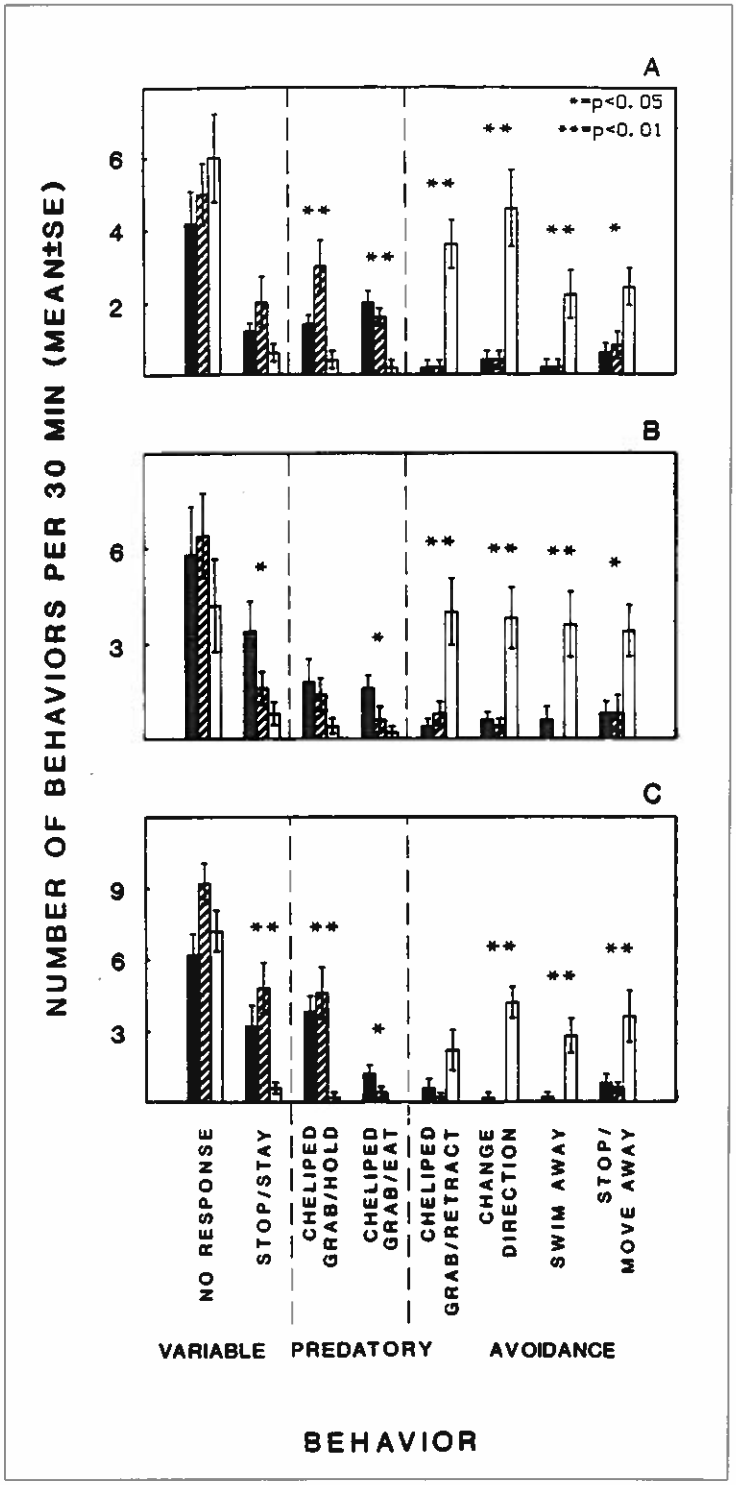


Figure 3. Frequency histograms of the behavioral responses of three portunid crab species (A=*P. sebae*; B=*P. spinimanus*; C=*P. ordwayi*) to interactions with luminescent and non-luminescent ophiuroids. Each bar represents the mean of that response from five 30-minute trials. Error bars represent ±1 SE. Non-luminescent preparations were *Ophioderma cinereum* (dark bars) and anesthetized *Ophiopsila riisei* (hatched bars). Normal *O. riisei* (open bars) was the single luminescent preparation. The means for the three ophiuroids were compared for each behavior using a one-way ANOVA. From Grober, 1988a.

all of these results were obtained during forced encounters between the predator and prey. To assess the responses of crabs to spontaneous interactions with luminescent brittle-stars, a crab was allowed to acclimate in an aquarium for 24 hours and then one of the three previously described prey types (*O. riisei*, anesthetized *O. riisei* and *Ophioderma*) was gently lowered into the aquarium at a point furthest from the crab. For the next 30 min, all interactions were observed using moonlight or red lighting and recorded on a tape recorder. Following the experiments, the tapes were transcribed with reference to a 30 min time base, and behaviors or pairs of behaviors were grouped into the following three functional categories: 1) predatory behaviors included Cheliped Grab/Hold and Cheliped Grab/Eat, and consistently had damaging effects on the ophiuroids; 2) avoidance behaviors included Cheliped Grab/Retract, Change Direction, Swim Away, and Stop/Move Away, and rarely resulted in harm to the ophiuroids and normally lead to the crab being displaced with respect to the ophiuroid; and 3) variable behaviors included No Response and Stop/Stay and were placed into this category because the lack of a response cannot be grouped as predatory or avoidance in nature, and Stop/Stay usually resulted in the crab touching the ophiuroid, but in no cases was the ophiuroid damaged.

All three species of crab showed significantly more avoidance behaviors in response to luminescent ophiuroids versus the non-luminescent controls (Fig. 3; except for Cheliped Grab/Retract in *P. ordwayi*). There were no significant differences in the number of avoidance behaviors elicited by the non-luminescent controls. Luminescent ophiuroids elicited significantly fewer predatory behaviors relative to the non-luminescent controls. Finally, there were no significant differences in the frequency of the No Response behavior, and crabs were more likely to produce the Stop/Stay behavior when interacting with non-luminescent prey. As in the previous experiments, luminescent *O. riisei* elicited more avoidance from predators, and incurred less predation than non-luminescent ophiuroids.

Luminescent signals affect ophiuroid survival

A final experiment assessed the amount of damage incurred by luminescent *O. riisei* after spending one night with either blind or normal crabs. During the day, all the arms on

Table 3. Mean overnight arm loss of Ophiopsila riisei in aquaria with sighted or blind portunid crabs.

Crab species	Crab Treatment Sighted (N)	Blind (N)	Probability*
P. sebae	0.13 (6)	3.00 (4)	<0.01
P. spinimanus	0.13 (6)	2.58 (9)	<0.02
P. ordwayi	0.05 (10)	1.16 (8)	<0.02

*The means for normal and blind crabs were compared using a Mann-Whitney U-test. From Grober,

five *O. riisei* were measured and then the ophiuroids were placed in an aquarium where they immediately took shelter beneath ceramic tiles. A normal or blind crab was added to the aquarium at dusk and then removed the following morning. The *O. riisei* were then removed from the aquarium and remeasured. For all three species, crabs that could perceive the luminescent flashes produced significantly less damage than crabs that could not detect the luminescence (Table 3).

To summarize the results from both the spontaneous interaction experiments, the production of luminescent flashes is associated with 1) decreases in predatory behaviors by crabs, 2) increases in avoidance behaviors by crabs, and 3) a decrease in crab-mediated arm loss to *O. riisei*.

Discussion

The results of this work on *Ophiopsila* demonstrate that luminescence functions as an aposematic signal to deter crustacean predators. This conclusion is based on the results of experiments which addressed several assumptions and predictions of theoretical studies (Harvey *et al.*, 1982; Sillen-Tullberg and Bryant, 1983; Turner *et al.*, 1984; Leimar *et al.*, 1986) regarding the evolution of aposematism. The present results can be used as 1) a basis for comparing aposematic signals that utilize reflected light acting against diurnal vertebrate predators with bioluminescence acting against nocturnal invertebrate predators, and 2) an assessment of the predictions from the theoretical studies.

Past studies have shown that conspicuous prey are initially taken at higher rates than cryptic prey (Gittleman and Harvey, 1980; Gittleman *et al.*, 1980). Therefore, it is reasonable to assume that the evolution of aposematic signals will be favored in unpalatable species whose behavior exposes them to predators. Field observations have shown that *O. riisei* is exposed to predators during the entire night. This is notable for a Caribbean brittle-star since the majority of both diurnal and nocturnal brittle-star species in this predator-rich environment exhibit cryptic behaviors (Aronson and Harms, 1985; and references therein). Thus, the extended exposure of *O. riisei* to nocturnal predators may have been a powerful driving force on the evolution of aposematic luminescent signals. Conversely, the ability of *O. riisei* to remain exposed to predators for long periods of time may be the result of their luminescent aposematism.

By definition, a prey species that utilized aposematic signals must be unprofitable in some way. A common form of unprofitability that has received the most attention in past studies is unpalatability. The results of the palatability tests on portunid crabs show clearly that *Ophiopsila riisei* is unpalatable to all three species of crab. Although the compound(s) responsible for unpalatability in this species are not known, previous studies have shown that a number of echinoderms contain noxious compounds such as saponins (Lucas *et al.*, 1979) and quinone pigments (Singh *et al.*, 1967). In addition, Lucas *et al.* (1979) demonstrated that saponins found in the eggs and larvae of a starfish function as a chemical defense against planktivorous fish. Finally, Fontaine (1964) suggested that copious production of highly acidic mucous by *Ophiocomina nigra* functions to deter predators.

Avian predators show an initial reluctance to sample conspicuous or novel prey versus cryptic or non-novel prey (Coppinger 1969, 1970; Shettleworth, 1972; Schlenoff, 1984; Sillen-Tullberg, 1985). Portunid crabs show a similar reluctance to sample luminescent prey at the outset of the experiments. However, it is possible that the crabs used in these experiments had previous experience with luminescent organisms in the field. In addition, the initial reluctance to handle luminescent prey may be a result of the crabs being startled by the light flash (Morin, 1983), in a manner similar to that reported for eyespot patterns on butterfly hindwings (Blest, 1957). A startle function has been proposed for the luminescent flashes produced by planktonic dinoflagellates, which cause erratic swimming and avoidance behaviors (Buskey *et al.*, 1983), and decreased feeding efficiency in herbivo-

rous copepods (Esaias and Curl, 1972; White, 1979).

Aposematic prey trigger more rapid avoidance learning in avian predators than do non-aposematic prey (Gittleman *et al.*, 1980; Sillen-Tullberg, 1985). Similarly, my results show rapid learning for crabs interacting with any unpalatable prey items, and more rapid avoidance learning when unpalatability is coupled with luminescence. In addition, luminescent *O. riisei* were always rejected more rapidly than non-luminescent controls. By the end of five trials, crabs no longer placed the luminescent *O. riisei* in their mouthparts to taste them, but discarded them within two seconds after the production of the luminescent flash. Thus, at that point, the crabs produce an avoidance response based only on the aposematic signal.

Two general selective mechanisms have been proposed to explain the evolution of aposematism. The primary difference between them is based on whether or not the aposematic prey is killed by the predator. Proponents of the traditional view (Harvey *et al.*, 1982; Turner *et al.*, 1984), which was initially formulated by Fisher (1930), suggest that aposematic species are generally represented by groups of related individuals, and the gene for aposematism is maintained via kin selection. Their primary reason for involving kin selection was the assumption that all animals died during a predation event, and thus should only give their lives if there was a payoff to related individuals in the form of decreased future predation. More recently, Guilford (1988, 1989) has shown that groups of individuals need not be related to favor the evolution of aposematism as long as they share the gene for the conspicuous coloration. Several recent studies showing that aposematic species often survive predator events suggest that individual selection could be a sufficient mechanism for the evolution of aposematism. For instance, aposematic insects experience a lower frequency of fatal interactions with vertebrate predators than do cryptic prey (Jarvi *et al.*, 1981; Wiklund and Jarvi, 1982; Sillen-Tullberg, 1985; Wiklund and Sillen-Tullberg, 1985), and aposematic tadpole larvae of urochodates can survive fish attacks and complete a normal metamorphosis (Young and Bingham, 1987). Similarly, since interactions between crab predators and *O. riisei* are rarely fatal, the evolution of luminescent aposematism in *O. riisei* may also be explained primarily on the basis of individual selection. The process of autotomizing arms is widespread amongst ophiuroids and may be an adaptation for surviving predator attacks. Luminescence provides an additional benefit since *O. riisei* experiences less damage than non-luminescent controls during interactions with crab predators.

An additional prediction of the theoretical models, which is supported by several empirical studies (Edmunds, 1974; Papageorgis, 1975; Sivinski, 1981) is that species which employ bright or conspicuous coloration only when disturbed by a predator (facultative aposematism) would have an advantage over those species that are always conspicuous (constitutive aposematism). The luminescent signal in *O. riisei* is facultative, being produced only upon mechanical stimulation. *Ophiopsila riisei* is generally cryptic when undisturbed and thus it gains the benefit of the aposematic signal without having to suffer the consequences of increased detection by predators. A constitutive signal utilizing bioluminescence would be very costly both in terms of the energetic requirements for continuous light production and the much greater probability of detection by a wide range of visually orienting predators. Moreover, some of these predators may not be deterred by the noxious chemicals, and this would select for a primary defense of crypsis that could be supplemented with aposematic luminescence when predators are in close proximity. Also, as mentioned earlier, there are a number of additional mechanisms whereby luminescence may function as a predator deterrent, and most of these are not mutually exclusive (for review see Morin, 1983). For instance, it is probably that luminescence in *O. riisei* functions to startle potential predators, as well as deterring them aposematically.

The ability to recover from partial predation is widespread in marine taxa, and may facilitate the evolution of aposematic signals. This type of protection against predators can be achieved by autotomy of certain body parts, as shown here in an echinoderm, but is also common in many arthropods and annelids. In addition, many taxa of invertebrates are colonial, including sponges, cnidarians, tunicates, and bryozoans. The ability of colonial taxa to recover from predation on a portion of the colony may account for their dominance on marine substrata in temperate and tropical waters. As a result of their competitive dominance for space and colonial habit, these animals are almost always sessile and are thus continually exposed to predators. Moreover, many of these species that are able to recover from predator attacks are also brightly colored and produce noxious chemicals (Bakus, 1981; Coll *et al.*, 1982; Pawlik *et al.*, 1987). These observations suggest that the marine realm is a fertile area for further studies of aposematic predator deterrents.

Acknowledgments

I thank the Division of Invertebrate Zoology for sponsoring the Symposium on Adaptive Coloration in Invertebrates, and Dr. Mary Wicksten for its conception and organization; Dr. James Morin and two anonymous reviewers for critical comments on

this manuscript; and the Smithsonian Tropical Research Institute, Sigma Xi, and the University of California, Los Angeles for financial support.

References

Aronson, R.B. and C.A. Harms, 1985. Ophiuroids in a bahamian saltwater lake: the ecology of a paleozoic-like community. **Ecology** 66(5):1472-1483.

Bakus, G.J. 1981. Chemical defense mechanisms on the Great Barrier Reef, Australia. **Science** 211:497-499.

Basch, L.V. 1988. Bioluminescent anti-predator defense in a subtidal ophiuroid. *In* Burke *et al.* (eds.), **Echinoderm Biology** pp. 503-515. Balkema Press, Rotterdam.

Berenbaum, M.R. and E. Miliczky. 1984. Mantids and milkweed bugs: Efficacy of aposematic coloration against invertebrate predators. **Am. Midl. Nat.** 111:64-68.

Blest, A.D. 1957. The function of eyespot patterns in the Lepidoptera. Behavior 11:209-255.

Buskey, E.J., L. Mills, and E. Swift. 1983. The effects of dinoflagellate luminescence on the swimming behavior of a marine copepod. **Limnol. Oceanogr.** 28:575-579.

Caldwell, G.A. and R.W. Rubinoff. 1983. Avoidance of venomous sea snakes by naive herons and egrets. **The Auk** 100:195-198.

Cameron, A.M. 1976. Toxicity of coral reef fishes. *In* O.A. Jones and R. Endean (eds.) **Biology and geology of coral reefs,** Vol. 3. pp. 155-176. Academic Press, New York.

Coll, J.C., S. LaBarre, P.W. Sammarco, W.T. Williams, and G.J. Bakus. 1982. Chemical defenses in soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef: a study of comparative toxicities. **Mar. Ecol. Prog. Ser.** 8:271-278.

Coppinger, R.P. 1969. The effect of experience and novelty on avian feeding behavior with reference to the evolution of warning coloration in butterflies. I. Reactions of wildcaught adult blue jays to novel insects. **Behaviour** 35:45-60.

Coppinger, R.P. 1970. The effect of experience and novelty on avian feeding behavior with reference to the evolution of warning coloration in butterflies. II. Reactions of naive birds to novel insects. **Am. Nat.** 104:323-334.

Darwin, C. 1871. **The Descent of Man, and Selection in Relation to Sex.** John Murray, London.

Edmunds, M. 1974. **Defence in Animals**. Essex: Longmans.

Edmunds, M. 1987. Color in Opisthobranchs. **Amer. Malac. Bull.** 5(2):185-196.

Endler, J. 1986. Defense against predation. *In* M.E. Feder and G.V. Lauder (eds.), **Predator-prey relationships, perspectives and approaches from the study of lower vertebrates,** pp. 108-134. University of Chicago Press.

Esaias, W.E. and H.C. Curl. 1972. Effect of dinoflagellate bioluminescence on copepod ingestion rates. **Limnol. Oceanogr.** 17:901-906.

Faulkner, D.J. and M.T. Ghiselin. 1983. Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. **Mar. Ecol. Prog. Ser.** 13:295-301.

Fisher, R.A. 1930. **The Genetical Theory of Natural Selection,** 2nd edn. New York: Dover.

Fontaine, A.R. 1964. The integumentary mucous secretions of the ophiuroid *Ophiocomina nigra*. **J. mar. biol. Ass. U.K.** 44:145-162.

Gittleman, J.L., and P.H. Harvey. 1980. Why are distasteful prey not cryptic? **Nature,** Lond. 286:149-150.

Gittleman, J.L., P.H. Harvey, and P.J. Greenwood. 1980. The evolution of conspicuous coloration: some experiments in bad taste. **Anim. Behav.** 28:897-899.

Goldsmith, T.H. and H. R. Fernandez. 1968. Comparative studies of crustacean spectral sensitivity. **Z. vergl. Physiol.** 60:156-175.

Grober, M.S. 1988a. Brittle-star bioluminescence functions as an aposematic signal to deter crustacean predators. **Anim. Behav.** 36:493-501.

Grober, M.S. 1988b. Responses of tropical reef fauna to brittle-star luminescence (Echinodermata: Ophiuroidea). **J. Exp. Mar. Biol. Ecol.** 155:157-168.

Grober, M.S. 1989. Bioluminescent aposematism: a reply to Guilford and Cuthill. **Anim. Behav.** 37:339-341.

Guilford, T. 1988. The evolution of conspicuous coloration. **Am. Nat.** 131:S7-S21.

Guildord, T. and I. Cuthill. 1989. Aposematism and bioluminescence. **Anim. Behav.** 37:339-341.

Harvey, P.H., J.J. Bull, M. Pemperton and R.J. Paxton. 1982. The evolution of aposematic coloration in distasteful prey: a family model. **Am. Nat.** 119:710-719.

Hazlett, B.A. 1971. Non-visual functions of crustacean eyestalk ganglia. **Z. vergl. Physiol.** 71:1-13.

Jarvi, T., B. Sillen-Tullberg and C. Wiklund. 1981. The cost of being aposematic. An experimental study of predation on larvae of *Papilio machaon* by the great tit *Parus major*. **Oikos** 36:267-272.

Leimar, O., M. Enquist, and B. Sillen-Tullberg. 1986. Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. **Am. Nat.** 128:469-490.

Lucas, J.S., R.J. Hart, M.E. Howden, and R. Salathe. 1979. Saponins in eggs and larvae of *Acan-*

thaster planci (Asteroidea) as chemical defenses against planktivorous fish. **J. Exp. Mar. Biol. Ecol.** 40:155-165.

Morin, J.G. 1983. Coastal bioluminescence: patterns and functions. **Bull. Mar. Sci.** 33:787-817.

Papageorgis, C. 1975. Mimicry in neotropical butterflies. **Am. Scient.** 63:522-532.

Pawlik, J.R., M.T. Burch, and W. Fenical. 1987. Patterns of chemical defense among Caribbean gorgonian corals: a preliminary study. **J. Exp. Mar. Biol. Ecol.** 108:55-66.

Rubinoff, I. and C. Kropach. 1970. Differential reactions of Atlantic and Pacific predators to sea snakes. **Nature** 228:1288-1290.

Schlenoff, D.H. 1984. Novelty: a basis for generalization in prey selection. **Anim. Behav.** 32:919-921.

Shettleworth, S.J. 1972. The role of novelty in learned avoidance of unpalatable "prey" by domestic chicks (*Gallus gallus*). **Anim. Behav.** 20:29-35.

Sillen-Tullberg, B. 1985. Higher survival of an aposematic than a cryptic form of a distasteful bug. **Oecologia** 67:411-415.

Sillen-Tullberg, B. and E.H. Bryant. 1983. The evolution of aposematic coloration in distasteful prey: an individual selection model. **Evolution** 37:993-1000.

Singh, H., R.E. Moore, and P.G. Scheuer. 1967. The distribution of quinone pigments in echinoderms. **Experientia** 23:624-626.

Sivinski, J. 1981. The nature and possible functions of luminescence in coleoptera larvae. **Coleopts. Bull.** 35:167-179.

Turner, J.R.G., E.P. Kearney, and L.S. Exton. 1984. Mimicry and the Monte Carlo predator: The palatability spectrum and the origins of mimicry. **Biol. J. Linn. Soc.** 23:247-268.

West, D.A. 1976. Aposematic coloration and mutualism in sponge-dwelling tropical zoanthids. *In* G.O. Mackie (ed.) **Coelenterate Ecology and Behavior.** pp. 443-452. Plenum Publishing Co., New York.

White H.H. 1979. Effects of dinoflagellate bioluminescence on the ingestion rates of herbivorous zooplankton. **J. Exp. Mar. Biol. Ecol.** 36:217-114.

Wiklund, C. and T. Jarvi. 1982. Survival of distasteful insects after being attacked by naive birds: a reappraisal of the theory of aposematic selection evolving through individual selection. **Evolution** 36:998-1002.

Wiklund, C. and B. Sillen-Tullberg. 1985. Why distasteful butterflies have aposematic larvae and adults, but cryptic pupae: evidence from predation experiments on the monarch and the european swallowtail. **Evolution** 39:1155-1158.

Williams, M. 1982. Natural food and feeding in the commercial sand crab *Portunus pelagicus* Linnaeus, 1776 (Crustacea: Decapoda: Portunidae) in Moreton Bay, Queensland. **J. Exp. Mar. Biol. Ecol.** 59:165-176.

Young, C.M. and B.L. Bingham. 1987. Chemical defense and aposematic coloration in larvae of the ascidian *Ecteinascidia turbinata*. **Mar. Biol.** 96 (4):539-544.

Analyzing Color Pattern as a Complex Trait: Wing Melanization in Pierine Butterflies

JOEL G. KINGSOLVER AND DIANE C. WIERNASZ

Synopsis

We analyze the wing melanization pattern of pierine butterflies as an example of a complex trait: A trait consisting of multiple, phenotypic characters that are interrelated functionally, developmentally, and genetically. The analysis has three parts. First, the melanization pattern is described quantitatively in terms of multiple wing melanin characters representing sets of serially homologous characters. Multivariate analyses identify the structure of phenotypic convariation among wing melanin characters at the within- and between-population levels. Second, we use comparative studies, field observations and experiments, and experimental manipulations of wing pattern to explore three potential functions of melanization pattern in pierines: Thermoregulation, mate choice, and predator-avoidance. The results suggest how thermoregulatory performance and mate choice by females are affected by different aspects of melanization pattern. Thermoregulatory function in different thermal environments may also explain some of the observed features of phenotypic covariation in melanization pattern within and among populations. Third, quantitative genetic studies show substantial genetic variation in most melanin characters, and reveal that serially homologous characters are highly correlated genetically. The existence of genetic correlations among most aspects of melanization pattern implies that selection on any melanin character will lead to indirect evolutionary responses in the rest of the pattern. We suggest that the multivariate approach used here may provide a useful model for analyzing the evolution of animal color patterns in particular, and of complex morphological traits in general.

Wing Pigment Pattern as a Complex Trait

The study of the evolution of wing color patterns in Lepidoptera has yielded some of our most complete demonstrations of neo-Darwinian evolution in natural populations (Turner 1977). These successes are the result of certain useful features of lepidopteran color patterns: Readily identifiable phenotypes, relatively simple genetics, and clear ecological significance, at least in some well-studied systems. In most studies, however, the description of the color pattern itself is qualitative and in terms of a few major aspects of pattern (see Endler (1984) for a partial exception). Clearly, the pattern of wing color of most lepidopterans is quite complex, and a quantitative description of the pattern would involve analysis of many phenotypic characters.

The study of complex traits — traits involving multiple, interrelated, phenotypic characters — has received increasing attention from evolutionary biologists in the past decade (Lande 1979, Lande and Arnold 1983, Arnold and Wade 1985). To analyze such complex traits, it is necessary to identify both the functional relationships among characters, determining the nature of selection on the trait, and their genetic relationships, determining the evolutionary response that may result from selection. While most features of organisms of interest to evolutionary morphologists involve such complex traits, it is often difficult to identify study organisms in which both functional and genetic organization of the trait can be studied experimentally (Endler 1986).

In this paper, we analyze the wing pigment pattern in one group of butterflies as an example of evolution of a complex trait. Using wing melanization patterns in pierine butterflies (family Pieridae; subfamily Pierinae) as a study system, our analysis involves three steps. First, we quantify the wing pattern as a set of measurable melanin characters within and among pierine species. Second, we use field observations, experimental manipulations of wing pattern, and modeling to examine several important functions of wing pattern, and show how different aspects of the melanization pattern serve different functions. Third, we describe the genetic organization of the pattern in relation to wing pattern development, and discuss the implications of this organization for the evolution of the pattern in response to selection.

Joel G. Kinsolver, Department of Zoology, University of Washington, Seattle, Wash. 98195; Diane C. Wiernasz, Department of Biology, University of Houston, Houston, Tex. 77204.

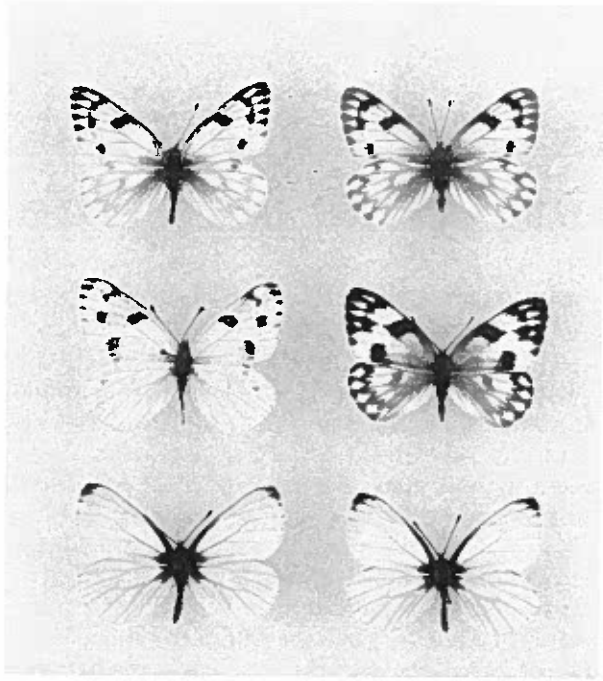


Figure 1. Melanin variation among three pierine species. For each species females are on the left, males on the right. *Pieris occidentalis* (top); *Pieris protodice* (middle); *Pieris napi* (bottom).

Wing Pattern Variation in Pierines

Wing color pattern in pierine butterflies is the result of the distribution of two classes of pigment: pteridine pigments of yellow, orange, white, or occasionally red; and black or brown melanic pigments (Fig. 1). The biochemistry and genetics of the pteridines are known in some detail (Descimon 1976, Watt 1964, 1967, Watt and Bowden 1966). The background color on the dorsal wing surface of most pierines is white; on the ventral surfaces it is yellow or white. The white pigment is primarily leucopterin, whereas the yellow pigment is sepiapterin. The concentrations of sepiapterin and leucopterin in pierines are controlled by single gene loci. In contrast, the pattern of wing melanization in most pierines is quite complex, and genetic determination of melanization pattern clearly involves multiple loci (Shapiro 1984a) (Fig. 1) (see Genetic Organization of Melanization Pattern).

Shapiro (1984b) has identified a 'bauplan' for the melanization pattern of Pierinae, which characterizes the wing pattern in terms of three features (Fig. 2A): 1) melanization baso-distally along wing veins, 2) a transverse band of melanin at the margin of the forewing and hindwing, and 3) an inner transverse band proximal to the first. The melanization pattern of nearly all pierines can be described in terms of a systematic reduction of melanin elements relative to this bauplan, which can be used to identify homologous sets of melanin characters (Nijhout

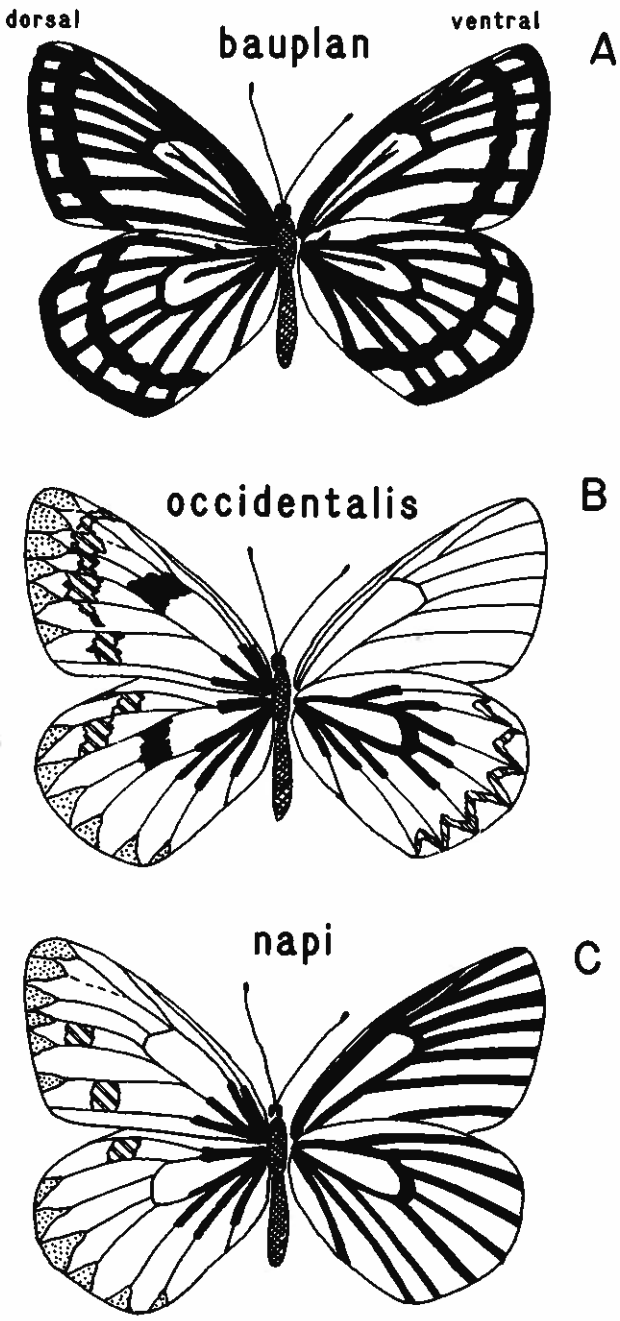


Figure 2. The pattern of wing melanization in pierines. A. The bauplan of Shapiro (1984b). B. *Pieris occidentalis* C. *Pieris napi*. In B and C, homologous characters (as suggested by the bauplan) are shaded similarly.

