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Fidelity to natal social groups and mating within and between social groups in an endangered false killer whale population

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ABSTRACT: Most mammals exhibit natal dispersal of one or both sexes, a behavior that likely evolved in part to reduce the chances of breeding with close relatives. When natal social group fidelity of both sexes has been documented, the risk of inbreeding is reduced by breeding among rather than within social groups. We investigated mating patterns in an endangered population of false killer whales *Pseudorca crassidens* from the main Hawaiian Islands (USA) using both genetic and photo-identification data. We tested the presence of the 2 most commonly observed inbreeding avoidance behaviors, i.e. natal dispersal and exogamy (mating occurring primarily among individuals from different social groups). Because not all mother–offspring pairs or individual ages were known prior to this study, we used re-sighting histories to determine plausible ranges of birth year for individuals, thereby limiting the pool of candidate parents and increasing analytical power. We identified 32 parent–offspring pairs, revealing strong natal social group fidelity for both sexes. Our results indicate that between 36 and 64% of matings involved individuals from the same social group. Because the population declined from over 400 to around 150 individuals between the 1980s and early 2000s, the intra-group matings may be the result of reduced opportunities for inter-group mating since the decline. Prior to the decline, social groups may have been sufficiently large that selective pressure to develop inbreeding avoidance mechanisms was low, or the population may have evolved alternate inbreeding avoidance mechanisms such as kin recognition.

KEY WORDS: Cetacean · *Pseudorca crassidens* · Social structure · Relatedness · Natal dispersal · Inbreeding · Photo-identification

1. INTRODUCTION

In most species of mammals, members of one or both sexes disperse from their natal group (Greenwood 1980). Natal dispersal can confer several benefits to dispersing individuals (Lawson Handley & Perrin 2007). By leaving their natal group, individuals reduce the likelihood of competing with close kin for access to food, territories, and mates (Greenwood 1980, Liberg & von Schantz 1985, Peacock 1997). Dispersal can also increase foraging success when food resources vary in time and space in an unpredictable manner (Johnson & Gaines 1990). Perhaps the greatest fitness benefit of natal dispersal is that it reduces

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the chances of inbreeding depression resulting from mating with close relatives (Pusey 1987, Pusey & Wolf 1996).

However, there are also costs associated with natal dispersal (Lawson Handley & Perrin 2007). Dispersers often have to traverse inhospitable territory, possibly facing an increased risk of predation or a lack of adequate food resources (Johnson & Gaines 1990). They may also lose the benefit of familiarity with their natal foraging habitat, and the foraging tactics best suited to the natal habitat may not be transferrable to new habitats (Greenwood 1980). In highly social species, dispersers could face aggression and hostility when attempting to join a new social group. The importance of familiarity with the habitat and the inclusive fitness benefits of foraging with kin can be especially high in species that hunt cooperatively and/or share food resources (Baird & Dill 1996, Wright et al. 2016).

In cases where the fitness costs of natal dispersal outweigh the benefits, an alternate mechanism for avoiding inbreeding is for members of both sexes to remain in their natal social groups, but mate primarily or exclusively with members of other social groups. This unusual social organization has been described in 2 species from the cetacean subfamily Globicephalinae: the killer whale *Orcinus orca* and the long-finned pilot whale *Globicephala melas*. Paternity analyses in several populations of killer whales and of a group of pilot whales killed in a drive hunt indicated that mating occurred almost entirely among individuals from different social groups, which was suggested as a mechanism to avoid inbreeding (Amos et al. 1993, Barrett-Lennard 2000, Pilot et al. 2010). However, studies of a small, endangered population of killer whales found that mating within social groups was common (Ford et al. 2011, 2018).

The distribution of mitochondrial DNA (mtDNA) haplotypes within a population of a third globicephalid species, the false killer whale *Pseudorca crassidens*, suggests that false killer whales may also have a social organization in which both sexes exhibit life-long philopatry to their natal social group (Martien et al. 2014). The small, isolated population of false killer whales that is resident to the main Hawaiian Islands (MHI), USA (Baird et al. 2008, Martien et al. 2014) has undergone a decline over the past 25 yr, from at least 400 (Reeves et al. 2009) to about 167 (CV = 0.14) individuals (Bradford et al. 2018). Using association data from a long-term photo-identification catalog, Baird et al. (2012) identified 3 large (40+ individuals) social clusters within the population, which we henceforth refer to as social groups. Although different social groups do interact with each other, individuals spend the majority of their time with members of their own social group. In a study that included samples from nearly two-thirds of the individuals in the population, Martien et al. (2014) found that some high-frequency mtDNA haplotypes were restricted to certain social groups, suggesting strong natal group fidelity for both sexes.

