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Inbreeding in an endangered killer whale population

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Abstract

There are genetic risks associated with small population sizes, including loss of genetic diversity and inbreeding depression . The southern resident killer whale (*Orcinus orca*) population is a group of ~80 whales listed as 'endangered' under the U.S. Endangered Species Act. Recovery efforts are focused on increasing prey and reducing impacts from environmental disturbance, but the population's small size and insularity suggest that inbreeding depression could also be important. We analyzed genotypes at 68-94 nuclear loci from 105 individuals to refine a population pedigree to evaluate inbreeding and the relationship between multi-locus heterozygosity and fitness. Our results expand upon an earlier study and shed new light on both inbreeding within this population and the mating patterns of killer whales. We found that only two adult males sired 52% of the sampled progeny born since 1990. Confirming earlier results, we found male reproductive success increased with age. Based on the pedigree, four sampled offspring were the result inbred mating – two between a parent and offspring, one between paternal half-siblings, and one between uncle and half-niece. There is no evidence to date that the survival or fecundity of these individuals is lower than normal. There was some evidence for inbreeding depression in the form of a weakly supported relationship between multi-locus heterozygosity and annual survival probability, but the power of our data to quantify this effect was low. We found no evidence of inbreeding avoidance in the population, but a late age of breeding success for males may indirectly limit the frequency of parent/offspring mating. The effective number of breeders in the population is currently ~26, and was estimated to have ranged from 12 – 53 over the past 40 years. The population that produced the oldest (pre-1970) sampled individuals was estimated to have 24 effective breeders. Overall, our results indicate that inbreeding is likely common in the population, but the fitness effects continue to be uncertain.

INTRODUCTION

Genetic risks associated with small population size include loss of genetic diversity and inbreeding depression (reviewed by Frankham, 1995). Inbreeding is mating among relatives, while inbreeding depression is the reduction in fitness often observed in inbred individuals (Frankham, Ballou & Briscoe, 2002). In the last few decades, molecular methods for estimating pedigrees have led to an improved understanding of inbreeding in wild populations (Crnokrak & Roff, 1999; Pemberton, 2008). Recently, genomic methods have allowed for direct estimation of relationships between heterozygosity (which is reduced by inbreeding) and fitness (Hoffman, Simpson, David *et al.*, 2014; Huisman, Kruuk, Ellis *et al.*, 2016; Kardos, Luikart & Allendorf, 2015; Wang, 2016a). These types of studies have revealed that inbreeding occurs frequently in wild populations (reviewed by Kardos, Taylor, Ellegren *et al.*, 2016), contributing to variation in individual fitness even in populations that are already inbred (e.g., Weiser, Grueber, Kennedy *et al.*, 2016) or growing (Taylor, Colbourne, Robertson *et al.*, 2017). Characterizing patterns of inbreeding is therefore an important step in evaluating population viability and understanding the factors that may be limiting population recovery (Frankham, 2010; O'Grady, Brook, Reed *et al.*, 2006).

Killer whales (*Orcinus orca*) are a widely distributed species found in all of the world's oceans (Taylor, Baird, Barlow *et al.*, 2013). Globally abundant, the species is highly subdivided into discrete populations characterized by dietary specializations and behavioral adaptations (de Bruyn, Tosh & Terauds, 2013; Ford & Ellis, 2006; Ford, Ellis, Barrett-Lennard *et al.*, 1998). The species has been well studied in the northeastern Pacific Ocean, where fish-eating populations are characterized by a matrilineal social structure in which offspring of both sexes remain associated with their mother while she lives and typically with her family thereafter (Ford, Ellis & Balcomb, 2000). With a life-span of >50 years and overlapping generations, this social structure has the potential for high levels of inbreeding.

The "southern resident" killer whales are ~80 individuals subdivided into three pods (social groups; "J", "K", and "L") that inhabit the coastal areas of the U.S. west coast and southern British Columbia (Ford *et al.*, 2000; Krahn, Ford, Perrin *et al.*, 2004). They are the southernmost of several fish-eating killer whale populations along the Pacific Rim (see map in Ford, Hanson, Hempelmann *et al.*, 2011) and are listed as endangered in the U.S. and Canada (COSEWIC, 2001; NMFS, 2005). The southern residents declined during the 1960s due to capture of 47 animals for aquaria (Bigg & Wolman, 1975), and likely declined earlier due to harassment and reduced salmon prey (Wiles, 2004). In contrast to other North Pacific killer whale populations, the southern residents have failed to recover after protection under the Marine Mammal Protection Act in 1972 (Krahn *et al.*, 2004; NMFS, 2017). The population faces several threats, including reduced prey abundance, disturbance, and chemical contamination (NMFS, 2008). The population may be also vulnerable to inbreeding depression due an effective population size of <30 and very limited gene flow with other populations (Ford *et al.*, 2011; Parsons, Durban, Burdin *et al.*, 2013; Pilot, Dahlheim & Hoelzel, 2010).