1985, Nijhout and Wray 1986)) (Fig. 2B, 2C). On this basis, we can identify a set of melanin characters, whose positions are identified relative to wing venation, that characterize the melanization pattern for a particular species (Fig. 2B, 2C). We have quantified the pattern in terms of linear measurements of the length or width of each melanin character, where a character is simply a 'blob' of melanin (or its absence) at a particular location on the wing surface (Kingsolver and Wiernasz 1987). Our expectations

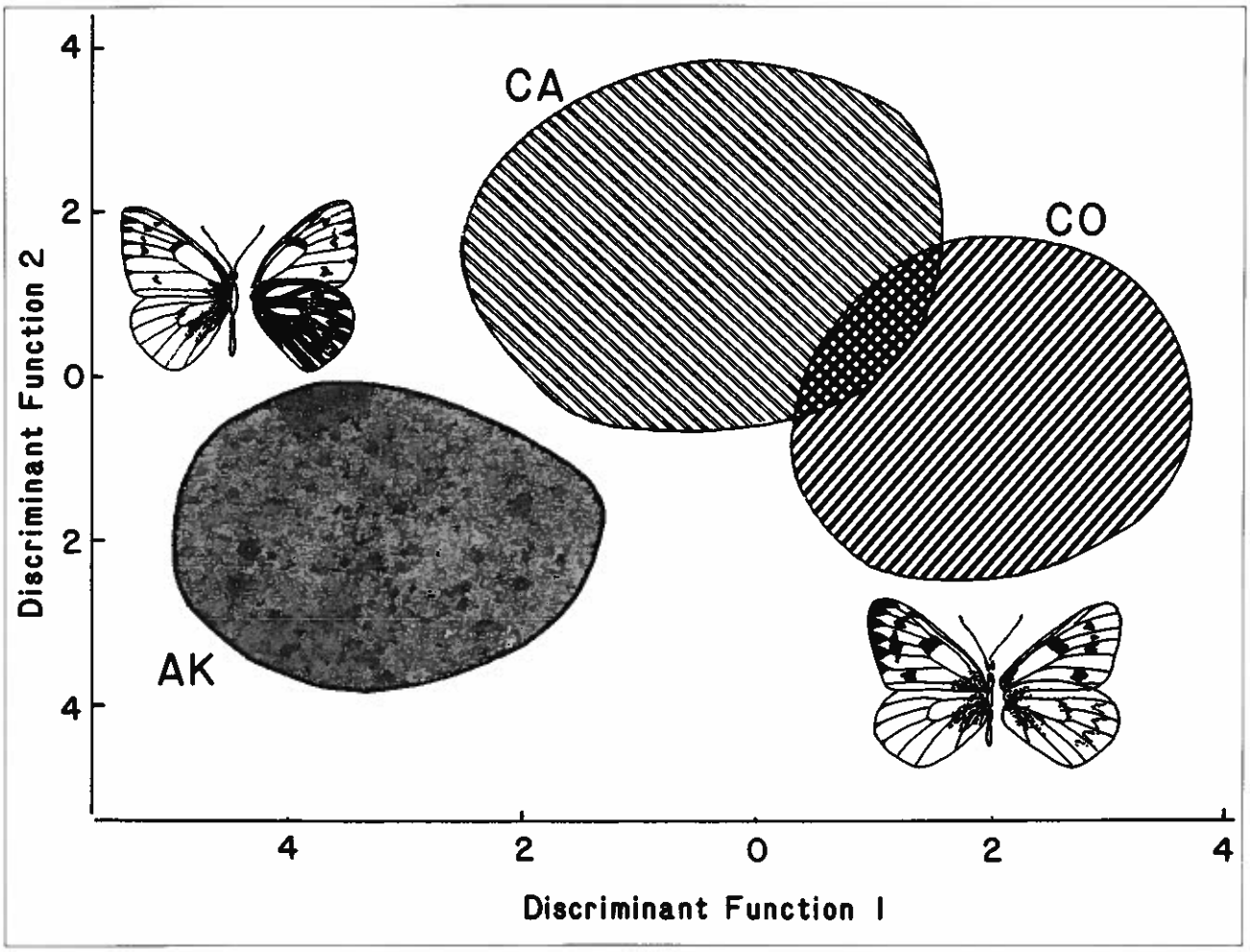


Figure 3. Discriminant function plot for three populations of *Pieris occidentalis*. AK=Fairbanks, Alaska; CA=Donner Pass, California; and CO=Gold Creek (Gunnison), Colorado.

about the pattern of covariation among melanin characters are shaped by functional, developmental, and genetic considerations.

Shapiro's extensive studies (reviews in Shapiro 1976, 1984a) document four important levels of variation in melanization pattern in pierines: among species (Fig. 1), biogeographic within species, seasonal within populations, and sexual dimorphism. Multivariate analyses of the pattern in terms of melanin characters can identify the principal parts of the pattern that contribute to pattern variation at each level (Kingsolver and Wiernasz 1987). For example, discriminant analyses of *Pieris occidentalis* populations from cool environments throughout North America reveal that high-latitude populations are distinguished from high-elevation populations by being more melanized (i.e., have larger melanin characters) on the ventral wing surfaces and less melanized (except on the wing bases) on the dorsal surfaces (Fig. 3). A similar result occurs for male *P. napi*. Similar analyses of sexual dimorphism in *P. occidentalis, P. protodice and P. napi* (Fig. 1) show that females are primarily distinguished from males by greater melanin in the medial and marginal areas of the dorsal surfaces.

Analyses of variation within North American populations of *P. occidentalis* and *P. napi* using principal components and orthogonal factor analyses reveal several consistent features of phenotypic covariation among melanin characters (Fig. 4). First, in all populations, ventral melanin characters are strongly and positively correlated with each other and with most dorsal basal melanin characters. Second, in many populations, these ventral and dorsal basal characters are negatively correlated with medial dorsal melanin characters. Third, the dorsal marginal characters are positively correlated with each other, but are generally uncorrelated with the ventral and other dorsal characters (Kingsolver and Wiernasz 1987). Analyses of variation across North American populations of *P.. occidentalis and P. napi* show qualitatively similar trends to the within-population results (Fig. 5).

Thus, analyzing melanization pattern as a com-

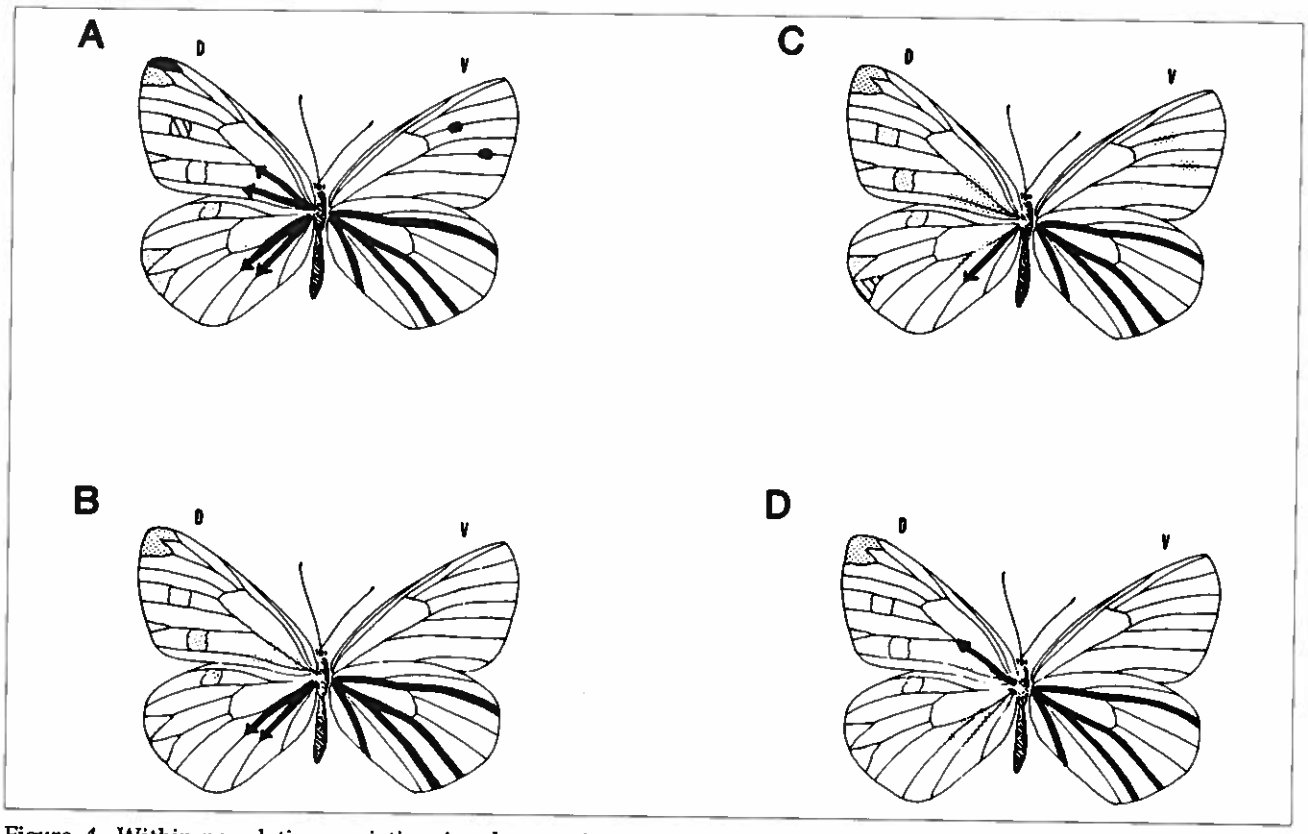


Figure 4. Within-population variation in phenotypic correlation of melanin characters in *Pieris occidentalis* males from Fairbanks, Alaska, (A) and Gold Creek, Colorado, (B), and for females from Donner Pass, California, (C) and Gold Creek, Colorado (D). Characters with similar shading are strongly positively correlated with each other; stripes are negatively correlated with black. Stippled characters are uncorrelated with either stripes or black-shaded characters.

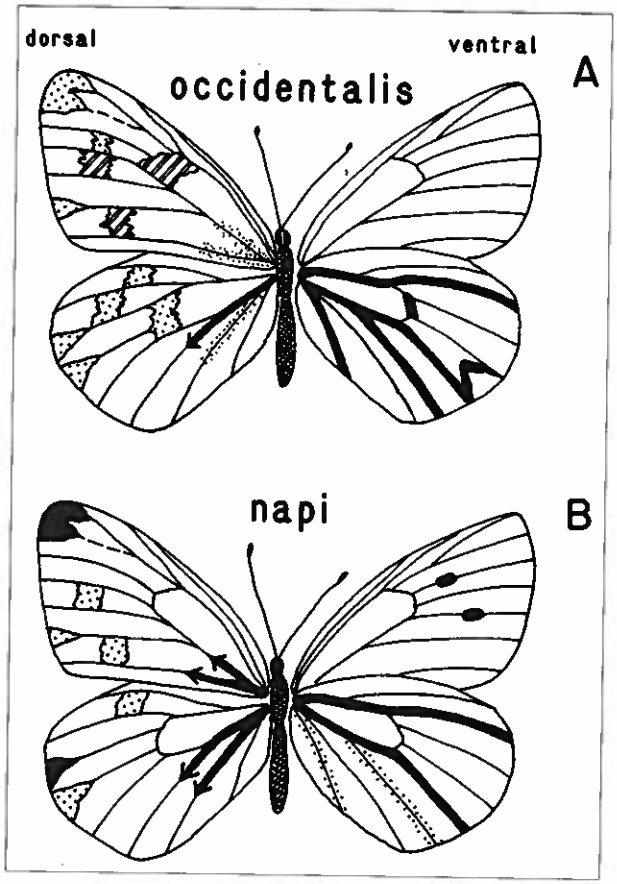


Figure 5. Across-population phenotypic correlation of melanin characters in (a) *Pieris occidentalis* males and (B) *Pieris napi* males. Significance of shadings as in Figure 4.

plex set of phenotypic characters reveals specific kinds of covariation among melanin pattern elements at the between-population, within-population and sexual-dimorphism levels. To demonstrate whether or not these aspects of covariation reflect the adaptive nature of melanization pattern, we need to explore the potential functions of wing pattern in pierines.

Functions of Wing Pattern: Thermoregulation

Most pierine butterflies require thoracic (body) temperatures of 26 to 40°C to take off and sustain flight (Kingsolver 1985a, Ohsaki 1986) and achieve these elevated temperatures by behavioral posturing, primarily orientation to solar radiation. Studies of the relationships between weather, body temperature, and flight activity in pierines (Courtney and Duggan 1983, Kingsolver 1985a and unpubl., Ohsaki 1986) reveal that in many temperate populations, weather strongly limits the amount of time available for flight activity. For example, in *Pieris* populations in Colorado and Alaska, the average available flight time during the flight season ranges from 2 to 10 h/day (Kingsolver, unpubl.), and similar values are reported for other pierids (Kingsolver 1983). Because mean adult lifespans in most pierid populations are only three to seven days (Courtney 1986), flight time may be a limiting resource in many temperate pierid populations.

Several lines of evidence (reviewed in Kingsolver (1988)) support the hypothesis that, because females must fly to find oviposition sites and lay eggs singly on host plants, limitations on flight activity time can reduce the realized fecundity of females. Indeed, Courtney (1986, p. 76) concluded that "reduced fecundity as a consequence of poor weather seems to be a recurrent theme in pierid biology." Similarly, male reproductive success may be limited by the time available for flight, since males must search for mates (Wiernasz unpubl.). These results suggest that we can use flight activity time (subject to certain overheating constraints: Kingsolver and Watt 1983, 1984) as a measure of thermoregulatory performance that is correlated with some component of fitness in both males and females in temperate pierines (Kingsolver 1988). We can then ask, how is this thermoregulatory performance in particular thermal environments related to wing pigmentation pattern and thermoregulatory behavior?

The key to answering this question is that nearly all pierines studied to date (*Infraphulia* is a possible exception: Shapiro 1985) use a unique thermoregulatory posture called reflectance basking as a mechanism for elevating body temperature prior to flight (Kingsolver 1985a,b). During reflectance basking,

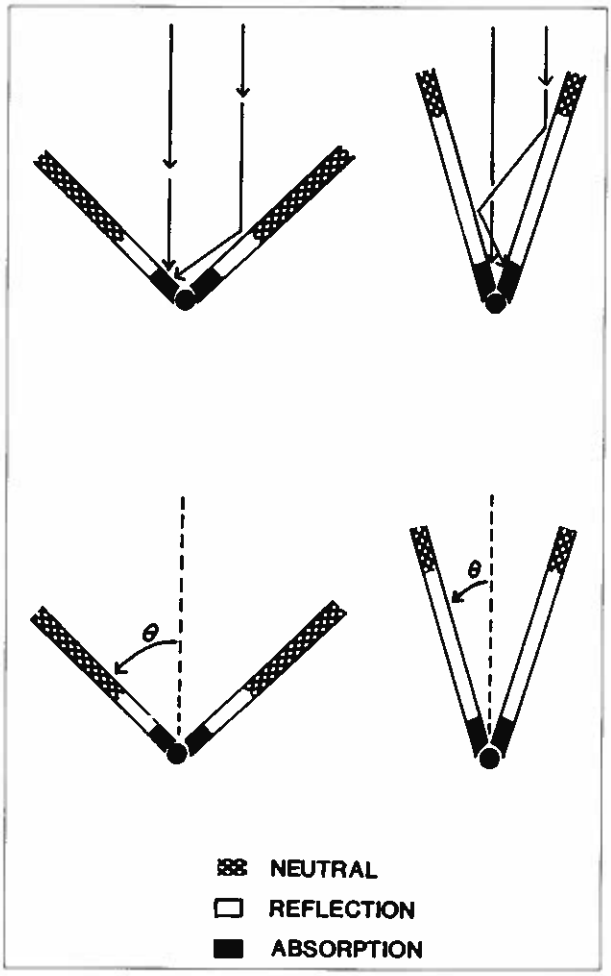


Figure 6. Diagram showing the different dorsal wing regions that can contribute to increased body temperature by absorption and reflectance heat transfer modes. As wing angle increases (left side), the region of the wing that can contribute via reflectance decreases.

the wings are used as solar reflectors: Increasing solar radiation is reflected from the white dorsal wing surfaces onto the body, increasing body temperature. When body temperature exceeds 40°C, the wings are closed and body and wings are oriented parallel to direct solar radiation, decreasing radiative heating. Certain pierine species appear to use a different basking posture, lateral basking, when they first emerge from roosting sites and before switching to reflectance basking prior to flight (Wiernasz, unpubl.). During lateral basking the wings are closed and the ventral wing surfaces oriented perpendicular to solar radiation, increasing radiative heating (Watt 1968).

Mathematical models, comparative analyses, and experimental manipulations of wing pigment pattern (Kingsolver 1985a, 1985b, 1987a, 1988) reveal that the thermoregulatory consequences of wing melanization depends on both the wing region and the angle of the wings during reflectance basking (Fig. 6). Radiation absorbed at the bases of the dorsal

forewings (DFW) and the anal regions of the hindwings (DHW) can be transferred as heat to the body, so that increased basal melanization increases body temperature. In contrast, increased melanization on the medial dorsal surfaces decreases the reflection of radiation to the body, and decreases body temperature. Comparative and experimental studies confirm these predictions about melanin effects on body temperature and flight activity. For example, experimental manipulations with *P. napi* show that increasing basal black pigment leads to earlier initiation of flight in the morning, whereas increasing medial black pigment leads to a delay in flight initiation (Kingsolver 1987a).

The effects of dorsal marginal melanization on body temperature and flight depend on the wing angle during basking (Fig. 6); and the wing angle at which basking body temperature is greatest depends on the degree of dorsal marginal melanization (Kingsolver 1985b, 1987a). Thus, pierine genera that differ in dorsal marginal melanization (e.g. Fig. 1) use significantly different basking wing angles (Kingsolver 1985a); and experimental increases in marginal black pigment significantly increase the wing angles used during basking (Kingsolver 1987a).

These and similar results can be conveniently summarized in a functional "map," illustrated here for *P. occidentalis*, that describes how increased melanin in specific wing regions affects body temperature (Fig. 7). For pierines, increased melanization can increase, decrease, or not affect body temperature depending on the wing region and wing angle. This functional map differs qualitatively from an analogous map for the sister group to the pierines, the subfamily Coliadinae, and results from the unique basking posture of the pierines (Kingsolver 1987a, 1988).

For pierine populations where limited flight time may reduce fitness, we can use this functional map to predict adaptive patterns of melanization for specific thermal environments. In an environment with low air temperatures, for example, an adaptive pattern would consist of heavy melanin on the basal dorsal forewings (DFW) and hindwings (DHW) and on the basal ventral hindwings (VHW), and little or no melanin on the medial DFW and DHW. In a warmer environment, the adaptive pattern would include less melanin on the basal DFW and DHW and on the basal VHW, and/or more melanin on the medial DFW and DHW. Environments with less or greater solar radiation intensity would lead to analogous adaptive predictions as those for cooler and warmer air temperatures, respectively.

Some of the important aspects of phenotypic covariation identified by our multivariate analyses of melanization pattern agree with these adaptive predictions based on thermoregulatory function

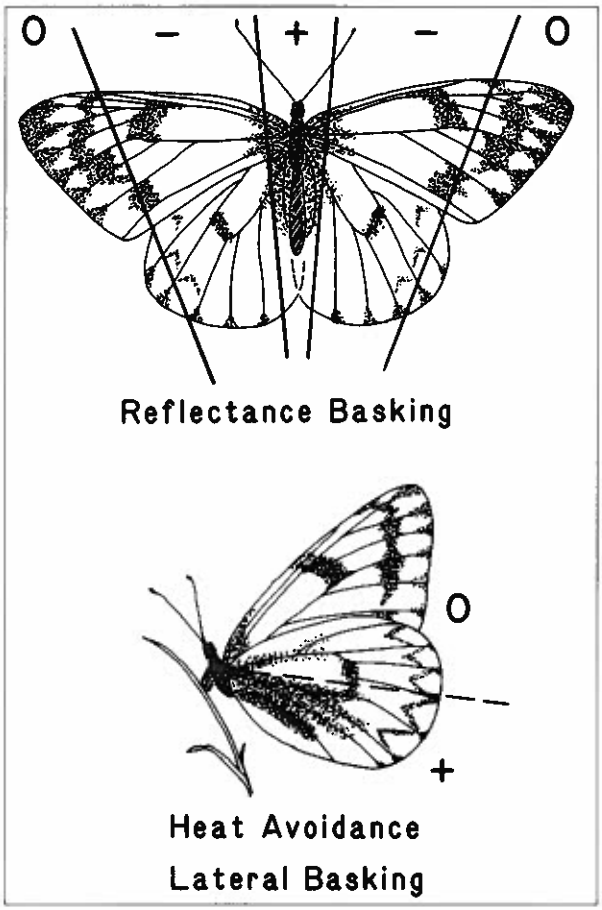


Figure 7. Functional map relating wing melanization in different wing regions to body temperature during basking (top) and heat-avoidance (bottom) behaviors. The map is given in terms of the effect of increased melanin in a wing region on body temperature: +, -, 0 represent increases, decreases, and no effects in body temperature, respectively.

(Kingsolver and Wiernasz 1987) (Fig. 4, 5). At both between-population and within-population levels, melanin on the basal DHW, DFW, and VHW are all positively correlated, and in most cases these are negatively correlated with melanin on the medial DFW. These covariance patterns are associated with biogeographic (between-population, Fig. 5) and seasonal (within-population, Fig. 4) variation in thermal conditions. furthermore, the differences in melanization pattern between high-latitude and high-elevation populations in several species (Fig. 3) are consistent with predicted patterns for environments with solar radiation levels of less (Alaska) or greater (montane Colorado and California) intensities (Kingsolver and Wiernasz 1987, Kingsolver unpubl.).

Several other aspects of melanin covariation are not consistent with the hypothesis of thermoregulatory adaptation. For example in all populations studied to date, all melanin characters on the VHW,

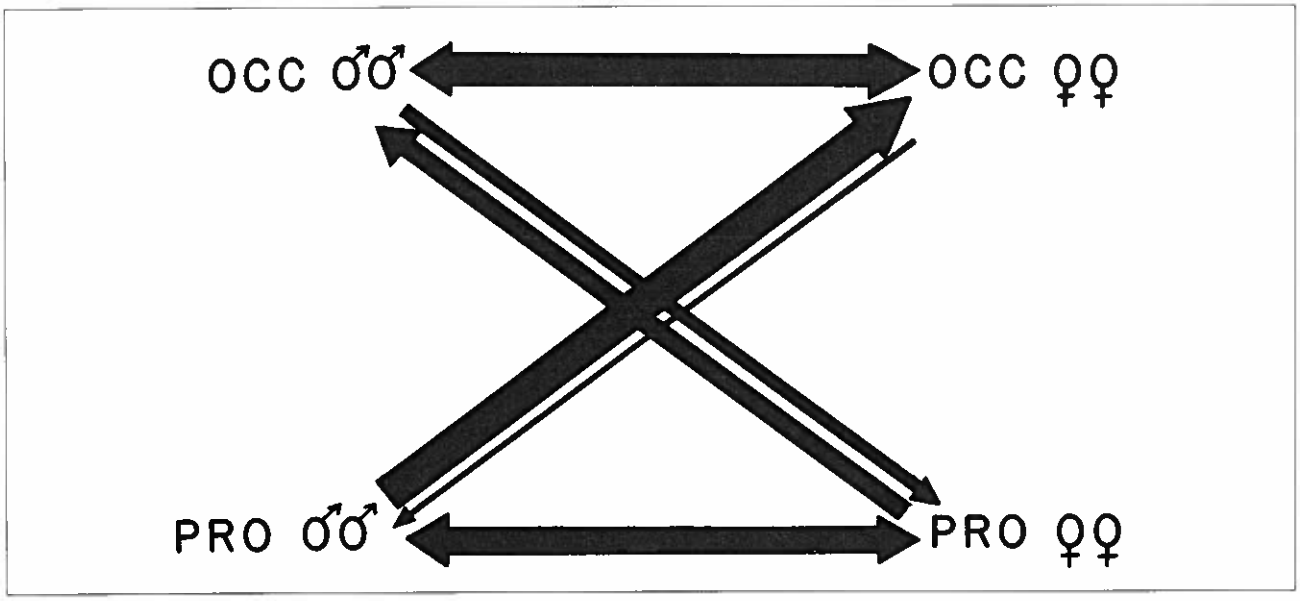


Figure 8. Asymmetries of mate choice in *Pieris occidentalis* and *P. protodice*. The width of the lines indicates the relative strength of the interaction.

not just the basal characters as predicted by thermoregulatory analyses, are strongly positively correlated. In addition, in sexually dimorphic species like *P. occidentalis* (see Fig.1), the pattern differences between sexes are not consistent with any hypothesis of thermoregulatory differences between the sexes (Kingsolver and Wiernasz 1987).

Thus our analyses of the thermoregulatory function of melanization pattern in pierines lead to adaptive predictions that agree with many, but not all, of the important qualitative aspects of phenotypic covariation within species that we identified by our multivariate analyses. Note, however, that we have not yet discussed what patterns of melanin covariation we might expect in the absence of thermoregulatory adaptation—that is, a null hypothesis for melanin covariation. For example, we might expect certain sets of melanin characters to be strongly positively correlated because of the developmental mechanisms that determine the patterns. As a result, pleiotropic effects of genes determining the pattern may generate genetic correlations among melanin characters, and influence the phenotypic correlations observed here (see Genetics of Melanization Pattern).

Functions of Wing Pattern: Mate Choice

Many species of pierids are sexually dimorphic in wing patterns of melanin, pteridine, and ultraviolet-absorbing pigments (et al 1952, Rutowski 1981, Silberglied and Taylor 1978). Experimental studies with several pierids have demonstrated that both males and females can discriminate on the basis of sexual differences in UYV reflectance. For example, in *P. protodice* the dorsal wing surfaces are more UV-reflective in females than in males, and the degree of UV-reflectivity in females increases with age (Rutowski 1981, 1982). Experimentally increasing the UV-reflectance of female wing models significantly decreases the duration of courtships by males (Rutowski 1981). Rutowski found no evidence for discrimination on the basis of melanization in male or female *P. protodice*.

To examine mate choice on the basis of melanization pattern, we have focused on female choice in the sexually dimorphic species *P. occidentalis* (Wiernasz, 1989). Newly eclosed virgin females were released in the field and approached and courted by a series of males. After each male was accepted or rejected (*P. occidentalis* females have a sterotypic rejection posture which is readily identified), the male and female were separated, the male was collected, and the female was allowed to continue. By measuring melanization pattern of the rejected and accepted males for each female, we can ask, Do accepted and rejected males differ in melanization pattern?

Discriminant function analyses for experiments conducted in 1986 and show that accepted males were significantly darker than rejected males for particular melanic characters on the medial and marginal dorsal forewing (DFW) (Wiernasz, 1989). This suggests that *P. occidentalis* females may choose males on the basis of specific aspects of the DFW melanization pattern, or with characters correlated with this pattern. Similar experiments were used to study species recognition in relation to melanization pattern in *P. occidentalis* and its close relative, *P.protodice*. These two species generally differ in

elevational range, but are sympatric at 1500-2100 m elevation in Western Colorado. The melanization patterns of *P. occidentalis* and *P. protodice* are similar, especially for females (Fig. 1), and the UV patterns of males are identical (Wiernasz unpubl.). Experimental tests of mate choice in these species revealed that both male and female choice are characterized by asymmetries in discrimination (Wiernasz, submitted) (Fig. 8). For example, in both species virgin females will accept 70 percent of nonspecific males that court them. However, *P. occidentalis* females will accept less than 10 percent of courting *P. protodice* males, whereas *P. protodice females* will accept nearly 50 percent of courting *P. occidentalis* males. Conversely, *P. protodice* females are courted significantly more often by conspecific than by heterospecific males, whereas *P. occidentalis* females are courted by conspecifics and heterospecifics in proportion to their frequency in the population, suggesting that *P. occidentalis*, but not *P. protodice*, males are discriminating among females.

These results are of special interest for three reasons. First, male and female *P. occidentalis* are primarily distinguished (See Wing Pattern Variation in Pierines) by differences in the melanization pattern on the medial and marginal DFW. The field results suggest that it is precisely those melanin characters that are sexually dimorphic which are involved in female choice. Second, discriminant function analyses of *P. occidentalis* and *P. protodice* males show that *P. occidentalis* males are significantly darker than *P. protodice* males for several melanin characters on the medial and marginal DFW. Again, the field results suggest that females choose *P. occidentalis* males that are darker for these same melanic characters: Female choice is in the same direction as species recognition by *P. occidentalis*. This is consistent with the result that *P. occidentalis* females rarely accept *P. protodice* males.

Finally, our results suggest that *P. occidentalis* males who are preferred by females are darker for some characters (specifically on the medial dorsal forewing) where increased melanization may decrease reflectance to the body and thus decrease body temperature. As a result, there is a potential conflict between natural and sexual selection on dorsal melanization pattern. However, these *P. occidentalis* populations in western Colorado experience relatively warm thermal environments, in which average flight activity times may exceed 8 to 10 h/day (Kingsolver, unpubl.). As a result, the relationship between melanin pattern and achieving high basking temperatures in males is less clear than in cooler thermal environments. At present we cannot predict quantitatively the most adaptive phenotype(s) for thermoregulation for these warmer environments.

Mate choice on the basis of melanization pattern has been carefully studied only in a few populations of *P. occidentalis* which occupy a similar thermal environment. However, the geographic range of this species is latitudinally and elevationally extensive, encompassing a variety of thermal environments. The generality of these results for populations of *occidentalis* in other climatic regimes, as well as for other sexually dimorphic pierine species, remains to be established.

Functions of Melanization Pattern: Predator-Avoidance

The importance of wing color pattern in avoiding predation has been demonstrated for a number of butterflies and moths (recent reviews in Turner 1977, Brower 1984, Endler 1984). Wing color in butterflies can serve a predator-avoidance function in two ways: Aposematic or warning coloration associated with unpalatability and/or mimicry, where conspicuous color patterns may deter predators from attacking; and cryptic coloration, where the match between the wing pattern and its background makes detection by potential predators more difficult.

Both of these functions have been suggested for pierine wing color patterns. Many observers have noted the striking white and black dorsal wing patterns in pierines, and suggested that it represents aposematic coloration (Wallace 1889, Jones 1932, Kettlewell 1965, Rothschild 1972). This suggestion is of particular relevance because the hostplants of nearly all pierines are in the plant families Cruciferae and/or Caparidaceae, many species of which are known to possess toxic mustard oils or glucosinolate derivatives (Aplin et al 1975, Marsh and Rothschild 1974, Chew and Rodman 1979). On the other hand, the ventral wing surfaces in pierines are rarely white, but are yellow- and black-pigmented. To human observers, at least, pierines at rest with their wings closed are often quite difficult to detect against background vegetation. Here we briefly review the current evidence for aposematic and cryptic coloration with respect to melanization pattern in pierines, as well as the relationship between predation and thermoregulation in these butterflies.