Strong natal group fidelity is also suggested by patterns of genetic differentiation between false killer whale populations in the North Pacific. The MHI population and nearby Northwestern Hawaiian Islands (NWHI) population are characterized by 3 closely related mtDNA haplotypes that are not found in any other populations, suggesting that the Hawaiian Islands share a common colonization history and are essentially closed to immigration from or emigration to the offshore population (Martien et al. 2014). However, patterns of variation in nuclear DNA (nuDNA) suggests that contemporary gene flow is higher between the NWHI and offshore populations than it is between the 2 insular populations, MHI and NWHI. Martien et al. (2014) concluded that the primary mechanism of gene flow between the MHI, NWHI, and offshore populations is through intergroup breeding (i.e. social group exogamy) without dispersal.

We examined parentage patterns within the MHI population of false killer whales using previously published (Martien et al. 2014) nuDNA microsatellite genotype data. We tested the hypotheses that both males and females remain in their natal social group for life and that mating occurs primarily between individuals belonging to different social groups. Genetic parentage analyses typically require large amounts of data. Most such studies in marine systems have involved cases where mother–offspring pairs can be identified observationally so that only paternity needs to be assessed, or where individual ages are known so that the pool of candidate parents for a focal individual is restricted to those known to be sexually mature when the focal individual was conceived (Clapham & Palsbøll 1997, Krützen et al. 2004, Frasier et al. 2007, Frère et al. 2010, Ford et al. 2011). Obtaining such data remains infeasible for most marine species, including false killer whales. However, we increased the number of parent–offspring pairs that could be confidently assigned by using long-term photographic identification data (Baird 2016) to restrict the pool of candidate parents in our parentage assessments.

2. MATERIALS AND METHODS

2.1. Data

We used published genetic data (Martien et al. 2014) generated from 107 false killer whales sampled between 2000 and 2011, all of which have been assigned to the MHI insular population based on analysis of association patterns from photo-identification studies (Baird et al. 2008, Baird 2009). Most samples were collected as part of the photo-identification study conducted by Baird et al. (2008), while the remainder were collected during Southwest (SWFSC) and Pacific Islands Fisheries Science Center (PIFSC) research surveys or opportunistically by other researchers. All samples were preserved frozen or in a 20% dimethylsulfoxide solution saturated with NaCl (Amos & Hoelzel 1991, Amos 1997) and archived in the SWFSC Marine Mammal and Sea Turtle Molecular Research Sample Collection (https://swfsc.noaa.gov/MMTD-TissueCollection/). Our data set represents a subset of the genetic data presented by Martien et al. (2014).

Each sample was genetically sexed, sequenced at a 947 base pair region of the mitochondrial control region, and genotyped for 16 nuclear microsatellite loci. Details of laboratory analyses and data review for quality control and assurance are reported by Martien et al. (2014). The microsatellite genotypes of all animals can be found on Dryad (http://dx.doi.org/doi:10.5061/dryad.2pq32), while mitochondrial sequences are available from GenBank (accession nos. EF601197, EF601198, EF601201, KJ567088).

We stratified the samples according to the social groups identified by Baird et al. (2012) through social network analyses of 12 yr of photo-identification data. Baird et al. (2012) used an analysis of network modularity to identify clusters of individuals that consistently associated with each other (maximum modularity = 0.63; Baird et al. 2012). They identified 3 main social groups, each containing more than 40 individuals. They also identified 4 small peripheral social groups (between 2 and 16 individuals). However, at least some of the peripheral social groups are likely sampling artifacts, as most of the animals contained in those groups were only sighted once (Baird et al. 2012, Martien et al. 2014). We therefore stratified samples into the 3 main social groups and treated the social group affiliation of samples from the 4 peripheral groups as unknown. We also classified as unknown some individuals that were not photographically identified at the time they were biopsied but are considered part of the MHI insular population because they were sighted with animals known to be part of that population. Sample size is relatively even among these 4 strata (Table 1).

2.2. Population differentiation

Martien et al. (2014) found significant differentiation among all 3 main social groups using nuclear markers. They also found significant differentiation in the mitochondrial data set between Group 3, in which all individuals possess Haplotype 1, and the other 2 groups, in which Haplotypes 1 and 2 occur at nearly equal frequencies. We recalculated the estimates of differentiation of Martien et al. (2014) due to the fact that 2 animals whose social group affiliation was unknown at the time of that publication have subsequently had their social group affiliation resolved through photo-identification (Tables 1 & 2). We estimated genetic differentiation and statistical significance using the 'strataG' (Archer 2016) package in the R programming language (R Development Core Team 2014). We further tested for evidence of

Table 1. Mitochondrial control region haplotype frequencies for each sex within each social group of false killer whales. F: female; M: male

Group	Group size (n)	Sex	Haplotype 1	2	5
1	34	F	8	10	0
		M	11	5	0
2	25	F	9	6	0
		M	5	4	1
3	24	F	13	0	0
		M	11	0	0
Unknown	24	F	13	1	0
		M	9	1	0

