Preliminary Report on Genomics Research Collaboration between the NOAA/NMFS/PIFSC/Hawaiian Monk Seal Research Program and the University of Wisconsin-Milwaukee for the Study of Hawaiian Monk Seal Conservation Genomics

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I. Executive Summary

Cutting-edge genomic methods promise to shed light on factors underlying population history and recent trends for the endangered Hawaiian monk seal (*Neomonachus schauinslandi*). In this report, we briefly review findings of previous conservation genetics research (based heavily on microsatellite markers), and then describe current research applying novel genomic methods as part of an ongoing contract with the University of Wisconsin-Milwaukee (UWM). With the genomic approach, this study yielded a data set of thousands of SNPs (single nucleotide polymorphisms) capable of finely differentiating Hawaiian monk seal individuals and populations. Given the limited representation of seals from the main Hawaiian Islands in previous genetics research, initial phases of this research focused on key questions regarding the population history and dynamics within the main Hawaiian Islands.

Objective 1: Investigate the genomic legacy of MHI population expansion

As a first step, we investigated the genomic signatures of the main Hawaiian Islands (MHI) vs Northwestern Hawaiian Islands (NWHI) to compare levels of genomic diversity and distinguish the contribution that migrants from NWHI or local remnant populations may have made in the expansion of the MHI population. To do this we:

  - Evaluated nucleotide diversity;
  - Evaluated linkage disequilibrium, a signal of recent population mixture;
  - Investigated the genetic ancestry of seals in the MHI.

- **Status: Ongoing —**The MHI-focused portion of this study is complete, and the successful proof of concept merits more detailed investigation of NWHI-MHI ancestry.
- **Key Preliminary Findings:**
  - Overall genetic diversity for the species was extremely low with heterozygosity values of 0.0593 (H_o) and 0.0678 (H_e). This echoes all previous genetic findings.
  - Genetic diversity metrics were similar between NWHI and MHI, indicating that there has not been long-term isolation between NWHI and MHI populations.
  - Linkage disequilibrium (LD), a signature that coincides with recent admixture of divergent alleles, was not apparent in this system. It is possible that our study missed a transient LD signature, which is quickly lost as populations homogenize.
  - With the improved resolution supplied by genomics data, we determined that the likely sources of MHI population expansion were a combination of migration from the NWHI as well as productivity within the MHI. The majority of MHI individuals exhibited some genomic ancestry from the NWHI.
  - Within the MHI, Molokai was the greatest source of migrants to the rest of the MHI. The widespread nature of Molokai ancestry may be linked to the importance of the Kalaupapa pupping site on Molokai.
- **Next Steps:**
  - In FY21 NOAA plans to add an additional 200 samples to expand coverage, but sample sharing has been greatly delayed by COVID-19 in FY20.

- The analysis of ancestry and population assignment will be expanded with better coverage of all of the NWHI sites as potential origins of MHI seals.

Objective 2: Investigate individual variation in reproductive success

We next investigated variation in reproductive success, an important variable in population monitoring and recovery. We sought to identify the contributions of various individuals by:

- Using genetic relatedness metrics to assign the parents of seal pups;
- Reconstructing the pedigree of MHI monk seals based on genome-wide SNP variation.

- **Status: Near Complete—**numerous analytical methods have been tested, and poor results suggest limited potential for genomic parentage analysis for monk seals.
- **Key Preliminary Findings:**
  - In our exhaustive parentage analyses, using both SNP and microhaplotype data, we were unable to accurately resolve any parent-offspring relationships, even for known mother-pup pairs.
  - Our inability to reconstruct parentage was not due to a lack of power in our data set; but rather, because genetic diversity was so low that all individuals, related or not, shared many alleles. The heterozygosity we observed for monk seals was far below levels recommended for accurate parentage assignment.
  - Difficulties in reconstructing parentage using molecular markers in Hawaiian monk seals highlights the value of observational data on maternity to provide essential information about monk seal reproduction.
- **Next Steps:**
  - UWM will run parentage test on samples previously tested by Minhovets and Gaughran to determine whether differing genetic markers, or different levels of background population sampling contribute to difficulty in distinguishing parent-offspring pairs.

Objective 3: Design high-resolution SNP panel for targeted, low-cost future analyses

While laboratory methods continue to become more cost effective, large-scale genome sequencing remains expensive, and many methods randomly target subsets of the genome. Thus, to maximize efficiency and repeatability of research, the genomic data generated in this study will be used to develop a high-resolution SNP panel capable of distinguishing individuals and populations of origin for Hawaiian monk seals.

- **Status: Early Stages**
- **Key Preliminary Findings:**
  - Initial sequencing runs generated gigabases (Gb) of high-quality sequence data revealing thousands of SNPs. Additional screening and panel design are ongoing.
- **Next Steps:**
  - UWM will continue the effort to develop a SNP panel for future analysis.

- The SNP panel will be validated in future analyses (Objective 1 Next Steps).
- SNP primer sequences will be developed and published in GenBank.
- Protocols will be produced to facilitate future use of the SNP panel in any qualified genomics lab.

II. Background

Conservation genetics has long played a central role in species conservation science; from defining biologically meaningful management units, to evaluating genetic variability crucial for adaptability, to using metrics of genetic variation and drift to assess dynamics within populations and connectivity between them (Frankham 2003). Recent developments in both laboratory and bioinformatic technologies (including next generation sequencing, whole genome scans) have made it possible to enormously increase the scope (functional genomics as well as neutral variation) and power (improved precision and accuracy of parameter estimates) of conservation genetic research (Allendorf et al. 2010; Ouborg et al. 2010). These advances will particularly aid studies of endangered species where numbers are small and consequences of population isolation, inbreeding depression or disease susceptibility can be dire (Steiner et al. 2013).

Cutting-edge genomic methods hold promise to help understand factors underlying population history and recent trends for the endangered Hawaiian monk seal (*Neomonachus schauinslandi*). The distribution and history of monk seals gives rise to numerous questions (regarding population fragmentation, bottlenecks, inbreeding) that are well-suited for genetic investigation. Hawaiian monk seals range throughout the Hawaiian Archipelago, characterized as a metapopulation with semi-isolated subpopulations distributed amongst a number of islands and atolls (Antonelis et al. 2006). Subpopulations at different sites are impacted by localized threats and exhibit variation in demographic rates (Baker and Thompson 2007). Of the ~1,400 Hawaiian monk seals estimated in 2019, ~1,100 seals inhabited the small islands and atolls of the NWHI, while ~300 seals inhabited the larger (and human-populated) MHI (Hawaiian Monk Seal Research Program 2019). Historically, monk seals inhabited the entire Hawaiian Archipelago with the population in the MHI being largely extirpated shortly after the first humans arrived (Rosendahl 1994). Commercial sealing, guano mining, and other human activities in the NWHI reduced the remainder of the population in the NWHI to near extinction in the mid-1800s (Cobb 1902; Kenyon and Rice 1959). More recently, the NWHI population declined precipitously after wartime activity in the Pacific (DeLong et al. 1976; Johnson et al. 1982; Kenyon 1973), then continued a slower decline in recent decades (4.2% per year from 1985 to 1993, then 1.9% per year from 1993 to 2003; (Carretta et al. 2004). Meanwhile, monk seals remained rare in the MHI until the mid-1990s, but rebounded considerably in the early 2000s (Baker and Johanos 2004). By 2013 the Hawaiian monk seals were showing signs of a stabilizing trend across their range (Baker et al. 2016). The MHI population has played a key role in this positive trend with the highest growth rate (Baker et al. 2011) and reproductive rate (Robinson et al. 2020) throughout the monk seals' range, making this a key area for examination of genetic signatures of population expansion.