Feeding studies on the unpalatability of pierines to bird predators have yielded conflicting results (review in Brower 1984). Several authors have reported that *Pieris brassicae* and *P. napi* are unpalatable to birds (Marsh and Rothschild 1974, Lane 1957), but studies with *P. rapae* concluded that this species is palatable (Wourms and Wasserman 1985) and unpalatable (Jones 1932, Lane 1957). Chai's (1986) results for tropical pierines using a specialized insectivorous bird predator indicated a low palatability relative to other butterflies.

Results on palatability of pierines may differ even at a single site in different years. Kingsolver (1987b) conducted paired-choice feeding trials using trained grey jays in montane Colorado to look at relative palatabilities of pierines (*Pontia occidentalis, Pieris napi*), coliadines (*Colias alexandra, C. philodice*), and several non-pierid butterfly species that are sympatric. Analyses of contingency tables showed that a) the two pierines had significantly lower palatabilities than the other butterflies, but *napi* and *occidentalis* were not significantly different from each other, and b) the pierine species had significantly lower palatabilities than the coliadine species. These data are consistent with the hypothesis that these white pierines are less palatable than other butterflies; however, the considerable differences in the extent and pattern of melanization between *napi* and *occidentalis* (Fig. 2) were not associated with significant differences in palatability. Interestingly, C. Lei and W. Watt (pers. comm.) recently conducted very similar feeding trials at this same Colorado site, and found no significant differences in palatability between pierines and coliadines, whereas these pierids still had lower palatabilities than the non-pierids they studied. The palatabilities found by Lei and Watt were considerably higher overall than those from Kingsolver (1987a), suggesting that perhaps the satiation levels and degree of discrimination of the birds differed in the two studies, which were conducted in different years (1984 and 1985 (Kingsolver) vs. 1987 (Lei and Watt)).

Thus the evidence for unpalatability and aposematic coloration for pierines remains unclear — even studies at the same sites gave conflicting results. Pierines are clearly not noxious in the sense that the monarch and many heliconiine butterflies are, but they are relatively less palatable than many other butterflies with which they co-occur. Of particular relevance to the present discussion, however, is the fact that there is no evidence from any study that differences in the degree or pattern of melanization between or within pierine species is associated with differences in palatability.

Evidence for cryptic coloration in pierines is largely anecdotal. The resting posture for pierines that are not basking is with the wings folded and the ventral wing surfaces (primarily the hindwings) exposed, and the yellow and black pigmentation of these surfaces make them difficult to detect while perched in vegetation. One other relevant observation is that several species of pierine (*Pieris occidentalis, Tatochila Vanvolxemii:* Wiernasz, unpubl.) use lateral basking (in which the ventral surfaces are exposed) when first emerging from roosting sites in the morning, and later switch to the more conspicuous reflectance basking posture before initiating flight. A crucial difficulty in examining the importance of cryptic coloration is that direct predation by birds on pierines, at least in temperate populations, is very rarely observed, suggesting that the frequency of visual predation may be low.

There is another, more indirect, way in which wing melanization in pierines may influence predation, via the effects of melanization on thermoregulation. Field studies in Colorado show that considerable predation may occur on both pierines and colidines as they emerge from roosting sites in the morning, before they are warm enough to actively fly (Kingsolver 1987b). In these studies the intensity of predation, at least some of which was due to microtine rodents, peaked about one hour before the majority of butterflies initiated flight. However, experimental manipulations showed that behavioral posture and wing pattern differences among pierid species had no significant effect on the predation rate, suggesting that these mammal predators were not locating butterflies primarily by visual cues.

These results on predator avoidance and wing color in pierines are still quite incomplete, but several general results do emerge. First, feeding studies with birds produce no evidence that differences in the degree or pattern of wing melanization between or within pierine species are related to differences in palatability or protection from predators. Second, important predation on pierines emerging from roosting sites may occur during thermally marginal periods when active flight is not possible, but wing color or posture does not appear to affect predation rate, except through their effects on thermoregulation.

Genetics of Melanization Pattern

Thus far we have considered functional aspects of melanization pattern and identified certain aspects of the pattern on which natural and sexual selection may be currently operating. To understand the rate and direction of evolutionary change that may result from such selection, however, we need to understand the genetic basis for each wing melanin character. Of particular interest are constraints on evolutionary change that may arise from genetic and developmental associations among characters. Such associations may limit or bias the range of possible phenotypes that the developmental system can produce; and genetic correlations among characters will determine the evolutionary response to selection (Lande 1979).

There are *a priori* reasons to believe that such developmental biases may be important in wing melanization pattern in pierines. Wing melanization in this group is the result of a unified process of melanin deposition during the pupal stage, involving the oxidation of tyrosine by the enzyme tyrosi-

Table 1. Broad-sense (both additive and non-additive genetic components) genetic correlations among 11 wing melanin characters for *Pieris occidentalis*, based on a analysis of 9 full-sib families (217 males, 181 females). Values above the diagonal are for males, below the diagonal are for females. Characters are named according to extent of malanin in the various wing locations. First letter in the character name indicates the wing surface: F=dorsal forewing; H=dorsal hindwing; V=ventral hindwing. The second (and third) letters indicate the wing region: B=vein-associated characters in the basal region; DS=discal cell spot; T=transverse (inner) band; M=marginal band; W=vein-associated characters in the anterior region. See text and Figure 2.

	FB	FDS	FT	FM	HB	HDS	HT	HM	VB	VW	VM
FB	---	-0.85	-0.02	0.37	0.85	0.61	0.28	0.32	0.76	0.49	1.06
FDS	-0.09	---	0.27	0.29	-0.34	0.10	-0.18	-0.21	-0.80	-0.82	-0.97
FT	0.01	0.22	---	0.36	0.01	0.75	0.75	0.71	0.18	0.32	0.47
FM	0.40	0.33	-0.33	---	0.29	0.80	0.54	0.58	-0.23	-0.32	0.30
HB	0.80	0.33	0.51	0.55	---	0.49	-0.16	0.20	0.69	0.14	0.43
HDS	0.65	-0.17	0.55	0.25	0.71	---	0.72	0.61	0.24	0.04	0.73
HT	0.66	0.31	0.79	0.09	0.96	0.75	---	0.79	0.03	0.36	0.85
HM	0.60	0.03	-0.40	0.86	0.53	0.19	0.08	---	0.21	0.47	0.66
VB	0.49	-0.63	0.07	-0.37	0.07	0.11	0.28	-0.04	---	0.88	0.80
VW	0.41	-0.30	0.36	-0.36	0.21	0.14	0.53	-0.10	0.90	---	0.65
VM	0.81	-0.32	0.18	0.46	0.62	0.57	0.57	0.38	0.64	0.51	---

nase, and the melanization pattern is determined during a restricted stage in late larval and early pupal development (Onslow 1916, Nihjout 1985). Thus, one might expect different melanin characters to be closely correlated, as an expression of a single developmental process. In particular, serially homologous melanin characters (Fig. 2A, 2B) may be closely correlated genetically.

We have been studying the quantitative genetics of melanization pattern for a population of *P. occidentalis* from western Colorado (Olathe; see Kingsolver 1987a). Families of full-sibs were reared under controlled conditions (at 25 C with continuous light) on a diet of greenhouse-grown *Lepidium perfoliatum*, one of the larval hostplants at Olathe. The melanization pattern of each adult then measured in terms of the 25 melanin characters described above (Kingsolver and Wiernasz 1987). Our preliminary experiments, based on 9 full-sib families (217 males and 181 females), enable us to estimate broad-sense (including both additive and non-additive genetic components) heritabilities and genetic correlations, with several important results.

First, 22 of 25 melanin characters showed significant heritabilities. Thus there is substantial genetic variation in melanization pattern on which selection may operate. Second, melanin characters that are serially homologous on a particular wing surface (see Fig. 2) were highly correlated genetically. For example, the genetic correlations among the eight vein-associated characters measured on the ventral hindwings of females ranged from 0.70 to 1.00, with a median value of 0.95. This supports the hypothesis that developmentally-associated aspects of the pattern are strongly correlated genetically.

The strong genetic correlations among homologous melanin characters enable us to reduce the number of characters needed to adequately describe the melanization pattern. For example, for each butterfly the values of the three melanin characters on the marginal dorsal forewing. By this process, we can reduce the number of melanin characters from 25 to 11 without loss of information. The genetic correlations among these 11 melanin characters (Table 1) reveal that many aspects of melanization pattern are correlated genetically, with values ranging from -0.97 to 1.06. These preliminary genetic results have two important implications for evolution of melanization pattern in *P. occidentalis*. First, the developmental processes of pigment determination and deposition influence the structure of genetic covariance of melanization pattern: Serially homologous characters are strongly associated genetically. Thus these homologous character sets will tend to evolve as integrated units. Second, the existence of genetic correlations among most aspects of the pattern means that directional selection on any melanin character will lead to correlated evolutionary responses in many aspects of the pattern. For example, (Table 1), selection for increased melanin on the basal dorsal forewings (character FB) will tend to lean to an evolutionary response of increased melanin on the entire dorsal and ventral hingwings, and decreased melanin on the medial

dorsal forewings. Clearly, the evolution of melanization pattern will be strongly affected by this complex genetic organization.

Conclusions

Our analyses of wing melanization pattern as a complex trait have yielded three important results. First, by using a multivariate approach we have obtained a quantitative description of the *pattern* of wing pigmentation, instead of a description of color or qualitative pattern alone. This description allowed us to identify the structure of phenotypic covariation among different aspects of melanization pattern at both the within- and between-population levels. Second, we have demonstrated that different aspects of melanization pattern affect different functions relevant to both natural and sexual selection. For example, melanin characters on the basal dorsal forewings and hindwings, medial dorsal forewings and hindwings, and posterior ventral hindwings all affect thermoregulatory performance (although in different ways: Fig. 7); whereas melanin characters on the medial and marginal dorsal forewings can affect mate choice and species recognition by females. Natural and sexual selection on melanization pattern resulting from these functional associations will likely be complex. Third, preliminary genetic studies suggest that developmentally homologous melanin characters are strongly correlated genetically, so that selection on any melanin character will likely lead to indirect evolutionary responses in other aspects of the pattern.

In analyzing melanization pattern as a complex trait, we have identified and distinguished the functional, developmental, and genetic interrelationships among melanin characters that will determine selection and the evolutionary response to selection on the pattern. The multivariate approach used here may provide a useful model for analyzing the evolution of animal color patterns in particular, and of complex morphological traits in general.

Acknowledgements

We thank John Endler and two anonymous reviewers for useful criticisms on an earlier version of the manuscript. Research was supported by NSF grants BSR 86-00485 and BSR 87-96193 to JGK.

Literature Cited

Aplin, R.T., R.A. Ward and M. Rothschild. 1975. Examination of the large white and small white butterflies (*Pieris* spp.) for the presence of mustard oils and mustard oil glycosides. **J. Entomol.** A 50:73-78.

Arnold, S.J. and M.J. Wade. 1985. On the measurement of natural and sexual selection: Theory. **Evolution** 38:709-719.

Brower, L.P. 1984. Chemical defense in butterflies. pp. 109-134. *In* **The Biology of Butterflies**, P. Ackery and R. Vane-Wright, ed. Academic Press, London.

Chai, P. 1986. Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galvula ruficauda*) to free-flying butterflies in a tropical rain forest. **Biol. J. Linn. Soc. Lond.** 29:161-189.

Chew, F. and J. Rodman. 1979. Plant resources for chemical defense. pp. 271-307 *In* **Herbivores: their interaction with secondary plant metabolites**, G. A. Rosenthal, D. H. Hanzed (eds.). Academic Press, New York.

Courtney, S. P. 1986. The ecology of Pierid butterflies: Dynamics and interactions. **Adv. Ecol. Res.** 15:51-131.

Courtney, S. P. and A. E. Duggan. 1983. The population biology of the Orange-Tip butterfly, *Anthocharis cardamines* in Britain. **Ecol. Entomol.** 8:271-281.

Descimon, H. 1976. Biology of pigmentation of Pieridae butterflies. pp. 805-840 *In* **Chemistry and Biology of Pteridines**, W. de Gruyter (ed.), Berlin.

Endler, J. A. 1984. Progressive background matching in moths, and a quantitative measure of crypsis. **Biol. J. Linn. Soc. Lond.** 11:187-231.

Endler, JH. A. 1986. **Natural selection in the wild.** Princeton Univ. Press, Princeton, NJ.

Jones, F. M. 1932. Insect coloration and relative acceptability of insects to birds. **Trans. R. Ent. Soc. Lond.** 80:345-385.

Kettlewell, H. B. 1865. Insect survival and selection for pattern. **Science** 148:1290-1296.

Kingsolver, J. G. 1983. Thermoregulation and flight in *colias* butterflies: elevation patterns and mechanistic limitations. **Ecology** 64:534-545.

Kingsolver, J.G. 1985a. Thermal ecology of *Pieris* butterflies:A new mechanism of behavioral thermoregulation. **Oecologia** 66:540-545.

Kingsolver, J. G. 1985b. Thermoregulatory significance of wing melanization in *Pieris* butterflies: Physics, posture and pattern. **Oecologia** 66:546-553.

Kingsolver, J. G. 1987a. Evolution and coadaptation of thermoregulatory behavior and wing pigmentation pattern in pierid butterflies. **Evolution** 41:472-490.

Kingsolver, J. G. 1986b. Predation, thermoregulation, and wing color in pierid butterflies. **Oecologia** 73:301-306.

Kingsolver, J. G. 1988. Thermoregulation, flight, and the evolution of wing pattern in pierid butterflies: The topography of adaptive landscapes. **Amer. Zool.** 28:899-912.

Kingsolver, J. G. and W. B. Watt 1983. Ther-

moregulatory strategies in *Colias* butterflies: Thermal stress and the limits to adaptation in temporally varying environments. **Amer. Natur.** 121:32-55.

Kingsolver, J. G. and W. B. Watt 1984. Optimality models and mechanistic constraints: Thermoregulatory strategies in *Colias* butterflies **Ecology** 65:1835-1839.

Kingsolver, J. G. and D. C. Wiernasz 1987. Dissecting correlated characters: Adaptive aspects of phenotypic covariation in melanization pattern of *Pieris butterflies*. **Evolution** 41:491-503.

Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: bodyt size allometry. **Evolution** 33:402-426.

Lande, R. and S. J. Arnold 1983. The measurement of selection on correlated characters. **Evolution** 37:1210-1226.

Lane, C. 1957. Preliminary note on insects eaten and rejected by a tame shama (*Kitticincla malabarica)* with the suggestion that in certain species of butterflies and moths females are less palatable than males. **Entomol. Monthly Mag.** 93:172-179.

Makino, K., K. Satch, M. Koike and N. Ueno 1952. Sex in *Pieris* rapae L. and the pteridin content of their wings. **Nature**, Lond. 170:933-934.

Marsh, N. and M. Rothschild 1974. Aposematic and cryptic Lepidoptera tested on the mouse. **J. Zool. Lond**. 174:89-122.

Nihjout, H. F. 1985. The developmental physiology of color patterns in Lepidoptera. **Anv. Insect Physiol.** 18:181-147.

Nijhout, H. F. and G. A. Wray 1986. Homologies in the color patterns of the genus *Charaxes* (Lepidoptera: Nyphalidae) **Biol. J. Linn. Soc. Lond**. 28:387-410.

Ohsaki, M. 1986. Body temperature and behavioral thermoregulation strategies of three *Pievis* butterflies in relation to solar radiation. **J. Ethol.** 4:1-9.

Onslow, H. 1916. On the development of the black markings on the wings of *Pieris brassicae*. **Biochem. J.** 10:26-30.

Rothschild, M. 1972. Secondary plant substances and warning coloration in insects. **Symp. R. Ent. Soc. Lond**. 6:59-83.

Rutowski, R. L. 1981. Sexual discrimination using visual cues in the checkered white butterfly (*Pieris protodice*). **Z. Tierpsychol**. 55:325-334.

Rutowski, R. L. 1982. Epigamic selection by males as evidenced by courtship partner preferences in the checkered white butterfly (*Pieris protodice*) **Anim. Behav.** 30:108-112.

Shapiro, A. M. 1976. Seasonal polyphenism. **Evol. Biol.** 9:259-333.

Shapiro, A. M. 1984a. Polyphenism, phyletic evolution and the structure of the *Pierid* genome. **J. Res. Lepid**. 23:177-195.

Shapiro, A. M. 1984b. Experimental studies on the evolution of seasonal polyphenism. pp. 297-307 *In* **The biology of butterflies**. P. Ackery and R. Vane-Wright (eds.). Academic Press, New York.

Shapiro, A.M. 1985. Behavioral and ecological observations of Peruvian high-Andean Pierid butterflies. **J. Res. Lepid**. 24:1-20.

Silberglied, R. E. and O. R. Taylor 1978. Ultraviolet reflection and its role in the courtship of the sulphur butterflies *Colias eurytheme* and *C. philodice* (Lepidoptera, Pieridae). **Behav. Ecol. Sociobiol**. 3:203-243.

Turner, J. R. G. 1977. Butterfly mimicry: The genetical evolution of an adaptation. **Evol. Biol.** 10:163-206.

Wallace, A. R. 1889. **Darwinism** (lst ed.), London.

Watt, W. B. 1964. Pteridine components of wing pigmentation in the butterfly *Colias eurytheme*. **Nature** 201:1326-1317.

Watt, W. B. 1967. Pteridine biosynthesis in the butterfly *colias eurytheme*. **J Biol. Chem**. 242:565-572.

Watt, W. B. 1968. Adaptive significance of pigment polymorphism in *Colias* butterflies. I. Variation in melanin pigment in relation to thermoregulation. **Evolution** 22:437-458.

Watt, W. B. and S. Bowden 1966. Chemical phenotype of pteridine color forms in *Pieris* butterflies. **Nature** 1210:304-306.

Wiernasz, D.C. 1989. Female choice and sexual selection of male wing melanin pattern in *Pieris occidentalis*. **Evolution** 43:1672-1682.

Wourms, M. and J. Wasserman 1985. Butterfly wing markings are more advantageous during handling than during initial strike. **Evolution** 39:845-851.

Photoprotective Pigmentation of Freshwater Zooplankton: A Phenomenon of Extreme Environments

CHRIS LUECKE AND W. JOHN O'BRIEN

Synopsis

Most populations of zooplankton are relatively transparent. However, in ponds and lakes on the coastal plain of northern Alaska two species of zooplankton, *Heterocope septentrionalis* and *Daphnia middendorffiana*, exhibit striking pigmentation patterns. Both species are polymorphic. *H. septentrionalis* exists as a pale green morph in large lakes and a red morph in ponds. *D. middendorffiana* exists as a melanic form in ponds and a translucent form in lakes. Results from field experiments indicated that the more deeply pigmented morph of both species survived better than the relatively unpigmented forms when both were exposed to sunlight. In lakes with visually feeding fish, only the pale morphs of both species exist. Predation experiments indicated that arctic grayling selectively fed on the darker more visible morphs. Deeply pigmented morphs of several species of zooplankton are found in extreme environments in other studies. In each instance, the pigmented morph occurs when abundance of visual predators is low. Production of photoprotective pigments in zooplankton seems only possible when predation risk is low.

Introduction

Limnologists have noted striking color patterns in freshwater zooplankton for decades. Around the turn of this century Sars (1903, cited in Nilsson and Pejler 1973) described the pigmentation of the calanoid copepod *Heterocope saliens* in lakes of northern Scandinavia: "Body generally of a beautiful ultramarine hue, antennae, oral parts and urosome often tinged dark orange."; and "Body semipellucid and generally of a light bluish green hue, anterior antennae and urosome in male tinged with orange." Yet in spite of the conspicuous pigmentation patterns exhibited by some populations, the majority of pelagic zooplankton are relatively colorless. With the exception of descriptions of hemoglobin in some cladoceran populations (Fox et al. 1951) explanations for body pigmentation in zooplankton were nonexistent until recently, when several researchers investigated the adaptive significance of carotenoid and melanic pigments in zooplankton (Hairston 1976, 1978, 1979, Siebeck 1978, Ringelberg 1980, Byron 1981, 1982 and Luecke and O'Brien 1981, 1983). In this paper we review these recent studies and summarize results of our research on photoprotective pigments of zooplankton in the lakes and ponds near Toolik Lake in northern Alaska.

Observations of a striking difference in coloration of *Diaptomus nevadensis* in two lakes in eastern Washington prompted Hairston (1976, 1979) to initiate research on the photoprotective properties of carotenoid pigments in diaptomid copepods. Hairston demonstrated in experiments that deeply pigmented copepods from Soap Lake survived better than pale individuals from Lake Lenore when both were exposed to blue fluorescent light. The pigment responsible for photoprotection was a keto-carotenoid, probably astaxanthin. Paanakker and Hallegraeff (1978) identified astaxanthin as the pigment responsible for the red coloration of *Acanthodiaptomus denticornus*.

Astaxanthin is known to have photoprotective properties in marine crustaceans and some fish eggs (Krinsky 1971). This carotenoid lessens the damage of UV radiation by absorbing oxygen radicals produced when oxygen molecules are exposed to this high energy radiation. The carotenoid content of *D. nevadensis* from Soap Lake was almost three times that found in same-sized animals from Lake Lenore. Hairston (1979) concluded that the greater amount of pigment in Soap Lake animals allowed these individuals to survive prolonged exposure to incident light.

Dark pigmentation permits diaptomids to remain in food-rich surface waters (Hairston 1980),

Chris Luecke, Department of Fisheries and Wildlife, Utah State University, Logan Utah, and W. John O'Brien, Department of Systematics and Ecology, University of Kansas, Lawrence, Kans.

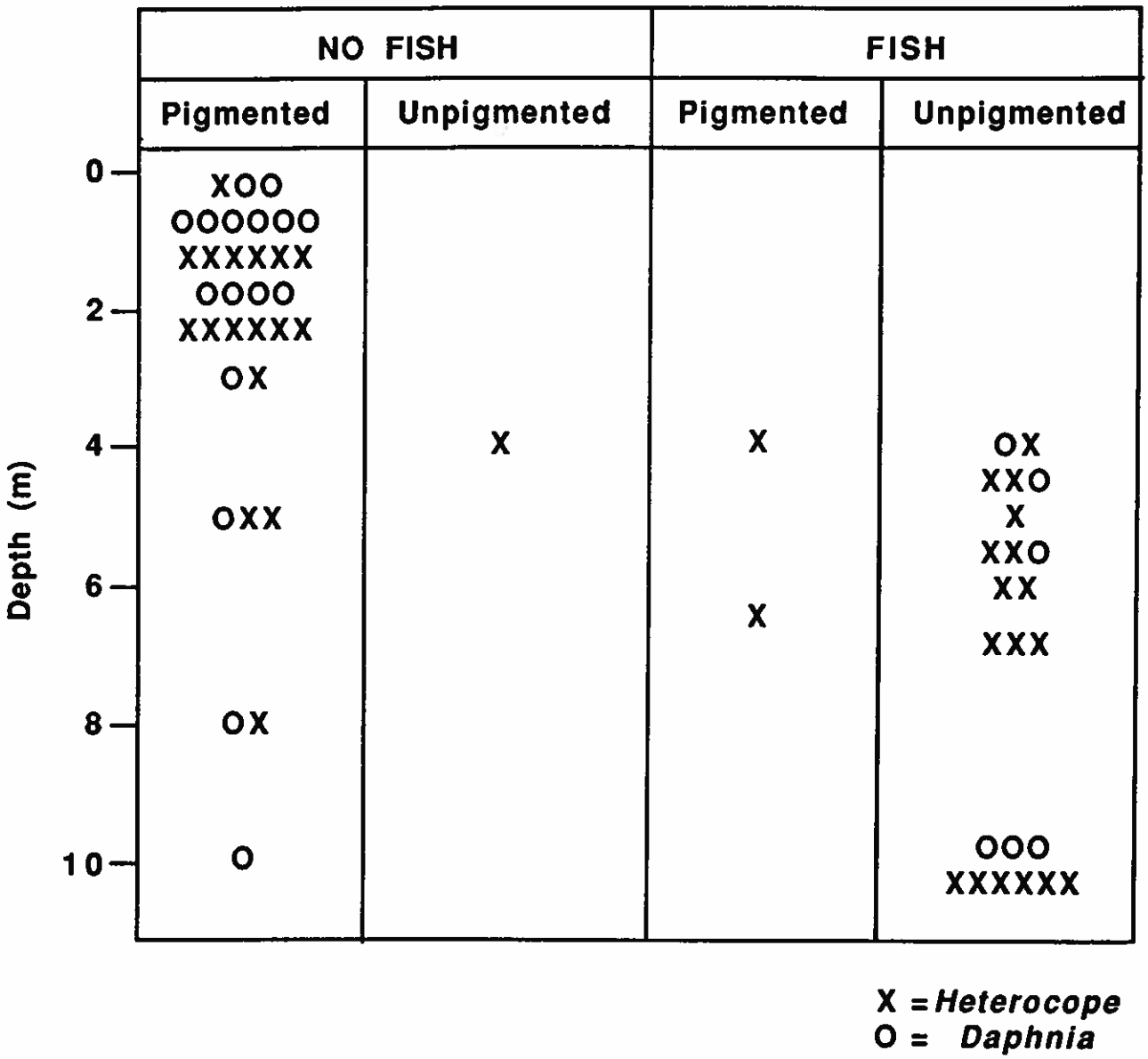


Figure 1. Distribution of pigmented and relatively unpigmented morphs of *H. septentrionalis* (X denotes presence) and *D. middendorffiana* (O) in lakes containing and lacking fish populations near Toolik Lake, Alaska. Depth of lake is given along vertical axis.

thus the presence of the relatively unpigmented *D. nevadensis* in the shallower Lake Lenore was perplexing. Hairston hypothesized that the presence of visually feeding odonates and salamanders in Lake Lenore made it impossible for the conspicuously pigmented morphs to survive, and subsequently proved that both of these predators preferentially consumed the pigmented morphs (Hairston 1979).

Byron (1981, 1982) hypothesized that carotenoid pigments in copepods could increase core body temperature and allow individuals to maintain higher metabolic rates. Field studies in alpine ponds in Colorado indicated that epilimnetic temperature variation explained more of the variation in copepod pigmentation than did variations in surface irradiation. Laboratory experiments indicated that metabolic rates of pigmented *Diaptomus leptopus* and *D. shoshone* were enhanced by exposure to light more than the relatively unpigmented *D. leptopus* (Byron 1981). Although this evidence suggests that high light intensities increase metabolic rates of pigmented copepods, subsequent research has indicated that darkly pigmented copepods would not be able to maintain body temperatures much above ambient levels (Hairston 1979).

Byron (1982) demonstrated that trout (*Salmo gairdneri*) in Colorado mountain lakes preferentially consumed pigmented calanoid copepods over unpigmented individuals in both field and laboratory trials. The absence of trout was correlated with

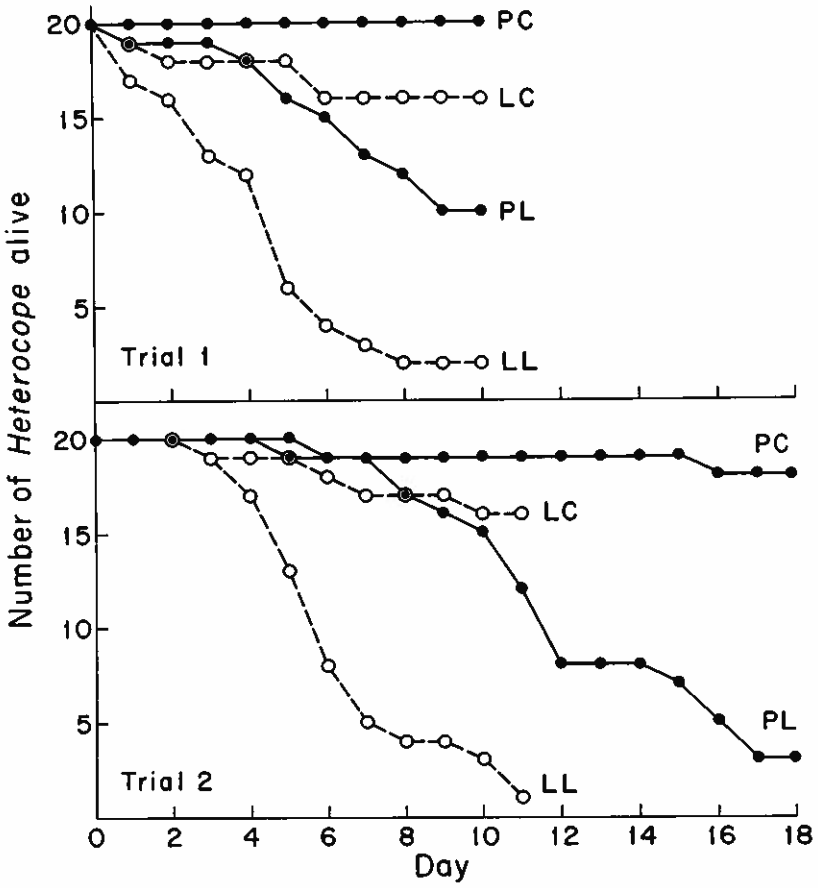


Figure 2. Survivorship of red and green color morphs of *H. septentrionalis* exposed to sunlight compared to shaded control treatments. PC refers to pond individuals (red) in the control treatment; LC to lake individuals (green) in the control treatment; PL to pond individuals in the light treatment; and LL refers to lake individuals in the light treatment. For both trials a Kolmogorow-Smirnoff test indicated that green individuals (LL) had lower survivorship than red individuals (PL) when both were exposed to full sunlight (Dmax-0.40, $p<0.01$ for trial 1; Dmax-0.55, $p<0.01$ for trial 2). Red individuals exposed to sunlight (PL) suffered greater mortality than red individuals in control treatments (PC) (Dmax=0.50, $p<0.01$ for trial 1; Dmax=0.75, $p<0.01$ for trial 2). Survivorship was similar for red and green individuals in control treatments (Dmax=0.20, $p>0.05$ for trial 1; Dmax=0.15 for trial 2). Modified from Luecke and O'Brien (1981).

the presence of deeply pigmented copepods in 30 different lakes. However, a high degree of covariation between low temperature, high light intensities and absence of trout in Byron's (1982) lakes made it difficult to separate these as causative factors in the distribution of copepod pigmentation patterns.

Pigmentation of Arctic Zooplankton

Two species of zooplankton exhibit distinct pigment polymorphisms in lakes and ponds near Toolik Lake in arctic Alaska (68°37'N, 149°35'W). *Heterocope septentrionalis* occurs as a bright red morph in ponds and a pale green morph in lakes; *Daphnia middendorffiana* is relatively translucent in lakes but individuals from pond populations possess a distinct black region on the dorsal part of the carapace. In 1979 we began a series of laboratory and field investigations designed to explain these spatial variations in pigmentation. We specifically tested the hypotheses that pigmented individuals would survive better when exposed to sunlight, and that pigmented morphs would be found more frequently in the absence of visually feeding planktivorous fish.

Distribution

At these latitudes, ponds less than 2 meters deep freeze solid during the winter months thus excluding fish from their fauna (Fig. 1). These shallow ponds contained both red H. *septentrionalis* and darkly pigmented *D. middendorffiana* (Fig. 1). Most lakes deeper than 4 meters contained planktivorous arctic grayling, *Thymallus arcticus*. Of the 18 lakes and ponds that contained *H. septentrionalis* but no fish, 17 of these populations were comprised of red individuals. In only two of 19 bodies of water that contain both *H. septentrionalis* and fish were the *H. septentrionalis* red. A similar distribution was observed for *D. middendorffiana* (Fig. 1). Lakes and ponds that contained fish had unpigmented *D. middendorffiana*, whereas fishless lakes generally contained the pigmented morph. Most lakes in which fish were present contained no populations of *D. middendorffiana*. In shallow fishless lakes, where solar radiation is high and risk to visual predators low, highly pigmented zooplankton morphs dominate.