Table 2. Estimates of genetic differentiation between social groups of false killer whales. Φ_{ST} was calculated based on the Tamura & Nei (1993) model with invariant sites. Note that values differ slightly from those reported by Martien et al. (2014) because 2 animals whose social group affiliation was unknown at the time of that publication have subsequently had their social group affiliation resolved through photo-identification

Group comparison	mtDNA Φ_{ST}	χ^2 p	Nuclear DNA F_{ST}	F'_{ST}	χ^2 p
1 vs. 2	−0.028	0.880	0.007	0.027	0.022
1 vs. 3	0.381	<0.001	0.014	0.048	0.001
2 vs. 3	0.328	0.001	0.013	0.055	0.002

sex-biased dispersal in the microsatellite data set using the sex-biased dispersal test of FSTAT (Goudet 2001, Goudet et al. 2002), which examines differences between males and females with respect to mean and variance of assignment indices, F_{IS}, F_{ST}, relatedness, and within-group gene diversity (H_S), and assessed significance through 1000 permutations. Because the sex-biased dispersal test in FSTAT looks for differences between the sexes in the number of immigrants present in the sample, it cannot detect gametic gene flow (i.e. gene flow due to intergroup mating without dispersal).

2.3. Relatedness

We estimated relatedness among individuals using the program 'Co-ancestry' (Wang 2011). To decide which relatedness estimator performed best for our data set, we first used the observed allele frequencies in the overall data set to simulate 100 dyads each of parent–offspring, full siblings, half siblings, first cousins, and unrelated individuals. We specified the proportion of loci typed as 0.966 to match our actual dataset, and set the genotyping error rate at 0.0258 based on Martien et al.'s (2014) estimated per-allele error rate of 0.013. 'Co-ancestry' then calculated the correlation coefficient between the true relatedness values for all dyads in the simulated data set and the relatedness estimate for each of 7 different estimators. We then used the estimator with the highest correlation coefficient to calculate the pairwise relatedness of individuals within each of the social groups and among all individuals in the data set. We calculated the difference between relatedness within a social group and relatedness for the entire data set and compared that difference to the upper 95th percentile of the null distribution (generated by the bootstrapping method implemented in 'Co-ancestry') to determine whether individuals from the same social group are significantly more related to each other than expected at random.

We also used the estimates of relatedness from 'Co-ancestry' (Wang 2011) to further test for evidence of sex-biased dispersal. We calculated mean pairwise relatedness between females from the same social cluster and compared it to the mean pairwise relatedness between males from the same social cluster. We again used the bootstrapping function in 'Co-ancestry' to assess the statistical significance of the difference in relatedness between female dyads and male dyads.

2.4. Parentage analyses

We used the maximum likelihood approach implemented in CERVUS 3.0.3 (Kalinowski et al. 2007) to identify putative parent–offspring pairs in our dataset. For most of our analyses, all males were considered candidate fathers, while the pool of candidate mothers was restricted to females that had the same haplotype as the focal individual. We refer to these as haplotype-restricted analyses. We conducted a sensitivity test in which all females, regardless of haplotype, were included in the candidate mother pool.

For many dyads, CERVUS was unable to determine which individual was the parent and which the offspring. We therefore conducted an age-restricted analysis in which we used photographic data and sighting dates to determine relative ages of individuals to further restrict the pool of candidate parents for each individual. Using the long-term photo-identification catalog (Baird et al. 2008), individuals were classified into stages (adults, sub-adults, or juveniles) based on markings on the dorsal fin, relative size in photographs, the number of years elapsed since they were first identified, and whether they have been observed with a small calf in close proximity (Table S1 in the Supplement at www.int-res.com/articles/suppl/n040p219_supp.pdf). Individuals were classified as juveniles if they were estimated to be less than 6 (females) or 9 (males) yr old, adults if they were estimated to be older than 10 (females) or 15 (males) yr (Ferreira et al. 2014), and sub-adults if they were estimated to be between the juvenile and adult age classes. Because individually identifying marks (e.g. dorsal fin notches and body scars) are typically not present in neonates or small calves (Baird et al. 2008), we were unable to identify mother–offspring pairs from photographic data. Once individuals had been assigned to life history stages, we estimated the latest year the individual could possibly have been born (birth_year) and the earliest year the individual could possibly have reproduced (repro_year). An individual whose repro_year was later than the birth_year for the focal individual could be excluded from the focal individual's pool of candidate parents. To avoid falsely excluding a true parent from the candidate pool, we purposely erred on the late side when determining birth_year and on the early side when determining repro_year. Details of how the life history stage, birth_year, and repro_year of an individual were determined are provided in the Supplement. For the age-restricted analysis, the candidate mother pool was again restricted to only those females that had the same haplotype as the focal individual.