A long history of conservation research has been dedicated to Hawaiian monk seals using a variety of genetic markers and techniques as technologies have evolved over the years. Here we briefly review findings of previous research (paying closest attention to studies that have occurred since a thorough 2011 review; Schultz 2011), and then describe the current frontier of Hawaiian monk seal research using novel genomic methods. While we will review the work of a number of collaborators, this report will focus on work performed under an ongoing contract with the University of Wisconsin-Milwaukee (UWM).

III. Review of Hawaiian Monk Seal Conservation Genetics Research

***Phylogenetic Context*—**Phylogenetic analysis has been used to resolve the evolutionary relationships among the monachine seals. Based on differences in DNA sequences (at both nuclear and mitochondrial genes), it has been estimated that the Monachus clade (including both *Monachus* and *Neomonachus* genera) was the earliest to diverge from the rest of the Monachinae subfamily (including *Lobrodontini* and *Miroungini*) around 11–14 million years ago (estimates vary in; Arnason et al. 2006; Fyler et al. 2005; Higdon et al. 2007). While Hawaiian (*N. shauinslandi*), Caribbean (*N. tropicalis*), and Mediterranean (*M. monachus*) monk seals were once considered a single genus, more recent genetic analysis (based on full cytochrome b gene sequence combined with skull morphology) has refined the classification within this clade (Scheel et al. 2014). Scheel et al. (2014) found that the level of divergence within the existing *Monachus* genus was of similar to magnitude to genus-level distinctions in other phocine and monachine taxa. This led to the formation of the new genus, *Neomonachus*, to include Hawaiian and Caribbean monk seals, leaving the Mediterranean monk seals the last extant member of the genus *Monachus*. Further, they refined estimates of divergence time within this clade, suggesting that Mediterranean and New World monk seals diverged around 6.30 million years ago (95% 4.98–7.64), while Caribbean and Hawaiian monk seals diverged around 3.67 Mya (95% 1.90–5.45) when the emergence of the Panamanian isthmus separated the Atlantic and Pacific ocean basins (Scheel et al. 2014). However, analysis of recently discovered fossils by Rule et al. (2020) questions the monophyly of the Neomonachus and places Hawaiian Monk Seals as the most basal group with Caribbean Monk Seals grouped with Mediterranean Monk Seals. This fossil-informed phylogeny would reverse the current biogeographical hypothesis, suggesting that monk seals diversified in the North Pacific before spreading to the North Atlantic 7.1 Mya (Rule et al. 2020).

***Low Genetic Diversity*—**A history of population declines and bottlenecks has led to losses of genetic diversity in both the *Monachus* and *Neomonachus* seals. Both extant species of monk seals have shown extremely low variability at all genetic markers tested to date. Perhaps surprisingly, while the Mediterranean monk seal has a lower population abundance (N <700; Karamanlidis et al. 2016) it has maintained higher genetic diversity than the more abundant Hawaiian monk seal (N ~1400; Hawaiian Monk Seal Research Program 2019). This is likely a product of distinct portions of diversity being maintained in the genetically isolated subpopulations of Mediterranean monk seals, whereas the Hawaiian monk seal population functions as a single unit (see discussion of population structure below).

As genetic markers have improved in resolution, researchers have only refined the conclusion of depauperate genetic diversity in Hawaiian monk seals. Early mitochondrial DNA sequencing showed that few lineages contributed to the sampled population (just 3 haplotypes in 50 seals; Kretzmann et al. 1997). Sequencing of MHC class I genes (where high diversity is expected to provide resistance to a variety of pathogens) showed a concerning lack of diversity with a total lack of variation detected among 80 seals sampled (Aldridge et al. 2006). Development of microsatellite markers was difficult in Hawaiian monk seals, with early efforts stymied by a dearth of variable (*polymorphic*) sites (*loci*). Gemmell et al. (1997) found just 3 polymorphic loci

out of 20 screened, Davis et al., (2002) found 0/10 polymorphic, Schultz et al. (2009; 2010) found 17/163 polymorphic, Minhovets et al. (2016) found 30/135 polymorphic. Analysis with microsatellites has shown that both allelic diversity (*A*: the number of unique variants per locus) and heterozygosity (*He*: the expected proportion of animals with two different alleles at a given locus) are exceptionally low in Hawaiian monk seals. A recent study of 785 NWHI seals genotyped at all 42 microsatellite loci available for Hawaiian monk seals (24; Mihnovets et al. 2016; 18; Schultz et al. 2010) found A= 1.93 alleles per locus and He = 0.38 (Mihnovets 2017; also see Schultz et al, 2009 for an excellent review table of earlier findings). Even recent work sequencing the complete Hawaiian monk seal genome supported the consistent conclusions of exceptionally low diversity in the species; finding that the genome of an adult male seal (RE74, "Benny") exhibited an overall heterogeneity just 5–10% that of a typical human (Mohr et al. 2017; https://www.dnazoo.org/assemblies/Neomonachus_schauinslandi).

Previous and ongoing work continues to investigate the underlying causes of the extreme lack of genetic diversity in Hawaiian monk seals. Shultz et al. (2010) genotyped 2,423 Hawaiian monk seals at 18 microsatellite loci to evaluate evidence of a selective sweep, a historical bottleneck, or persistently small population size. While they found strong evidence for a historic population bottleneck, likely reaching a nadir after overhunting in the 1800s, this bottleneck was not of sufficient severity and duration to explain the genome-wide depletion of genetic diversity in monk seals, leading to the conclusion that long-term population size restriction, likely associated with the ecology of isolated populations on small islands, must be a key factor (Schultz et al. 2010). On-going work with partners at Yale University and the American Museum of Natural History is further investigating the demographic history and evolutionary patterns of the species (Gaughran, personal communication). They plan to sequence the genomes of 15 additional seals to get more precise estimates of previous population size fluctuations. Additionally, they will calculate the rates of mutation accumulation in Hawaiian monk seals compared to other species with larger population sizes. With recent advances in evolutionary modelling, they plan to model the proportion of beneficial versus deleterious alleles in the Hawaiian monk seal genome. We would expect that, in order to persist with such low levels of diversity, Hawaiian monk seals may have a more favorable balance of beneficial:deleterious mutations compared to larger populations.

Limited Population Structure— Genetic population structure, defined by the organization of genetic variation within or among groups, is driven by forces including mutation, genetic drift, demographic history and natural selection. Because conservation legislation like the Endangered Species Act (ESA) and Marine Mammal Protection Act (MMPA) aim to preserve evolutionary potential as well as protecting species from extinction, genetic population structure is a key consideration in the definition of units listed for conservation and management (though exact criteria may vary under each act). Significant reproductive isolation leading to divergent population structure is required for listing conservation units for protection under the ESA (NMFS 2008). However, smaller subunits may be listed for different levels of management based on more recent divergence (e.g. Recovery Units under the ESA; NMFS and FWS 2004) and/or demographic independence (e.g. Demographic Independent Populations under the MMPA; NMFS 2008). Key factors in evaluating population structure relevant to conservation units include 1) testing for genetic heterogeneity between units, 2) estimating degrees of divergence, 3) estimating rates of migration between units, and 4) conducting mixture analysis

(Waples et al. 2018). It is important to define the level of genetic divergence that is biologically impactful to a species (for instance leading to demographic independence or localized adaptation), rather than relying on statistical significance alone (Palsboll et al 2007).