To date, however, inbreeding within the population is poorly characterized. Based on observational studies dating from the 1970s to the present, maternal relationships are well known (Ford *et al.*, 2000). An initial paternal pedigree (Ford *et al.*, 2011) detected no instances of inbreeding, but the number of paternities (12) was small, suggesting the lack of inferred inbreeding could be due to insufficient sampling. Here, we build upon the earlier study with the goals of 1) evaluating the degree of inbreeding in the population using a larger sample of parents and offspring, 2) quantifying the relationship between inbreeding, heterozygosity and fitness, and 3) evaluating trends in the effective number of breeders by comparing estimates made from older and younger individuals.

METHODS

Sample collection and DNA extraction - Skin and fecal samples were collected and DNA was extracted as previously described (Ford *et al.*, 2011). All skin samples were from whales that were field-identified based on visible markings (Bigg, Ellis, Ford *et al.*, 1987). For whales born after 1973, year of birth and mother were known from direct observation (Ford *et al.*, 2000). Whales born prior to 1973 had estimated birth years (Ford *et al.*, 2000). We also included samples from three carcasses. Samples were collected under NMFS General Authorization No. 781–1725, and Scientific Research Permits 781-1824-01, 16163, 532-1822-00, 532– 1822 and 10045.

Genotyping – We developed assays for a 68 single nucleotide polymorphism (SNP) loci using the allele-specific Fluidigm "SNP Type" method (Fluidigm, 2016; see supplemental information for details). Some individuals were also genotyped for 26 microsatellite loci as described in (Ford *et al.*, 2011). Tests for Hardy-Weinberg equilibrium and linkage disequilibrium were done using Genepop 4.4 (Rousset, 2008). Inbreeding coefficients were calculated from the pedigree using Wright's path method (Wright, 1922) using the 'pedantics' R package (Morrissey & Wilson, 2010) in the R environment (version 3.3.1; R Core Development Team, 2017). Relatedness coefficients were estimated from the pedigree and from the genotypic data using the COANCESTRY program (Wang, 2011) and the 'related' R package (Pew, Muir, Wang *et al.*, 2015).

Parentage analysis – Parentage analysis was conducted using maximum-likelihood methods in the COLONY and FRANZ computer programs (Riester, Stadler & Klemm, 2009; Wang & Santure, 2009). For COLONY, we employed the full-likelihood approach to find the maximum likelihood pedigree of the entire sample, considering both parent-offspring and sibling relationships. FRANZ was used as a comparison by identifying the most likely father for each sampled mother/ offspring pair. Computer simulations of the population were used to evaluate pedigree accuracy. See supplemental information for details.

Reproductive success and inbreeding depression – The relationship between male age and probability of paternity was evaluated using log link Poisson generalized additive models (GAMs) with a smooth spline over age in the 'mgcv' R package (Wood, 2011). We examined the relationship between standardized multi-locus heterozygosity (MLH) and annual survival and fecundity. Variance in MLH due to inbreeding was evaluated using the g2 statistic (correlation of homozygosity among loci) (David, Pujol, Viard *et al.*, 2007; Szulkin, Bierne & David, 2010) in the inbreedR package (Stoffel, Esser, Kardos *et al.*, 2016). The SNP genotypes from the two individuals used for SNP discovery were excluded from this analysis because their MLH was upwardly biased due to ascertainment of heterozygous sites in these individuals. To evaluate the relationship between MLH and survival or fecundity rates, we used a modification of the generalized linear modeling approach described in Ward *et al.* (2013) that uses life-history information from both the southern and closely related northern resident population. See supplemental information for details.

Effective breeders – The effective number of breeders (N_b) was estimated using the using the sibship method of Wang (2009), assuming Hardy-Weinberg equilibrium (Wang's eqn 10 with $\alpha = 0$). To evaluate trends, we estimated N_b for whales grouped by birth date in 10-year sliding windows. Whales born prior to 1970 were included in a single 'old' window. Uncertainty in these estimates was characterized by using the 1000 most likely pedigree configurations saved by the COLONY program and by bootstrapping over individuals. This method assumes the sampled older whales are a random sample from the population as it existed when they were born. Estimates of N_b may be biased if they contain individuals from more than one cohort (Wang, 2016b; Waples, 2016), so these estimates should be interpreted as an approximation of N_b useful for examining trends.

RESULTS

Pedigree construction – We obtained multi-locus SNP genotypes at 105 unique samples, 100 of which were from known whales, 2 from unidentified fecal samples, and 3 from unidentified stranded calves (Table S1). Seventy-nine samples also had genotypes at up to 26 microsatellite loci. SNP genotypes were in Hardy-Weinberg proportions, with an average heterozygosity of 0.425 (Table S2). The SNP-only and combined SNP and microsatellite data sets produced very similar pedigrees using the full-likelihood (COLONY) method, with 43 of 46 high posterior probability ($p > 0.9$) paternity assignments identical between the two data sets (Table S3). The FRANZ paternity results were also very similar to the COLONY pedigree, with only 3 conflicts among the 105 parentage tests (Table S4). Two of these conflicts involved the same male (L57), who was identified as the father of two offspring by FRANZ, while COLONY inferred the father to be absent from the sample (with L57 as a paternal sib of the offspring in question), while the other involved an uncertain maternal relationship among two older whales.