Phototoxicity experiments

Although Hairston demonstrated that pigmented copepods survived better under artificial

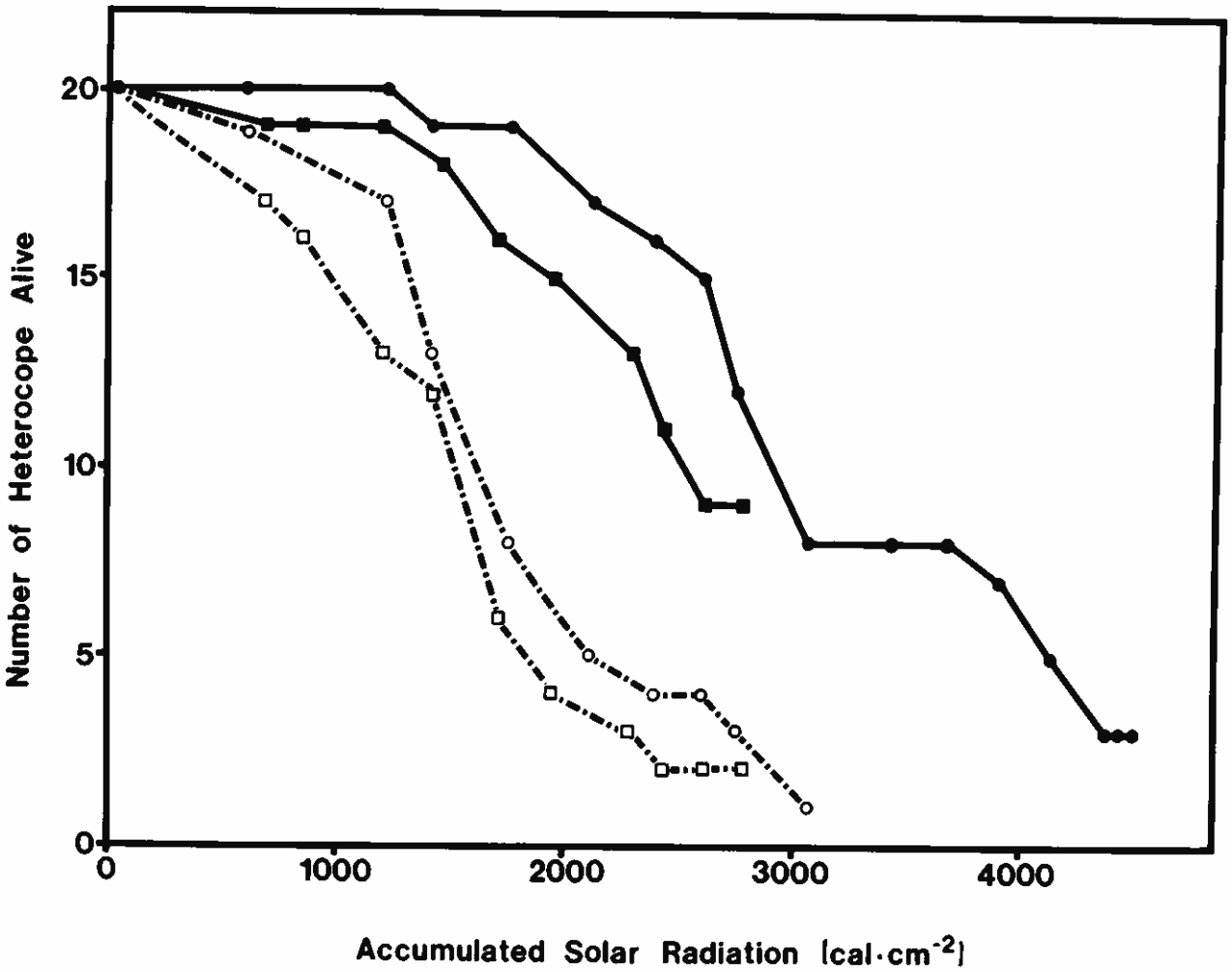


Figure 3. Survivorship of *H. septentrionalis* as a function of accumulated solar radiation in the two experimental trials. Solid lines refer to red individuals and dashed lines refer to green individuals. Numbers 1 and 2 refer to results from the first trial; 3 and 4 refer to results from the second trial. Modified from Luecke and O'Brien (1981).

blue light, no study had demonstrated that natural intensities of sunlight were damaging to zooplankton. To examine the effect of sunlight on survivorship of red and pale green *H. septentrionalis* we performed a field experiment (Luecke and O'Brien 1981). In two separate trials 40 individuals of each color morph were collected (from Camp Pond-red and Toolik Lake-green) and placed individually in shallow plexiglass cylinders containing 30 ml of water 30mm deep. These cylinders were partially submerged in a small wading pool (1.6 m diameter) with water from Toolik Lake circulating through it. Each cylinder had a small hole covered with 156 um plankton netting to allow water to circulate from the wading pool into the cylinder. Half (20) of the individuals of each color morph were shaded as controls with an aluminum coated blanket; the other half were exposed to natural sunlight. The circulating water system kept both shaded and unshaded cylinders at the same temperatures (within 2°C of the lake). Each day the number of H. *septentrionalis* remaining alive in each treatment was recorded. Solar radiation was measured with an integrating Licor quantum photometer.

In both trials the red *H. septentrionalis* survived better than green individuals when exposed to natural sunlight (Fig. 2). In the first trial 90 percent of the green animals had died on Day 8 while greater than 50 percent of the red individuals were still alive. Results from the second trial were similar except the red *H. septentrionalis* also suffered high mortality when exposed to sunlight for long periods of time (more than 2 weeks). Individuals of both color morphs survived well in the shaded treatment. When plotted against accumulated solar radiation, the mortality curves for both trials was similar (Fig. 3). The similarity in the mortality curves suggests that damage from exposure to sunlight

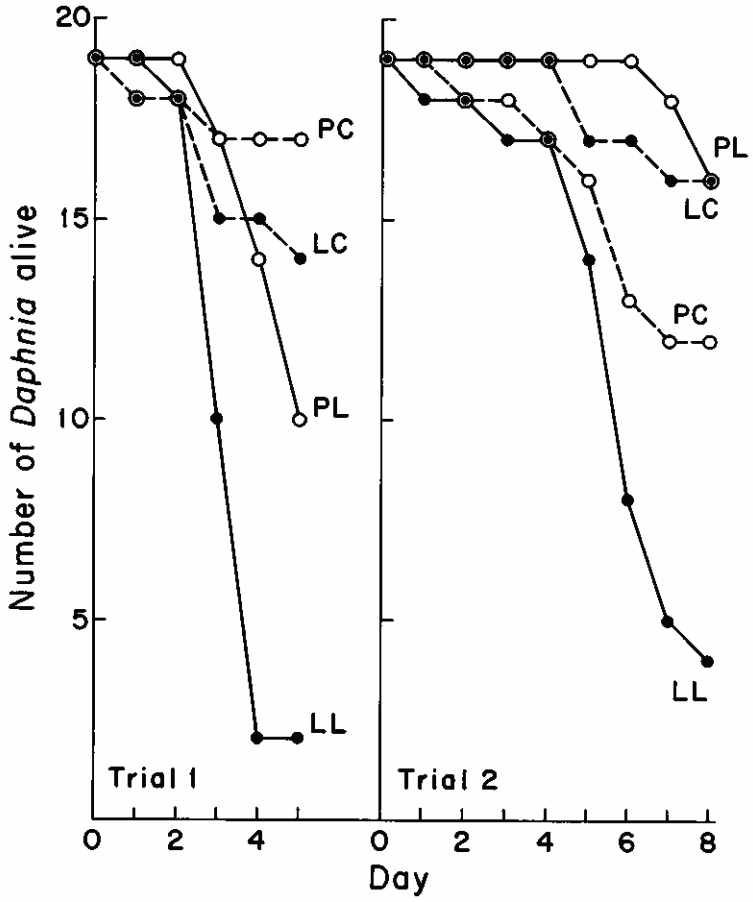


Figure 4. Survivorship of pigmented and unpigmented *D. middendorffiana* exposed to sunlight compared to shaded control treatments. PC refers to pond individuals (pigmented) in the control treatment; LC to lake individuals (unpigmented) in the control treatment; PL to pond individuals in the light; and LL to lake individuals in the light treatment. A Kolmogorov-Smirnoff test indicated that unpigmented individuals suffered higher mortality than pigmented individuals when both were exposed to sunlight (Dmax=0.63, $p<0.01$ in trial 1; Dmax=0.58, $p<0.01$ in trial 2). In the first trial both pigmented and unpigmented individuals had lower survivorship in the sun compared to respective controls ($Dmax_{pig}$= 0.36, $p<0.05$; $Dmax_{unpig}$=0.68, $p<0.01$), but in the second trial no significant differences were observed between pigmented individuals in control and lighted treatments ($Dmax_{pig}$=0.26, $p>0.05$). Modified from Luecke and O'Brien (1983).

accumulates over time, and that under conditions of these experiments the copepods may not be able to repair this damage.

The pigment responsible for the red coloration in *H. septentrionalis* is a keto-carotenoid, probably astaxanthin, that absorbs light most strongly at 465 nm (Luecke and O'Brien 1981). All attempts to extract the green pigment from lake *H. septentrionalis* failed. When treated with organic solvents, the green coloration immediately turned red. The green color is most likely due to a carotenoid-protein complex (Ringleberg 1980). Perhaps breaking the carotenoid-protein bond by the solvent changed the reflectance of the pigment. Extracts of this red color also absorbed light most strongly at 465 nm. It is likely that the green coloration confers some photoprotection to lake *H. septentrionalis* but the quantity of carotenoid pigment in the pond morphs was much greater. *H. septentrionalis* from Camp Pond contained 50 percent more of the carotenoid pigment than an equal mass of *H. septentrionalis* from Toolik Lake (Luecke and O'Brien 1981).

Pigmented *Daphnia middendorffiana* from Camp Pond also survived exposure to sunlight better than did unpigmented individuals collected from Toolik Lake (Luecke and O'Brien 1983). In an experiment similar to that described for *H. septentrionalis*, greater than 50 percent of the *D. middendorffiana* with a melanic carapace survived several days of exposure, whereas more than 75 percent of the unpigmented individuals had died after a few days of being exposed to direct sunlight (Fig. 4). Extensive cloud cover during the first two days of the second trial may have been responsible for the low mortality for both morphs during this period.

The pigment responsible for the dark coloration of *D. middendorffiana* has not been isolated. Dodson (1984) described it as a structural pigment and examined whether the pigment patch reduced the vulnerability of these daphnids to predation by copepods. It is interesting to note that Siebeck (1978) described a similar pigment patch for another cladoceran, *Scaphaloberis kingi*. He hypothesized that the pigmentation pattern may confer protection from light in this species. Results of our phototoxicity experiment with *D. middendorffiana* support Siebeck's hypothesis.

Long-term exposure

In the phototoxicity experiments, one lake *H. septentrionalis* that was initially pale green had become a pale orange by the end of the experiment. This individual was the only lake animal that survived the experiment. This observation raised the question as to whether the pigmentation patterns of *H. septentrionalis* and *D. middendorffiana* are genetically determined or induced by environmental cues. To examine this question we exposed individuals of each morph to a gradient of natural light intensities for 27 days. Each treatment consisted of either lake

Table 1. Results of fish predation experiments with arctic grayling feeding on red and green colored *H. septentrionalis*. In the first trial grayling consumed all of the red individuals. In the second trial, grayling preferentially consumed red over green individuals (X^2=9.46, df=1, p<0.01).

	Number of *H. septentrionalis*			
	Trial 1 (30-minute feeding)		Trial 2 (10-minute feeding)	
	Initial	Final	Initial	Final
Red morph	200	0	200	21
Green morph	200	24	200	46

or pond zooplankton assemblages contained in 20-liter glass aquaria placed in a small wading pool through which Toolik Lake water was circulated to maintain temperature conditions. In the high light treatment, more than 100 pond and 100 lake H. *septentrionalis* and *D. middendorffiana* were exposed to direct sun in 20-liter aquaria. In the medium light treatment, the aquaria was wrapped in two layers of plastic window screening that reduced light levels to 6 percent of ambient. A third treatment consisted of wrapping the aquarium in black plastic and styrofoam insulating material. Less than 1 percent of ambient was present in this treatment. Every three days, half the water in each aquarium was replaced with lake water and *H. septentrionalis* were provided with *Daphnia pulex* as food.

In the high light treatment mortality of the green *H. septentrionalis* was high but five of the 17 individuals that survived had lost the green coloration and appeared reddish-orange. *H. septentrionalis* from Camp Pond remained bright red in this treatment. *H. septentrionalis* morphs in the intermediate light treatment had not changed color. In the no light treatment, both morphs were their original color but appeared somewhat paler. *H. septentrionalis* survivorship was relatively high in all but the high light treatment with the green morph. After the 27 days, at least 50 individuals were recovered from each of these treatments.

Unpigmented *D. middendorffiana* from Toolik Lake did not survive well in any of the treatments. No animals remained at the end of the experiment and only five and eight *D. middendorffiana* were alive in the intermediate and no light treatments, respectively, on day 15. These individuals remained unpigmented, similar in appearance to *D. middendorffiana* from Toolik Lake. Some pigmented *D. middendorffiana* survived in all treatments. In full sunlight the individuals maintained their pigmentation pattern. In both the intermediate and no light treatments the pigmented *D. middendorffiana* became much paler, having lost most of the melanic pigmentation associated with the dorsal carapace.

Results from these longer term experiments indicated that the differences in coloration of *H. septentrionalis* and *D. middendorffiana* morphs are not due to genetic differences alone. The ability of green *Heterocope* to become red may allow these individuals to survive if they were introduced into shallow ponds. For both *H. septentrionalis* and *D. middendorffiana* the change in pigmentation involved the loss of a pigment or structure. Green *H. septentrionalis* became red, presumably by separation of a carotenoid-protein bond. The melanic pigment in *D. middendorffiana* was lost after several body molts. Results from this long-term exposure suggest that the loss of a pigment characteristic may be environmentally induced but that the production of a new pigmentation pattern does not occur with short-term exposure to high amounts of solar radiation.

Fish predation

Although the pigmentation of the pond morphs of *H. septentrionalis* and *D. middendorffiana* provided protection from solar radiation, the relative lack of pigmentation in lake morphs has not been addressed. The presence of planktivorous fish correlates well with the presence of the unpigmented morphs of both zooplankton species (Fig. 1). Even in the few deep fishless lakes, in which the pigmentation pattern is not a requisite for survival, the pigmented morph of both species occurred. Several authors have examined the hypothesis that grayling (*Thymallus arcticus*) (Schmidt and O'Brien 1982, Luecke and O'Brien 1981) and juvenile lake trout (*Salvelinus namaycush*) (Kettle and O'Brien 1978) feed selectively on the pigmented morphs of both species in a variety of laboratory and field experiments.

We performed a field experiment in which 200 red and 200 green *H. septentrionalis* were placed in a 54-liter plexiglass container with two small grayling (90 mm standard length) and suspended two meters deep in Toolik Lake (Luecke and O'Brien 1981). After either 10 or 30 minutes, the container was retrieved and the number of *H. septentrionalis* of each morph was enumerated. A control container

without fish was also run for 30 minutes. Grayling consumed more red than green individuals in both trials (Table 1). In fact, in the 30-minute trial no red *H. septentrionalis* remained. Also Schmidt and O'Brien (1982) reported that small arctic grayling were able to locate and pursue red *H. septentrionalis* at much greater distances than green individuals. These observations indicate that grayling feed selectively on darkly pigmented red *H. septentrionalis*.

Kettle and O'Brien (1978) examined the ability of juvenile lake trout to locate a variety of zooplankton species that occurred in lakes and ponds of the Toolik region. In laboratory experiments, these fish were able to locate pigmented daphnids at 1.4 times the distance of which unpigmented individuals of a similar size were located. Results of these laboratory and field predation experiments implicate selective feeding by planktivorous fish as a causal mechanism in determining the distribution of pigmented zooplankton morphs.

Discussion

Hairston (1979) proposed that the extent of pigmentation in calanoid copepods was determined by a balance between the risk of being eaten by visual feeding predators and the enhanced ability to survive exposure of solar radiation. The spatial distribution of different pigmentation patterns in *Heterocope septentrionalis* and *Daphnia middendorffiana* in arctic lakes supports this idea. In lakes containing planktivorous fish, deeply pigmented forms of zooplankton are selectively consumed and eliminated if fish densities are high. In bodies of water lacking fish, deeply pigmented morphs survive better than relatively unpigmented individuals. Zaret (1980) reached a similar conclusion concerning the presence of fish and the degree of pigmentation of cladocerans.

The spatial distribution of *H. septentrionalis* color morphs in arctic lakes does not support the metabolic enhancement hypothesis of Byron (1982). In contrast to the alpine lakes of Byron's study, high levels of solar radiation, low water temperatures and lack of visual predators were not positively correlated in lakes and ponds of the Alaskan tundra. The fishless arctic ponds were much warmer than deeper lakes during the summer months. The presence of deeply pigmented *H. septentrionalis* and *D. middendorffiana* in these warm fishless ponds and the presence of unpigmented forms in colder lakes weakens the argument that copepods in cold environments need more pigmentation to enhance metabolic rates.

The close association between the absence of visual planktivores and the presence of pigmented morphs of *H. septentrionalis* and *D. middendorffiana* suggests that predation is a more powerful selective force than phototoxicity in determining the distribution of zooplankton color morphs. In lakes and ponds where no fish were present, almost all (33 out of 34) *H. septentrionalis* and *D. middendorffiana* populations consisted of pigmented individuals (Fig. 1). Pigmented individuals can only persist in habitats with no or low densities of visually feeding predators. In the absence of predators the intensity of solar radiation will select for individuals containing these photoprotective pigments.

This scenario can also explain the distribution of zooplankton color morphs in other areas. In saline desert lakes (Hairston 1979) and alpine ponds (Loffler 1969, Byron 1982) the presence of pigmented zooplankton is common. In each of these extreme environments fish or other visual feeding planktivores are rare. The lack of predators in these environments allows the production of photoprotective pigmentation patterns in a variety of freshwater zooplankton. These photoprotective pigments allow individual zooplankters to increase their growth and reproductive output by remaining in surface waters where food resources are abundant (Hairston 1980, Sticht and Lampert 1983).

Acknowledgments

We thank D. Schmidt, D.I. Wright and P. Skvorc for help with the field sampling, M. Vanni, J. Post, R. Nero and an anonymous reviewer for providing useful suggestion to the manuscript, and C. Hughes for drafting the figures. This research was supported by NSF grant DPP-7828041 to W.J. O'Brien.

References

Byron, E.R. 1981. Metabolic stimulation by light in a pigmented freshwater invertebrate. **Proc. Nat. Acad. Sci.** 78:1765-1767.

Byron, E.R. 1982. The adaptive significance of calanoid copepod pigmentation: a comparative and experimental analysis. **Ecology** 63:1871-1886.

Dodson, S.I. 1984. Predation of *Heterocope septentrionalis* on two species of *Daphnia*: morphological defenses and their costs. **Ecology** 65:1249-1257.

Fox, H.M., B.M. Gilchrist, and E.A. Phear. 1951. Functions of haemoglobein in *Daphnia*. **Proc. R. Soc. Lond.** Ser. B 139,514-527.

Hairston, N.G.,Jr. 1976. Photoprotection by carotenoid pigments in the copepod *Diaptomus nevadensis*. **Proc. Nat. Acad. Sci.** 73:971-974.

Hairston, N.G.,Jr. 1978. Carotenoid photoprotection in *Diaptomus kenai*. **Verh. Int. Ver. Limnol.** 20:2541-2545.

Hairston, N.G.,Jr. 1979. The adaptive significance of color polymorphism in two species of *Diaptomus* (Copepoda). **Limnol. Oceanogr.** 24:15-37.

Hairston, N.G.,Jr. 1980. The vertical distribution of diaptomid copepods in relation to body pigmentation. p. 98-110 *In* W.C. Kerfoot [ed], **Evolution and ecology of zooplankton communities.** University Press of New England, Hanover.

Kettle, D. and W.J. O'Brien. 1978. Vulnerability of arctic zooplankton to predation by small lake trout (*Salvelinus namaycush*). **J. Fish. Res. Bd. Can.** 11:1495-1500.

Krinsky, N.I. 1971. Function. p. 669-716. *In* 0. Isler [ed], **Carotenoids.** Birkhayser, Basel.

Loffler, H. 1969. High altitude lakes in Mt. Everest region. **Verh. Verein. Limnol.** 17:373-385.

Luecke, C. and W.J. O'Brien. 1981. Phototoxicity and fish predation: selective factors in color morphs in *Heterocope*. **Limnol. Oceanogr.** 26:454-460.

Luecke, C, and W.J. O'Brien. 1983. Photoprotective pigments in a pond morph of *Daphnia middendorffiana* **Arctic** 36:365-368.

Nilsson, N. and B. Pejler. 1973. On the relationship between fish fauna and zooplankton composition in north Swedish lakes. **Rept. Inst. Freshwat. Res. Drottningholm** 53:51-77

Paanakker, J.E., and G.M. Hallegraeff. 1978. A comparative study on the carotenoid pigmentation of the zooplankton of Lake Maarsseveen and of Lac Pavin. I. Chromatographic characterization of carotenoid pigments. **Comp. Biochem. Physiol.** 60B:51-58.

Ringleberg, J. 1980. Aspects of red pigmentation in zooplankton, especially copepods. p 91-97. *In* **Evolution and ecology of zooplankton communities.** W.C. Kerfoot [ed.] University Press of New England, N.H.

Schmidt, D. and W.J. O'Brien. 1982. Planktivorous feeding ecology of arctic grayling (*Thymallus arcticus*). **Can. J. Fish. Aquat. Sci.** 39:475-482.

Siebeck, 0. 1978. Ultraviolet tolerance of planktonic crustaceans. **Verh. Int. Ver. Limnol.** 20:2469-2473.

Stich, H.B. and W. Lampert. 1984. Growth and reproduction of migration and nonmigrating *Daphnia* species under simulated food and temperature conditions of diel vertical migration. **Oecologia** 61:192-196.

Adaptive Coloration in Texas Fiddler Crabs (*Uca*)

CARL L. THURMAN

Synopsis

Five species of fiddler crabs occupy a variety of intertidal niches along the Texas coast. Each *Uca* is adapted to a specific array of physical factors in the environment. Some aspects of their adaptations are reflected by body color. Interspecific differences in morphological coloration are correlated with camouflage and substrate characteristics. Intraspecific color variation is expressed through neurosecretion-mediated physiological change in cellular pigment distribution. Adaptation to a dark or light colored background reveals different "secondary" chromomotor capabilities for each species. In addition, pigments in melanophores, leucophores and erythrophores exhibit circadian rhythms of dispersion and aggregation.

During a "primary" chromomotor response to light or temperature, chromatophores act as independent effectors without endocrine mediation. Generally, logarithmic changes in luminosity from 12- to 1,000-foot candles disperse chromatophore pigments in species from the *Minuca* subgenus but not members of the *Celuca* subgenus. In the very terrestrial *Celuca*, *U. subcylindrica*, erythrophores and melanophores were observed to aggregate. Since this does not occur in eyestalk-less crabs, the response is augmented by light activating a visual-neurosecretory reflex. Changing temperature stimulated thermoregulatory chromomotor activity in the *Celuca*, *U. panacea* and *U. subcylindrica*, but not the *Minuca*. In *Celuca*, the carapace darkens as temperature decreases and lightens as it increases. Based on these chromatophore studies, the pigmentary systems of the subgenus *Celuca* appear to be predisposed for better short-term thermoregulation than those in the subgenus *Minuca*.

Introduction

The forces affecting the evolution of invertebrate pigmentation are numerous (Burtt, 1979). As pointed out by other contributors to this symposium, coloration plays an important role in (1) communication, (2) camouflage, and (3) thermoregulatory behavior. A large number of studies have addressed the role played by pigmentation in cryptic coloring, inter- and intraspecific communication (e.g. courtship, aposematic and parasematic signaling). In addition, dermal and hypodermal pigments may regulate the absorption of radiation through either behavioral, physical or biochemical changes. Through these modifications, color may control body temperature or rates of evaporative water loss. Thus, pigmentation can have considerable significance where radiation, temperature and moisture are critical factors limiting the distribution of species.

These physical factors appear to have influenced both evolution (Bliss, 1979) and pigmentation in terrestrial crustaceans. In aquatic Crustacea with thick, strongly calcified exoskeletons, body color is conferred by a pigment layer in the integument below the epicuticle (Bagnara and Hadley, 1973; Ghidalia, 1985). If the crustacean possesses a thin, translucent exoskeleton, like the semi-terrestrial fiddler crabs, body color is due to pigments occurring inside the body or in special cells called chromatophores. Regardless of their localization in a layer or a cell, the same pigment compounds are responsible for crustacean color: carotenoids, ommochromes and pterins (Ghidalia, 1985). The distribution of these pigments may be modified to alter body color.

Modifications in the display of a pigment are classified as either morphological or physiological color change (Rao, 1985). The integumental color of a crab is determined by the number, types and distribution of both epidermal and chromatophore pigments. Quantitative changes in these components will lead to a morphological color change. Typically it occurs in response to environmental changes or development and takes place over several days, weeks or months. For example some herbivorous crustaceans may incorporate different carotenoids into their exoskeleton with each molt and growth cycle (Lee, 1966). Altering the number of chromatophores or quantitative changes in cellu-

Department of Biology, University of Missouri, St. Louis, Mo. 63121.

lar pigment content or arborescence may contribute to morphological color change (Green, 1964).

Color transformations due to pigment migration within chromatophores are commonly known as physiological color change. During these chromomotor responses (Needham, 1974), colors are displayed or obscured by the respective dispersion or aggregation of chromatophore pigment. Physiological color change may be either slow, predictable and rhythmic, or rapid and spontaneous. Slow chromomotor responses are usually expressed as daily, tidal, lunar or seasonal rhythms (Palmer, 1974). Rapid responses are displayed immediately in response to background color, fluctuating illumination and changes temperature. Some species of fiddler crabs may use rapid physiological color change in courtship (Crane, 1944; 1975).

Physiological color change occurs by one of two basic mechanisms: a "primary" or a "secondary" response. Primary responses are primitive chromatophore changes independent of nerve and hormone control (Brown, 1973). These responses are elicited by illumination or temperature directly stimulating chromatophore pigment migration. The cellular basis of the primary response is unknown (Weber, 1983). Secondary responses occur through indirect routes involving neurosecretory tracts in the eyestalk. The primary response persists in the absence of neurosecretory components while the secondary will not.

Generally, secondary responses are mediated by neurosecretory hormones known as chromatophorotropins (Rao, 1985; Fingerman, 1987). The sinus gland, located in the crustacean eyestalk, is a neurohemal organ engaged in the storage and release of material elaborated by cells in the nervous system. Brown and his associates (Brown and Ederstrom, 1940; Brown and Wulff, 1941; Brown and Klotz, 1947) demonstrated that chromatophore pigment migration is regulated by a dual hormone mechanism. Antagonistic pairs of pigment-dispersing and pigment-aggregating hormones released from the sinus gland regulate chromatophore movements. However, removing the sinus gland-nervous system complex may not block entirely a secondary response. In some natantians, reptatians and stomatopods, a caudal photoreceptor may entrain light-dependent responses by an extraocular pathway (Page and Larimer, 1976; Wilkens and Larimer, 1976). Since pigment-affecting hormones are synthesized in the nervous system, removal of the sinus gland does not prevent the release of hormones from neurosecretory cells located in other parts of the crab (Webb et al., 1954; Fingerman et al., 1967, 1969).

Light intensity, temperature and water loss are important factors influencing the evolution of semiterrestrial crabs (Bliss, 1979). Much of our basic understanding about crustacean color physiology has been developed using the fiddler crab (Fingerman, 1970; 1987; Rao, 1985). Consequently, it appears that color change plays a significant role in the physiological adjustment of semiterrestrial *Uca* to some environmental factors. This discussion will focus on cryptic coloration, circadian rhythms in pigment movement and subgeneric differences in the primary responses of Texas *Uca*. Their color patterns and physiological responses are similar to those expressed by other *Uca* around the world.

An Ecological Perspective for Color

From a recent biogeographic survey (Barnwell and Thurman, 1984), seven species of *Uca* occur in Texas. Two, *Uca minax* (Le Conte) and *Uca vocator* (Herbst), are rare in the region. The five remaining species are frequently encountered in either semiarid, riverine or intertidal areas. Only these common species will be addressed (Plate 7). From a systematic perspective, two species, *Uca longisignalis* Salmon and Atsaides and *Uca rapax* (Smith), are recognized as members of the subgenus *Minuca* according to Crane (1975). Two others, *Uca panacea* Novak and Salmon and and *Uca spinicarpa* Rathbun are considered to be *Celuca*. The fifth species, *Uca subcylindrica* (Stimpson), was recently transferred from the *Minuca* to the *Celuca* subgenus (Barnwell and Thurman, 1984). All species except *U. rapax* are endemic to the western Gulf of Mexico. *Uca rapax* is found throughout the Gulf and distributed into Central and South America.

Climate

Fiddler crabs are inhabitants of the intertidal zone throughout tropical and temperate latitudes. Neotropical species, in particular those from the western Gulf of Mexico, are unique in their adaptation not only to temperate latitudes but also to relatively arid coastal conditions. In terms of climate, the Texas coastline is divided sharply into two distinct zones (Hedgpeth, 1953). Between the Sabine and the Guadalupe rivers along the northern coast, the climate is moist with more than 35 inches of precipitation annually. South of the Guadalupe river, the climate becomes subhumid and semi-arid with less than 25 inches of precipitation per year. Because of low rainfall, few rivers, barrier-islands, and a high transevaporation coefficient, coastal lagoons in the south are often hypersaline (salinity >> 35 o/oo). Along the northern Texas coast the average winter-minimum temperature is about 4.4°C. Fiddler crab populations in this region may be inactive during the winter months. In south Texas, the average winter-minimum rises to near 10°C. During most of the winter, periodic warming periods are not uncommon along the southern coast

and crab activity is often seen during the day. To inhabit this region, intertidal *Uca* must tolerate temperature extremes, high salinity and low humidity (Thurman, 1984; Rabalais and Cameron, 1985).

Intertidal distribution

The ecological distribution of fiddler crabs in the western Gulf of Mexico has been described by Thurman (1982, 1984, 1987). A description of the ecology of *Uca* in the northern Gulf is forthcoming (Thurman, in preparation). A typical distribution of fiddler crabs in a brackish-water habitat is shown in Figure 1. Between the Louisiana border and the Laguna Madre near Corpus Christi, Tex., the shores of embayments and lagoons usually contain *U. longsignalis, U. rapax, U. spinicarpa* and *U. panacea*. The fifth species, *U. subcylindrica*, is ecologically distinct from the others (Figure 2a). It is endemic to hypersaline, brackish, and freshwater niches in semi-arid south Texas. This species expresses a greater degree of terrestriality than other species of *Uca*.