The distribution of Hawaiian monk seals as a metapopulation spread across isolated islands generates natural questions about fragmentation and genetic isolation. However, field observations have shown that Hawaiian monk seals move widely throughout the archipelago, with ~14% of animals dispersing from their natal site (Johanos et al. 2014; Stewart et al. 2006). In light of such dispersal rates, it may be little surprise that a number of studies have found limited evidence of population genetic structure (spatial segregation of genetic variation) across the monk seals' range. Kretzmann et al. (1997) found no evidence for population differentiation, but compared only 10 individuals each from five locations in the Northwestern Hawaiian Islands. Schultz et al. (2011) analyzed approximately 85% of seals born between 1994 and 2007 in both the NWHI and MHI (n = 1842 NWHI, 55 MHI) at 18 microsatellite loci, and still found no evidence of spatial or temporal partitioning of genetic variation, providing a measure of confidence that seals could be translocated between subpopulations without detrimental genetic impacts to the population. Even with such extensive sample coverage, questions have remained as to whether these genetic markers have the power to differentiate between populations given the low amount of variation available. Thus, more recent investigations of population structure have increased the number of microsatellite loci and conducted a power analysis (using POWSIM; Ryman and Palm 2006), concluding that 30 loci should be sufficient to detect population differentiation with 95% confidence (Minhovets unpublished data, NOAA collaboration).

In recent efforts, Minhovets (2017) used both Bayesian population assignment tests (STRUCTURE; Paetkau et al. 1999) and principal component analysis (Jombart et al. 2010) to further investigate population structure among NWHI locations. This data set relied on 37 of the best-performing loci from previous studies (Mihnovets et al. 2016; Schultz et al. 2010), genotyped in 1,244 individuals from the NWHI (as well as 8 individuals from the MHI, but conclusions based on so few microsatellite genotypes are not considered reliable). STRUCTURE runs were conducted assuming correlated allele frequencies in an admixture model with an a priori designation of origin based on sample collection location (LOCPRIOR; Hubisz et al. 2009). Results from both STRUCTURE and the principal component analysis indicated a single admixed population. Minhovets further investigated rates of migration between islands. This approach, designed to estimate the number of individuals moving between genetically distinct populations (termed "first-generation migrants"), had mixed success given the limited genetic distinction among sites. Using GENECLASS v2 (Piry et al. 2004), 40 individuals were identified as first-generation migrants. However, it is valuable to note that, although the sample set included two animals that had been translocated from French Frigate Shoals to Kure Atoll, neither of the two were identified by GENECLASS as potential migrants between these subpopulations. In total, all of these analyses further support the notion that sites throughout the NWHI maintain genetic connectivity through the frequent exchange of migrants.

Genetic variation of course, may be structured more subtly without leading to genetically distinct population units. Given that observed monk seal dispersal has exhibited distance-based trends (high movement between sites separated by up to 100 km, but very little between locations

separated by > 400 km; Johanos et al. 2014), we might expect genetic variation to be loosely partitioned along a gradient between increasingly distant sites. To evaluate genetic isolation-by-distance, Minhovets (2017) used correlograms to plot spatial autocorrelation among allele frequencies, and tested for correlation of genetic and geographic distance using a Mantel test (GenAlEx v6.5; Peakall and Smouse 2006). Spatial autocorrelation analysis showed significantly positive correlations in the first distance class at 50 km, suggesting genetic similarity was clustered within sites more than between them. When correlograms were run separately for males and females, they were not significantly different, suggesting that dispersal in Hawaiian monk seals is not sex-biased. The Mantel test indicated minor, but not statistically significant, isolation-by-distance indicating that more distant sites likely share less genetic exchange. Such patterns are corroborated by sightings data indicating that, while long distance do occur, dispersals between closer sites are more common (Johanos et al. 2014).

***Parentage Analysis*—**Genetic parentage analysis has the potential to help recreate pedigrees and evaluate individual reproductive success. Various methods have been used to genetically determine parent-offspring relationships in Hawaiian monk seals. To investigate the relationship of putative mother-twins sets (one mother observed nursing two pups), Schultz et al. (2011) manually compared the 18-locus genotypes of pups and putative mothers for 5 mother-twin sets. Presence of at least 1 shared allele at every locus was considered confirmation of maternity. The analysis confirmed that each of the sets were twins (previous analysis of allele frequencies in the broader population suggested that the probability of mistaking parentage based on the 18 loci was low; Schultz et al. 2009). The genotypes of twin pups were also compared, and all pairs were determined to be dizygotic (non-identical, some differing alleles) (Schultz et al. 2011). In another highly targeted parentage analysis, Gaughran (unpublished) used genetic relatedness (Rxy; Goodman 1997) measures to determine whether a deceased pup could have been the offspring of either of two deceased females (all found in close spatial and temporal proximity; Harting et al. 2020). The data set consisted of 8 seals (the pup, 2 putative mothers, and 5 others for reference) genotyped at 30 microsatellite loci (a subset of; Mihnovets et al. 2016; Schultz et al. 2010). Pairwise relatedness was tested using the R package 'related' (Pew et al. 2015), and parentage was assigned to the female with the highest relatedness measure 0.45 (95% CI: 0.26 – 0.68; this is close to the theoretical expectation that parent-offspring pairs share 0.50 genetic material). While the low genetic diversity in monk seals means that many seals have high relatedness measures, this was also the only potential mother for which the 95% confidence interval of relatedness did not include zero (indicating potential of no relationship).

We have seen signs of success in assigning parentage within a larger pool of potential parents and offspring. As part of an unpublished collaborative project with the Hawaiian Monk Seal Research Program, Minhovets used the pedigree reconstruction program CERVUS (Marshall et al. 1998) to search for fathers siring pups within a large sample of NWHI seals. The samples analyzed included a subset of the large data set genotyping 85% of NWHI seals (Schultz 2011); putative mother-pup pairs (females and pups that were part of an observed nursing pair in the NWHI from 1994 to 2007) and candidate fathers (males born between 1984 and 2002, making them reproductive age for at least one cohort of sampled pups) for which at least 30 microsatellite loci had been successfully genotyped. Thirty-seven female-pup pairs and 544

males met these criteria. Of the 37 female-pup pairs, 22 mothers were assigned, including 4 females assigned as the mother of a pup other than the one she was nursing (likely an unidentified pup-switch). However, 15 pairings could not be resolved (females not assigned as the mother of any pup, and pup not assigned as the offspring of any female). It is unknown whether this could indicate a failing of the maternity assignment test, or whether each of these cases could have represented an undetected pup-switch for which the other member of the pair was not genotyped. In the paternity assignment tests using 544 candidate males that were of viable reproductive age during the timespan of the mother-pup data set, only 7 were assigned as fathers of one of the pups in the study (including both the 95% and 80% confidence levels). This low assignment rate could reflect low instance in sampling true fathers but more likely (given high sampling intensity) indicates poor assignment ability with these tests. It will be valuable to test whether increasing marker resolution increases parentage assignment success.

Remaining Questions for Conservation Genomics Research—While Hawaiian monk seal science has benefited greatly from the genetic research reviewed above, many opportunities still remain for further research.

- Given the long history of low population size, genetic diversity is exceptionally low in this species, and previous research (primarily based on microsatellites) has suffered from low marker resolution and the inability to adequately address many questions regarding monk seal populations. Newer genomics technologies will be essential to broadly characterize variation throughout the monk seal genome and improve resolution for both evolutionary and population genetic questions. The recently available annotated genome sequence will provide an excellent reference for putting future genomic loci in context.
- To date, the MHI population has been scarcely covered genetic investigations, most of which were conducted while the MHI population was at very low numbers. While Schultz et al. (2011) included MHI samples, the much greater representation of NWHI animals, and cohort-level comparisons from years with few MHI pups could have limited potential conclusions. While the Minhovets data set started with 120 MHI samples, most were eliminated (for various quality issues or comparability to earlier datasets) leaving the final data set with only 8 MHI seals. The recent rebounding of seals in the MHI, and the geographic and demographic distinction of the MHI from the NWHI, make this area particularly ripe for genetic investigations of population history and connectivity.