COLONY also estimates full- and half-sib families for samples with no identified parents. The combined SNP/microsatellite data and the SNP only dataset produced similar results, typically differing by the inclusion or exclusion of a single individual (Table S5). Based on the combined SNP/microsatellite dataset, we developed a consensus pedigree based on highly supported ($p > 0.9$; most were 1; Table S3) paternities and very highly supported ($p > 0.95$) families without two identified parents, with uncertain relationships treated as unknown (Table S6). There were four identifiably inbred offspring in the consensus pedigree: one from a mother-son mating

(J26+J16 -> J42), one from a father-daughter (J1+J28 -> J46), one between half-sibs (L41 + K13 -> K34) and uncle/ half-niece (L41 + K22 -> K33) (Table S6). Simulation results indicated that rate of incorrect paternity assignment was <3%, and that any errors are likely to be a failure to assign a father when he is in fact in the sample (Table S7).

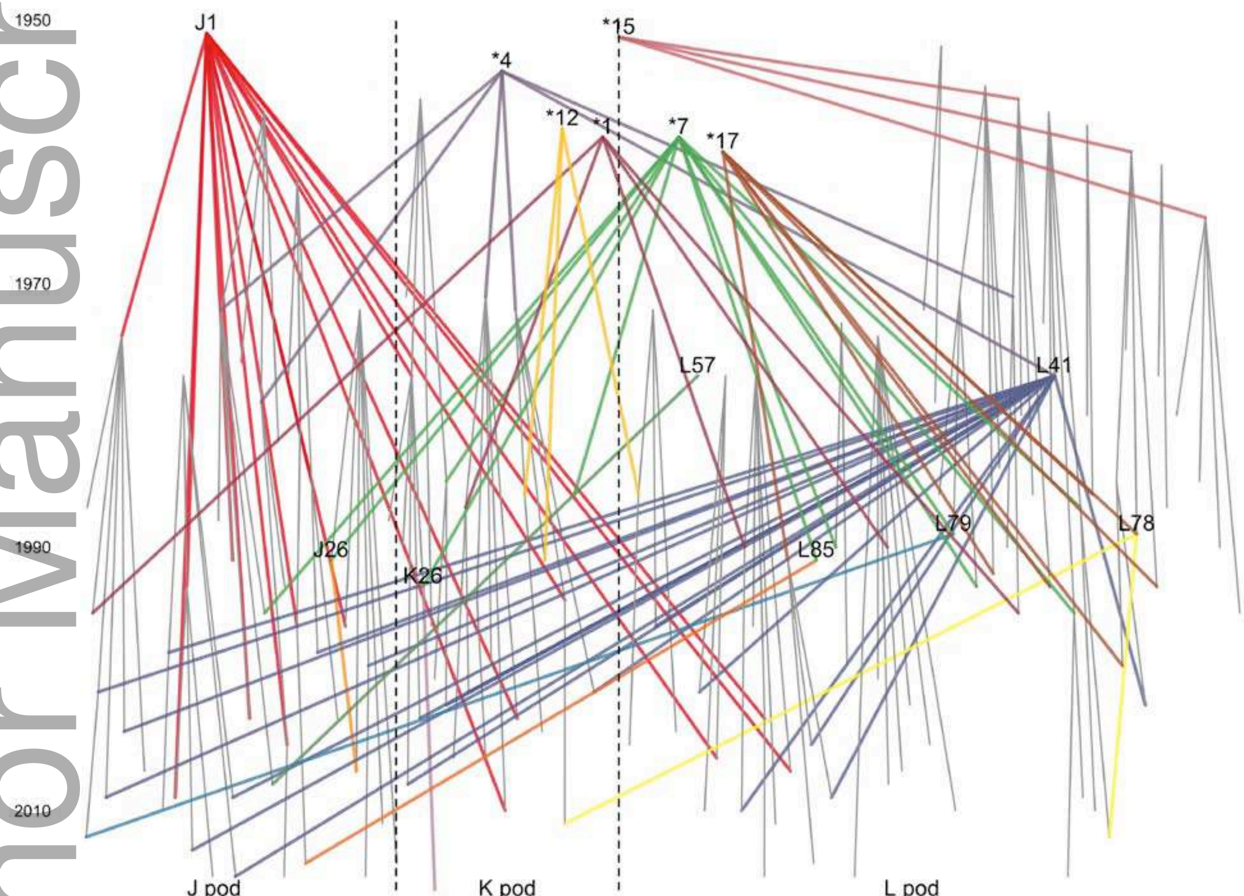

Figure 1 – Inferred southern resident killer whale pedigree, 1950 to 2016, focusing on paternal relationships. Maternal relationships are illustrated by gray lines; paternal relationships are illustrated by colored lines. Line end points correspond to birth years. Sampled males with at least one inferred offspring are labeled with their pod and identifier. Six inferred but unsampled males ("*") are also

labeled, with birth years estimated as the average year of birth for unsampled males in the population that are potential candidates for the inferred fathers (Table S5).