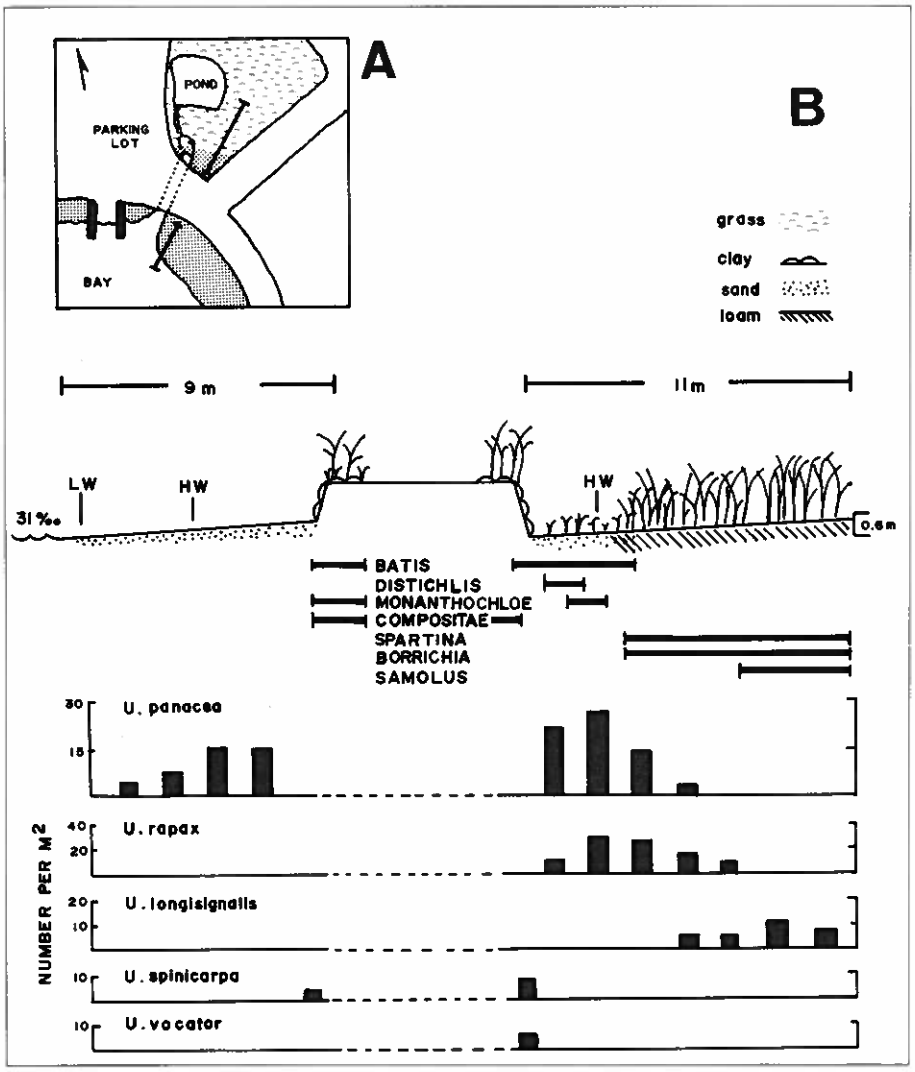


Figure 1. Transect of *Uca* habitat at Ingleside Cove, Tex. (Thurman 1984). A. Overall view of location. B. Transect through marsh. Position of transect in A indicated by bars. LW = low tide water mark. HW = high tide water mark.

Throughout their distributions, each species of *Minuca*, *U. longisignalis* and *U. rapax*, typically inhabits substrates with an average particle diameter of less than 109 μ (Thurman, 1982; 1987). Both live on dark-colored soils with vegetation coverage. Although they are commonly sympatric, *U. longisignalis* is often collected in areas with lower salinity (5 o/oo) than *U. rapax* (Thurman, 1982; 1984). However, due to their ability to osmoregulate over a broad range of salinities (Rabalais and Cameron, 1985), populations of *U. longisignalis* can be euryhaline in their distribution. Although its osmoregulatory abilities have not been assessed, colonies of *U. rapax* are commonly limited to habitats with salinities greater than 15 o/oo. Segregation into two different microhabitats appears to be due to differences in the physiological capabilities of these *Minuca*.

Among the *Celuca*. *U. panacea* typically inhabit exposed, sandy substrates in euryhaline environments (Rao and Fingerman, 1968; Powers, 1975; Thurman, 1984). They are found on intertidal substrates having a mean particle diameter of 112 μ between western Florida and Tabasco, Mexico. *Uca spinicarpa*, on the other hand, occur in habitats with low salinity. Water near or in the crab's burrow has a salinity between 1 and 30 o/oo. They often burrow in clay or loam banks with an average particle diameter greater than 110 μ (Thurman, 1984). These habitats are covered with low but thick vegetation. The third *Celuca*, *U. subcylindrica*, is found near hypersaline and freshwater lagoons in Texas and northeastern Mexico with very low vegetational coverage. Since they possess the ability to burrow exceptionally deep (Figure 2b) and have special larval characteristics, this species can maintain populations in nontidal, isolated lagoons several kilometers from the coast (Thurman, 1984; Neck, 1987). Because of its terrestriality, *U. subcylindrica* is

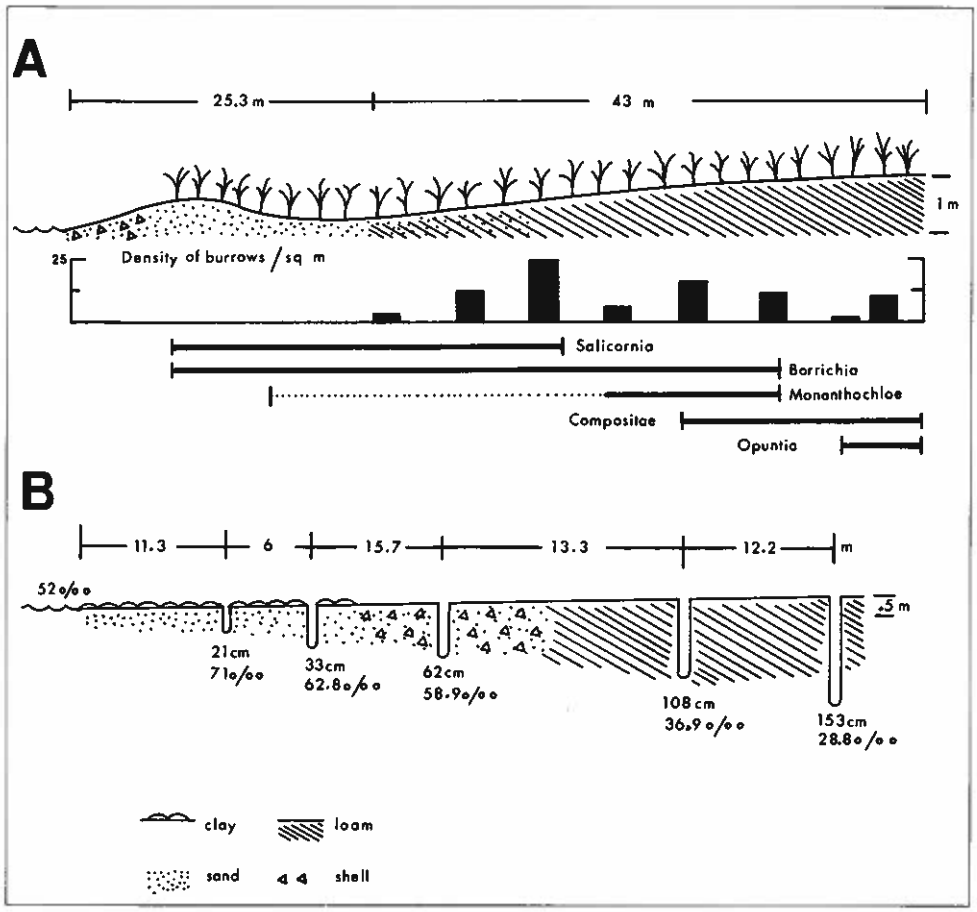


Figure 2. Transect of *Uca subcylindrica* habitat at Laguna Salado, Riviera, Kleberg County, Tex. (Thurman 1984). A. Burrow distribution. B. Burrow depth and salinity profile along transect.

often exposed to high levels of evaporation, insolation, and elevated temperatures (Thurman, 1984).

Environmental rhythms

Cycles of color change in the fiddler crabs usually correlate with specific environmental periodicities such as photoperiod or tidal inundation and ebb (Brown, 1973). Daily solar and temperature cycles appear to be among the more important "zeitgebers." In the western Gulf, these appear more important than tides for regulating fiddler crab activities (Barnwell, 1968a; 1976; Powers, 1975) During summer months, high surface temperatures may restrict crabs to crepuscular and nocturnal activity where as during the winter, daily warming may encourage diurnal crab activity (Thurman, 1984).

Color Variation in *Uca*

Morphological coloration

Usually each species of *Uca* can be identified by its color (Plate 7). However, this sole criterion should not be used to identify a taxon. The general pigmentation of both male and female *Uca subcylindrica* is remarkably constant throughout the range of the species. They possess a gray-mottled carapace with gold, yellow, and orange flecks (Plate 7a). The carapace of an individual crab can vary from pearl-white to tan depending upon its physiological state. The large cheliped of the male is white. Although the ambulatories are heavily setose, they are usually gray or white.

The carapace of *U. panacea* varies from light gray to olive-brown. It is similar in color to *U. pugilator* (Bosc), a close relative from the eastern Gulf (see Rao and Fingerman, 1968; Novak and Salmon, 1974). The H-depression in the mesogastric region is permanently dark (Plate 7b). Unlike *U. pugilator. U. panacea* never possess a purple spot in the anterio-mesogastric region. The fingers of the male's large cheliped are white and the outer propodus is orange to purple. Ambulatories are usually colored creamy-tan or brown-black.

The carapace of *U. spinicarpa* is ash-gray mottled with small white, black and brown specks (Plate 7c). Some individuals have a cream-colored carapace. Individuals with green pigmentation on the interocular lobe were collected in Ocean Springs, Miss. (Barnwell and Thurman, 1984). This color variety is rare. The major cheliped has gray to white

fingers; the propodus is yellow to ochre. Walking legs are usually mottled with black, brown and white spots.

Morphological coloration in the *Celuca* correlates closely with edaphic characteristics (Plate 7). First, *U. subcylindrica* inhabit a dry, gray-colored clay. The gray carapace undoubtedly contributes to cryptic coloration. *U. panacea* live on sandy soils with sparse vegetation. The brown-gray body color of this species aids in substrate-matching camouflage. Likewise, *U. spinicarpa* are cryptically colored for inhabiting gray to black-colored clay-loam soils.

Minuca are generally darker in color than *Celuca*. They possess a gray, brown or buff-colored carapace (Crane, 1975). This "broad-fronted" *Minuca* may have a light-colored anterior edge on the carapace (Plate 7d). The most anterior third of the carapace can vary in color from apple-green to blue-green or turquoise (Salmon and Atsaides, 1968). The carapace of the female, in cases, may be void of this bright coloration. Her carapace may be simply brown-black. Fingers of the male's large cheliped are yellowish white with brown to dark-yellow pigment spots on the articulations. The upper carpus and merus are often yellow-green with dark-yellow and brown specks. The ambulatories are black-brown in color.

In *U. rapax* from the western Gulf of Mexico carapace color is invariant over a wide geographic range. Anterio-frontal and orbital regions are creamy-white to rose (Plate 7e). The anterior third of the carapace is blue with purple-red specks. Dactyl and exopodite of the large cheliped are white while the propodus is gray to blue with yellow lining. The ambulatories are always dark.

Coloration in the *Minuca* is also correlated with edaphic characteristics. Both *U. longisignalis* and *U. rapax* possess dark pigmentation on the lower portions of the carapace and legs that provides cryptic coloration in muddy habitats. However, the bright colors around frontal and orbital regions of carapace in both sexes are not correlated with any obvious environmental parameter.

Physiological coloration

Individual variation in body color is achieved by altering chromatophore pigment distribution. Experiments examining physiological color change were conducted with fiddler crabs measuring at least 14 mm across the carapace within 24 hours of their capture. The crabs were kept individually in pans with saltwater under constant temperature (22°C) and illumination (32-foot candles) unless otherwise indicated. The dispersion index or stage of melanophores, leucophores and erythrophores on the anterior merus of the third pereopod was estimated using the method of Hogben and Slome (1931). An index of one (1) describes a chromatophore in which the pigment is completely aggregated; while five (5) indicates one in which pigment is maximally dispersed. To examine the direct effect of illumination and temperature on chromatophore dispersion, the methods of Brown and Sandeen (1948) and Barnwell (1968b) were used. Chromomotor datum is reported as the mean and standard error of dispersion index.

Background adaptation. To chromatically blend with a background, the chromatophores of an organism are physiologically adjusted to adapt its body color to that of the substrate. Optimum physiological color adaptation to a light substrate is the complete dispersion of leucophores and aggregation of melanophores and erythrophores. On the other hand, dark adaptation involves the dispersion of melanophores and erythrophores and a concentration of leucophores. Physiological color change in these cases usually takes about one hour.

The magnitude of the color-change associated with background adaptation was determined for each species of *Uca* by indexing chromatophores on dark- and light-background adapted crabs. Crabs were placed in either a white or black plastic container for one hour to adjust or adapt their body color to a dark (DBA)- or light (LBA)-background under constant conditions. The chromatophores were initially staged at 0900 CST, then once every hour for three hours. Mean dispersion index (±SEM) for black, white and red chromatophores following the adaptation of each species (N = 20) is shown in Figure 3.

Except for erythrophores in *U. longisignalis* (Figure 3b), the chromatophores in all species exhibit dispersion patterns indicating statistically significant background adaptation ($P<0.05$). The difference in average chromatophore stage between black and white background-adapted crabs can be used as a measure of physiological flexibility or capability. The difference in average chromatophore index is minimum in *U. rapax* (1.3 units) and maximum in *U. subcylindrica* (2.6 units). Intermediate physiological capabilities are seen in *U. spinicarpa* (1.7), *U. panacea* (1.9) and *U. longisignalis* (2.1). In general, *Minuca* tend to remain dark when placed on either dark or light substrata. Except for leucophores in *U. longisignalis*, chromatophores of the *Minuca* do not exhibit extensive physiological adjustment to background color. The amplitude of chromatophore background responses is greater in the *Celuca* (Figure 3c,d,e).

Chromatophore rhythms. Chromomotor adjustments for background adaptation are not constant. The fiddler crabs are well known for their circadian rhythms of color change even though their adaptive significance is unknown (Brown, 1973; Webb, 1983). Chromatophore pigment migration in *Minuca* under constant temperature and illumination (LL) for 48

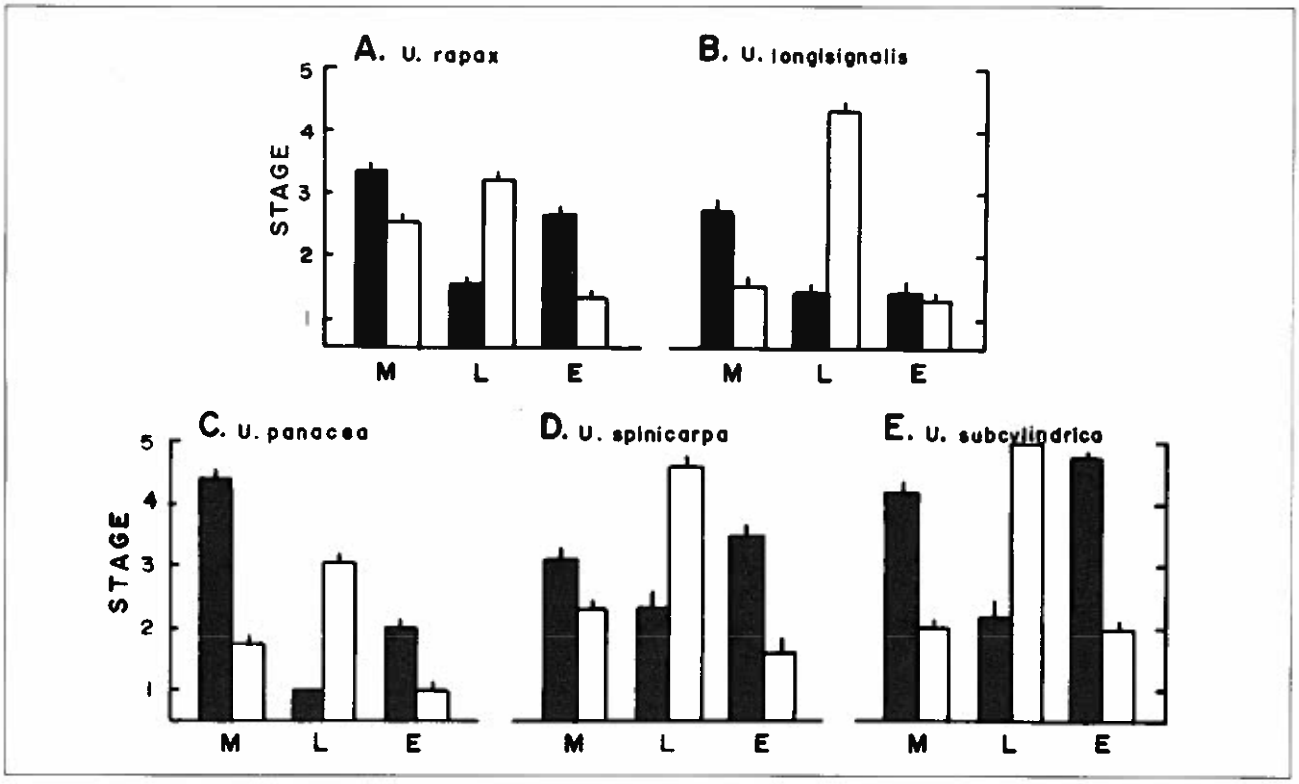


Figure 3. Mean (±SEM) chromomotor response of *Uca* following dark (solid bar) and light (open bar) background adaptation. N = 20 individuals/species. M = melanophore, L = leucophore, E = erythrophore.

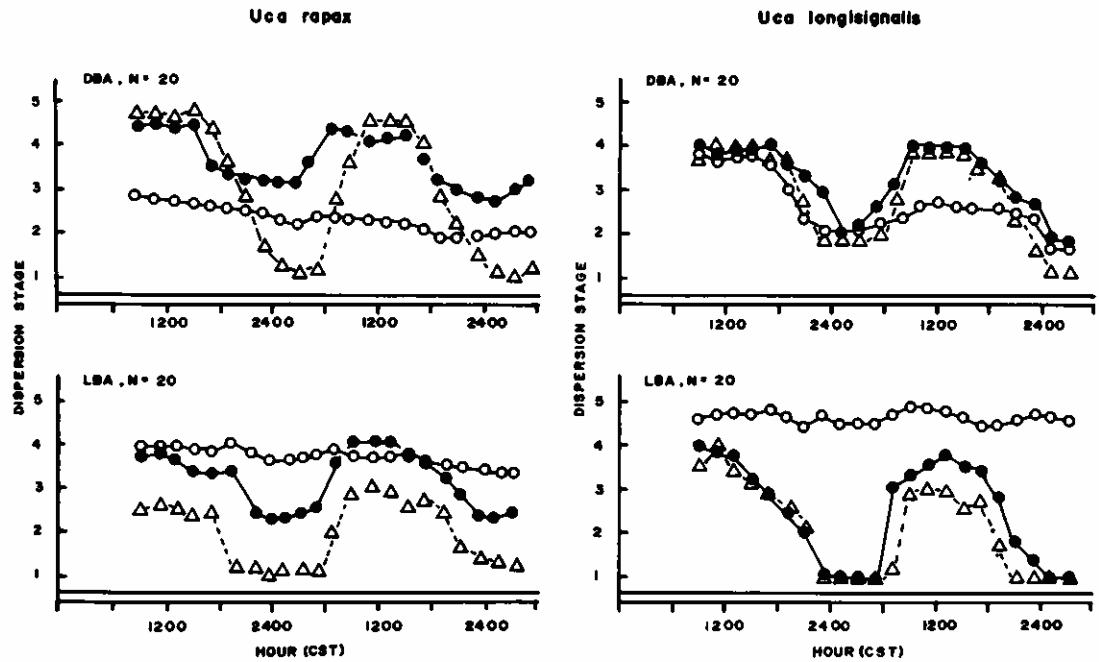


Figure 4. *Minuca* chromomotor rhythms in constant illumination (LL, 32-foot candles). DBA - dark background, LBA - light background. • - melanophores, o - leucophores, Δ - erythrophores.

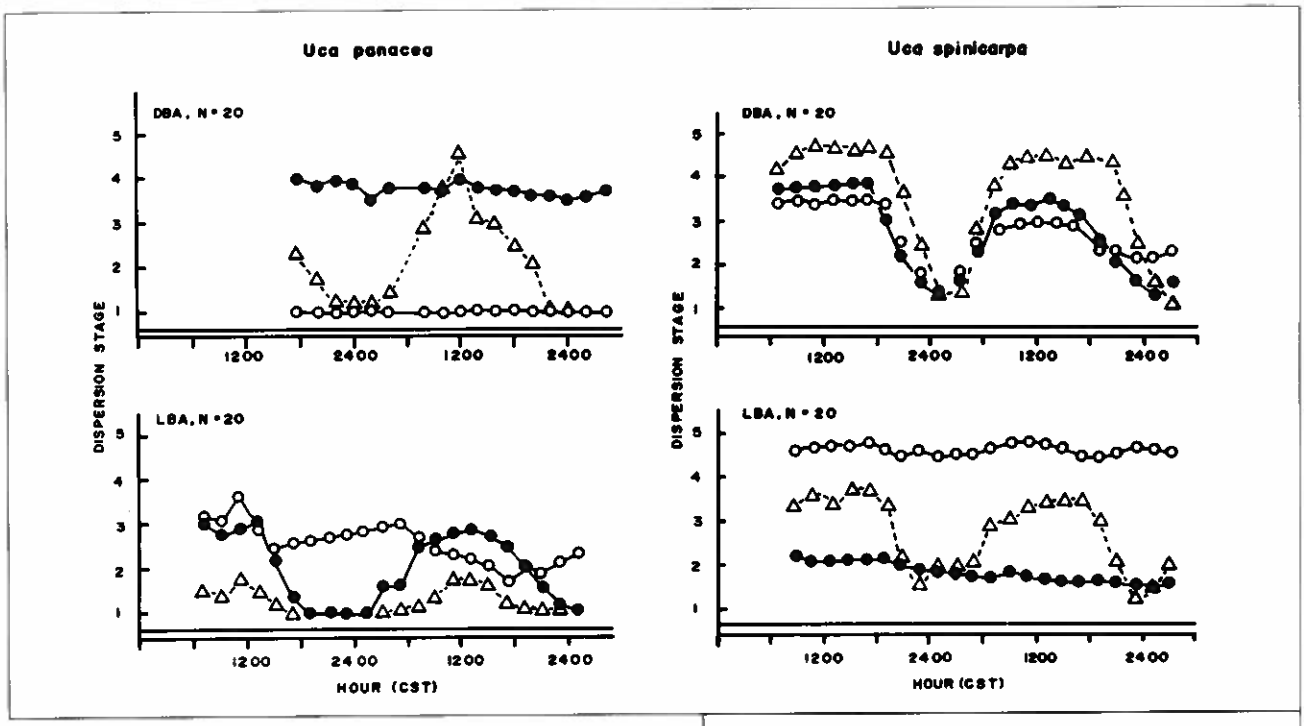


Figure 5. *Celuca* chromomotor rhythms in constant illumination (LL). Symbols same as in Figure 4.

hours is shown in Figure 4 while *Celuca* rhythms are shown in Figures 5 and 6. Both *Minuca* species darken between 0600 and 1800 CST regardless of background. This is due primarily to endogenous black and red chromatophore rhythms. The amplitude of the black pigment cycle is greater in *U. longisignalis* than *U. rapax* (e.g. Figure 4). Although white chromatophores initially exhibit a circadian rhythm during dark background adaptation in *U. longisignalis*, the oscillation dampens. Illumination is reported to inhibit chromatophore rhythms in *U. pugilator*. *U. pugnax* and *U. thayeri* (Brown, 1950; Brown and Hines, 1952; Barnwell, 1963). Black and red pigment background adaptation in constant illumination is overridden by a circadian rhythm of either chromatophorotropin release or chromatophore responsiveness to regulating hormones.

The *Celuca* chromatophore rhythms under constant conditions are diverse (Figures 5 and 6). Melanophores exhibit circadian rhythms in LBA-ed *U. panacea* and *U. subcylindrica* and DBA-ed *U. spinicarpa*. In general, leucophores adapt. However, a white pigment rhythm is expressed in DBA-ed *U. spinicarpa*. Erythrophores express a circadian rhythm in all species regardless of background.

All chromatophore rhythms under constant conditions are low amplitude in *U. subcylindrica* (Figure 6). Erythrophore and melanophore rhythms are not seen in LBA-ed specimens. In constant darkness (DD), melanophores and erythrophores adapt while leucophores express a circadian rhythm

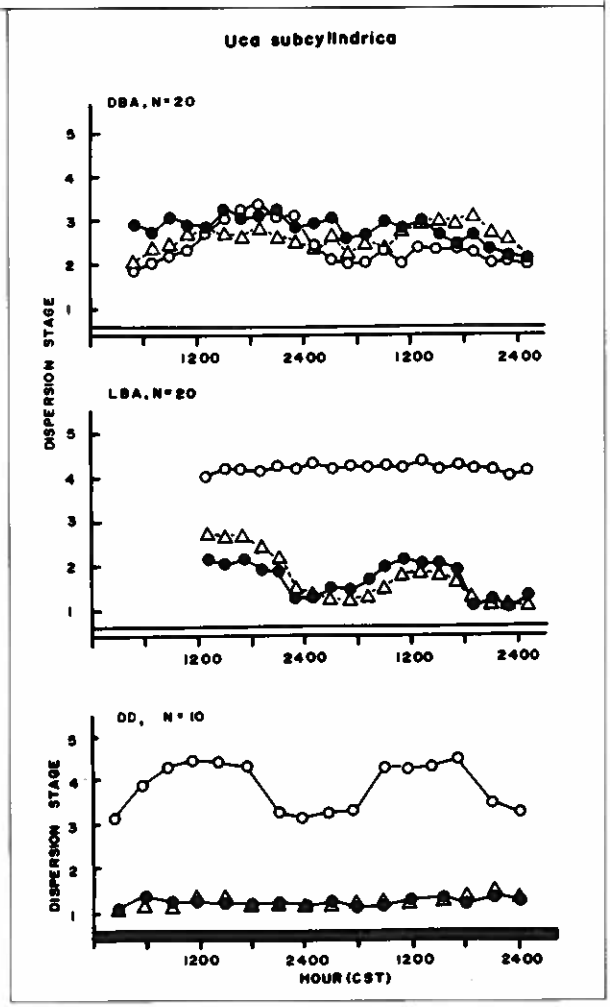


Figure 6. *U. subcylindrica* (*Celuca*) chromomotor rhythms in constant illumination (LL) and darkness (DD). Symbols same as in Figure 4.

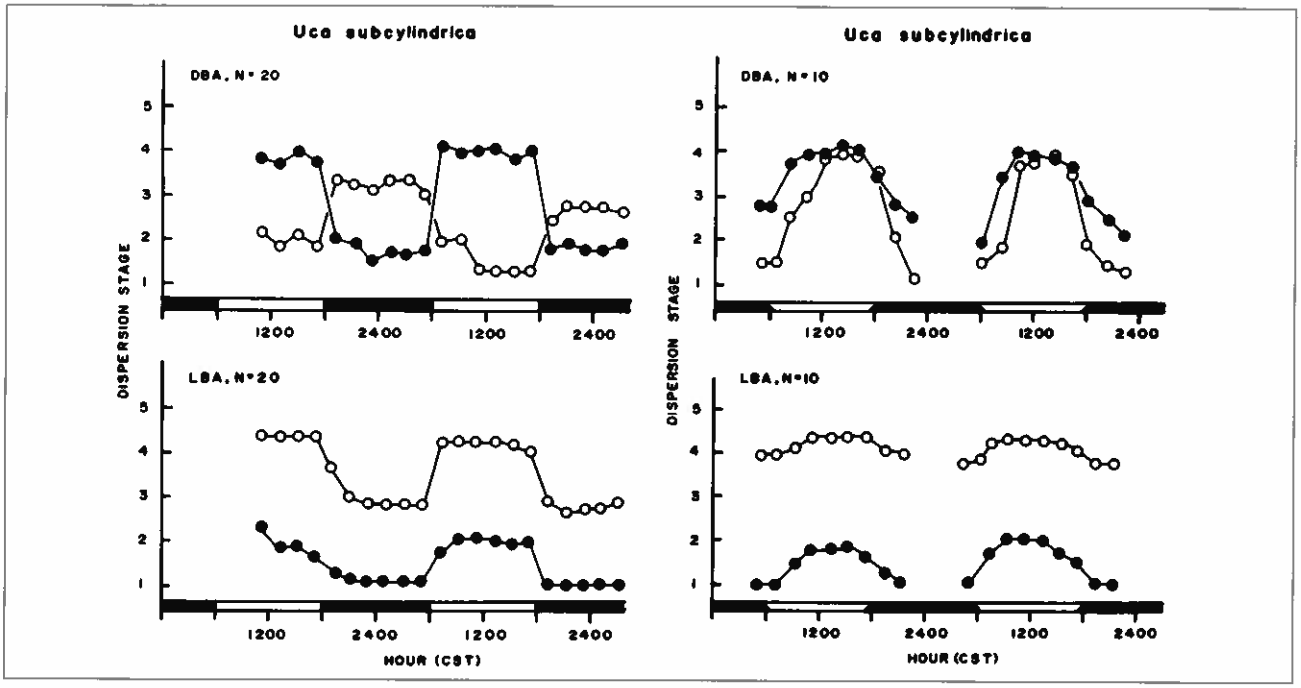


Figure 7. Photoperiod-induced chromomotor disperion in *U. subcylindirca*. Left graph — artificial LD cycle (32-foot candles). Right graph — natural light cycle (= 900-foot candles). Symbols same as Figure 4.

(Figure 6). To examine the effects of photoperiod on color change rhythms, *U. subcylindrica* were exposed to cycles of either natural (NL) or artificial (LD) illumination (Figure 7). DBA-ed crabs experiencing LD cycles darkened during illumination and lightened in the dark. In LBA-ed crabs, the leucophore rhythm was phase-shifted 180 degrees to produce white pigment dispersion during the photoperiod. Under indirect natural illumination (app. 900-foot candles), melanophores and leucophores disperse and aggregate in synchrony.

From these experiments, several conclusions can be drawn about color change physiology in *U. subcylindrica*. First, the expression of periodic color change is influenced by light intensity and substrate color. Constant illumination inhibits the expression of chromatophore rhythms (Figure 6). Second, periodic illumination encourages the expression of color change rhythms. Leucophore rhythms are expressed in constant darkness and natural illumination. Leucophore dispersion in DBA-ed crabs experiencing NL-cycles indicates the photoperiod response may override background adaptation in this species. Third, in these experiments natural illumination was 28 times more intense than the artificial light. The dispersion of leucophores in DBA-ed crabs could be due to a strong primary response to illumination.

Primary responses to illumination. A primary chromatophore response requires the direct dispersion of pigments with increased illumination. In secondary chromatophore responses, pigment migration is mediated visually through a neurosecretory reflex. For this albedo or background response, the eye measures, simultanously, the intensity of incident light from above and reflected light from below. Neurosecretory activity is regulated by visual assessments. Light backgrounds have high albedo while dark backgrounds have low. Due to albedo and neurosecretory coupling, black and red chromatophores will have greater dispersion on a dark than a light background. Leucophores will have greater dispersion on a light than a dark background. Since the reflectance of a light background is greater at any given illumination intensity, a strictly primary response produces a greater degree of pigment dispersion on a light than on a dark back-ground. In many species, the primary response may be antagonized by the secondary.