IV. Objectives of Genomics Research Collaboration with UWM

This study employed a genomic approach to yield, for the first time, a data set of thousands of SNPs (single nucleotide polymorphisms) capable of finely differentiating Hawaiian monk seal individuals and populations. Given the limited representation of MHI seals in previous genetics research, initial phases of this research focused on key questions regarding the population history and dynamics within the MHI (detailed below), relevant to the Hawaiian Monk Seal Recovery Plan priority to "*Ensure the natural recovery of the Hawaiian monk seal in the MHI*" (National Marine Fisheries Service 2007). Focusing on population expansion and reproduction within the MHI, this study included NWHI for appropriate context, but did not sample to fully investigate stock structure between the MHI and NWHI (future studies will expand on this question). This first population-level genomics study of Hawaiian monk seals has the potential to serve as a

proof of concept for using genomics research to tackle complex questions in the future, including differential disease susceptibility (Storfer et al. 2020), adaptation to environmental change (Allendorf et al. 2010; Benestan et al. 2016; Franks and Hoffmann 2012), and management of conservation breeding efforts (Roca and Schook 2010; Willoughby et al. 2017; Wright et al. 2020).

Objective 1: Investigate the genomic legacy of MHI population expansion

As a first step we investigated the genomic signature of the recent MHI population expansion. Population genomic methods are well suited to detect the legacy of population expansion; they can help to distinguish the contribution that migrants from NWHI or local remnant populations made in the expansion of the MHI population. Further, evaluation of genomic diversity can help determine whether founder effects or potentially deleterious decreases in diversity persist in this population. Addressing this objective required three activities:

1) **Evaluation of nucleotide diversity—**We analyzed nucleotide diversity of seals in the MHI relative to those at other sites. An influx of new individuals from NWHI would bring in new genetic variation, increasing genetic diversity in MHI and reducing structure across the metapopulation. Meanwhile, if reductions in nucleotide diversity were observed in the MHI relative to NWHI, it could indicate founder effects (indications that the MHI was established by a limited number of NWHI migrants).
2) **Evaluation of linkage disequilibrium—**We analyzed linkage disequilibrium across all pairs of loci as well as in specific genomic regions. The presence of linkage disequilibrium would indicate recent admixture of previously distinct populations.
3) **Assignment of genetic ancestry—**We compared genetic signatures among areas of origin to assign MHI individuals' genetic ancestry to one or more source populations (NWHI or MHI). First-generation migrants and their descendants should be detectable based on signatures such as linkage and Hardy-Weinberg disequilibrium.

Objective 2: Investigate individual variation in reproductive success

We next investigated variation in reproductive success, an important variable in population monitoring and recovery. If only a few individuals breed, their genes become over-represented and founder representation can quickly erode genetic variation, threatening long-term population persistence. By identifying the reproductive contributions of various individuals, we can begin to understand factors influencing reproductive success (age, body condition, habitat use, dominance), and determine whether any management interventions can help preserve representation of the gene pool. Pedigree reconstruction is a powerful tool for monitoring population demography and genetics, and the use of genome-wide SNP markers in reconstructions has the potential to improve accuracy considerably over other molecular markers such as microsatellites

1) **Detect individual variation in reproductive success—**We conducted a pilot study using genome-wide SNP variation in an attempt to reconstruct the pedigree for the Hawaiian monk seal population in the MHI. We focused on a temporal and spatial subset of the MHI population including pups from Oahu (where some of the best sample coverage

exists), and their potential parents. Multiple algorithms and software programs were tested and compared to evaluate the most promising analytical methods.

Objective 3: Design high-resolution SNP panel for targeted, low-cost future analyses

While laboratory methods continue to become more cost effective, large-scale genome sequencing remains expensive, and many methods randomly target subsets of the genome. Thus, a defined panel of variable markers is helpful to maximize efficiency and repeatability of research. Genomic data generated in the above analyses will aid the development of a high-resolution SNP panel capable of distinguishing individuals and populations of origin for Hawaiian monk seals. The data generated in Objectives 1 and 2 will provide a basis for marker selection and panel development using custom amplicon sequencing methods such as "genotyping-in-thousands by sequencing" (GT-seq).

1) **Design SNP panel—**We investigated the structure of genotyped groups to identify approximately 300 markers that exhibit high levels of differentiation between populations (data from Objective 1) and have the best individual discriminatory power (data from Objective 2). These markers will be used to design the SNP panel.
2) **Validate SNP panel performance—**We will validate the above SNP panel by using it to genotype additional samples and evaluate the power of the panel to assign individuals back to populations of origin and/or assign parentage.

V. Laboratory and Analytical Methods for Genomics Research Collaboration

Study Area and Sampling

The Hawaiian Monk Seal Research Program (HMSRP) has been monitoring monk seal populations, recording sightings and behavioral data, and consistently tagging weaned monk seal pups since the 1980s (Antonelis et al. 2006). In addition to pups, older animals are tagged as opportunities arise. The tissue plugs generated during the application of flipper tags have been archived along with information on age, sex, and location of each seal, creating a rich source of material for genomic research. For the initial stages of this study, we selected a sample set heavily focused on the MHI, with lesser representation of the NWHI for reference (after pilot analysis, future additions to this research will include more thorough coverage of NWHI seals). For purposes of parentage analysis, we included samples from all available mother-pup pairs from the Island of Oahu, and males (potential fathers) from throughout the MHI. The sample set for this study included 192 monk seal individuals (suitable for running in two genomic library preparations with 96 unique barcodes each) (see Appendix I for details).

Laboratory Methods

***DNA extraction*—**We extracted high-quality genomic DNA from tissue samples using the Qiagen DNeasy Blood and Tissue Kit. DNA in each extraction was quantified using a Qubit fluorometer and visualized on a 1% agarose gel stained with ethidium bromide. All DNA samples were normalized to 20 ng/ul.

***Genome library preparation*—**We followed the BestRAD library preparation as per Ali et al. (2016). Standardized DNA samples were cut with the restriction enzyme SbfI on the 5' end and adaptors with 96 unique barcodes were ligated to the cut site. Samples were pooled, and further fragmented to 300–350 bp through sonication. Targeted DNA fragment sizes were confirmed through 2% agarose gels. DNA fragment sizes outside of the desired size range were removed through 0.7× and 1.0× AMPureXP purification, removing large (> 400 bp) and small DNA fragments (< 200 bp) and any impurities. Further purification with Dynabeads M-280 Streptavidin removed any DNA without a barcode by targeting the 5' end of the adaptor. A final AMPureXP purification (1.5×) removed any remaining SbfI enzyme, impurities, salts, and small fragments of DNA from the libraries. A library-specific adaptor was ligated to the DNA and then amplified using the NEBNext Ultra DNA library Prep Kit for Illumina, following the standard protocol. The libraries were sequenced an Illumina Novaseq lane producing 223 Gb of high-quality data.

***Sequencing & Genomic Data Set*—**Sequence reads were demultiplexed, filtered for quality and trimmed to 140 bp using the *process_radtags* function with the best-rad flag in STACKS v2.0 (Rochette et al. 2019), yielding 59,498,365 reads (83.6% of total sequence data). The filtered reads were then aligned to the Hawaiian monk seal reference genome (Mohr et al., 2017) using BOWTIE (Lemel et al. 1997), and SNP genotypes were called using the reference-alignment pipeline in STACKS v2.0 (min_maf = 0.02; Rochette et al. 2019). Of the 192 samples processed, 166 individuals aligned to the reference genome, and 44,583 variant sites (SNPs) were

discovered through the STACKS pipeline. We further filtered the data in VCFTOOLS for minor allele count (mac = 3) and genotyping rates for loci and individuals (50% each; Danecek et al. 2011). After filtering, 3,451 SNPs for 90 seals from 11 islands remained (Table 1, Table 2). We did not filter for Hardy-Weinberg equilibrium because our system violates the assumptions and such filtering would cede valuable genetic information.