CERVUS determines whether a parent should be assigned to an individual by calculating the difference in the log likelihoods of the most likely and second most likely putative parents (ΔLOD). We used CERVUS's simulation function to determine the threshold value of ΔLOD at which parent–offspring pairs could be assigned with 95% confidence. As in the 'Co-ancestry' analyses, we specified the proportion of loci typed as 0.966 and set the per-genotype error rate at 0.0258. We ran paternity and maternity analyses separately and calculated the population allele frequencies based on all MHI samples. We set the proportion of candidate parents sampled at 0.5. Although our sample size (n = 107) is more than two-thirds of the current estimated population size (N ~ 150), the proportion of sampled parents is lower because some of the oldest animals in our sample set will have parents that died prior to the start of sample collection. We examined sensitivity of the results to this parameter by re-running the haplotype-restricted analysis with the proportion set to 0.35 and 0.65.

To account for the large number of close relatives expected within the population, we ran the CERVUS simulations assuming that a proportion p of the population was related to the focal offspring with relatedness coefficient of r. The 'Co-ancestry' analysis revealed that 7.5% of the pairwise relatedness values within the population were equal to or greater than the expected value for half siblings (0.25) and 22.5% were equal to or greater than the value expected for first cousins (0.125). We therefore ran the CERVUS analyses with p = 0.075 and r = 0.25 and conducted a sensitivity test for the haplotype-restricted analysis in which p = 0.225 and r = 0.125.

2.5. Effective population sizes

We estimated the effective population size (N_e) of different strata with the program 'LDNE' (Waples 2006, Waples & Do 2008), which uses estimates of linkage disequilibrium (LD) to infer N_e. We excluded alleles with frequencies less than 0.05 and used the jackknife procedure described by Waples & Do (2008) to calculate 95% confidence intervals.

3. RESULTS

The tests we conducted in FSTAT for sex-biased dispersal between social groups did not reveal significant differences between males and females for any of the metrics we examined. Haplotype frequencies by sex and group membership are shown in Table 1.

'Co-ancestry' identified TrioML, a relatedness estimator that takes into account inbreeding (Wang 2007), as having the highest correlation with true relatedness values in our data set. We therefore used TrioML for all further relatedness calculations. The mean pairwise relatedness value between individuals was 0.077 ± 0.012, with a range from 0 to 0.737 (Fig. S1). The mean and variance of pairwise relatedness values within each of the social groups (Group 1: r = 0.0906 ± 0.0162; Group 2: r = 0.0873 ± 0.0177; Group 3 r: = 0.0971 ± 0.0166) was higher than mean pairwise relatedness for the population as a whole, although the difference was only statistically significant for Groups 1 and 3. The relatedness between females from the same social group (0.0863 ± 0.0154) was higher than that between males from the same social group (0.0754 ± 0.0115), but the difference was not statistically significant.

The haplotype-restricted CERVUS analysis identified 32 parent–offspring dyads (Table 3). For 19 of these dyads, CERVUS was able to unambiguously determine which individual was the parent and which the offspring, as the ΔLOD threshold was only exceeded for 1 of the 2 possible polarities of the pair. For the remaining 13 dyads, however, the ΔLOD value for the pair was above the threshold value regardless of which individual was presumed to be the parent and which the offspring. The age-restricted analysis identified the same 32 dyads as were identified in the haplotype-restricted analysis. However, by using age data to further restrict the candidate parent pools, the age-restricted analysis was able to distinguish the parent from the offspring for an additional 4 dyads (Table 3).

Of the dyads for which we could not determine which individual was the parent and which was the offspring, 3 were female–female dyads that could therefore be classified as mother–daughter and 1 was a male–male dyad that we classified as father–son (Table 3). For the 5 remaining mixed-sex dyads, we were unable to determine whether they were mother–son or father–daughter.

Thirteen of the 16 mother–offspring pairs identified by CERVUS involved individuals from the same social group, with the remaining 3 mother–offspring pairs involving individuals that were not photographed when they were biopsied (Table 3). In contrast, only 3 of 11 father–offspring pairs included individuals known to belong to the same social group. There were 2 father–offspring pairs whose members are known to belong to different social

Table 3. Parent–offspring dyads of false killer whales identified by CERVUS in haplotype-restricted and age-restricted analyses. Parents are indicated in **bold** font. Dyads where the parent is marked with an asterisk (*) are those for which CERVUS was unable to determine which individual was the parent based only on genetic data, but for which we could resolve the polarity of the relationship with ages inferred from photo-identification. Dyads for which we were unable to resolve the polarity of the relationship with either genetic or photo-identification data are in the bottom 9 rows. The group membership of individuals assigned to one of the peripheral social groups (Baird et al. 2012) is marked as P, while individuals that are not linked to a photograph are marked 'No photo'. F: female; M: male