To examine illumination and albedo responses in the fiddler crabs, a variety of light intensities was achieved by placing a light bulb (60 or 100 watt) or G.E. flood lamp at various distances from crabs adapted to either a dark or light background. Light intensities at the level of the crabs was estimated with a G.E. color-cosine corrected light meter to be 1,000, 320, 120, 32 and 12-foot candles. White containers reflected 40 percent of the incident light while black reflected 14 percent. The chromatophores on each crab were staged after one hour under a particular illumination. The crabs were then rotated to another bath for an hour until they had experienced all five light intensities. The experiments were carried out twice during the day

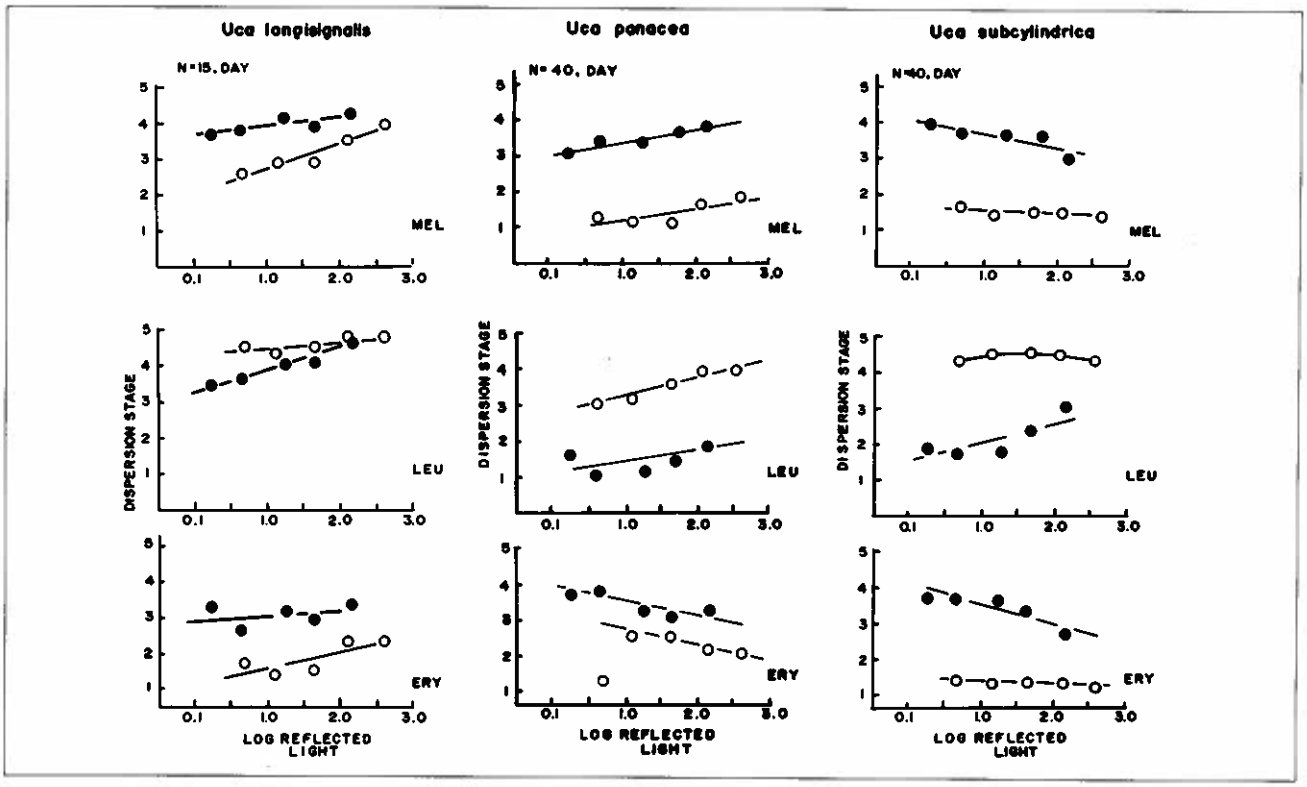


Figure 8. Relation between reflected light intensity and chromatophore dispersion in *Uca*. • - DBA, o - LBA. Mel = melanophores, Leu = leucophores and Ery = erythrophores.

(0900 and 1400 CST) and night (2100 and 0200 CST). Bath temperatures were maintained at 21° ± 1°C. Figure 8 illustrates the relationship between reflected light and pigment dispersion in three species of fiddler crabs: *U. longisignalis*. *U. panacea* and *U. subcylindrica*. Due to the similarity between day and night reactions, only the results of daily studies are shown.

The albedo or secondary response is clearly evident in the chromatophores in all three species (Figure 8). Red and black chromatophores are always more dispersed on a dark than a light background. The opposite is always true for leucophores. In *U. longisignalis*, the albedo response is susceptible to the primary illumination response. As light intensity increases, melanophores, leucophores and erythrophores disperse to augment the albedo response. However, the ability of the primary response to override background adaptation prevents *U. longisignalis* from chromatically adapting to light backgrounds under high intensity illumination. That is, the light-background adapted crab becomes black due to the primary chromomotor effects.

Similar results are seen in *U. panacea* (Figure 8). The primary response is seen in the chromomotor behavior of black as well as white pigment. Unlike the other chromatophore systems, the albedo response of the erythrophores antagonizes the primary response. Consequently, red chromatophores concentrate with increasing illumination. The net chromatic effect of the albedo and primary responses in *U. panacea* produces a pale crab under high intensity illumination on either light or dark substrates.

In *U. subcylindrica*, a strong albedo-mediated antagonism of the primary response occurs in both melanophores and erythrophores (Figure 8). The primary response augments the albedo response of the leucophores. The net effect of the chromomotor system is to produce a white crab on a black background under high intensity illumination. This response is enhanced in *U. subcylindrica* over *U. panacea* or *U. longisignalis*.

The illumination responses of the *Celuca* appear better controlled than those in the *Minuca*. In *U. longisignalis*, primary responses can override the albedo response. As a result, the crab darkens as illumination intensity is increased. In the *Celuca*. the secondary response is better developed and antagonizes the light-mediated dispersion of black and red pigments. Consequently, the crabs do not darken under high intensity illumination. The melanophores and erythrophores of *Uca subcylindrica* possess the most efficient albedo response. This secondary response inverts the primary response in black and red pigment cells.

To test the strength of the primary chromomotor

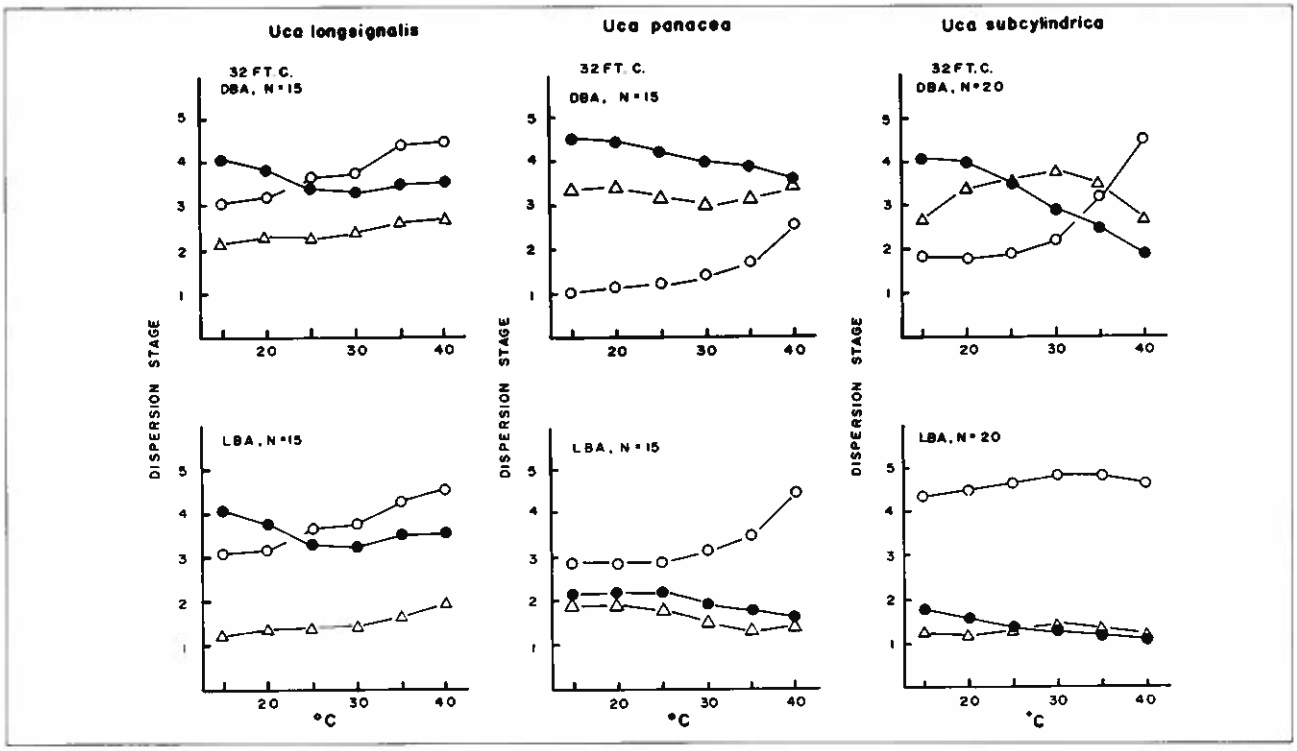


Figure 9. The response of *Uca* chromatophores to temperature. Symbols same as Figure 4.

response, eyestalk-less *U. subcylindrica* were subjected to increasing illumination intensity. Melanophores and erythrophores aggregated while the leucophores dispersed to near maximum following eyestalk ablation. None of the chromatophores exhibited a primary response. Consequently, the aggregation of black and red chromatophores with elevated illumination in *U. subcylindrica* is mediated completely by the neuroendocrine system. In addition to evolving an exceptional albedo response, it has ameliorated itself from the direct reaction to light. Since color may be related to body temperature, this chromomotor behavior can be interpreted as evidence for thermoregulation.

Primary response to temperature. Another factor influencing physiological color change is temperature. In addition to altering chromomotor activity, extreme temperature alters hormone secretion from the sinus gland and nervous system (Fingerman et al., 1969). Consequently, changes in the dispersion of chromatophores induced by temperature may be secondary rather than primary responses to stress. *Uca longisignalis, U. panacea* and *U. subcylindrica* were held under constant illumination and subsequently exposed to six different temperatures between 15°C and 40°C. After an hour at each temperature, black, white and red chromatophores were indexed. The experiments were carried out twice between 0800 and 1600 CST on the same crabs.

The pigments of the *Minuca, U. longisignalis,* exhibit little in the way of a thermoregulatory response to temperature (Figure 9). The reactions of melanophores and leucophores are virtually identical regardless of dark or light background. Temperature promotes the dispersion of leucophores and erythrophores and the concentration of melanophores. The crab darkens slightly at low temperature while blanching a little at high temperatures.

In the *Celuca, U. panacea,* the erythrophore response to temperature is minimal (Figure 9). Melanophores concentrate as temperature increases for crabs on both black and white backgrounds. The leucophores in both DBA-ed and LBA-ed crabs disperse with elevated temperature.

Since LBA-ed crabs adjust their chromatophores to a maximum extreme, thermal regulation is obvious only in DBA-ed *U. subcylindrica* (Figure 9). At low temperatures, the albedo response keeps the DBA-ed crabs dark. As temperature increases, the crabs blanch. Both erythrophores and melanophores concentrate significantly while leucophores disperse at elevated temperatures. At 40°C, the crabs are pearl-white on a black background. In this species, the thermal response overrides the albedo response at high temperatures.

In general, the pigments of *Celuca, U. panacea* and *U. subcylindrica* exhibit greater thermoregulatory behavior than those of the *Minuca*. Under elevated illumination, dark chromatophores in *Celuca* may contract rather than dispersing (e.g. Figure 8). Dark-background adapted *Celuca* abandon the al-

bedo response to enhance their pale color at elevated temperatures (e.g. Figure 9). Blanching under high temperature and light intensity presumably increases body reflectance and decreases absorption of solar radiation (Wilkens and Fingerman, 1965; Coohill *et al*, 1970). In the case of *U. longisignalis* with its strong primary response, only slight changes in the dispersion of the chromatophores are observed as temperature is elevated. The chromatophores appear to contribute little toward thermoregulation.

Discussion

Body color in the fiddler crabs results from the combined effects of morphological (chromogenic) and physiological (chromomotor) processes. Morphological pigmentation is generally stable over long periods of time. Chromogenic variation results from changes in development or the environment occurring over a period of weeks, months or years. Physiological color change occurs in minutes or hours due to alteration in the distribution of pigments within chromatophores. Chromogenic and chromomotor mechanisms have evolved to satisfy both long-term ecological and short-term physiological needs, respectively.

Of the two, our understanding about morphological coloration is the most limited. The genetic and nutritional factors regulating morphological pigmentation are essentially unknown. Comparing representatives from two subgenera indicates that the *Minuca* are more variable in morphological coloration than the *Celuca* (Plate 7). Except for the anterior carapace, major body portions of the *Minuca* are usually drab. The dark-brown to black coloring of walking legs and lower carapace blend well with dark, muddy environments. This apparently confers cryptic coloration to these marshland inhabitants.

However, the anterior portion of the carapace in male *Minuca* may be brightly colored. It is difficult to see how this bright color could serve a cryptic function. Perhaps it is involved in species recognition. In the "narrower-fronted" *U. rapax*, the pink and blue anterior carapace does not vary along the Texas coast. In one estuary, *U. longisignalis* may possess carapaces colors ranging from solid brown to bright turquoise. Usually crabs with a brown or dark-green carapace are collected in low salinity while those with brighter green and turquoise are found in more brackish habitats. *Uca longisignalis* is related to the North American "broad-fronted" species *U. minax* and *U. pugnax* (Barnwell and Thurman, 1984). The colors of the Gulf species are reminiscent of those seen on anterior portions of the carapace in *U. pugnax* from the Atlantic coast, which ranges from green to blue or turquoise (Crane, 1975). Since there are no green chromatophores, dietary and/or genetic differences may account for this pigment polymorphism in the *Minuca*.

In general, the morphological color of the Texas *Celuca* are somewhat lighter than that of the *Minuca* (Plate 7). Members of the subgenus usually inhabit light-color substrates in tidal or supratidal zones. Excepting *U. spinicarpa*. the lower carapace and walking legs are lighter than those of the *Minuca*. In addition, the anterior carapace lacks the bright pigments seen in the other subgenus. *Uca panacea* from the western Gulf of Mexico never possess a tan-white carapace like *U. pugilator* from the eastern United States. The carapace of *U. panacea* is usually gray, brown or dark-tan matching the color of the beach sands in the western Gulf. This distinction is evident even in areas of sympatry between the two species (Rao and Fingerman, 1968). The mottled brown and tan colors of both *U. subcylindrica* and *U. spinicarpa* appear typical for most *Celuca* according to Crane's assessment (1975). The general morphological coloration of these *Celuca* make them less conspicuous on the gray or black soils they inhabit. Like the *Minuca*, the morphological color of *Celuca* correlates with substrate. For long-term ecological adaptation, their coloration is probably determined either by genetic selection or diet.

On the other hand, physiological color change or chromomotor physiology has been studied in a large number of crustaceans. Color change in the fiddler crabs was first reported in *U. pugnax* and *U. pugilator* by Megusar (1912) and Abramowitz (1937). Since then, our knowledge concerning chromatophore physiology has developed into a sophisticated biochemical and biophysical science (Thurman, 1988). Chromomotor processes affect short-term adaptation through rapid physiological adjustments. This review summarizes chromatophore (1) endogenous rhythms, (2) thermal reactions, and (3) illumination responses in *Uca*.

The cyclic physiological process(es) driving periodic color change overrides background adaptation in some chromatophore systems. Counting the *Uca* species discussed here, rhythms of color change have been described in 15 species (Table 1). Details of these rhythms differ among species. In view of the ecological diversity exhibited by the genus, this variety may correlated with the habitat difference of each species (e.g. Barnwell, 1976). Generally, rhythms are considered to be either circadian or tidal depending upon their characteristics. For the most part, circadian rhythms have been well documented. Daily rhythms of black pigment dispersion have been recorded in all species except *U. thayeri* (Table 1). Erythrophore rhythms do not occur in *U. thayeri* , *U. mordax* or *U. pugnax*. Although leucophore rhythms are common among to all species, they may be expressed only in constant

Table 1. Chromatophore rhythms in *Uca*.

Subgenus, Species	Reference	Locale	Lighting	Cell	Rhythm	
					Circadian	Tidal
Uca						
U. maracoani	Barnwell, 1963	Brazil	NL/LL	M	X	?
			LL	E	X	-
Boboruca						
U. thayeri	Barnwell, 1963	Brazil	NL/LL	M	-	-
			LL	E	-	-
Minuca						
U. mordax	Barnwell, 1963	Brazil	NL/LL	M	X	?
			NL/LL	E	-	-
U. pugnax	Brown and Webb, 1949;	Mass	DD/LL	M	X	X
	Brown *et al*, 1953;		DD	L	X	X
	Barnwell, 1968b)		LL	E	-	-
U. longisignalis	Fingerman *et al*,	Miss/Texas	DD/LL	M	X	?
	1958		LL	L	X	-
			LL	E	X	-
U. zacae	Barnwell, 1968b	Costa Rica	LL	M	X	-
			LL	L	X	-
U. rapax	Barnwell, 1963;	Brazil,	NL/DD/LL	M	X	?
	Delft, 1968	Curacao,	LL	L	X	-
		Texas	NL/DD/LL	E	X	-
U. herradurensis	Barnwell, 1968b	Costa Rica	LL	M	X	-
			LL	L	X	-
			LL	E	X	-
Celuca						
U. pugilator	Brown and Webb,	Mass	DD/LD	M	X	-
	1948; Brown, 1950;		DD	L	X	-
	Webb, 1983		DD	E	X	-
U. panacea	Fingerman, 1956;	Miss/Fla	DD/LL	M	X	?
	Fingerman *et al*, 1958;		DD	L	X	-
	Fingerman and Yamamoto, 1967; Rao *et al*, 1967		LL	E	X	-
U. crenulata	Bates, 1966	W. Mexico	NL	M	X	?
			DD	E	X	?
U. spinicarpa	Fingerman, 1956	Miss/Texas	DD/LL	M	X	?
			LL	L	X	-
			LL	E	X	-
U. uruguayensis	Martin *et al*, 1959	Brazil	NL/LD	M	X	-
			LD	L	X	-
			LL	E	X	-
U. annulipes	Nagabhushanam,	India	DD	M	X	-
	1963; 1964; Rao and		DD	L	X	-
	Nagabhushanam, 1967		DD	E	X	-
U. subcylindrica		Texas	NL/LL/LD/DD	M	X	-
			NL/LL/LD/DD	L	X	-
			NL/LL/LD/DD	E	X	-

NL—natural illumination, LL—constant light, LD—light/dark cycle, DD—darkness. M—melanophore, L—leucophore, E—erythrophore. X—present, - — absent, ?—possibly present.

darkness. Since melanophores and erythrophores are often incomplete in their adjustment to a background, their daily rhythms may be seen under constant but low intensity illumination. Higher intensities of light may inhibit rhythmic expression through either primary or albedo reflexes (Brown and Webb, 1949; Brown and Hines, 1952). Low amplitude daily chromomotor rhythms are seen in *U. thayeri* and *U. subcylindrica*. However, rhythmic expressions for *U. subcylindrica* are promoted by DD, LD or NL lighting regimens. Circadian chromatophore rhythms are synchronized with each other regardless of pigment content or species. Generally, they exhibit peak dispersion during the day and aggregation at night.

The presence of tidal rhythms has not been reported as frequently in the *Uca*. Although circatidal rhythms have been attributed to at least seven species, they remain to be confirmed in all but *U. pugnax*. Since tides are unpredictable along the Texas coast where *Uca* live, it is not surprising that overt tidal rhythms are not readily apparent in their chromatophore behavior. Tidal rhythms of both color change and locomotor activity have been observed in *U. pugnax* and *U. maracoani* (Brown et al, 1953; Barnwell, 1963; 1966). Although considered intertidal (Crane, 1967), there are no reports of tidal locomotor or chromatophore rhythms in *U. rapax*. When kept in the dark for 30 days, a circadian melanophore rhythm develops in *U. rapax* which has a 24.8 h period (Delft, 1968). However, it is not clear that this "free-running" rhythm is correlated with the local tides (e.g. Barnwell, 1976). Strong daily rhythms of locomotor activity have been observed in *U. longisignalis*, *U. minax*, *U. pugnax*, *U. pugilator*, and *U. mordax*. Tidal rhythms of locomotor activity are known in *U. minax*, *U. pugilator*, and *U. pugnax* (Barnwell, 1963; 1966; 1968a,b). Each of the five Texas species expresses daily rather than tidal rhythm of color change.

In addition to the rhythms, the direct effects of light and temperature can modify neurosecretion-mediated chromatophore movement. In those species with limited physiological regulation, environmental factors control pigment dispersion. In other species, an internal mechanism regulates color change conferring independence from the environment. Primary responses have been observed in four *Minuca* and four *Celuca* species. In general, the *Minuca* exhibit strong primary responses that conflict with thermoregulation. *Celuca* exhibit greater physiological control over chromatophore activity. Some members of this subgenus may manipulate body color in a limited attempt to become homeothermic.

Celuca frequently show unusual adaptations for extreme semi-terrestrial life (Crane, 1975: p 219). They often live in open sandy or muddy habitats surviving high temperatures and severe desiccation. The chromomotor systems of four *Celuca* exhibit responses to light and temperature that are interpreted as thermoregulatory (Brown and Sandeen, 1948; Barnwell, 1968b). Several investigators have examined primary chromatophore activity in *Celuca*. The responses of *U. pugilator* in the laboratory were first reported by Brown and Sandeen (1948). Wilkens and Fingerman (1965) examined the role played by color change, evaporation and burrow-retreating behavior in the thermoregulation of the crab. In addition to the present study, the responses of *U. panacea* to illumination have been analyzed by Fingerman and Yamamoto (1967) and Rao and Fingerman (1968). Nagabhushanam (1963; 1964) and Rao and Nagabhushanam (1967) found chromatophore dispersion in the tropical species *U. annulipes* to be influenced temperature and light. *Uca subcylindrica*, another member of the subgenus, posseses chromomotor reponses to light and temperature that physiologically facilitate thermal regulation.

In general, responses to light are regulated better in *Celuca* than *Minuca* (Figure 8). The primary response of *Minuca* chromatophores enhances adaptation to dark, muddy backgrounds. In addition to *U. longisignalis* in the present study, the dispersion of dark chromatophores by a primary reflex has been documented in *U. pugnax* and *U. g. heradurensis* by Barnwell (1968b). When exposed to elevated light, light background-adapted (LBA-ed) *Minuca* become conspicuously dark. In *Celuca*, the primary response of melanophores and erythrophores but not leucophores is antagonized by the neurosecretory system. This behavior is apparent in the temperate species *U. panacea*, *U. pugilator* and *U. subcylindrica* but lacking in the tropical form *U. annulipes*. Temperate species lighten even on dark substrata. Increasing light intensity to high levels will eventually disperse melanophores and erythrophores in *U. pugilator* and *U. annulipes*. Using eyestalk-less crabs, a strong primary response has been found in melanophores of *U. annulipes*, *U. pugilator* and *U. panacea*. (Brown and Sandeen, 1948; Nagabhushanam, 1963; Rao and Fingerman, 1968). Black pigment dispersion in eyed-crabs proceeds at illumination intensities too low to stimulate melanophores in eyestalk-less *U. pugilator* (Coohill and Fingerman, 1976). This light-dependent behavior is lacking altogether in eyestalkless *U. subcylindrica*. Unlike the *Minuca*, chromomotor behavior in the *Celuca* is mediated internally to a greater degree by neurosecretion and chromatic cell physiology than by external environmental factors.

Within the *Celuca*, hormonal control of melanophore responses is more completely developed in *U. panacea* and *U. subcylindrica* than in *U. pugilator*. *U. subcylindrica* have apparently abandoned the

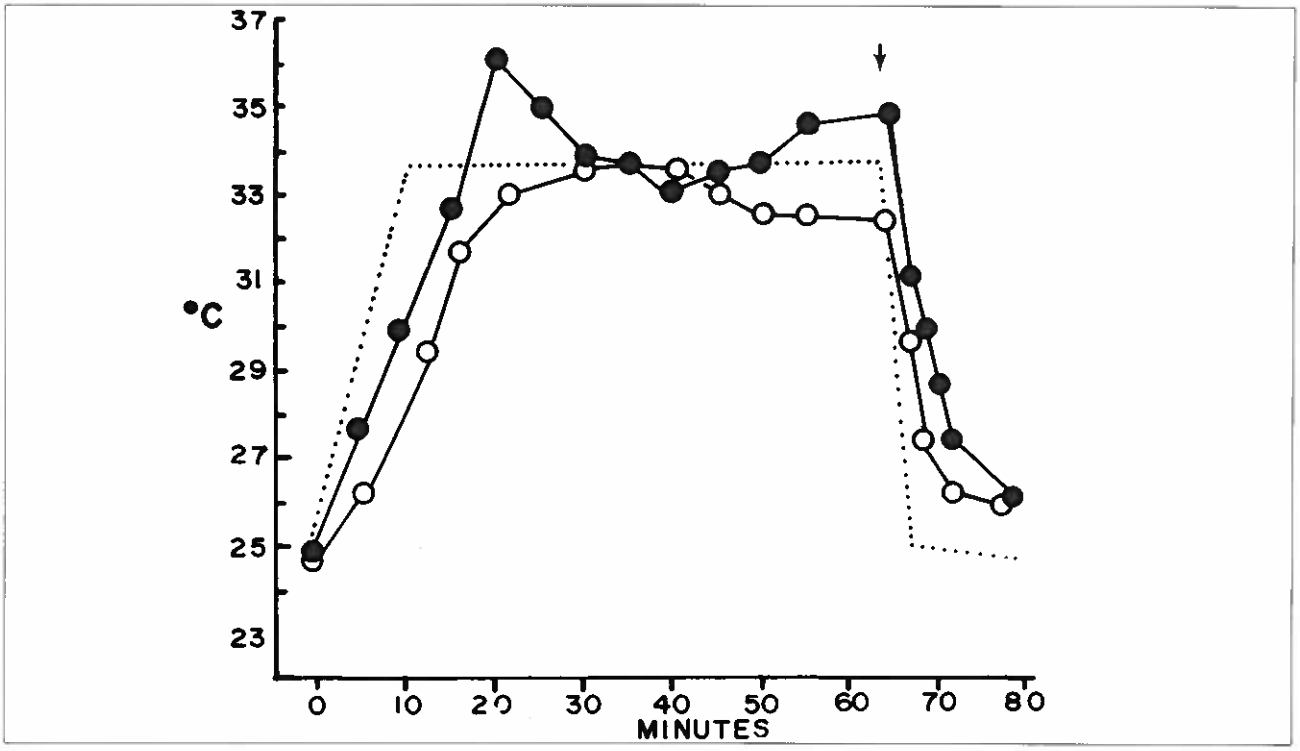


Figure 10. Branchial chamber temperature of DBA and LBA *U. subcylindrica* in sunlight. • - DBA, o - LBA, ... - box temperatures. Arrow - moved into shade.

primary illumination response for internal neurosecrtory control over the black and red chromophores. Both pigment cells may contract with increased illumination. This is apparently mediated by factors released from the sinus gland in the eyestalk. A similar trend is seen in the red pigment of *U. panacea*. From this perspective, the *Celuca* species may be ranked according to their control over the primary illumination response: *U. subcylindrica* > *U. panacea* > *U. pugilator* > *U. annulipes*.

Chromatophore dispersion with elevated temperature also appears to be better controlled in *Celuca* than *Minuca*. As ambient temperature is increased, leucophores disperse in all species. However, the dark chromatophores of the *Minuca* also disperse. In the two tropical species, *U. g. herradurensis* and *U. zacae* (Barnwell, 1968b), increasing temperature increased melanophore and erythrophore dispersion. In this case, temperature stimulates the albedo response. The apparent lack of a thermoregulatory response may be correlated to the narrow range of environmental temperatures confronting these species. Although the chromatophores of *U. pugnax* from the temperate latitudes respond in a thermoregulatory fashion, the amplitude of the reaction is small (Barnwell, 1968b). Smith and Miller (1973) found that color change in another *Minuca*, *U. rapax*, contributed a minimum to thermoregulation. In the present study, the chromatophores of *U. longisignalis* respond minimally to thermal changes.

The paling of *Minuca* at high temperatures is antagonized by a strong, inflexible, albedo response cancelling any thermoregulatory benefits of blanching. Under these conditions, the *Minuca* tend to remain a black-body absorbing heat.

In the *Celuca*, red and black chromatophores tend to aggregate with elevated temperature. This is best illustrated in dark-background adapted *U. subcylindrica*. As temperature is increased, the background respond is reduced as black and red chromatophores aggregate. Leucophores expand to shade internal organs from insolation and increase reflectance. Paling of the carapace in sunlight can lower corporal temperature 2°C below that of a dark crab (Wilkens and Fingerman, 1965). The thermal role associated with chromomotor blanching can be demonstrated in *U. subcylindrica* (Figure 10). Crabs were placed in high humidity, transparent, containers and copper-constantan thermocouples were inserted into their branchial chambers as described by Wilkens and Fingerman (1965). The crab that adjusted its body color to the dark background initially gained body temperature more rapidly than the one on the white substrate. However, the dark adapted crab began to blanch after 20 minutes. Its body temperature fell to equal that of the white adapted crab in 10 minutes. Afterwards, the corporal temperature rose above that of the chamber as well as the white crab. A change in body coloration may bestow some thermoregulatory capability to

U. subcylindrica at elevated temperatures for a short period of time.

To colonize terrestrial habitats, *Uca* have evolved adaptations to: (1) avoid ionic and osmotic stress, (2) survive thermal extremes and (3) assure reproductive success. Undoubtedly, color has played an important role in the adaptation of aquatic crabs to habitats with high temperature and little water. Both morphological and physiological color change mechanisms appear to contribute to the ecological success of the genus. In south Texas where the environment is hostile to marine organisms, both *Minuca* and *Celuca* are common along the nontidal coast. In terms of thermal regulation, the *Celuca* to possess better color-mediated capabilities than the *Minuca*. Based on a comparison to other species around the world, these subgeneric differences may be determined by phylogeny. The *Celuca* are far more adaptable in extreme habitats and express considerable plasticity in their chromomotor responses.

Physiological coloration does not appear to confer a thermoregulatory advantage to *Uca* of the *Minuca* subgenus. Both *U. longisignalis* and *U. subcylindrica* are very terrestrial species endemic to the western Gulf of Mexico. *Uca longisignalis* has been found to be a better ionic/osmotic regulator than *U. subcylindrica* (Rabalais and Cameron, 1985). Assuming other homeostatic mechanisms equal, the strong primary and albedo response may contribute to the inability of the *Minuca* to inhabit hot, dry exposed habitats. The *Minuca* tenaceously hold to the albedo response preventing dark background-adapted crabs from increasing their reflectance by blanching. This reduces the ability of their pigments to aid in thermoregulation. Since the crabs remain dark even on light substrates, this could be a liability under intense insolation and heat.