Analytical Methods

***Genetic Diversity*—**We quantified genetic diversity by estimating allelic richness, observed and expected heterozygosity (H_o and H_e, respectively), inbreeding coefficient (F_{IS}), and the number of private alleles (with bootstrapping) for the total data set, within each region (NWHI and MHI), and for each island using the *basic.stats* function in the R package 'hierfstat' (Goudet 2005) and *allel.rich* function in the R package 'PopGenReport'(Gruber and Adamack 2017).

***Linkage Disequilibrium*—**We tested for linkage disequilibrium (LD) with a Bonferroni correction for multiple comparisons (Hauser et al. 2019; Sethuraman et al. 2019) in VCFTOOLS (Danecek et al. 2011) across all SNP loci across islands and regions.

***Genetic Ancestry*—**We characterized island-level population structure using DAPC and STRUCTURE. For both the DAPC and STRUCTURE analyses, we used a hierarchical approach to look at (a) all islands, (b) islands with sample size of 5 or higher, and (c) the MHI of Kauai, Oahu and Molokai, the areas of recent demographic expansion. DAPC was run using the R functions *DAPC*, *compplot*, and *assignplot* in the 'adegenet' R package (Jombart 2008; Jombart et al. 2010) using the islands as the grouping prior. In STRUCTURE, we used the admixture model with 500,000 burn-in and 500,000 MCMC iterations to evaluate *K* (number of genetically distinct population segments) values from 1 to 11 with 10 replicate runs at each *K*. We used the ΔK (Evanno et al. 2005) and MedMeaK and MaxMeaK (Puechmaille 2016) methods to determine the appropriate number of clusters (*K*) and produced summary barplots for all values of *K* in STRUCTURESELECTOR (Li and Liu 2018).

To determine the genomic ancestry of MHI individuals, we performed two population assignments using the R package "assignPOP" (Chen et al. 2018): 1) assign MHI individuals to NWHI, and 2) assign MHI individuals to all islands (throughout NWHI and MHI). For both assignments, all NWHI individuals were used as training data for their respective island, i.e., as a reference for the genetic signature of their respective islands to which we assigned individuals. The NWHI were classified as French Frigate Shoals, Necker, Nihoa, and NWHI-Combined. NWHI-Combined is the pooled set of individuals from Midway, Kure, and Pearl and Hermes, combined to bolster sample sizes. For the all-islands assignment, we chose 5 MHI training individuals per island from Kauai, Oahu, and Molokai; those individuals with the oldest and most consistent observational sighting data at only their respective islands and those who were not part of known mom-pup pairs or known translocations. Hawaii (n=1) and Maui (n=1) had insufficient sample sizes to include in the training data. The remaining MHI individuals (non-training individuals; n=57) were then assigned to their most probably island of genetic origin from the pool of all islands.

***Reproductive Success: Parentage*—**Given the anticipated challenge of assigning parentage in a species with extremely low genetic variation, we exhaustively attempted to reconstruct parentage using three software programs: COLONY (Jones and Wang 2010), R package "Sequoia" (Huisman 2017), and CERVUS (Kalinowski et al. 2007). Sequoia runs very quickly and is specifically tailored for SNP data and may useful in studies with incomplete population sampling (Flanagan and Jones 2019; Thrasher et al. 2018). CERVUS has been found to outperform COLONY in systems with widespread inbreeding (Jones and Wang 2010) but might require more loci to do so (Karaket and Poompuang 2012). We also attempted to reconstruct parentage and paternity by region (restricting the set of potential parents to those sampled in the same region as the query pup), to assign paternity for pups with known moms, and to assign maternity among known mom-pup pairs in CERVUS. The full data set contained 90 offspring, 18 candidate mothers, and 72 candidate fathers. For the parentage per region, we assigned 72 offspring to 14 candidate mothers and 58 candidate fathers in the MHI and 18 offspring to 4 candidate mothers and 14 candidate fathers in the NWHI. To assign paternity for pups with known moms, we assigned 31 mom-pup pairs to 72 candidate fathers. Lastly, we assigned the 31 pups to their known moms (n = 31).

We repeated this comprehensive set of parentage assignment approaches with alternative subsets of our molecular data predicted to be more informative for parentage: a subset of SNP data and two microhaplotype data sets (Table 1). The subset of SNP data (SNP Data Set #2) had a more stringent filter for locus genotyping rate (60%) than the full SNP data set (SNP Data Set #1) and a relaxed individual genotyping rate (30%) to generate a more informative set of loci across more individuals. We developed 2 microhaplotype data sets, one with no filtering (Microhaplotype Data Set #1) and one with a stringent genotyping rate filter (60%) with no filtering on individuals (Microhaplotype Data Set #2). Microhaplotypes, short regions with multiple SNPs, can be especially useful for parentage because they are multi-allelic and thus more informative per locus than bi-allelic SNPs (Baetscher et al. 2018). We calculated the proportion of shared alleles (Dps) between all pairs of individuals using all 4 data sets to evaluate our power to resolve parentage (Gómez-Romano et al. 2013).

VI. Preliminary Results of Genomics Research Collaboration

Objective 1: Investigate the genomic legacy of MHI population expansion

***Genetic Diversity*—**Overall genetic diversity for the species was extremely low with heterozygosity values of 0.0593 ± 0.198 (H_o) and 0.0678 ± 0.138 (H_e). Across the archipelago, we observed a global deficiency of heterozygotes relative to expectations that likely reflects population structure across islands (F_{IS} = 0.1302). Genetic diversity between regions was comparable based on heterozygosity values (NWHI H_o: 0.060 ± 0.206, H_e: 0.068 ± 0.167; MHI H_o: 0.059 ± 0.186, H_e: 0.067 ± 0.094; Table 2), private alleles (NWHI: 1019, MHI: 988; Table 2), and F_{IS} (NWHI = 0.116; MHI: 0.126; Table 2). We detected a deviation from Hardy-Weinberg equilibrium, observing fewer heterozygotes than we expected. The magnitude of the deviation observed was similar in the MHI (F_{IS} = 0.126) and NWHI (F_{IS} = 0.116).

Similar genetic diversity between the two regions, NWHI and MHI, indicates that there has not been long-term isolation between NWHI and MHI populations. If the populations were isolated, we would expect to see genetic drift erode genetic variation in the smaller MHI population more quickly than in the larger NWHI population. Extremely low genetic diversity in monk seals species-wide does limit our discriminatory power (Wang 2018), but in concert with our observation of low population structure between NWHI and MHI (below), our similar diversity estimates are consistent with the hypothesis that the MHI population expansion was bolstered by regular immigration from the NWHI.

Our estimates of diversity based on a genome-wide survey of variation echo low values reported for microsatellites (Schultz et al., 2009), mitochondrial DNA sequences (Kretzmann et al., 1997), and MHC markers (Aldridge et al., 2006). Although low genetic diversity does not necessarily beget species extinction, as seen in species capable of long-term persistence despite extremely low genetic diversity (Reed 2010), it does increase the risk of inbreeding depression and erosion of evolutionary potential (Frankham 2005; Hedrick and Garcia-Dorado 2016; Keller et al. 1994). Accordingly, this extremely low genetic diversity is cause for concern, and maintaining genetic variation through connectivity should be a top conservation priority for the Hawaiian monk seal.