Male reproductive success – The 46 high confidence paternities involved males mating with females from all three pods (Table S6; Figure 1). Two males, L41 and J1, were responsible for 80% of the paternities where a sampled father was identified, and 52% of all sampled offspring born since 1990. J1 was the sire for 16 progeny from 9 different matrilines from all three pods, including all J-pod matrilines except the J10 matriline. L41 was the sire of 20 progeny from 11 matrilines from all three pods, including 4 L-pod matrilines. The remaining seven sampled males with assigned progeny had only 1-2 progeny each. There were also at least 10 unsampled fathers, several of which were inferred to have produced > 5 progeny (Table S6). Based on the ages of the family members, there were typically known but deceased males from the population that are candidates for these unsampled fathers (Table S5). Females produced progeny with up to four different males (Figure 1). Based on the paternities, male age at reproduction ranged from 16 to 59, with a median age of 31. There was a strong positive relationship between paternity and age (Figure 2).

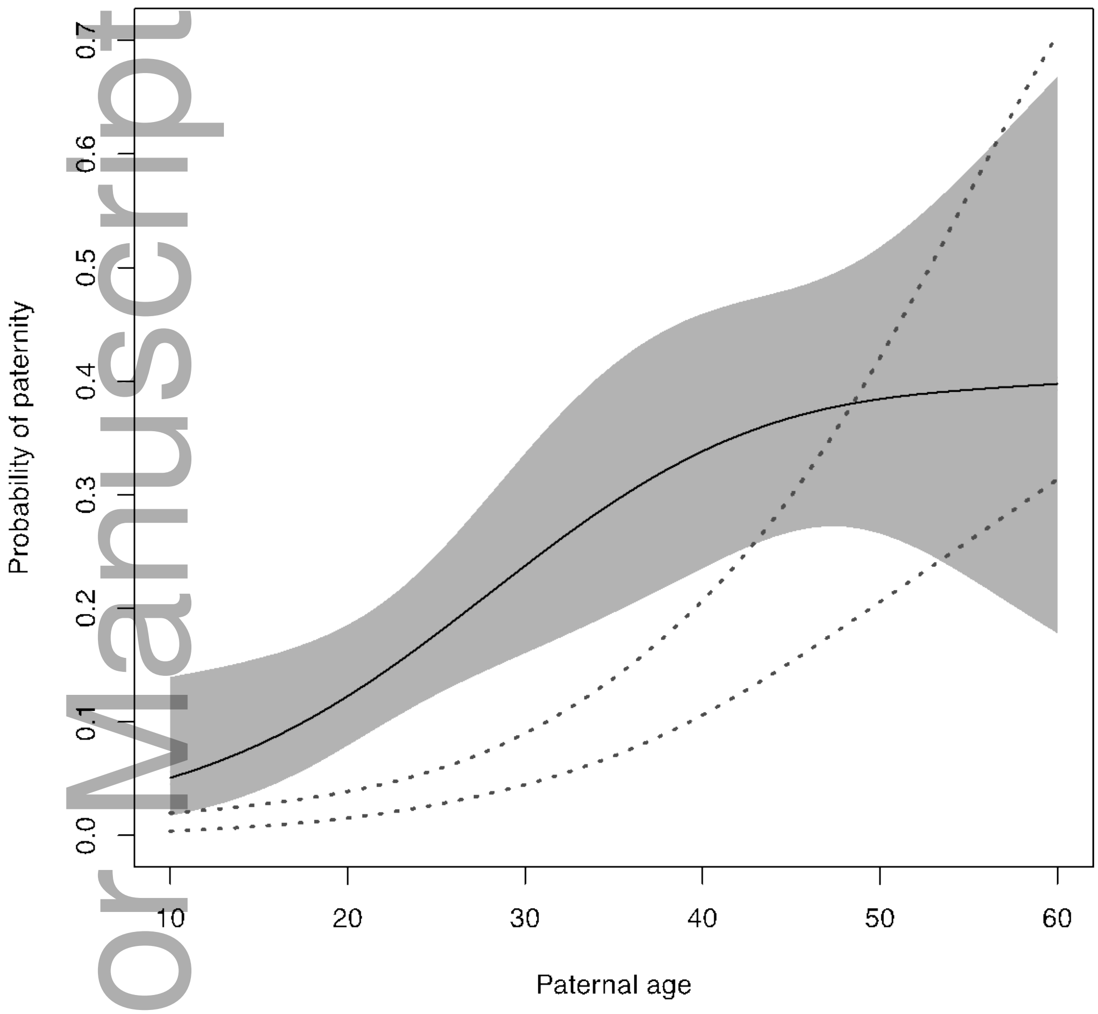

Figure 2 – Fitted relationship between male age and reproductive success, estimated as the annual probability of a male having an offspring. Mean and 95% credible intervals for model results from confirmed sires are indicated with the sold line and shaded region, respectively, and 95% credible results from a model considering all males including those with no known offspring are indicated with dashed.

Trends in effective population size – Estimated N_b varied over time, but was generally <25, with a peak in the late 1970's and trough in mid 1990's (Figure 3; Table S8). Estimated N_b for the 14 individuals born prior to 1970 was 24 (95% CI: 17 – 40). There was almost no uncertainty in estimated N_b due to pedigree uncertainty based on the 1000 best COLONY configurations (Figure S1). The N_b/census size ratio varied from 0.11 to 0.66, and averaged 0.28 (Figure 3).