Uca subcylindrica, a *Celuca*, has evolved unusual reproductive and developmental strategies, desiccation tolerance and behavioral patterns that make it distinct from other members of the genus (Rabalais and Cameron, 1983; Thurman, 1984). Adaptive coloration may contribute, in part, to their unique distribution in the harsh supratidal habitats of the western Gulf of Mexico. Flexible chromomotor capabilities may represent a physiological advantage evolved in the *Celuca* but not the *Minuca*.

Acknowledgments

The encouragement and support of F.H. Barnwell, University of Minnesota, is gratefully acknowledged. Appreciation is offered to John Judd, University of Missouri, for his assistance in preparing illustrations. Financial support for this investigation was provided by grants from the Dayton Natural History Fund, Bell Museum of Natural History, the Department of Zoology at the University of Minnesota and the Sigma-Xi Research Foundation of North America. Symposium travel expenses were deferred, in part, by the Graduate School and the Extension Division, University of Missouri - St. Louis.

References

Abramowitz, A.A. 1937. The chromatophorotropic hormone of the Crustacea: standardization, properties and physiology of the eyestalk gland. **Biol. Bull.** 72:344-365.

Bagnara, J.T. and M.E. Hadley. 1973. **Chromatoohores and color change: The comparative physiology of animal pigmentation.** Prentice-Hall, Inc., Englewood Cliffs, N.J.

Barnwell, F.H. 1963. Observations on daily and tidal rhythms in some fiddler crabs from equatorial Brazil. **Biol. Bull.** 125:399-415.

Barnwell, F.H. 1966. Daily and tidal pattern of activity in individual fiddler crab from (genus *Uca*) the Woods Hole region. **Biol. Bull.** 130:1-7.

Barnwell, F.H. 1968a. The role of rhythmic systems in the adaptation of fiddler crabs to the intertidal zone. **Amer. Zool.** 8:569-583.

Barnwell, F. H. 1968b. Comparative aspects of chromatophoric responses to light and temperature in fiddler crabs of the genus *Uca*. **Biol. Bull** 134:221-234.

Barnwell, F.H. 1976. Variation in the form of the tide and some problems it poses for biological timing systems. *In* P.J. DeCoursey (ed.), **Biological Rhythms in the Marine Environment.** pp 161-187. University South Carolina Press, Columbia.

Barnwell, F.H. and C. L. Thurman. 1984. Taxonomy and biogeography of the fiddler crabs (Ocypodidae: genus *Uca*) of the Atlantic and Gulf coasts of eastern North America. **Zool. J. Linnean Soc.** 81:23-87.

Bates, E. J. 1966. Tidal and diurnal rhythms in the fiddler crab, *Uca crenulata*. **Biol. Studies Gulf. of California** 4:12-26.

Bliss, D. 1979. From sea to tree: Saga of a land crab. **Amer. Zool.** 19:385-410.

Brown, F.A., Jr., 1950. Studies on the physiology of *Uca* red chromatophores. **Biol. Bull.** 98:218-226.

Brown, F.A., Jr., 1973. Chromatophores and color change. *In*: C.L. Prosser, **Comparative Animal Physiology**. pp 915-950. W.B. Saunders, Co., Philadelphia.

Brown, F.A., Jr. and H.E. Ederstrom. 1940. Dual control of certain black chromatophores of *Crago*. **J. Exp. Zool.** 85:53-69.

Brown, F.A., Jr., M. Fingerman, M.I. Sandeen and H.M. Webb. 1953. Persistent diurnal and tidal

rhythms of color change in the fiddler crab, *Uca pugnax*. **J. Exp. Zool.** 123:29-60.

Brown, F.A., Jr. and M.N. Hines. 1952. Modifications in the diurnal pigmentary rhythm of *Uca* effected by continuous illumination. **Physiol. Zool.** 25:56-70.

Brown, F.A., Jr. and I.M. Klotz. 1947. Separation of two mutually antagonistic chromatophorotropins from the triocerebral commissure of *Crago*. **Proc. Soc. Exp. Biol.** 64:310-333.

Brown, F.A., Jr. and M. Sandeen. 1948. Responses of the chromatophores of the fiddler crab *Uca* to light and temperature. **Physiol. Zool.** 21:361-371.

Brown, F.A., Jr. and H. M. Webb. 1948. Temperature relations of an endogenous daily rhythmicity in the fiddler crab, *Uca*. **Physiol. Zool.** 21:371-381.

Brown, F.A., Jr. and H. M. Webb. 1949. Studies on the daily rhythmicity of the fiddler crab, *Uca*. Modification by light. **Physiol. Zool.** 22:136-148.

Brown, F.A., Jr. and V.J. Wulff. 1941. Chromatophore types in *Crago* and their endocrine control. **J. Cell Comp. Physiol.** 18:339-353.

Burtt, E.H., Jr. 1979. **The Behavioral Significance of Color.** Garland STPM Press, New York.

Coohill, T.P., C.K. Bartell and M. Fingerman. 1970. Relative effectiveness of ultraviolet and visible light in eliciting pigment dispersion directly in melanophores of the fiddler crab, *Uca pugilator*. **Physiol. Zool.** 43:232-239.

Coohill, T.P. and M. Fingerman. 1976. Comparison of the effects of illumination on the melanophores of intact and eyestalkless fiddler crabs, *Uca pugilator*, and inhibition of the primary response by cytochalasin B. **Experientia** 32:569-570.

Crane, J. 1944. On the color changes of fiddler crabs (genus *Uca*) in the field. **Zoologica** 29:161-168.

Crane, J. 1967. Combat and its ritualization in fiddler crabs with spicial refence to *Uca rapax*. **Zoologica** 52:49-77.

Crane, J. 1975. **Fiddler Crabs of the World, Ocypodidae: Genus *Uca*.** Princeton University Press, Princeton.

Delft, A.M.L. van. 1968. The daily color rhythm of the fiddler crab *Uca rapax* on Curacao. **Natuurwetschft. Studkrng. Suriname Ned.** 25:58-72.

Fingerman, M. 1956. Phase difference in the tidal rhythms of color change of two species of fiddler crab. **Biol. Bull.** 110:274-290.

Fingerman, M. 1970. Comparative Physiology: Chromatophores. **Ann. Rev. Physiol.** 32:345-372.

Fingerman, M. 1987. The endocrine mechanisms of crustaceans. **J. Crust. Biol.** 7:1-14.

Fingerman, M., M.E. Lowe and W.C. Mobberly. 1858. Environmental factors involved in setting the phases of tidal rhythm of color change in the fiddler crabs *Uca pugilator* and *Uca minax*. **Limnol. Oceanogphy.** 3:271-282.

Fingerman, M., K.R. Rao, and G. Ring. 1969. Restoration of a rhythm of melanophoric pigment dispersion in eyestalk-less fiddler crabs, *Uca pugilator*, at a low temperature. **Crustaceana** 17:97-105.

Fingerman, M. and Y. Yamamoto. 1967. Daily rhythms of melanophore pigment migration in eyestalkless fiddler crabs, *Uca pugilator*. **Crustaceana** 12:303-319.

Ghidalia, W. 1985. Structural and biological aspects of pigments. *In* D.E. Bliss and L. H. Mantel (eds.), **The Biology of Crustacea Vol. 9: Integument. Pigments. and Hormonal Processes.** pp. 301-394. Academic Press, Inc., New York.

Green, J.P. 1964. Morphological color change in the fiddler crab, *Uca pugnax* (S.I. Smith). **Biol. Bull.** 127:239-255.

Hedgpeth, J. 1953. An introduction to the zoogeography of the northwestern Gulf of Mexico with reference to the invertebrate fauna. **Publ. Inst. Mar. Sci.** Univ. Texas 3:110-224.

Hogben, L. and D. Slome. 1931. The pigmentary effector system. VI. The dual character of endocrine co-ordination in amphibian colour change. **Proc. R. Soc. London,** Ser. B 108:10-53.

Lee, W.L. 1966. Color change and ecology of the marine isopod *Idothea (Pentidotea) montereyensis*-Maloney, 1933. **Ecology** 47:930-941.

Martins, T., W. Ladosky and B.M. Castro. 1959. A regulacao dotempo biologico inversao experimental do ritmo diurno dos cromatoforos de *Uca olimpioi*. **An. Acad. Brasileira de Ciencias** 31:87-90.

Megusar, F. 1912. Experimente uber den Fasbwechsel der Crustacean. (I. Gelasimus. II. Potamobius. III. Palaemonetes. IV. Palaemon.). **Arch. Entwmech. Org.** 33:462-665.

Nagabhushanam, R. 1963. Physiology of the black chromatophores of *Gelasimus annulipes*. **Indian J. Physiol.** 17:67-72.

Nagabhushanam, R. 1964. Physiology of red chromatophores of *Gelasimus annulipes*. **Indian J. Exp. Biol.** 2:69-71.

Neck, R.W. 1987. Two noteworthy populations of the fiddler crab, *Uca subcylindrica*, in south Texas. **Texas J. Sci.** 39:196-197.

Needham, A.E. 1974. **The Significance of Zoochromes.** Springer-Verlag, New York.

Novak, A. and M. Salmon. 1974. *Uca panacea*, a new species of fiddler crab from the gulf coast of the United States. **Proc. Biol. Soc. Wash.** 87:313-326.

Page, T.L. and J.L. Larimer. 1976. Extraretinal photoreceptors in entrainment of crustacean rhythms. **Photochem. Photobiol.** 23:245-251.

Palmer, J.D. 1974. **Biological Clocks in Marine Organisms.** John Wiley & Sons, New York.

Powers, L.W. 1975. Fiddler crabs in a nontidal environment. **Contri. Mar. Sci.** Univ. Texas 19:76-78.

Rabalais, N.N. and J.N. Cameron. 1983. Abbreviated development in *Uca subcylindrica* reared in the laboratory. **J. Crust. Biol.** 3:519-541.

Rabalais, N.N. and J.N. Cameron. 1985. Physiological and morphological adaptations of adult *Uca subcylindrica* to semi-arid environments. **Biol. Bull.** 168:35-146.

Rao, K.R. 1985. Pigmentary effectors. *In* D.E. Bliss and L.H. Mantel (eds.). **The Biology of Crustacea. Vol. 9: Integument. Pigments and Hormonal Processes.** pp 395-462. Academic Press, Inc., New York.

Rao, K.R. and M. Fingerman. 1968. Dimorphic variants of the fiddler crab *Uca pugilator* and their chromatophore responses. **Proc. Louis. Acad. Sci.** 31:27-39.

Rao, K.R., M. Fingerman and C. Bartell. 1967. Physiology of white chromatophores in the fiddler crab, *Uca pugilator*. **Biol. Bull.** 133:606-617.

Rao, K.R. and R. Nagabhushanam. 1967. The responses of the white chromatophores of the crab *Uca annulipes* to light and temperature. **Crustaceana** 13:155-160.

Salmon, M. and S.P. Atsaides 1968. Behavioral, morphological and ecological evidence for two new species of fiddler crabs (genus *Uca*) from the Gulf coast of the United States. **Proc. Biol. Soc. Wash.** 81:275-290.

Smith, W. K. and P.C. Miller. 1973. The thermal ecology of two south Florida fiddler crabs: *Uca rapax* and *U. pugilator*. **Physiol. Zool.** 46:186-207.

Thurman, C.L. 1982. On the distinctness of the fiddler crabs *Uca minax* (LeConte) and *Uca longisignalis* Salmon & Atsaides in their region of sympatry. **Crustaceana** 43:37-50.

Thurman, C.L. 1984. Ecological notes on fiddler crabs of south Texas, with special reference to *Uca subcylindrica*. **J. Crust. Biol.** 4: 665-681.

Thurman, C.L. 1987. Fiddler crabs (genus *Uca*) of eastern Mexico (Decapoda, brachyura, ocypodidae). **Crustaceana** 53: 94-105.

Thurman, C.L. 1988. A review: rhythmic physiological color change in Crustacea. **Comp. Biochem. Physiol.** 91C:171-185.

Webb, H.M. 1983. Persistent rhythms of decapod crustaceans. *In* S. Rebach and D. Durham (eds.), **The Behavior of Higher Crustacea.** pp. 197-216. John Wiley & Sons, Inc., New York.

Webb, H.M., M.F. Bennett and F.A. Brown. 1954. A persistant diurnal rhythm of chromatophoric response in eyestalk-less *Uca pugilator*. **Biol. Bull.** 106:371-377.

Weber, W. 1983. Photosensitivity of chromatophores. **Amer. Zool.** 23:495-506.

Wilkens, J.L. and M. Fingerman 1965. Heat tolerance and temperature relationships of the fiddler crab, *Uca pugulator*, with reference to body coloration. **Biol. Bull.** 128:133-141.

Wilkens, L.A. and. J. L. Larimer. 1976. Photosensitivity in the sixth abdominal ganglion of decapod crustaceans: a comparative study. **J. Comp. Physiol.** 106:69-75.

Special Resemblance, Aposematic Coloration and Mimicry in Opisthobranch Gastropods

TERRENCE M. GOSLINER AND DAVID W. BEHRENS

Synopsis

Color has played a major role in the adaptive radiation and evolution of opisthobranch gastropods. Crypsis is common among generalized grazers, while more specialized predators often exhibit special resemblance to their prey. Aposematic coloration is common in most opisthobranch taxa and is more predominant in tropical faunas than in temperate ones. Examples of Batesian mimicry, involving opisthobranch models and other invertebrate mimics, are presented. Several examples of Mullerian mimicry involving different species of opisthobranchs are presented. Mullerian mimicry involving opisthobranchs, polyclad flatworms and a sea cucumber is discussed. Mimicry appears to be far more widespread in opisthobranchs than previously indicated.

Introduction

Opisthobranch gastropods are among the most brilliantly colored organisms inhabiting the world's oceans. The myriad of colors and patterns exhibited by these organisms has fascinated naturalists for centuries, yet little documentation exists as to the role of color in the adaptive radiation of the opisthobranchs.

The opisthobranch mollusks are known to have reduced or entirely lost their shells independently within several distinct lineages (Pelseneer, 1894, Fretter and Graham, 1962; Morton, 1963; Ghiselin, 1966; Gosliner, 1981a, in press; Gosliner and Ghiselin, 1984). It has been demonstrated that primitive, shelled opisthobranchs evolved toxins (Fretter and Graham, 1954; Thompson, 1960) that provided protection from predation, thus permitting the reduction or loss of the shell (Faulkner and Ghiselin, 1983). Toxicity or reduced palatability in opisthobranchs is well documented and involves the utilization of a wide variety of organic and inorganic compounds (Edmunds, 1966a, 1966b, 1968; Thompson, 1960, 1969, 1983; Faulkner and Ghiselin, 1983; Mebs, 1985). Most of these compounds are derivatives of chemicals produced by the prey of opisthobranchs. Only a few species of notaspideans (Thompson and Colman, 1984) and the dorid nudibranch, *Dendrodoris limbata* (Cuvier, 1904), are known to manufacture toxins *de novo* (Cimino *et al.*, 1982), though it is reasonable to expect that many more opisthobranchs do this.

Different clades of opisthobranchs feed on a variety of toxic prey. The Sacoglossa feed primarily on siphonaceous chlorophytes, especially species of *Caulerpa* (Jensen, 1980; Clark and Defreese, 1987). Doty and Aguilar-Santos (1970) have shown that toxins derived from algal prey are transferred to opisthobranch predators.

Cryptobranch and porostome dorid nudibranchs are predatory on sponges and often derive toxins from their prey (see Faulkner and Ghiselin, 1983 for a review of sponge-derived toxins in dorids).

Aeolid nudibranchs feed largely upon cnidarians and utilize undischarged nematocysts derived from their prey for their own defense (Kepner, 1943; Edmunds, 1966a). The nematocysts are stored in specialized cnidosacs at the apex of each ceras. There appears to be a terminal pore at the apex of the ceras, through which the nematocysts can be fired. The physiological mechanisms of nematocyst manipulation and utilization by aeolids are poorly understood, though recent work by Greenwood and Mariscal (1984) indicates that immature nematocysts mature within the cnidosac of the aeolids.

The various categories of color in opisthobranch mollusks have been reviewed recently (Edmunds, 1987). Examples of crypsis and special resemblance were described by Edmunds and are relatively well documented. Additional examples are provided in

Terrence Gosliner, Department of Invertebrate Zoology and Geology, California Academy of Sciences, Golden Gate Park, San Francisco, Calif. 94118; David Behrens, Pacific Gas and Electric Company, Research and Development, 3401 Crow Canyon Rd., San Ramon, Calif. 94583.

this paper. Edmunds (1987) also discussed the criteria necessary to establish that aposematic coloration exists in opisthobranchs. Additional examples of aposematic coloration being widespread in tropical opisthobranchs are provided, followed by a critical discussion of warning coloration in opisthobranchs.

Mimicry in the terrestrial environment, especially involving insect prey and avian predators, is well documented and has an extensive literature (Wickler, 1969; Edmunds, 1974). However, there are few documented cases of mimicry in the marine environment, with even less experimental evidence to support anecdotal accounts of its existence. A few cases, such as aggressive mimicry in blennies that resemble cleaner wrasses (Randall and Randall, 1960) have been well documented. To date only a handful of cases of mimicry of marine invertebrates has been suggested, this despite the abundance of brightly colored species, particularly in tropical habitats. Most of these instances involve pleustid amphipods and prosobranch gastropods (Carter and Behrens, 1980; following discussion). Mimicry has been suggested to occur between different species of nudibranchs (Ros, 1974, 1976, 1977; Rudman, 1982, 1983; Goddard, 1987) and between a nudibranch and an amphipod (Goddard, 1984). Edmunds (1987) has emphasized the speculative nature of these examples of mimicry, since little or no information is available on visual acuity of predators or palatability of prey. Recent investigations on the opisthobranchs of southern Africa, California and Papua New Guinea have revealed dramatic examples of mimicry between different species of opisthobranchs and between opisthobranchs and other marine taxa. These data, together with information on palatability, enable us to present several documented cases of Batesian and Mullerian mimicry in marine invertebrates.

It should be emphasized that mimicry in opisthobranchs, and most marine invertebrates, for that matter, is poorly known and is only now in the descriptive phase. As a result study of mimicry in these organisms suffers from the lack of experimental rigor that characterizes the study of terrestrial organisms. Nevertheless, it is important to describe and document these examples, and to begin experimental work to verify the types of mimicry that appear to occur in the marine environment.

Color in Opisthobranchs

Any intraspecific role of color in opisthobranchs has long been discounted (Edmunds, 1987). Darwin (1871) stated that it was unlikely that bright colors in opisthobranchs were a result of sexual selection as in cephalopods and vertebrates. Intraspecific signals in opisthobranchs are almost entirely chemical, involving the sensory appendages of the cephalic region, the rhinophores and oral tentacles. Opisthobranch eyes are generally small and function largely as light-dark receptors. In many taxa the eyes are dermal, covered by a thick, opaque layer of epidermis and may not even function as light receptors in these organisms. The largest eyes in opisthobranchs are found in aglajid opisthobranchs that are predatory upon other opisthobranchs and other large invertebrates (Rudman, 1974; Gosliner, 1980). Even these opisthobranchs track the mucus trail of their prey by means of compound sensory cilia located on the anterior surface of the head. There is no evidence that the eyes of any opisthobranchs are image forming or are able to discern colors. A far more plausible explanation is that colors produced by opisthobranchs serve as cryptic or aposematic signals to predators with good color vision.

Crypsis

The difference of extreme cases of crypsis versus mimicry has been the subject of considerable controversy and discussion (see Edmunds, 1981, 1987; Endler, 1981 for references). Edmunds distinguished crypsis from special resemblance, following Cott (1940). Although the two intergrade, it is useful to distinguish cases where organisms blend in with a generally confused, disruptive background from those cases where an organism resembles a specific, largely inedible substance or organism. Mimicry as employed here, and most recently by Edmunds, refers to organisms that are resemble an aposematic model.

Crypsis or disruptive coloration has been well documented in a wide variety of opisthobranchs, but most commonly in dorid and aeolid nudibranchs (Edmunds, 1986). Crypsis also appears to be present in members of the other orders of opisthobranchs, the Cephalaspidea, Anaspidea, Sacoglossa and Notaspidea. Many infaunal representatives of the Cephalaspidea and Sacoglossa retain well developed shells. These infaunal organisms are most commonly uniformly whitish in color and are cryptic on the clean, sandy substrata they inhabit. Epifaunal members of these taxa are often brown or green in color and are well camouflaged in the algal-dominated habitats in which they reside. Small cephalaspideans that reach a maximum size of a centimeter or less, such as *Haminoea* spp., blend in exceedingly well with mixed sand, rock and algal substrata.

Crypsis is the most common form of coloration in the herbivorous Anaspidea. Some species, such as *Dolabella auricularia* (Solander, 1786) and *Aplysia vaccaria* (Winkler, 1955) may exceed 400 mm in length, yet are exceedingly difficult to differentiate from the background on which they are crawling. Some anaspideans, such as *Bursatella* and *Stylocheilus*

spp., bear ramified appendages over the surface of their bodies to further disguise their appearance.

Members of the Notaspidea are generally large, brightly colored and conspicuous, but species of the primitive genus *Tylodina* are cryptically colored on yellowish sponges (Faulkner and Ghiselin, 1983; present study). Some species of *Berthella* are cryptically colored when on the species of tunicates on which they prey.

Members of the dendronotid nudibranch genus *Melibe* are filter feeders that blend in well with their backgrounds (Gosliner, 1987a). Most tropical members of the genus have highly branched processes that contain zooxanthellae (Kempf, 1984) which further disguises them in areas where brown algae are abundant.

Special Resemblance

Following Cott (1940) and Edmunds (1987), this form of crypsis or mimicry applies to extreme cases of resemblance between organisms and unpalatable substances such as tree bark, bird droppings, sticks, etc. Hailman (1977) has distinguished this "concealing imitation" from "animal mimicry." He also noted that this type of resemblance is essentially intermediate in a continuum from crypsis to strict mimicry.

Among the opisthobranch gastropods there are relatively few examples of special resemblance. Edmunds restricted his examples to the Nudibranchia, such as members of the genera *Doridella* and *Phyllodesmium*.

Members of several other opisthobranch orders also exhibit special resemblance. Carlson and Hoff (1973) described two species of the cephalaspidean genus *Sagaminopteron* that closely match the color and texture of their sponge prey. Many species of the anaspidean genera *Phyllaplysia* and *Petalifera* closely resemble the algal or sea grass habitat on which they reside (Beeman, 1968; Williams and Gosliner, 1973; Behrens, 1980; Bertsch and Johnson, 1981). *Phyllaplysia engeli* Marcus, 1955, feeds on diatoms growing as epiphytes on turtle grass, *Thalassia* in the Caribbean. Its longitudinal lines match the veins of the sea grass and it bears pinkish white blotches that are virtually identical to the coralline algae that grow in profusion on the sea grass (Plate 8:1).

Many of the herbivorous sacoglossans are specialized grazers on a single genus or species of algae. It has already been mentioned that many of these taxa are cryptic and feed on such poisonous algae as *Caulerpa*. Many of them also bear special resemblance to their algal food. *Elysia halimedae* Macnae, 1954, is a flattened green slug that feeds only upon the algal genus *Halimeda* in the Indo-Pacific tropics. The arrangement of pigment spots is virtually identical to that of the algae (Gosliner, 1987b: fig. 39). Specimens are exceedingly difficult to detect in the field and are generally collected only by dislodging them from the algae by vigorous shaking. Numerous other species of *Elysia* are specialized herbivores and bear special resemblance to their food. Some of the cerata-bearing sacoglossans are also remarkably similar in appearance to their food. Species of *Costasiella* are associated with the green algal genus *Avranvillea* and are exceedingly cryptic on their food. *Caliphylla* species feed on the filamentous algae *Bryopsis* spp., and are seen only after careful searching through algal samples.

Edmunds (1987) noted several aeolid nudibranchs that resemble their prey. Most of these are members of the genera *Cuthona*, *Phestilla* and *Phyllodesmium*. The most striking example was *Cuthona kuiteri* Rudman, 1981, whose cerata are identical in shape to the polyps of its hydroid prey. He stated that *Phestilla lugubris* (Bergh, 1870) and *P. melanobrachia* Bergh, 1874, were simply cryptic on their coral prey, but had no special resemblance. In fact, the shape, color and texture of the cerata of the aeolids precisely match the extended tentacles of the coral polyps. Harris (1968) demonstrated how the color of the *P. melanobrachia* is dependent upon the species of dendrophyllid coral on which it feeds. We consider all three of these species of *Phestilla*, plus a fourth undescribed species that feeds on the coral *Goniopora* sp., to represent cases of special resemblance.

Among the dorid nudibranchs, Edmunds mentioned the resemblance of members of the Corambidae to their bryozoan prey (see Behrens, 1980: figs. 53,59; Gosliner, 1987b: fig. 166) and two other taxa to their respective sponge prey. The Corambidae are especially good examples of several aspects of resemblance cited by Hailman (1977). They are appressed to the substrate to reduce shadow, are transparent and have distinct white lines that closely match their bryozoan prey. There are many other cases of special resemblance among dorids, particularly among species that are predatory upon sponges. The Californian dorid, *Aldisa sanguinea* (Cooper, 1863), feeds on red sponges (Plate 8:2) that have evenly spaced, well separated oscula. The dorsal surface of the nudibranch bears two darker red spots that precisely match the diameter and spacing of the oscula. In another species of *Aldisa*, *A. pikokai* Bertsch and Johnson, 1982, and in species of *Scleroderis* Eliot, 1904 (Rudman, 1978) there are circular pits in the notum that mimic sponge oscula.

Members of the phanerobranch dorid genus *Goniodoris* feed on colonial ascidians and are often the same color and texture as their prey.

Many dendronotacean nudibranchs also exhibit special resemblance with their prey. *Tritonia nilsodhneri* Marcus, 1984, was mentioned by

Edmunds (1987) for its resemblance to its gorgonian prey, *Eunicella*. In southern Africa, this same nudibranch feeds on two species of *Eunicella*, one with orange colonies and the other white (Gosliner 1987b: 107, fig. 204). The color of the nudibranch and its eggs always match the color of the colony on which it resides. If the nudibranch is switched to the other color prey it will change its color over a two week period as Harris (1968) has described in *Phestilla melanobrachia*. Another undescribed species of *Tritonia* from southern Africa feeds on the variably colored soft coral *Alcyonium* and varies from white to brown to purple depending on its prey (Gosliner, 1987b: 108, fig. 205). Most other species of tritoniids exhibit special resemblance on their alcyonarian prey.

Members of the dendronotid genera *Doto* and *Lomanotus* are generally cryptic on the hydroids upon which they feed. In *Lomanotus vermiformis* O'Donoghue, 1929, which feeds on the plumularid hydroid, *Lytocarpus philippinus* and in *Doto* sp., which feeds on an unidentified plumularid hydroid, there is special resemblance between predator and prey (Plate 8:3 and 8:4). The animals in both cases are far more slender and elongate than other members of their respective genera. In both cases the center of the notum of the nudibranchs bears longitudinal brown markings that correspond to the central axis of the hydroid colony while the outer margins bear opaque white, evenly-spaced gills or cerata that correspond to the pinnate hydranth-bearing portions of the hydroids. These are the marine analogs of walking sticks.

The aeolid nudibranch *Pleurolidia juliae* Burn, 1966, feeds upon the hydroid genus *Solanderia* and is exceedingly cryptic when on its prey (Willan and Coleman, 1984: fig. 136).

There are many cases of special resemblance within the opisthobranchs, particularly in taxa which are stenotrophic and are found in intimate association with their prey. Special resemblance is certainly more widespread in opisthobranchs than previously indicated and may be the dominant defensive strategy in entire clades, such as the Sacoglossa and Dendronotacea.

Aposematic Coloration

Warning coloration in opisthobranchs has been suggested since the end of the 19th Century (Garstang, 1889, 1890; Hecht, 1896). Edmunds (1974) cautioned against making assumptions of aposematic coloration in opisthobranchs and stressed the need for experimental data. More recently, he (1987) suggested a list of four criteria that must be met for there to be a selective advantage in being brightly colored. He noted that the first two, that opisthobranchs are brightly colored and that they are unpalatable to some predators, were well established, as was the fact that most shallow water fishes have good color vision. Color vision is also widespread in decapod crustaceans (Waterman, 1961), also known predators of opisthobranchs (Harris, pers. comm.). Bursey (1984) has shown that the portunid crab *Callinectes sapidus* Rathbun, 1896, can differentiate blue, red and yellow pigments.

Edmunds suggested that the second two criteria, that some predators avoid attacking a particular species because of its color and an aposematic species is better protected than a cryptic one, had not been demonstrated for opisthobranchs.

Goddard (1984) presented data from experiments trying to induce predation upon the nudibranch *Flabellina trilineata* (O'Donoghue, 1921) by rosy-lipped sculpins. In no instance were specimens of *Flabellina* attacked by the fish. Harris (1987) cited feeding experiments where labrid fish predators on both the Atlantic and Pacific coasts of North America would avoid feeding upon individuals of the brightly colored nudibranchs *Flabellina verrucosa* (Sars, 1829) and *Hermissenda crassicornis* (Eschscholtz, 1831). Repeated stimulation of feeding responses in fishes by smashing sea urchins failed to elicit feeding upon these toxic and colorful nudibranchs. When cryptic opisthobranchs were placed in the vicinity of the same predators and the fish were stimulated in the same manner, the cryptic species were readily consumed. It has been previously shown (Allen, 1976) that the nematocysts stored by *Hermissenda* are capable of causing extensive tissue damage to fish which consume the nudibranch. Predation upon *Hermissenda* by fishes in the field is rare. Lavenberg (pers. comm.) has observed the mosshead warbonnet fish, *Chirolophis nugator* (Jordan and Williams) to feed upon healthy individuals of *Hermissenda crassicornis*. In the process of engulfing the nudibranchs the fish bends the body of the nudibranch in half so that all the cerata are appressed to each other. This behavior prevents the nematocysts from coming in direct contact with any of the tissue of the oral cavity or digestive tract of the fish. Predation upon these nudibranchs has also been confirmed by dissection of mosshead warbonnet stomachs, revealing specimens of *H. crassicornis*.

These experiments prove that brightly colored species are avoided because of their color and that brightly colored, toxic species are better protected than are cryptic taxa. Thus, it can be reasonably concluded that aposematic coloration has been proven to occur in opisthobranch mollusks. Baylis (1979) predicted that stabilizing selection should be important in reducing varibility of color pattern in aposematic species. The relatively low degree of intraspecific varibility of color pattern in most aposematic species of opisthobranchs is supportive of this suggestion, but requires quantification.

Experiments testing the criteria for aposematic coloration have been conducted in temperate conditions, where predation pressure is considerably lower and where there are fewer species of brightly colored, toxic opisthobranchs. If one examines the proportion of cryptic (including crypsis and special resemblance) versus aposematic species in several temperate versus tropical environments (Table 1) several interesting patterns are apparent. The majority of species in temperate environments are cryptic while in the tropics the majority of species are brightly colored. These differences between levels of aposematic coloration in temperate versus tropical environments are consistent in the regions where adequate data are available. Though these findings are intriguing, they must be treated as preliminary, and tested with data from other temperate and tropical regions.