Dyad type	ID Offspring	ID Parent	Sex		Group membership		Haplotype	
			Offspring	Parent	Offspring	Parent	Offspring	Parent
Mother–offspring	33890	**123188**	F	**F**	3	3	1	1
	33904	**33903**	M	**F**	3	3	1	1
	33908	**33902**	M	**F**	3	3	1	1
	49044	**30072**	F	**F**	No photo	2	1	1
	49049	**30081**	M	**F**	No photo	2	1	1
	75676	**75679**	F	**F**	1	1	1	1
	75678	**75679**	F	**F**	1	1	1	1
	98743	**30072**	F	**F**	2	2	1	1
	98746	**49051**	M	**F**	2	2	2	2
	132632	**132631**	F	**F**	2	2	1	1
	75677	**75666***	F	**F**	1	1	2	2
	92256	**75679***	M	**F**	1	1	1	1
	98737	**98732***	F	**F**	1	1	2	2
Father–offspring	33890	**30078**	F	**M**	3	No photo	1	2
	33907	**18954**	M	**M**	3	P	1	1
	92256	**23317**	M	**M**	1	1	1	1
	98736	**33907**	F	**M**	1	3	2	1
	98740	**30078**	F	**M**	1	No photo	1	2
	98744	**30078**	M	**M**	2	No photo	1	2
	98745	**49052**	M	**M**	2	2	2	5
	98746	**23316**	M	**M**	2	1	2	1
	102500	**30078**	M	**M**	1	No photo	1	2
	91277	**23317***	F	**M**	P	1	1	1
	Ind1	Ind2	Ind1	Ind2	Ind1	Ind2	Ind1	Ind2
Mother–offspring	27453	27454	F	F	3	No photo	1	1
	33902	45928	F	F	3	3	1	1
	49043	132642	F	F	2	2	1	1
Father–offspring	33886	45932	M	M	3	3	1	1
Unresolved mixed-sex	45932	33895	M	F	3	3	1	1
	71016	71017	M	F	1	1	2	2
	91083	18955	M	F	P	P	1	1
	98738	23320	M	F	1	1	2	2
	102485	98743	M	F	3	2	1	1

groups (23316/98746 and 33907/98736) and 2 for which 1 individual was not assigned to 1 of the main social groups (23317/91277 and 18954/33907). One male that was not photographically identified at the time he was biopsied (30078) was identified as the father of 4 individuals spread across each of the 3 main social groups.

We calculated the maximum and minimum number of intra-group father–offspring dyads that are consistent with our results. The maximum value is obtained by assuming that male 30078 is from Group 1, along with 2 of his offspring, and the 2 other unassigned dyads (23317/91277 and 18954/33907) represent intra-group dyads. These assumptions result in 7 out of 11, or 64%, of the father–offspring dyads involving individuals from the same group. Making the opposite assumptions, that Male 30078 is from either Group 2 or 3 along with only 1 of his offspring and that dyads 23317/91277 and 18954/33907 represent inter-group dyads, results in a minimum value of only 4 out of 11, or 36%, of father–offspring dyads involving individuals from the same group.

Several individuals appear in more than 1 dyad identified by CERVUS. In addition to 30078, who fathered 4 individuals in the data set, 23317 was identified as the father of 2 individuals, 30072 as the mother of 2 individuals, and 75679 as the mother of 3 individuals. CERVUS was able to assign both a mother and a father to 3 individuals (33890, 92256, and 98745). Finally, CERVUS identified 1 multigenerational triad, with 18954 having fathered 33907, who in turn fathered 98736.

One of the dyads identified by CERVUS involved the only individual (49052) in the MHI insular population that did not possess 1 of the 2 haplotypes that characterize the population. Instead, he possessed haplotype 5, a haplotype also detected in animals sampled in offshore waters near Australia. This individual, a male first sighted as an adult in 1986 and a member of Social Group 2, was identified as the father of individual 98745, a male first sighted in 2010 also with Social Group 2. In an assignment test, Martien et al. (2014) found that 49052 assigned more strongly to the offshore population than to the MHI insular population, and that the exclusion probability for eliminating the MHI insular population as a potential source population for 49052 ($p = 0.027$) was close to their threshold for statistical significance ($\alpha = 0.01$). We repeated the exclusion test for 49052, but removed 98745's genotype when calculating the allele frequencies for the MHI insular population. The resulting exclusion probability was $p = 0.013$, still slightly higher than the recommended significance threshold of $\alpha = 0.01$ (Piry et al. 2004).

In our sensitivity tests, the number of parent–offspring dyads identified by CERVUS varied depending on the way the candidate parent pool was identified and the settings used in the simulation to determine the threshold value of ΔLOD above which parentage could be assigned with 95% confidence (Table 4). However, the patterns with respect to group membership were consistent across all of the CERVUS analyses we conducted (Table 4). There were no female–female dyads identified in any of the analyses involving individuals known to belong to different social groups, while 20–33% of the female–male and male–male dyads for which the social group membership of both individuals was known involved individuals from different social groups. All of the female–female dyads identified in the unrestricted analysis involved individuals with the same haplotype (Table S2).