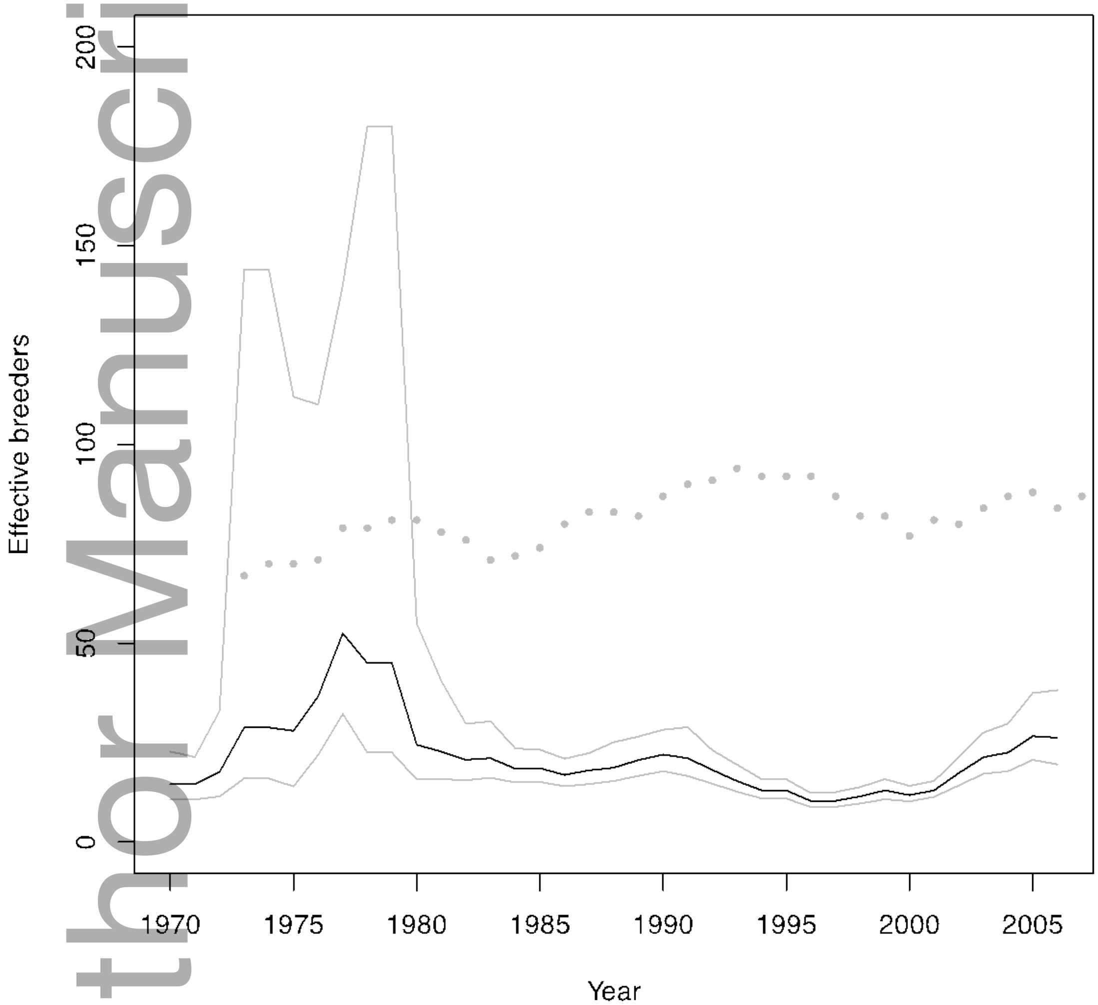

Figure 3 – Trends in the estimated effective number of breeders (*N_b*), estimating using the approach of Wang (2009) in a 10-year sliding window. Dark line is the point estimate and light lines are the 95% confidence intervals based on bootstrapping over pairs of individuals within each 10-year window. The dotted grey line is the observed number of individuals in the population for each year.

MLH-fitness correlations -- MLH varied among individuals, although confidence intervals were wide (Figure S2). The MLH values for the four inbred individuals did not differ significantly from the rest of the population (t-test, t = 0.085, p = 0.94). Identity disequilibrium was not significantly greater than zero for the SNP loci alone (g2 = 3.386e-05, 95% CI: -0.00554 – 0.0058, p > 0 = 0.467) or for the combined data (g2 = 0.0032, 95% CI: -0.0043 – 0.010, p > 0 = 0.077). Based on simulations using the 'related' package, all seven relatedness estimators tested were similar and highly correlated with the true (simulated) relatedness (Figure S3); here we focus on the relatedness estimator of Wang (2002). The mean estimates of pairwise relatedness among individuals corresponded well with the relationships in the pedigree, with values near 0.5 for parent/offspring and full-sib relationships and 0.25 for half-sib relationships (Figure S4). The expected (based on random mating) and observed relatedness coefficients among identified parent pairs were not significantly different from each other (Figure 4), and the number of matings within and between pods did not differ from that expected by chance (Table S9).