It is interesting to note that these proportions are not uniformly distributed between different taxonomic groups of opisthobranchs. For example, all anaspideans are cryptic, whether temperate or tropical. The majority of sacoglossans are cryptic, though there are more brightly colored species in the tropics. Even within the Doridacea different families have markedly different proportions of cryptic versus aposematic species. Discodorids and rostangids tend to be cryptic while chromodorids, phyllidiids (Plate 9) and polycerids are aposematic regardless of whether they are temperate or tropical. Even within a family such as the Asteronotidae species of *Sclerodoris* are cryptic, while species of *Halgerda* are aposematic.

The facts that the proportion of brightly colored species and predation pressure are higher in the tropics, suggest that aposematic coloration is likely to be even more important as a defense mechanisms in tropical environments. However, experiments in the tropics have not yet been undertaken to augment the strong circumstantial evidence in support of this hypothesis.

Behavioral Enhancement of Color

Flight and flash coloration and deimatic or frightening behavior were discussed by Edmunds (1987). We have chosen to combine these as behavioral enhancements of coloration. In all cases, the behavior of the organism modifies the impact of the color of the animal to startle a potential predator. Many organisms, especially reptiles, cephalopods and fishes possess chromatophores that are employed to alter the color of the organisms physically to either increase its camouflage or to startle its predator. In opisthobranchs, only the cephalaspidean *Haminoea navicula* (da Costa, 1778) is known to possess chromatophores (Edlinger, 1982).

Other opisthobranchs with aposematic color alter their behavior to enhance their color. When disturbed, many aeolid nudibranchs that store nematocysts in their cerata, bristle their cerata in a fashion analogous to that of a porcupine. This effectively increases the area protected by nematocysts, and perhaps makes the nudibranch appear larger to a potential predator.

The large dorid *Hexabranchus sanguineus* (Rüppell and Leuckart, 1831), is widespread in tropical oceans and is brightly colored with a mottled red, orange and white pigment pattern. The edges of its mantle are typical rolled tightly along the sides of the animal. When disturbed, these margins are unfurled, revealing vivid spots of intense red pigment. Should a predator continue to disturb the dorid, it will swim for prolonged periods of time by dorsoventrally flexing its body. Edmunds (1987) stated that swimming behavior exhibited by many species of nudibranchs is an effective means of escaping slow moving predators. This would be particularly effective against benthic crustaceans and predatory echinoderms that are incapable of elevating themselves into the water column, but may actually increase the risk of being attacked by fishes and portunid crabs.

Mimicry

There are relatively few documented cases of mimicry known for any marine invertebrates. Edmunds (1987) stated that examples of mimicry are poorly established for opisthobranch gastropods, but presented a few examples of what Ros (1976; 1977) has termed mimicry circles, involving similar color patterns between different species of sympatric opisthobranchs. Rudman (1982, 1983, 1984, 1985, 1986) and Harris (1987) have also suggested other cases of mimicry between various species of Indo-Pacific chromodorid nudibranchs and red and white sympatric nudibranchs in New England. Little discussion has focused on whether these cases are more likely to represent Batesian or Mullerian mimicry complexes. However, Harris suggested that most cases of mimicry involving similar color patterns in different species of nudibranchs are more likely to represent Mullerian mimicry, owing to the widespread toxicity present in members of the Nudibranchia. The evidence for Batesian and Mullerian mimicry involving opisthobranchs will be discussed separately.

Batesian Mimicry

Batesian mimicry has been suggested for only a few species of marine invertebrates. Most of these involve prosobranch gastropod models and pleustid amphipod mimics. Crane (1969) and Carter and Behrens (1980) reported mimicry complexes involv-

Table 1. Proportions of cryptic and aposematic opisthobranchs in temperate and tropical habitats.

	California Temperate				South African Temperate				South African Tropical				Papua New Guinea Tropical			
	Cryptic		Aposematic		Cryptic		Aposematic		Cryptic		Aposematic		Cryptic		Aposematic	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cephalaspidea	12	86	2	14	6	75	2	25	7	50	7	50	14	34	27	66
Anaspidea	3	100	0	0	2	100	0	0	8	100	0	0	0	100	0	0
Sacoglossa	8	100	0	0	4	80	1	20	12	57	9	43	19	56	15	44
Notaspidea	4	80	1	20	4	80	1	20	2	29	5	71	3	43	4	57
Doridacea	38	61	24	39	27	55	22	45	20	25	59	75	50	33	102	67
Dendronotacea	11	61	7	39	8	80	2	20	2	40	3	60	13	81	3	19
Arminacea	4	50	4	50	1	14	6	86	2	100	0	0	4	80	1	20
Aeolidacea	21	50	21	50	15	56	12	44	11	48	12	52	30	45	36	55
Total	101	63	59	37	67	59	46	41	64	40	95	60	139	43	188	57

ing two species of pleustid amphipods, *Pleustes platypa* Barnard and Given, 1960, and *P. depressa* Alderman, 1936, with the gastropod *Alia carinata* (Hinds, 1844). The specimens discussed by Crane were similar in appearance and banding pattern to a distinct morph of the polymorphic snail. Carter and Behrens described two distinct morphs of *P. depressa* resembling two distinct morphs of *Alia carinata*. Field (1974) described mimicry of three morphs of the amphipod *Stenopleustes* sp., which resemble three sympatric species of the gastropod genus *Lacuna*. One of these snails, *L. vincta* (Montagu, 1803), is dimorphic and had a distinct amphipod morph mimicing each snail model. Field presented experimental data showing that the models were largely unpalatable to fish while the pleustids were eaten by the same fish, usually after they began swimming, a behavior that was divergent from that of the model.

The only other examples of possible Batesian mimicry in marine invertebrates involve opisthobranch models. Goddard (1984) described a striking example of Batesian mimicry between the nudibranch *Flabellina trilineata* and an amphipod, *Podocerus* sp.

Another species of *Podoceros*, *P. cristatus* (Plate 10:2) (Thompson, 1879), is present along the coast of California and resembles another species of *Flabellina*, *F. iodinea* (Cooper, 1862) in its coloration (Plate 10:1). Both species are vivid purple with orange appendages and are sympatric on hydroid colonies in southern California.

Harris (1987) suggested that another possible case of Batesian mimicry involving a nudibranch model, the aeolid *Hermissenda crassicornis*, and an arminacean nudibranch mimic, *Janolus barbarensis* (Cooper, 1863). Harris believed that the mimic was likely to be palatable since it fed on bryozoans rather than the nematocyst-bearing hydroid prey of the model. However, Gosliner (1981b) has shown that other zephyrinid arminaceans are not ingested by fish predators and have some other form of toxicity. This case is far more likely Mullerian rather than Batesian mimicry.

Another Batesian mimicry complex involves species of prosobranch genus *Trivia* and nudibranchs of the genus *Doriopsilla* in southern Africa. *Trivia* spp. are frequently found in the guts of fishes off southern Africa (Liltved, pers. comm.) and are therefore palatable. Individuals of *Doriopsilla*, as adults, are rarely, if ever, eaten by fish. Two species of *Trivia*, *T. millardi* (Cate, 1979), and *T. ovulata* (Lamarck, 1810), are polymorphic with respect to color of the mantle (Gosliner & Liltved, 1982, 1987). Some color morphs of both species bear special resemblance to their compound tunicate prey species. One morph of *T. ovulata* (Plate 10:3) and one of *T. millardi* (Gosliner and Liltved, 1982: fig. 1 E) mimic the sympatric nudibranch *Doriopsilla* sp. Another morph of *T. millardi* is orange with irregular opaque white lines and mimics the dorid *Doriopsilla miniata* (Alder and Hancock, 1864) (Plate 10:4). When the animal is actively crawling, the mantle of *Trivia millardi* entirely obscures the shell and the resemblance between the two is even more pronounced.

Similar Batesian mimicry also appears to exist between opisthobranch models and prosobranch mimics in the Indo-Pacific tropics. *Phyllidia* sp. (Plate 9:8) is a brightly colored, distasteful nudibranch that commonly occurs in the western Pacific. Sympatric with this nudibranch is the egg shell, *Ovula ovum* (Linnaeus, 1758). Juveniles of this species have a black mantle with scattered tubercles containing bluish white bases and yellow-orange apices (Tan, Pai and Hsha, 1986: pl. 2), and are the same size as the adult nudibranchs. When mature, *Ovula ovum* is about twice the size as the nudibranch, lacks tubercles and is uniformly black with scattered white spots. This case of Batesian mimicry appears to be size-dependent, but offers considerable protection to the juveniles of this snail.

Mullerian Mimicry

All of the previously reported cases of Mullerian mimicry in marine organisms involve convergent coloration between various species of opisthobranch mollusks. Ros (1976, 1977) was the first to report similar bright color patterns in different complexes of Mediterranean opisthobranch mollusks. He considered species of white nudibranchs with red, orange or yellow spots or markings to represent a complex of Mullerian mimics. However, the resemblance of several of these species is rather superficial and one must be cautious not to generalize the mimetic importance of superficial similarity of appearance. Similarly, Goddard (1987) suggested that three species of nudibranchs, *Triopha catalinae* (Cooper, 1863), *Laila cockerelli* MacFarland, 1905, and *Crimora coneja* Marcus, 1961, represent a similar mimetic complex on the Pacific coast of North America. While the similarity in appearance of *T. catalinae* and *L. cockerelli* is indeed striking, *C. coneja* has black pigment on the tips of its papillae, which give the animal a very different appearance than the other two species. Harris (1987) cited a possible mimetic complex of aeolid nudibranchs bearing red cerata with white tips. Most of these species are brightly colored and are often seen crawling in the open. However, *Catriona gymnota*, which does have a color pattern that is similar to the other species, was cited by Edmunds (1987) as an example of special resemblance, because of its similarity to its hydroid prey. *Catriona gymnota* is usually found in the stolon mat of its prey and is reclusive in its behavior. One must place the similarity of organ-

isms in the proper contexts of natural habitat and predator perception. Rudman (1982, 1983, 1984, 1985, 1986) has described chromatic convergence between different chromodorid species in several genera throughout the Indo-Pacific tropics. These appear to be some of the best examples of Mullerian mimicry complexes known from the marine environment. The fact that closely related congeners often have dramatically different color patterns, suggests that the similarity in color pattern in chromodorids of different genera is due to convergence rather than common descent.

It is not necessary to possess bright colors to suggest aposematic coloration and Mullerian mimicry. On sandy subtidal slopes in Papua New Guinea, there is a common species of black and white opisthobranch with an elongate caudal tail. This undescribed species of *Chelidonura* is very conspicuous on its clean, white sand substrate. It is polymorphic in color. Individuals may be all black, except for a white transverse stripe on the head, black with scattered white spots or predominantly white with black spots. All three morphs are sympatric, but their relative abundances vary from one locality to another. Resembling the black form with white spots, are two other rarer sympatric opisthobranchs (Plate 10:5 through 10:7), *Gastropteron bicornutum* Baba and Tokioka, 1965, and an undescribed genus and species of haminoeid opisthobranch. Members of these three species crawl quickly over the sand surface in a very conspicuous manner. All three of these species produce toxic secretions but are in different families of opisthobranchs. The haminoeid and *G. bicornutum* are the only members of their respective families that possess elongate tails, like *Chelidonura* sp. This probably represents another case of Mullerian mimicry, but no palatability studies have yet been conducted, owing to the rarity of the latter two species.

Perhaps one of the best cases of Mullerian mimicry involving opisthobranchs is between the phyllidiid nudibranch *Phyllidia nobilis* Bergh, 1869, and an undescribed cryptobranch dorid (Plate 11:1). Phyllidiids are extremely toxic opisthobranchs (Johannes, 1963) and are aberrant dorids in that they lack a circle of gills around the anus. Their secondary gills are situated ventrally, forming a row on either side of the animal, between the mantle and foot. The cryptobranch mimic possesses a circle of gills around the anus, which is small in size compared to other dorids. When a shadow is cast over the cryptobranch it entirely withdraws its gills into the branchial pocket, further enhancing its resemblance to *P. nobilis*. All species of *Phyllidia* that have been examined, secrete a toxic liquid from notal glands. This liquid forms a white precipitate upon contact with sea water. The cryptobranch also produces white precipitate from notal glands. The toxicity of the secretions of the cryptobranch were not tested, however. Further experimentation is required to establish that this case of mimicry is Mullerian rather than Batesian. In either case, it represents one of the best cases of mimicry with behavioral enhancement reported from the marine environment.

There have not been any recorded cases of Mullerian mimicry between opisthobranchs and other marine organisms. Several cases have recently been discovered in the shallow, tropical waters involving opisthobranchs and polyclad flatworms. In the Gulf of California two opisthobranchs, the aglajid *Navanax inermis* (Cooper, 1862) and dorid *Hypselodoris ghiselini* Bertsch, 1973, and the polyclad *Pseudoceros bajae* all are blackish with yellow and blue spots ornamenting the notum. In Papua New Guinea, the dorid nudibranch *Chromodoris geometrica* (Risbec, 1928) closely resembles an unidentified species of polyclad flatworm (Plate 11:3 and 11:4). Two species of aglajid opisthobranchs, *Chelidonura varians* Eliot, 1903 (Plate 9:2), and *Philinopsis gardineri* (Eliot, 1903) and another unidentified polyclad all have black ground color with vibrant blue marginal lines. All of these opisthobranchs are known to be members of highly toxic taxa. Toxicity in polyclads has been suggested by many workers, but little direct evidence exists. Thompson (1965) has reported that some British polyclads secrete acids that have been recorded as having a pH of 1. Also, many brightly colored polyclads that have unique color patterns, and like the opisthobranchs they resemble, are very obvious on reef flats and overt in their behavior. It is therefore likely that the above described resemblances between polyclads and opisthobranchs also represent cases of Mullerian mimicry, but experimental evidence of the palatability of the specific flatworms involved is presently lacking.

One of the best cases of probable Mullerian mimicry is between three phyllidiid nudibranchs, *Phyllidia varicosa* Lamarck, 1801, *P. coelestis* Bergh, 1905, and *Fryeria ruppelli* Bergh, 1869, and juvenile specimens of the sea cucumber *Bohadschia graeffei* (Semper, 1868) (Plate 11:2). In all three taxa the animals are bluish gray with black longitudinal lines and yellow tubercles. Phyllidiids are notoriously toxic opisthobranchs and another species of *Bohadschia*, *B. argus* Jaeger, 1833, is known to be toxic to fish and crustaceans (Bakus, 1981). The three nudibranchs reach a maximum size of about 100 mm in length, while *Bohadschia* grows to 500 mm in length. When the sea cucumber begins to exceed the maximum length of the nudibranchs, it begins to alter its color pattern. Adult individuals of *Bohadschia graeffei* are mottled grayish with large brown spots and smaller black spots. The large tubercles that

characterize juvenile specimens entirely disappear with growth and adults appear smooth dorsally. The adult cucumbers bear no resemblance to the juveniles or the three phyllidiids.

From these examples of probable Mullerian mimicry, it appears that mimicry complexes may involve different species of opisthobranchs only, or may include other invertebrate organisms such as sea cucumbers and polyclad flatworms. In the cases involving only opisthobranchs, the mimics are generally not closely allied to each other and appear to have developed their color patterns through convergent and parallel evolution, rather than through common ancestry.

Discussion

Until recently, the role of color in the evolution and adaptive radiation of opisthobranch gastropods had been poorly understood. Edmunds' (1987) treatment of the subject, provided an excellent review and much new information on the array of documented and potential roles of color in opisthobranch evolution.

The presence of cryptic coloration and special resemblance in opisthobranchs is relatively uncontroversial. Numerous examples are now known and many additional examples are likely to be discovered with continued observation. The presence of special resemblance, as opposed to more generalized crypsis, is highly correlated with increased habitat and trophic specialization of the opisthobranchs. Virtually all the examples of special resemblance cited by Edmunds and presented here, involve opisthobranchs that are intimately associated with a single genus or species of prey.

Edmunds (1987) correctly expressed the need for exercising caution in making statements regarding aposematic coloration and mimicry, and stressed the necessity for rigorous experimental evidence to support circumstantial observations of bright or convergent color patterns. Predation experiments conducted by Goddard (1984) and Harris (1987) provide the necessary experimental evidence to firmly establish that aposematic coloration exists in at least some species of opisthobranchs. More experiments are required to determine how widespread aposematic coloration is within the opisthobranchs. The circumstantial evidence, together with increasing knowledge about the presence and nature of biochemical toxins produced by opisthobranchs, strongly suggests that aposematic coloration has played a dominant role in the adaptive radiation of the opisthobranchs. It is interesting to note that close relatives of brightly colored opisthobranchs often possess equally bright, but strongly divergent patterns of coloration(Plate 9). The preliminary evidence presented here suggests that aposematic coloration is more abundant in tropical environments and this is supported by the view that predation pressure by visual predators is more intense in the tropics than in temperate environments.

Mimicry in marine organisms is not well documented. There are relatively few reported cases of its probable existence. Batesian mimicry is known from only a few cases reported in the literature. Apart from three cases involving prosobranch gastropod models and pleustid amphipod mimics, all other cases involve opisthobranch models. As noted above, closely related aposematic prosobranchs and opisthobranchs tend to have divergent color patterns. This has had interesting consequences in the coevolution of Batesian mimicry. The cases of Batesian mimicry in marine invertebrates involve convergence in color between different species of models and different mimics of certain taxa. Different species of pleustid amphipods have evolved the same color patterns as different species of prosobranchs. Different species of podocerid amphipods have evolved convergent color patterns with different species of flabellinid nudibranchs. Southern African species of *Trivia* have evolved the same color patterns of different species of the nudibranch genus *Doriopsilla*. These cases also seem to have occurred in discrete geographical areas. More comprehensive study of these coevolutionary Batesian mimicry examples is required to determine how these taxonomic and geographical factors characterize marine Batesian mimicry in general.

Mullerian mimicry in the marine environment was previously known only from chromatic convergence in sympatric opisthobranchs. All of the species of the mimicry complexes presented by Ros (1976, 1977), Goddard (1987) and Harris (1987) may not be Mullerian mimics. As noted above, a few species bear only a superficial similarity to the other taxa they are reputedly mimicing. One must exercise the caution suggested by Edmunds (1987). These species must be viewed in their natural habitat and in the context of their predators' perceptions of their color, rather than human ones. Edmunds also suggested that some of these mimics may be palatable to prey, representing Batesian mimics. Field experiments are necessary to test relative palatability of these similarly colored opisthobranchs to various prey. Additional examples of probable Mullerian mimicry between different species of nudibranchs are presented here. Also, several examples of extreme morphological and chromatic similarity between opisthobranchs, several polyclad flatworms and a sea cucumber, represent the first probable cases of Mullerian mimicry involving different phyla of animals.

The study of the role of coloration in the evolution of terrestrial organisms, especially insects, has

a long and distinguished history (Bates, 1862; Wickler, 1969). Circumstantial evidence of similarity between organisms has been supported by numerous classical experiments documenting color perception of predators and palatability of prey (Brower, 1960; Brower & Brower, 1965). Still, many areas of controversy remain. Semantic arguments exist (Edmunds, 1981; Endler, 1981) and concepts such as industrial melanism as a selective force remain in dispute.

In contrast to terrestrial organisms, our knowledge about the role and importance of coloration in marine organisms is in its infancy. Nevertheless, the examples that are presently known are intriguing and strongly suggestive that crypsis, special resemblance, aposematic coloration, Batesian and Mullerian mimicry are effective defensive strategies in the marine environment, as well. Opisthobranch mollusks, by virtue of the facts that they contain diverse and widespread toxins and exhibit an array of distinct and vibrant color patterns, are ideal organisms for further study and experimentation.

Acknowledgments

Numerous individuals shared their field observations and knowledge with us. Jeff Goddard, Bill Liltved and Richard Willan kindly permitted us to include unpublished observations of mimicry involving opisthobranchs. Bob Lavenberg kindly permitted us to cite unpublished feeding observations of fish upon nudibranchs. Michael Ghiselin and Larry Harris kindly read the manuscript and provided many valuable suggestions for its improvement. Marc Chamberlain and Bill Liltved permitted us to use their photographs of some of the species included in the paper. To these individuals we extend our sincere appreciation.

References

Allen, J. 1976. Function of nematocysts in eolid nudibranchs. **West. Soc. Malac. Ann. Rep.** 9: 50.

Bakus, G. 1981. Chemical defense mechanisms on the Great Barrier Reef, Australia. **Science** 211:497-499.

Bates, H. 1862. Contributions to an insect fauna of the Amazon Valley (Lepidoptera: Heliconidae). **Trans. Linn. Soc.** 23: 495-566.

Baylis, J. 1979. Optical signals and interspecific communication. *In*: **The behavioral significance of color**, E. H. Burtt, ed., Garland Press: New York.

Beeman, R. The Order Anaspidea. **The Veliger 3** (supplement 2): 87-102.

Behrens, D. 1980. **Pacific coast nudibranchs. A guide to the opisthobranchs of the northeastern Pacific.** Sea Challengers: Los Osos. 112 pp.

Bertsch, H. and S. Johnson. 1981. **Hawaiian nudibranchs.** Oriental Publishing: Honolulu. 112 pp.

Brower, J. 1960. Experimental studies of Mimicry. 4. **Amer. Nat.** 44: 271-282.

Brower, L. & J. Brower. 1965. Experimental Studies of Mimicry. 8. **Amer. Nat.** 49: 173-188.

Bursey, C. 1984. Color recognition by the blue crab, *Callinectes sapidus* Rathbun (Decapoda, Brachyura). **Crustaceana** 47: 278-284.

Carlson C. and P. Hoff. 1973. Two new species of Gastropteridae from Guam, Marianas Islands (Opisthobranchia: Cephalaspidea). **Publ. Seto mar. biol. Lab.** 21(2): 141-151.

Carter, J. and D. Behrens. 1980. Gastropod mimicry by another pleustid amphipod in central California. **The Veliger** 22: 376-377.

Cimino, G., S. de Rosa, S. De Stefano and G. Sodano. 1982. The chemical defense of four Mediterranean nudibranchs. **Comp. Biochem. Physiol.** (B) 73: 471-474.

Clark, K. and D. Defreese. 1987. Population ecology of Caribbean Ascoglossa (Mollusca: Opisthobranchia): a study of specialized algal herbivores. **Amer. Malac. Bull.** 5: 259-280.

Cott, H. 1940. **Adaptive coloration in animals.** Methuen: London.

Crane, J. 1969. Mimicry of the gastropod *Mitrella carinata* by the amphipod *Pleustes platypa*. **The Veliger** 12: 200.

Darwin, C. 1871. **The descent of man and selection in relation to sex.** Murray: London.

Doty, M and G. Aguilar-Santos. 1970. Transfer of toxic algal substances in marine food chains. **Pac. Sci.** 24: 351-355.

Edlinger, K. 1982. Colour adaption in *Haminea navicula* (da Costa) (Mollusca-Opisthobranchia). **Malacologia** 22: 593-600.

Edmunds, M. 1966a. Protective mechanisms in the Eolidacea (Mollusca Nudibranchia). **J. Linn. Soc. Lond.** (Zool.) 46: 27-71.

Edmunds, M. 1966b. Defensive adaptations of *Stiliger vanellus* Marcus, with a discussion on the evolution of 'nudibranch' molluscs. **Proc. Malac. Soc. Lond.** 37: 73-81.

Edmunds, M. 1968. Acid secretion in some species of Doridacea (Mollusca, Nudibranchia). **Proc. Malac. Soc. Lond.** 38: 121-133.

Edmunds, M. 1974. **Defence in Animals. A survey of anti-predator defences.** Longman: Harlow. 357 pp.

Edmunds, M. 1981. On defining 'mimicry'. **Biol. J. Linn. Soc.** 16:9-10.

Edmunds, M. 1987. Color in opisthobranchs. **Amer. Malac. Bull.** 5:185-196.

Edmunds, J. 1981. An overview of the relationship between mimicry and crypsis. **Biol. J. Linn. Soc.** 16: 25-31

Faulkner, D. and M. Ghislelin 1983. Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. **Mar. Ecol. Prog. Ser.** 13: 295-301.

Field, L. 1974. A description and experimental analysis of Batesian mimicry between a marine gastropod and an amphipod. **Pac. Sci.** 28: 439-447.

Fretter, V. and A. Graham. 1954. Observations on the opisthobranch mollusc *Acteon tornatilis* (L.), **J. Mar. Biol. Ass.** U. K. 33: 565-583.

Fretter, V. and A. Graham. 1962. **British Prosobranch Molluscs**. Ray Society: London.

Garstang, W. 1889. Report on the nudibranchiate Mollusca of Ply:nouth Sound. **J. Mar. Biol. Assn.** U. K. 1: 73-98.

Garstang, W. 1890. A complete list of the opisthobranchiate Mollusca found at Plymouth. **J. Mar. Biol. Assn.** U. K. 1: 399-457.

Ghiselin, M. 1966. Reproductive function and the phylogeny of opisthobranch gastropods. **Malacologia** 3: 325-378.

Goddard, J. 1984. Presumptive Batesian mimicry of an aeolid nudibranch by an amphipod crustacean. **Shells Sea Life** 16: 220-222.

Goddard, J. 1987. Observations on the opisthobranch mollusks of Punta Gorda, California, with notes on the distribution and biology of *Crimora coneja*. **The Veliger** 29: 267-273.

Gosliner, T. 1980. Systematics and phylogeny of the Aglajidae (Opisthobranchia: Mollusca). **Zool. J. Linn. Soc.** 325-360.

Gosliner, 1981a. Origins and relationships of primitive members of the Opisthobranchia (Mollusca: Gastropoda). **Biol. J. Linn. Soc.** 16: 197-225.

Gosliner, T. 1981b. The South African Janolidae (Mollusca: Nudibranchia) with the descriptions of a new genus and two new species. **Ann. S. Afr. Mus.** 86: 1-42.

Gosliner, T. 1987a. Review of the nudibranch genus *Melibe* (Opisthobranchia: Dendronotacea) with a description of two new species. **The Veliger** 29:P 400-414.

Gosliner, T. 1987b. **Nudibranchs of southern Africa. A guide tothe opisthobranch mollusks of southern Africa.** Sea Challengers: Monterey. 136 pp.

Gosliner, T. 1990 (in press). Morphological parallelism in opisthobranch gastropods. Malacological Symposium.

Gosliner, T. and M. Ghislin, 1984. Parallel evolution in opisthobranch gastropods and its implications for phylogenetic methodology. **Syst. Zool.** 33: 255-274.

Gosliner, T. and W. Liltved. 1982. Comparative anatomy of three South African Triviidae (Gastropoda: Prosobranchia) with description of a new species. **Zool. J. Linn Soc.** 74: 111-132.

Gosliner, T. and W.Liltved, 1987. Further studies on the morphology of the Triviidae (Gastropoda: Prosobranchia) with emphasis on species from southern Africa. **Zool. J. Linn. Soc.** 90: 207-254.

Greenwood, P. and R. Marischal. 1984. Immature nematocyst incorporation by the aeolid nudibranch *Spurilla neapolitana*. **Mar. Biol.** 80: 35-38.

Hailman, J. 1977. **Optical signals. Animal communication and light.** Indiana University Press, Bloomington.

Harris, L. 1968. Notes on the biology and distribution of the aeolid nudibranch *Phestilla melanobrachia* Bergh, 1874. **Publ. Seto Mar. Biol. Lab.** 16: 193-198.

Harris, L. 1987. Aeolid nudibranchs as predators and prey. **Amer. Malac. Bull.** 5: 287-292.

Hecht, E. 1896. Contribution a l'etude des nudibranches. **Mem. Soc. Zool.** France 8: 537-711.

Jensen, K. 1980. A review of sacoglossan diets with comparative notes on radular and buccal anatomy. **Malac. Rev.** 13: 55-77.

Johannes, R. 1963. A poison-secreting nudibranch (Mollusca: Opisthobranchia). **The Veliger** 5: 104-105.

Kempf, S. 1984. Symbiosis between the zooxanthella *Symbiodinium* (=*Gymnodinium*) *microadriaticum* (Freudenthal) and four species of nudibranchs.

Kepner, W. 1943. The manipulation of the nematocysts of *Pennaria tiarella* by *Aeolis pilata*. **J. Morph.** 73: 297-310.

Mebs, D. 1985. Chemical defense of a dorid nudibranch, *Chromodoris quadricolor*, from the Red Sea. **J. Chem. Ecol.** 11 (6): 713-716.

Morton, J. 1963. The molluscan pattern: evolutionary trends in a modern classification. **Proc. Linn. Soc. Lond.** 174: 53-72.

Pelseneer, P. 1894. Recherches sur divers opisthobranches. **Mem. Cour. Cl. Sci. Nat. Acad.** Roy. Belgique 53: 1-160.

Randall, J. and H. Randall. 1960. Examples of mimicry and protective resemblance in tropical marine fishes. **Bull. Mar. Sci. Gulf Carib.** 10:444-480.

Ros, J. 1974. Competencia i evolucio en especies veines de gasteropodes marins. **Coll. Soc. Catalana Biol.** 7: 101-121.

Ros J. 1976. Sistemas de defensa en los opisthobranquios. **Oceo. aquat.** 2: 41-77.

Ros, J. 1977. La defensa en los opisthobranquios. **Invest. Cien.** 12: 48-60.

Rudman, W. 1982. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Chromodoris quadricolor, C. lineata* and *Hypselodoris nigrolineata* colour groups. **Zool J. Linn. Soc.** 76: 183-241.

Rudman, W. 1983. The Chromodorididae (Opisthobranchia:Mollusca) of the Indo-West Pacific: *Chromodoris splendida, C. aspersa* and *Hypselodoris placida* colour groups. **Zool. J. Linn. Soc.** 78: 105-173.

Rudman, W. 1984. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: a review of the genera. **Zool J. Linn. Soc.** 81: 115-273.

Rudman, W. 1985. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Chromodoris aureomarginata, C. verrieri* and *C. fidelis* colour groups. **Zool. J. Linn. Soc.** 83: 241-299.

Rudman, W. 1986. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Noumea flava* colour group. **Zool. J. Linn. Soc.** 88: 377-404.

Rudman, W. 1987. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Chromodoris epicuria, C. aureopurpurea, C. annulata, C. coi* and *Risbecia tyroni* colour groups. **Zool. J. Linn. Soc.** 90: 305-407.

Tan, T., J. Pai and K. Hsha. 1986. A survey on seashells (Gastropod and Bivalvia) of northeastern coast, Taiwan, **R.O.C. Bull. Malac. Repub. China** 12: 27-4

Thompson, T. 1960. Defensive adaptations in opisthobranchs. **J.Mar. Biol. Assn. U. K.** 39: 123-134.

Thompson, T. 1965. Epidermal acid-secretion in some marine polyclad Turbellaria. **Nature** 206: 954-955.

Thompson, T. 1969. Acid secretion in Pacific Ocean gastropods. **Aust. J. Zool.** 17: 755-764.

Thompson, T. 1983. Detection of epithelial acid secretions in marine molluscs: review of techniques and new analytical methods. **Comp. Biochem. Physiol.** 74A: 615-621.

Thompson, T. and J. G. Colman. 1984. Histology of acid glands in Pleurobranchomorpha. **J. Moll. Stud.** 50 (1): 65-67.

Waterman, T. 1961. **The Physiology of the Crustacea. 2. Sense organs, integration and behavior.** Academic Press: New York.

Wickler, W. 1969. **Mimicry in plants and animals.** McGraw-Hi11: New York. 255 pp.

Willan, R. and N. Coleman. 1984. **Nudibranchs of Australasia**. Australian Marine Photographic Index: Sydney.

Williams, G. and T. Gosliner. 1973. A new species of anaspidean opisthobranch from the Gulf of California. **The Veliger** 16: 216-232.