The estimated effective population size of the MHI insular population is $N_e = 57.6$ with a 95% confidence interval of 47.2–71.8. Because inclusion of first-generation immigrants in the sample set results in a Wahlund effect that can positively bias the results of 'LDNE' (Waples & Smouse 1990), we also calculated N_e with individual 49052 excluded from the data set, resulting in an estimate of 57.9 (95% CI: 47.0–72.6).

Table 4. Number of parent–offspring pairs of false killer whales identified by CERVUS using different candidate parent pools and different assumptions regarding the proportion of parents in the sample and average relatedness among individuals in the population. Threshold ΔLOD scores are the values of the change in log likelihood at which a parent–offspring pair can be identified with 95% confidence and were determined by CERVUS simulation. Pair type indicates whether a pair includes 2 females (F/F), 2 males (M/M), or a female and a male (F/M)

		Candidate parent pool restricted by:			Sensitivity analyses		
		Unrestricted	Haplotype	Age	35% parents sampled	65% parents sampled	Low relatedness
Maternity threshold ΔLOD		8.26	6.59	6.79	7.44	5.27	4.28
Paternity threshold ΔLOD		7.33	7.33	7.44	8.52	6.11	4.95
Pair type	Group membership						
F/F	Same	6	9	9	8	10	11
	Different	0	0	0	0	0	0
	Unknown	1	2	2	1	2	2
F/M	Same	5	7	7	4	9	11
	Different	2	2	2	2	3	3
	Unknown	4	5	5	4	6	9
M/M	Same	3	3	3	2	3	3
	Different	1	1	1	0	1	1
	Unknown	3	3	3	1	5	6
Total		25	32	32	22	39	46

4. DISCUSSION

4.1. Parentage and patterns of mate choice

Our parentage analyses indicate that social structure within the MHI population is based on philopatry to natal social groups. We identified 9 mother–daughter dyads and 4 mother–son dyads in which the social group membership of both individuals was known. In all of these cases, both individuals were from the same social group, indicating that offspring of both sexes remain in the same social group as their mothers. This conclusion is further supported by the absence of Haplotype 2 from Group 3, indicating a lack of movement of individuals from Groups 1 and 2 into Group 3, and the non-significance of the tests for sex-biased dispersal. Although the statistical power of the sex-biased dispersal test from FSTAT is low (Goudet et al. 2002), the comparison of relatedness between female versus male dyads that we conducted in 'Co-ancestry' has been used successfully in other studies to detect subtle sex-biased dispersal that was not detected using other methods (Phillips et al. 2014).

The number of dyads identified by CERVUS varied depending on how we defined the pools of candidate parents and the assumptions we made regarding the proportion of parents sampled and the degree of relatedness among individuals within the population. However, this variation was due to variation in the threshold value of ΔLOD above which parentage could be assigned with 95% confidence, not due to changes in the most likely parent identified for each candidate offspring. Thus, although the certainty surrounding a specific dyad may depend on the parameters used in the analysis, the overall pattern that members of both sexes remain in their natal social group and that mating occurs both between and within groups is robust.

In the majority of mammals with a social organization consisting of social groups that are stable across multiple generations, individuals of one sex leave their social group prior to reaching sexual maturity (Greenwood 1980). Philopatry to natal social groups evolves when the benefits of remaining outweigh the potential costs, such as inbreeding and competition with close relatives (Greenwood 1980). For false killer whales, the benefits of natal social group fidelity may stem from the fact that they exhibit food sharing (Baird et al. 2008, Baird 2016), a behavior that increases the inclusive fitness in resident killer whales (Wright et al. 2016). False killer whales also engage in cooperative hunting by spreading out to search for prey. When one individual finds and captures a large prey item, the others in the hunting group converge and share the prey (Baird 2018). The high level of cooperation required for cooperative hunting to be successful could serve as a barrier to an individual joining a new social group with which it has not previously hunted. This would be especially true if the social groups use slightly different hunting strategies. Although the ranges of the 3 social groups are almost entirely overlapping, satellite telemetry has shown that they differ in terms of habitat use. Specifically, the social groups have different distributional 'hot spots' in which they spend a majority of their time, and they exhibit significant differences in the median depths they inhabit (Baird et al. 2012, Baird 2016). No data are available to assess possible dietary differences between the social groups, but it is possible that these differences in habitat preference have led to different hunting strategies.

The most commonly observed mating pattern in species with natal social group fidelity by both sexes is exogamy, in which individuals mate primarily with members of other social groups. This pattern has been observed in several odontocete species that are closely related to and have similar social structure as false killer whales (Amos et al. 1991, Barrett-Lennard 2000, Pilot et al. 2010). Of the 5 father–offspring dyads we identified where the social group affiliation of both dyad members was known, 3 (60%) involved individuals from the same social group and 2 (40%) from different social groups. Considering all of the father–offspring dyads, the frequency of intra-group dyads could be as low as 36% or as high as 64%. Thus, our data suggest that mating within MHI false killer whales does not follow a pattern of exogamy. Rather, MHI false killer whales mate within their social group at least one-third of the time, possibly closer to two-thirds of the time.