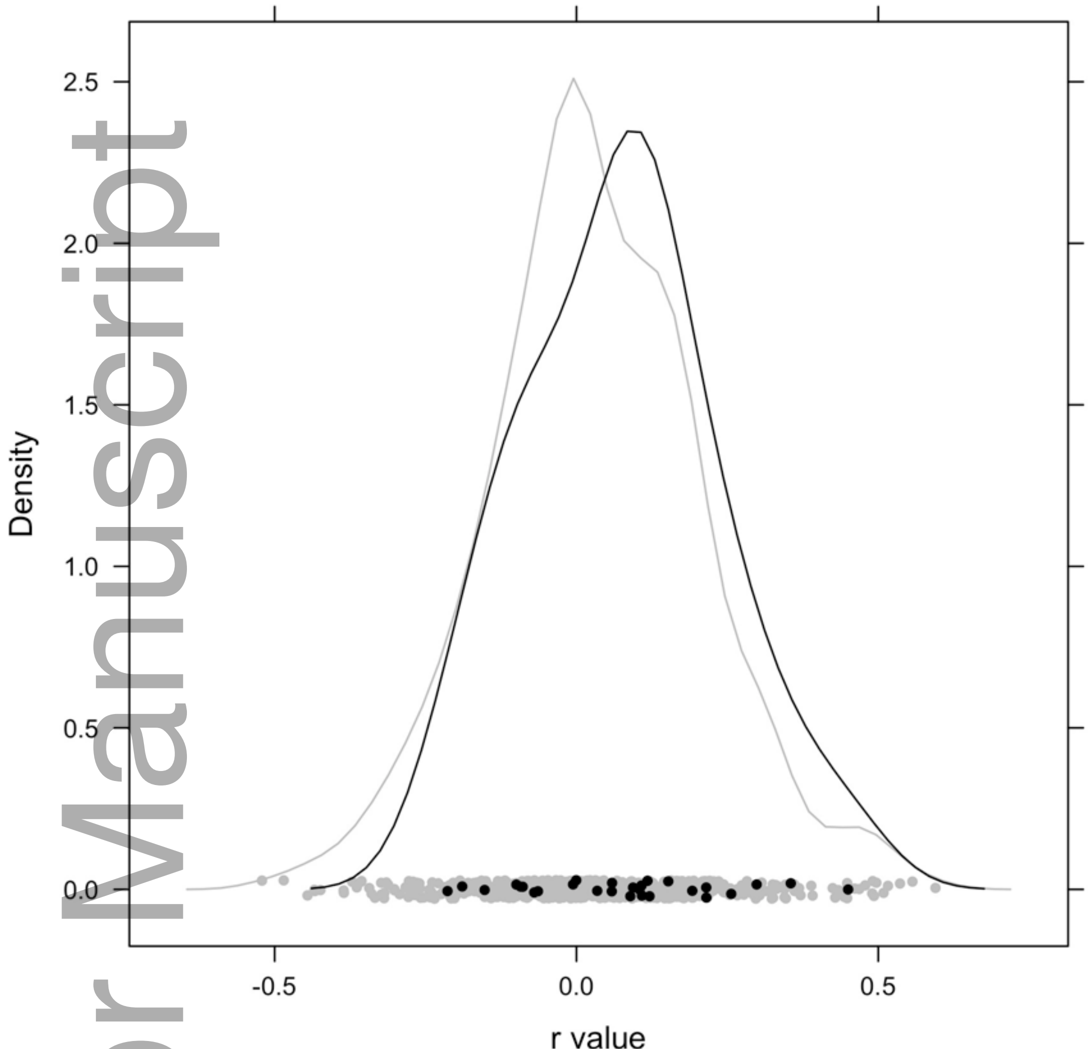

Figure 4 – Observed (black) and expected (gray) distributions of pairwise relatedness among potential mates. The means of the two distributions are not significantly difference (ANOVA; F = 1.569, df = 720, p = 0.21).

For models predicting survival as a function of year, age, sex and MLH, the best-fitting model for the combined dataset included time, age and sex but not MLH (Table 1). MLH was included in the second-ranked model, with a modest effect size (Figure S5). There was less model support for a relationship between MLH and female fecundity (Table 2). Similar results were obtained when the SNP data were analyzed separately (Tables S10 and S11).

Table 1 – Model fits for alternative GAM models describing survival as a function of time, age, sex and MLH, for population censuses 1979-2016 and the combined SNP and microsatellite data set. To allow comparison between models with and without MLH, the subset of animals with MLH data were used (n = 84). These models either include time as a smoothed term (Y/N), include sex as a fixed effect ('Factor', offset) or fits separate splines to age effects by sex ('Smooth'), and include MLH or not as a predictor (Y/N). The best models (ΔAIC <2) are highlighted in bold.

Model	Time	Age	Sex	MLH	ΔAIC	df
1	**Y**	**Y**	**Factor**	**N**	**0**	**4.59**
5	**Y**	**Y**	**Factor**	**Y**	**0.09**	**5.6**
3	**Y**	**Y**	**Smooth**	**N**	**0.75**	**5.16**
7	**Y**	**Y**	**Smooth**	**Y**	**1.23**	**6.01**
6	N	Y	Factor	Y	6.17	4
2	N	Y	Factor	N	6.26	3
4	N	Y	Smooth	N	7.96	3.94
8	N	Y	Smooth	Y	8.5	4.89

Table 2 -- Model fits for alternative GAM models describing female killer whale fecundity as a function of time, age and MLH, for population censuses 1979-2016 and the combined SNP and microsatellite dataset. To allow comparison between models with and without MLH, the subset of females with MLH data were used (n = 35). The best models (ΔAIC) are highlighted in bold.