***Linkage Disequilibrium*—**We did not detect any deviations from linkage equilibrium before or after a Bonferroni correction for multiple comparisons. Linkage disequilibrium (LD), a signature that coincides with recent admixture of divergent alleles (Weir 1979), was not apparent in this system. It is possible that our study missed the transient LD signature, which appears immediately after initial admixture but then is lost as populations homogenize. Low genetic diversity in this system could also explain why we see evidence of immigration yet no LD. Admixture between populations that share many alleles due to low species-wide genetic diversity would produce a minor LD signature that could be too weak to observe (Silio et al. 2016).

***Genetic Ancestry*—**With the improved resolution supplied by genomics data, we determined that the likely sources of MHI population expansion were a combination of migration from the NWHI as well as productivity within the MHI. The majority of MHI individuals exhibited some genomic ancestry from the NWHI (most heavily represented by French Frigate Shoals in the current sample set). Island-level population structure was resolved via DAPC analyses (Figure

2), particularly for populations with larger sample sizes including Kauai, Oahu, Molokai, and French Frigate Shoals (Figure 2b). The assignment tests performed in STRUCTURE were less able to accurately resolve island-level population structure (Figure 3) an outcome that was likely due to limitations in STRUCTURE software for resolving fine-scale population structure (Janes et al. 2017; Latch et al. 2006). When weak differentiation between populations is exacerbated by low genetic diversity, as observed in the Hawaiian monk seal, the multivariate analysis used in DAPC may be more efficient at resolving structure (Jombart et al. 2010).

When attempting to assign MHI individuals to sites in the NWHI using assignPOP, 73% of individuals showed ancestry from the nearest atoll (French Frigate Shoals), while a smaller set (25%) showed ancestry to the farther northwest atolls (Pearl and Hermes Reef, Midway Atoll, Kure Atoll; "NWHI-Combined" in Table 3). When assigning MHI individuals to all islands, including both MHI and MWHI sites, 63% still showed some ancestry from French Frigate Shoals and 36% assigned to Molokai (Table 4). We also found evidence for French Frigate Shoals ancestry in the NWHI-combined atolls which coincides with records that NWHI-combined (Kure) was previously supplemented with individuals translocated from French Frigate Shoals (Baker et al., 2011; Johanos et al., 2014; NMFS, 2007). French Frigate Shoals was well-sampled in this study, potentially upwardly biasing the input from French Frigate Shoals relative to other, less-well sampled islands or those that were not sampled at all (e.g., Laysan and Lisianski). Thus, migration from NWHI could have been from French Frigate Shoals specifically, from one of these un- or under-represented islands, or both. Overall, our finding of connectivity between NWHI and MHI corroborates previous observational movement data (Johanos et al., 2014), and adds net directionality (from NWHI to MHI) to our understanding of Hawaiian monk seal dispersal patterns.

We inferred migration from the NWHI to the MHI and between islands within the MHI, particularly from Molokai to the rest of the MHI. Within the MHI, Molokai and Oahu exhibit signatures of migration including assignment of individuals to the other island in the DAPC (Figure 2c) and assignment of an individual sampled in Molokai to Oahu in assignPOP (Table 4). There is also some evidence of unidirectional migration between Molokai and Kauai, as individuals found on Kauai were assigned to Molokai but none of the Molokai individuals assigned to Kauai (Figure 2c; Table 4). The widespread nature of Molokai ancestry may be linked to the importance of one particular pupping site, Kalaupapa on Molokai. This pupping site was among the first areas of heavy pupping use in the MHI, and continues to be the most concentrated pupping site, contributing as many as 13 out of 30 pups observed in the MHI in 2018 (HMSRP, unpublished 2019).

***Management Implications*—**While this study was not specifically designed to evaluate stock structure of the Hawaiian monk seal population, initial findings from our investigation of MHI population expansion limited structure and high connectivity between NWHI and MHI monk seals. The similar genetic diversity metrics in the NWHI and MHI suggest populations that have undergone processes of genetic drift in tandem rather than in isolation. The lack of linkage disequilibrium suggests that admixture events between NWHI and MHI were not mixing widely divergent groups. And, finally, the genetic ancestry analysis showed substantial shared ancestry (63% of MHI samples with some NWHI ancestry), suggesting frequent migration and breeding between regions.

Objective 2: Investigate individual variation in reproductive success

Reproductive Success: Parentage—In our exhaustive parentage analyses, using both SNP and microhaplotype data, we were unable to accurately resolve any parent-offspring relationships, even for known mother-pup pairs. Across the 4 genomic data sets (Table 1) and 3 analytical approaches (CERVUS, COLONY and Sequoia) we used to reconstruct parentage, we were unable to accurately assign any parents for any pups. This even included assigning maternity to pups using only the set of their known moms as potential parents. Our absolute inability to reconstruct any parentage was not due to a lack of power in our data set; rather, it was because genetic diversity was so low in this species that all individuals, related or not, shared many alleles. Our Dps values revealed that known parents and offspring shared as many alleles as presumably unrelated pairs of individuals (Figure 4). This pattern of completely overlapping Dps values for known parent-offspring relationships and for unknown relationships was observed in both our SNP data sets and our microhaplotype data sets (Figure 4). Microhaplotype Data Set #2 contained few loci and had poor resolution for parent-offspring pairs with a large range of Dps values (Figure 4).

The power to assign parentage depends upon the number and variability of loci (Morin et al. 2004). The heterozygosity we observed for monk seals was far below levels required for accurate parentage assignment (0.20–0.40 for SNPs; Morin et al. 2004), suggesting that accurate parentage analysis may not be possible in a species with such low genetic diversity. It is possible that the multi-allelic microsatellite loci could be more informative for parentage assignment in monk seals than our SNP data sets (Hammerly et al. 2013), as the Minhovets study reviewed above had some success in assigning parentage with relatively comprehensive sampling from a breeding population. However, genomic data sets and microhaplotypes in particular generally outperform microsatellites in parentage even with sparse sampling (Hauser et al. 2019; Huisman 2017; Kaiser et al. 2017; Labuschagne et al. 2015), suggesting that differences in the scope of sampling likely contribute to parentage assignment outcomes in the current study. Regardless, difficulties in reconstructing parentage using molecular markers in Hawaiian monk seals highlights the value of observational data on maternity to provide essential information about monk seal reproduction.

VII. Future Directions for Genomics Research Collaboration

Work under the collaboration between HMSRP and University of Wisconsin, Milwaukee is still ongoing, and several steps will be taken to build on the preliminary work described above.

Objective 1: Investigate the genomic legacy of MHI population expansion

- HMSRP will provide additional samples for thorough ancestry analysis of MHI relative to NWHI; we plan to provide an additional 200 samples to provide even coverage from all possible sites (due to lack of staff presence, limited samples are available from Ni'ihau in the MHI, and Nihoa and Mokumanamana in the NWHI).
- The analysis of ancestry and population assignment will be expanded to include all of the NWHI sites as potential origins of MHI seals.
- Anticipated final deliverables will include a database of genomic data, and a manuscript on genomic diversity and NWHI-MHI migration (which has potential to provide important communication points regarding the history of MHI population expansion).

Objective 2: Investigate individual variation in reproductive success

- We have established contact between the labs at UWM and Yale so that labs working with microsatellites and genomic data could compare methodologies and cross-test samples in parentage analysis.
- UWM will run parentage test on samples previously tested by Minhovets and Gaughran to determine whether differing genetic markers, or different levels of background population sampling contribute to difficulty in confidently distinguishing parent-offspring pairs.
- Anticipated final deliverables will include a report on analysis completed and limitations of molecular parentage analysis for a low-diversity species (potential for manuscript publication is uncertain).

Objective 3: Design high-resolution SNP panel for targeted, low-cost future analyses.