There is growing recognition that inbreeding avoidance behaviors, such as exogamy, will only develop when they confer a selective advantage (Szulkin et al. 2013). The small size of the current MHI false killer whale social groups suggests that intra-group mating may carry a high risk of breeding with a close relative, but the population is believed to have been much larger a generation ago (Reeves et al. 2009, Oleson et al. 2010). If the social groups were much larger prior to the decline, inbreeding risk may have been much lower. Thus, there would have been little selective pressure to favor extra-group mating. Alternatively, females may avoid inbreeding by preferentially mating with unrelated males, regardless of their social group affiliation, an inbreeding avoidance mecha-

nism that has been suggested to occur in northern resident killer whales (Barrett-Lennard 2000), but was recently shown not to be operating in the endangered southern resident killer whales (Ford et al. 2018). Note that one of the intra-group father–offspring dyads we identified involves a male (49052) that appears to be an immigrant from the surrounding offshore population, and therefore represents an example of an intra-group mating between individuals that are unrelated.

It is also possible that MHI false killer whales used to mate primarily between social groups, but the recent decline in abundance has resulted in a partial breakdown of that pattern. The reduced size of the social groups may have reduced the rate at which they encounter each other, in turn reducing the opportunity for inter-group mating. Ford et al. (2011) suggested a similar explanation for the unexpectedly high rate of intra-group paternity they found in the endangered southern resident population of killer whales. Ford et al. (2018) detected weak evidence of inbreeding depression in the population, although their statistical power was low.

We cannot rule out the possibility that there were more social groups when the MHI false killer whale population was larger, but that some groups were either extirpated or subsumed by other groups. This could explain the existence of several small, satellite social groups identified by Baird et al. (2012), which could be remnants of largely extirpated groups. If true, the consolidation of social groups following the decline could mean that some of the apparent intra-group dyads we identified involve individuals that originally belonged to different social groups. However, the short sighting histories of most of the individuals in these satellite groups suggest that most of these groups are more likely artifacts of low sighting rates for the individuals involved rather than evidence that there were more social groups in the recent past (Baird et al. 2012).

The substantially higher number of father–offspring pairs, as opposed to mother–offspring pairs, for which the social group affiliation of one member of the pair is unknown could occur if some older males leave their natal social group to become 'rovers' that move among social groups. Such males might be less likely to assign to one of the main social groups, either due to lower encounter rates reducing their chances of being photographed or because they are more likely to be assigned to a peripheral group if they were photographed. However, our data are not consistent with this idea. In our data set as a whole, the proportion of individuals with unknown group affiliation either because they were assigned to a peripheral group (0.128 for males, 0.117 for females) or were not photographically identified when they were sampled (0.085 for males, 0.117 for females) is similar between the sexes, which contradicts the idea that males are less likely than females to belong to a main social group. Among individuals involved in parent–offspring dyads, there were 3 females (1 daughter and 2 females in dyads with unresolved polarity) and 3 males (1 father, 1 son, and 1 male in a dyad with unresolved polarity) that could not be assigned to social group. The large number of father–offspring dyads for which the father is not assigned to a social group is due to the fact that the individual who fathered 4 offspring (30078) was not photographed at the time he was genetically sampled. Thus, none of the dyads involving that individual can be classified as coming from the same or different groups. However, because 30078 has offspring in all 3 social groups, resolving the group affiliation of 30078 will result in an increase in the number of both same-group and different-group father/offspring pairs. Nonetheless, further photo-identification work could help to resolve the relative social group fidelity of males versus females in this population.

Breeding structure varies among populations in a variety of bird and mammal species. For example, the rate of extra-group paternities (EGPs) in African lions *Panthera leo* varies between populations depending on the mean ratio of males to females within prides (Lyke et al. 2013). Intraspecific variation in EGPs has also been found in common mole rats *Cryptomys hottentotus hottentotus* (Bishop et al. 2004) and killer whales (Barrett-Lennard 2000, Pilot et al. 2010, Ford et al. 2011). Thus, the patterns we identified in the MHI population may not generalize to other populations of false killer whales. The MHI population is unique in many ways. It and the NWHI population are the only known island-associated populations of false killer whales. Oceanographic conditions result in a strong ecological discontinuity between the nearshore and offshore environments surrounding the Hawaiian Archipelago, particularly around the MHI (Doty & Oguri 1956, Polovina et al. 2001, 2008, Seki et al. 2002, Baird et al. 2009, Martien et al. 2014, Baird 2016). The unique habitat that the MHI false killer whale population occupies has likely led to the evolution of unique behavioral adaptations, possibly including social organizations and breeding structures that differ from offshore populations. Given its ecological uniqueness and the fact that the MHI population has undergone a dramatic decline in abundance in recent decades that could have

affected its social organization, our results cannot be assumed to be representative of any other false killer whale population.