Model	Time	Age	MLH	ΔAIC	df
1	**Y**	**Y**	**N**	**0**	**5.93**
2	**N**	**Y**	**N**	**0.44**	**4.58**
3	**Y**	**Y**	**Y**	**1.78**	**6.94**
4	N	Y	Y	2.06	5.58

DISCUSSION

PEDIGREE AND MATING PATTERNS

The pedigree is considerably expanded compared to prior results (Ford *et al.*, 2011). We made 46 confident paternity assignments, compared to only 12 in the earlier analysis. The increase was greater than might be expected based on the increase in total samples (105 compared to 78) due to more young animals in the current study, and because nearly all of the current samples were from known animals. Two paternities were changed from the prior analysis based on new data. One involved an incorrectly identified sample (J42); in the other a missing father was inferred in the prior analysis but a sampled father was inferred in the current analysis (J1/J14). COLONY is sensitive to inclusion/exclusion of samples that may alter the inferred family structures within the population (Wang & Santure, 2009), so some changes with increasing sample size are not surprising. The fact that our results were generally stable with the addition of new samples and additional loci, along with the results of our computer simulations (Table S7), indicates that our pedigree is robust.

Our results strengthen two primary conclusions from the earlier study. First, we confirmed that offspring produced by mating within pods are common. Ford *et al.* (2011) based this conclusion primarily on intra-pod mating by one male. Our study adds substantially to this result, with two males (J1 and L41) clearly inferred to have sired offspring from all three pods and two others (J26 and L78) inferred to have sired at least one progeny within their own pod (Figure 1, Table S6). In addition, 3 of the 8 inferred paternal half-sib families contain members from all three pods (Table S6). Pilot *et al.* (2010) found intra-pod mating in an Alaskan killer whale population, further suggesting that social association does not appear to be related to patterns of breeding. In contrast, no mating within pods was found in the closely related northern resident killer whale population (Barrett-Lennard, 2000), suggesting considerably behavioral plasticity among populations.

Second, our results also support Ford *et al.*'s (2011) finding that male breeding success increases with age (Figure 2), and extend the age range of identified paternities, ranging from 16 to 59 compared to 21 to 55 in the earlier study. The dominance in breeding by older (and larger - (Fearnbach, Durban, Ellifrit *et al.*, 2011)) males confirms that male mating success is highly skewed in this population.

One area where our results differ from the earlier study is in the degree of inbreeding. None of the paternities identified by Ford *et al.* (2011) or Barrett-Lennard (2000) involved mating among closely related individuals. In contrast, of the 81 progeny in the current study where both the mother and father were identified (including the inferred but unknown parents from COLONY; Table S6), 4 were inbred. Of these 81 progeny, 42, 44, 37 and 19 had an identified paternal grandmother, maternal grandfather, either two paternal grandmothers or two maternal grandfathers, or all grandparents, respectively, resulting in rates of mother-son, father-daughter, half-sib, and full-sib mating of 2.4% (1/42), 2.3% (1/44), 2.7% (1/37) and 0% (0/19), respectively. The lack of outbreeding based on genetic relatedness (Figure 4) or pod membership (Table S9) suggests there is little inbreeding avoidance in the population.

Inbreeding via parent/offspring mating requires overlapping generations, and a late age of male reproduction in the population may prevent some inbreeding (Wright, Stredulinsky, Ellis *et al.*, 2016). To test this, we calculated the expected probability of parental age based on the predicted effects of age on survival and fecundity (Figure 5). Progeny produced from mother/offspring mating are expected to be rare but father/offspring mating is not precluded. Many of the observed offspring had a father whose age allowed for a parent/offspring relationship, similar what has been observed in many other mammal populations (e.g., Krutzen, Barre, Connor *et al.*, 2004; Rioux-Paquette, Festa-Bianchet & Coltman, 2010; Smith, 1979; Stopher, Nussey, Clutton-Brock *et al.*, 2012).