- UWM will continue the effort to develop a SNP panel appropriate for future analysis.
- Anticipated final deliverables will include SNP primer sequences developed and published in GenBank.
- Anticipated final deliverables will include protocols produced to facilitate future use of the SNP panel in any qualified genomics lab.

VIII. Tables

Table 1. Summary of data sets used with their respective genotyping rate (%) filter for loci and individuals (Ind) and the resulting number of individuals (NInd), loci (NLoci) and islands (NIslands) included in subsequent analyses.

Data Set	Loci (%)	Ind (%)	N_{Ind}	N_{Loci}	$N_{Islands}$
SNP #1	50	50	90	3,451	11
SNP #2	60	30	135	151	13
Microhaplotype	0	0	163	47,616	13
Microhaplotype Panel	60	0	161	77	13

Table 2. Genetic diversity per population (top) and region (bottom) as per observed and expected heterozygosity (Ho and He, respectively), inbreeding coefficient (FIS), and number of private alleles corrected for sample size via resampling (PA). Sites in gray are those with low sample sizes (n=1) and subsequently H_e and F_{IS} were unable to be calculated.

	Island	N	H_O	H_e	F_{IS}
NWHI	Kure	1	0.063	NA	NA
	Midway	1	0.054	NA	NA
	Pearl and Hermes	2	0.063	0.069	0.089
	French Frigate Shoals	6	0.057	0.072	0.202
	Necker	4	0.059	0.063	0.072
	Nihoa	4	0.062	0.069	0.100
MHI	Kauai	17	0.058	0.065	0.111
	Oahu	31	0.060	0.066	0.101
	Molokai	22	0.059	0.071	0.166
	Maui	1	0.061	NA	NA
	Hawaii	1	0.055	NA	NA

Region	PA	N	H_O	H_e	F_{IS}
NWHI	1019	18	0.060	0.068	0.116
MHI	988	72	0.059	0.067	0.126

NWHI = Northwest Hawaiian Islands, MHI = main Hawaiian Islands

Table 3. Population assignment of individuals sampled in the MHI to the NWHI: NWHI-Combined (Kure, Midway, and Pearl and Hermes), French Frigate, Necker, and Nihoa. Values represent the number of MHI individuals from their respective islands (Sampled MHI) that were assigned to NWHI. NWHI assignment subtotals and percentages of the total number of MHI individuals were tabulated at the bottom.

		NWHI Assignment			
		NWHI-Combined	French Frigate Shoals	Necker	Nihoa
Sampled MHI	Oahu	9	21	0	0
	Molokai	5	17	0	1
	Kauai	4	13	0	0
	Maui	0	1	0	0
	Hawaii	0	0	0	1
Total		18	52	0	2
%		25	72	0	3

Table 4. Population assignment of MHI individuals to the all islands: Kauai, Oahu, Molokai, NWHI combined (Kure, Midway and Pearl and Hermes), French Frigate Shoals, Necker and Nihoa. Values represent the number of MHI individuals from their respective islands (Sampled MHI) that were assigned to the islands. Islands that received no assignment were omitted from the table. Population assignment subtotals and percentages of the total number of MHI individuals were tabulated at the bottom.

		Island Assignment		
		French Frigate Shoals	**Oahu**	**Molokai**
Sampled MHI	Oahu	15	0	11
	Molokai	9	1	6
	Kauai	9	0	3
	Maui	1	0	0
	Hawaii	1	0	0
Total		35	1	20
%		63	2	36

IX. Figures

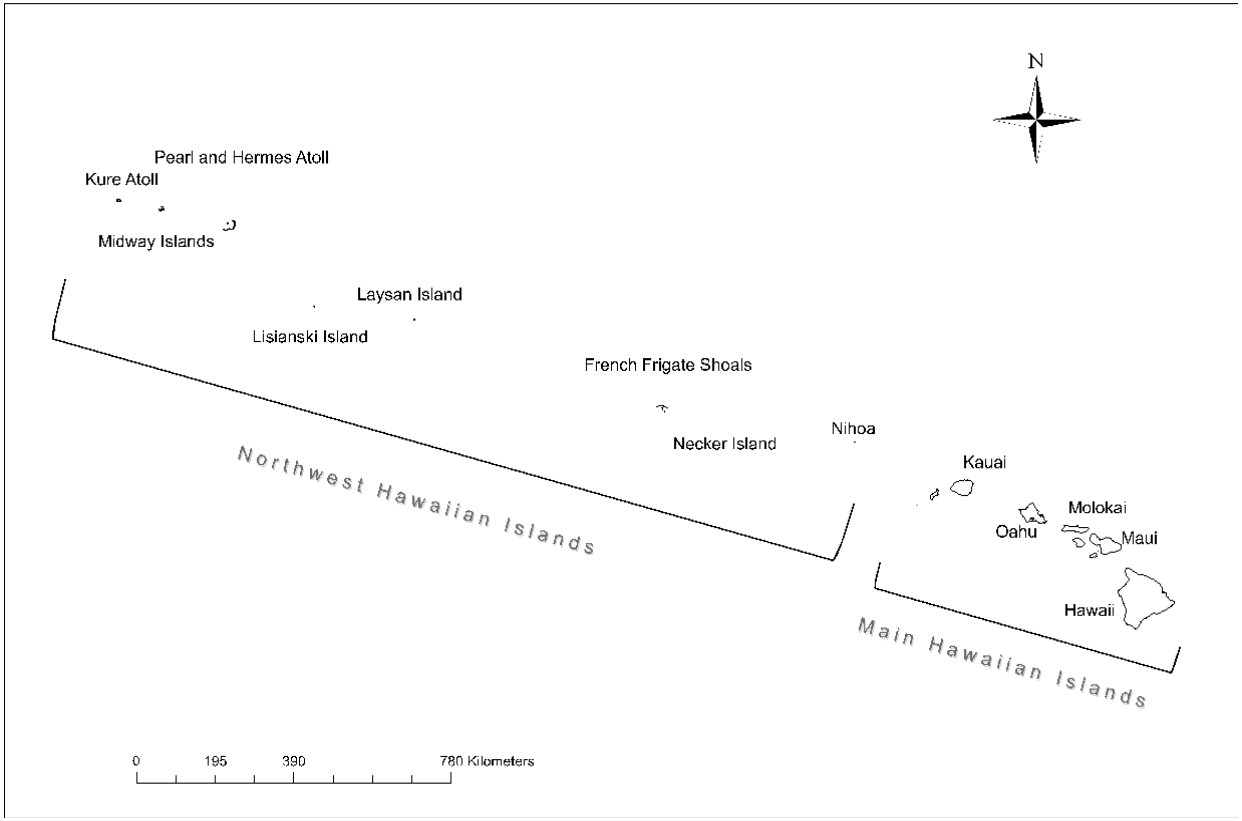

Figure 1. The 13 Hawaiian Islands (black text) and 2 regions (gray text) from which Hawaiian monk seal were sampled. Brackets encircle islands included in each region. Northwest Hawaiian Islands (aka NWHI) include Kure, Midway, Pearl and Hermes, Lisianski, Laysan, French Frigate Shoals, Necker, and Nihoa. The main Hawaiian Islands (aka MHI) include Kauai, Oahu, Molokai, Maui, and Hawaii.