4.2. Interplay between social structure and population structure

Our results provide insight into the primary mechanism of gene flow between the MHI false killer whale population and adjacent false killer whale populations. In a study of false killer whale population structure in the North Pacific, Martien et al. (2014) reported that the difference in estimates of differentiation in the mitochondrial versus nuclear genome were substantially greater than would be expected if gene flow occurred at equal rates in both genomes (Larsson et al. 2009). They interpreted the discrepancy as evidence of male-mediated gene flow between populations. Although male-mediated gene flow is often invoked as an explanation for greater differentiation in the mitochondrial genome than the nuclear genome, it is rarely confirmed. Stochasticity can result in the ratio of mitochondrial to nuclear differentiation deviating substantially from the expected value even in the absence of sex-biased gene flow.

The fact that individuals do not disperse from their natal social groups means that they also do not leave their natal population. Thus, our results indicate that mating between populations without organismal dispersal is the likely driver of the patterns of differentiation reported by Martien et al. (2014). Despite being statistically significant, the magnitude of differentiation among social groups is only one-third of that between MHI and NWHI populations, despite the fact that the social groups likely have a smaller effective population size than the populations. Our results therefore suggest substantially lower rates of gene flow between populations than among social groups within a population, which is consistent with the fact that social groups have been seen to interact frequently, creating opportunities for mating, while no interactions have been documented between populations (Baird 2016).

Martien et al. (2014) noted that individual 49052, the only animal from an insular population that shared a haplotype with offshore animals, assigned most strongly to the offshore population in an assignment test. Furthermore, 49052 had a p-value of 0.027 in an analysis designed to determine whether the MHI population could be excluded as its source population. Although this p-value did not meet their significance threshold of 0.01, Martien et al. (2014) concluded that 49052, which at the time of that sighting had not been matched to any other individuals in the catalog, probably represented an offshore animal that had been sampled in association with the MHI population. Subsequently, that individual has been matched to the catalog, having first been documented with the insular population in 1986 and resighted as recently as 2015. This individual indicates that dispersal among populations can occur, and our finding that 49052 is the father of an individual within the MHI population suggests that gene flow from the pelagic to the MHI population does occur at some level.

The case of 49052 provides a unique opportunity to document the impact of infrequent gene flow events. The presence of one or more offspring of 49052 in the reference data set used to calculate allele frequencies in the exclusion analysis will affect the estimated likelihood of 49052 having originated in the MHI population, thereby reducing the probability of the population being excluded as a possible source population. Indeed, when we removed an individual identified by CERVUS as 49052's offspring from the reference data set, the exclusion p-value went down, albeit not quite enough to reach the significance threshold. This result highlights the impact that very low levels of gene flow can have on our ability to detect migrants.

4.3. Effective population size

We estimate that the N_e of the MHI insular false killer whale population is about 58 animals. This population is probably naturally small with strong social structure that limits genetic diversity. Nonetheless, such a low estimate of N_e is cause for concern, as domestic animal studies show that lethal or semi-lethal genetic traits begin to be displayed when N_e declines to about 50 individuals (Franklin 1980). The estimate of N_e produced by 'LDNE' reflects the effective size of the population in the generations immediately prior to the collection of samples (Waples 2005). Although no data are available for calculating trends in abundance, observational data suggest that the abundance of the MHI insular population declined from over 400 to around 150 between the 1980s and the early 2000s, starting approximately 15 yr before our sample collection began (Baird 2009, Reeves et al. 2009, Oleson et al. 2010). If such a decline has occurred and the population remains at its current size, then N_e is likely to decline in the

coming decades as the population comes into equilibrium at a new, lower population size. However, if the decline is reversed, the population could recover quickly enough to avoid a reduction in N_e.

There are several potential sources of uncertainty in our estimate of N_e. 'LDNE' has a slight (<5%) negative bias (Waples 2006) and was developed and tested under the assumption of a closed population with non-overlapping generations. The bias, if any, introduced by overlapping generations has not been well studied, although Waples (2006) noted that analyses of populations with overlapping generations will estimate the effective number of breeders that produced the sample, which is related to N_e.

If a population is not completely closed, the estimate of N_e will be positively biased due to a Wahlund effect created by the presence of first-generation immigrants in the population. The amount of linkage disequilibrium introduced by this effect is small (Waples & Smouse 1990) and therefore unlikely to significantly impact estimates of N_e. Furthermore, the strong natal social group fidelity reflected in our data and the results of our assignment and exclusion tests suggest that first-generation immigrants to the MHI population are rare and will have a negligible effect on the estimate of N_e. If the MHI population receives occasional low-level gametic gene flow (i.e. gene flow due to inter-group mating without dispersal), the genetic effects of such gene flow are reflected in our estimates of N_e.

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