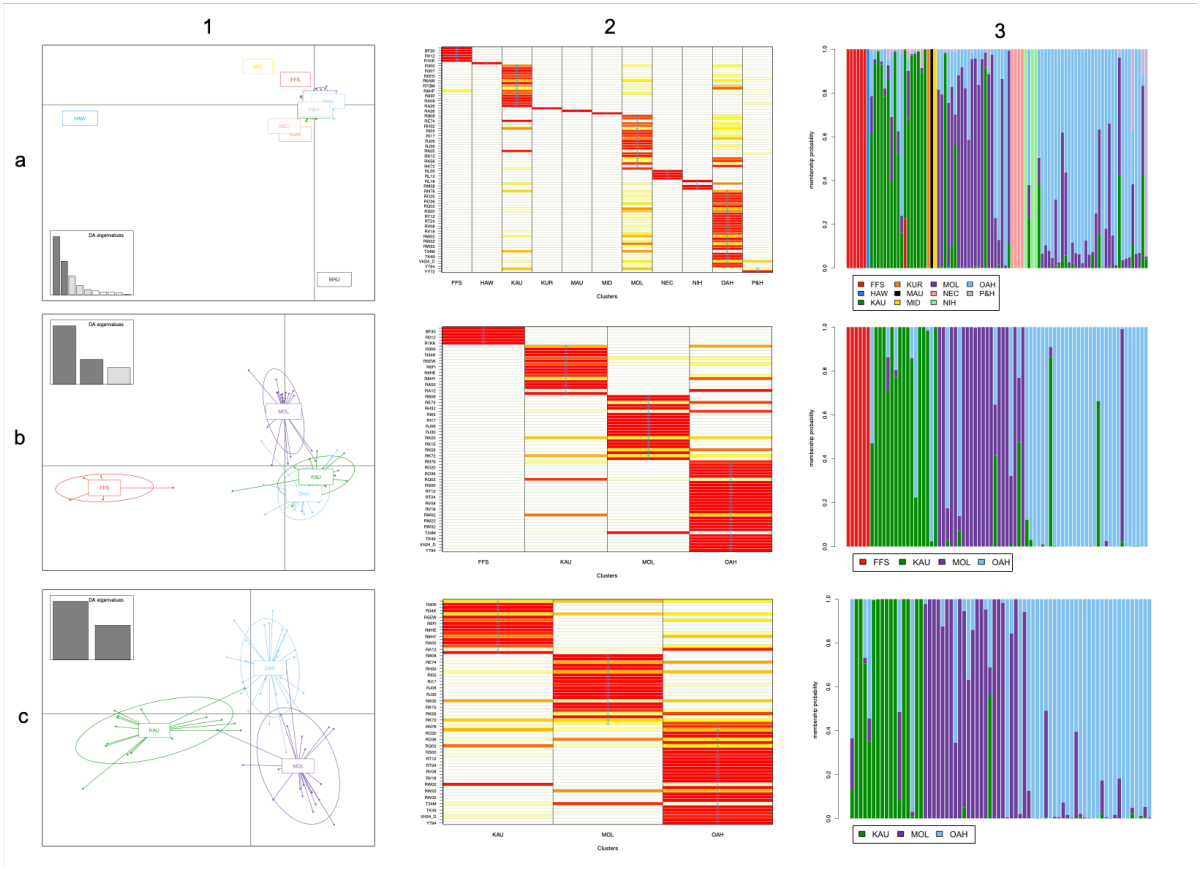

Figure 2. Island-level population structure from DAPC analyses for (A) 11 islands with their representative number code in parentheses: Nihoa (NIH), Kauai (KAU), Oahu (OAH), Molokai (MOL), Maui (MAU), Hawaii (HAW), Necker (NEC), French Frigate Shoals (FFS),), Pearl and Hermes (P&H), Midway (MID), and Kure (KUR) (B) 4 islands with >5 samples: Kauai (KAU), Oahu (OAH), Molokai (MOL) and French Frigate Shoals (FFS), and (C) 3 main Hawaiian Islands with >5 samples: Kauai (KAU), Oahu (OAH), Molokai (MOL). Columns 1 through 3 show DAPC plot, assignplot of DAPC results, and barplot of DAPC results, respectively.

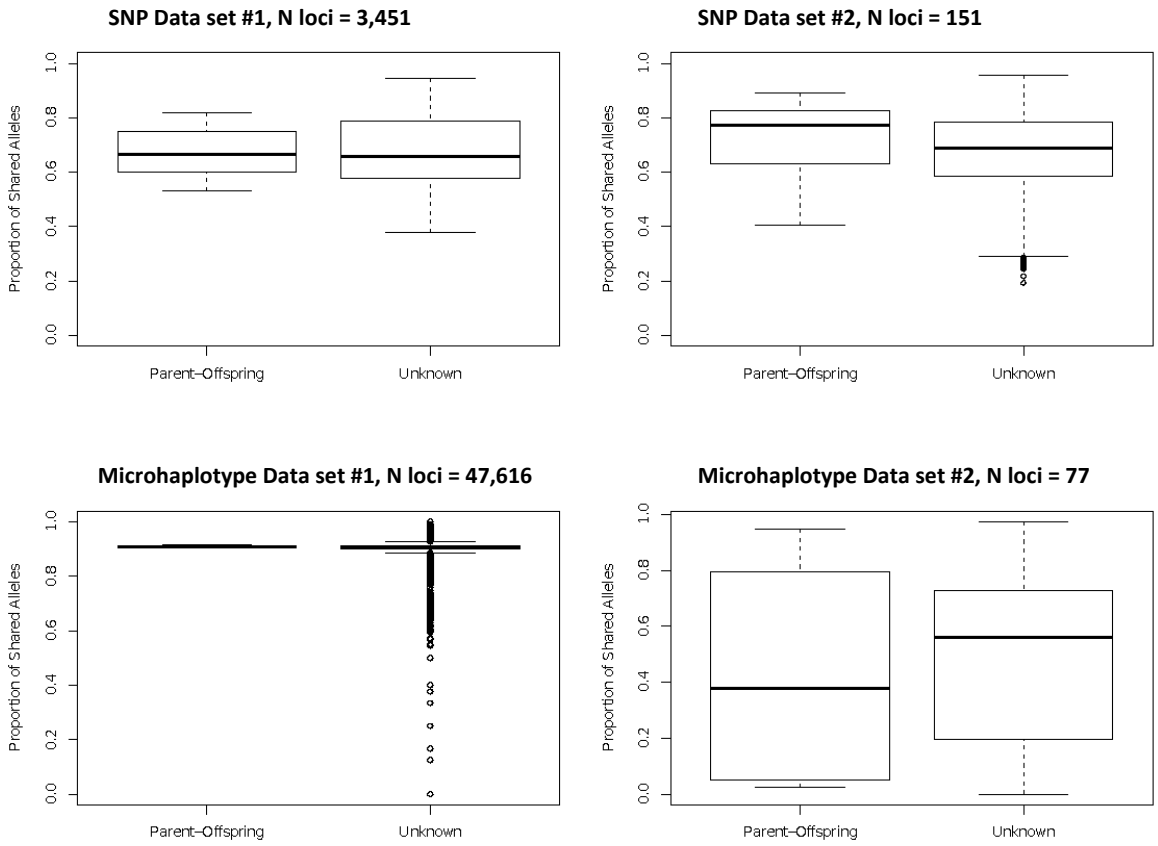

Figure 3. Proportion of shared alleles (aka Dps) for the 4 molecular data sets used to reconstruct parentage for Hawaiian monk seals (see Table 1 for more details on each data set): SNP Data Set #1 with 3,451 loci, SNP Data Set #2 with 151 loci, Microhaplotype d Data Set #1 with 47,616 loci, and Microhaplotype Data Set #2 with 77 loci. Boxplots depict the distribution of values for known parent-offspring pairs (left) and unknown relationships (right); thick black lines represent mean, upper and lower lines of the boxes represent the 2nd and 3rd quantiles, error lines denote the 1st and 4th quantiles, and points denote data values that lie outside of the quantiles.

X. References

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