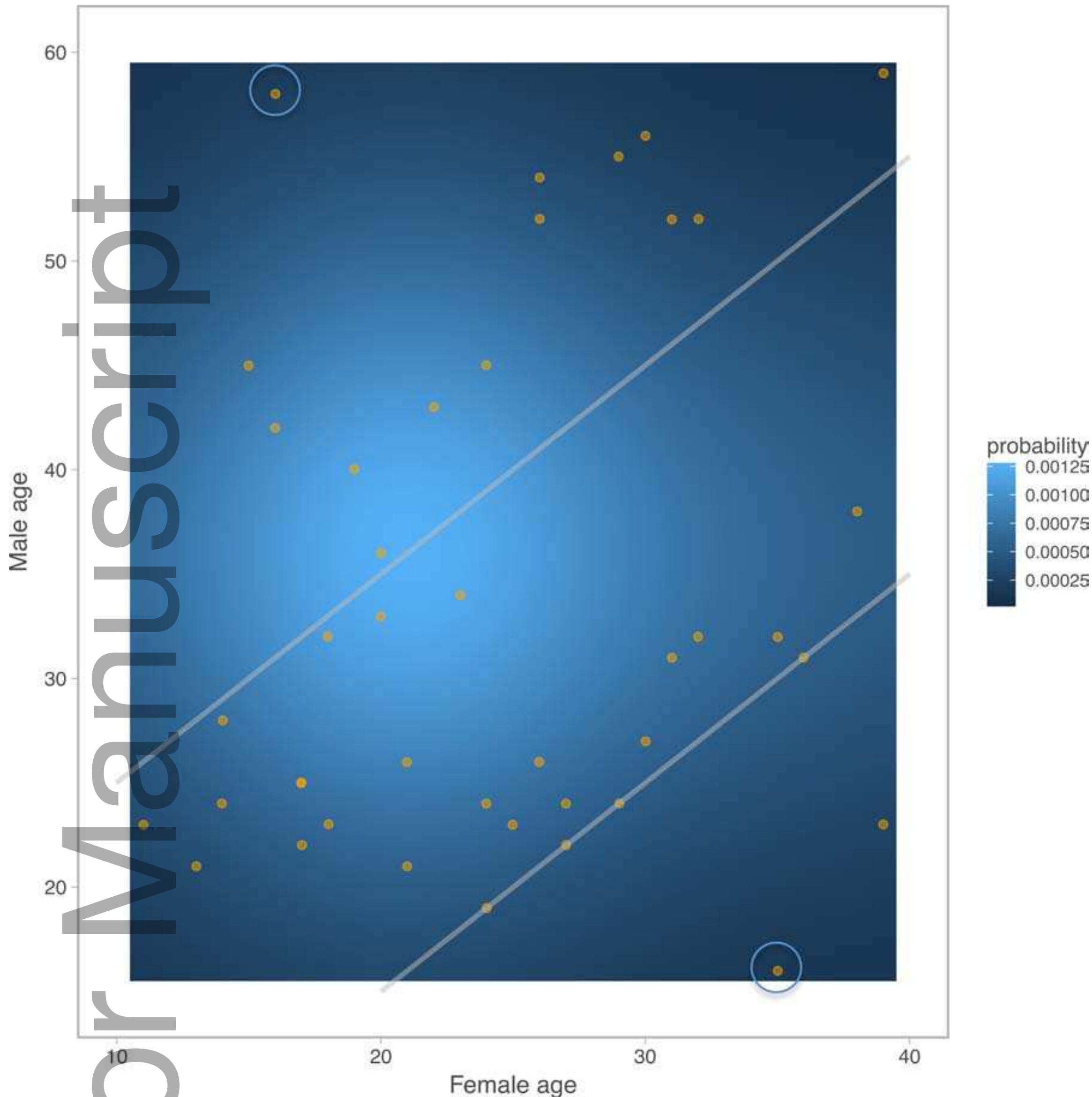

Figure 5 -- Probabilities of different parent age combinations based expected survival and fecundities at age. Points are actual parent ages based on paternity analysis. The two lines indicate the boundaries where parent/offspring mating is possible, assuming sexual maturity at age 10 for females and 15 for males; in the area between the two lines parent/offspring mating is not possible due to age constraints. All of the points above the upper line are associated with a single older male (J1). Two cases of apparent parent/offspring mating are circled.

INBREEDING DEPRESSION

All four of the inbred offspring were still alive in 2017, at ages 16 for two males (K33 and K34), and 10 and 8 for two females (J42 and J46). This small sample size does not imply a lack of inbreeding depression. We also only had the opportunity to sample observed animals. The rate of fetal loss has been estimated to be >50% in this population (Wasser, Lundin, Ayres *et al.*, 2017), and inbreeding depression could be expressed as fetal loss.

We also evaluated MLH as an indicator of inbreeding, a metric that has been shown to be useful (Hoffman *et al.*, 2014; Szulkin *et al.*, 2010). A weakly supported relationship between MLH and annual survival (Table 1) suggests some inbreeding depression, consistent with findings in other species (Huisman *et al.*, 2016; Keller & Waller, 2002; Ralls, Ballou & Templeton, 1988; Szulkin *et al.*, 2010). However, our data were insufficient to evaluate variance in inbreeding (non-significant g2 statistic), indicating that power to detect a relationship between MLH and fitness is low. The four individuals identified as inbred from the pedigree did not have low MLH (Figure S2), suggesting our sample of loci is not sufficient for MLH to be a good metric of even close inbreeding in this population. Several theoretical (e.g., Kardos *et al.*, 2015) and empirical (Hoffman *et al.*, 2014; Huisman *et al.*, 2016) analyses have found that MLH-fitness correlations are difficult to detect with small numbers of loci. The finding of some support for a MLH/survival relationship despite surveying a small number of loci suggests that inbreeding depression could be a factor influencing survival in this population.

Populations of fish-eating killer whales in the northeastern Pacific have the unusual characteristic of social philopatry for both sexes, where offspring spend their lives with their mother and her maternal relatives and never disperse to join other populations (Ford *et al.*, 2000). This is in contrast to male-biased dispersal typical of mammals (Clutton-Brock, 2009; Handley & Perrin, 2007; Smith, 2014), and dispersal of both sexes in some mammal-eating killer whale populations (Baird & Whitehead, 2000). Inbreeding avoidance is often invoked as a cause of sex-biased dispersal (reviewed by Handley & Perrin, 2007), but there are benefits to social philopatry. For example, Wright *et al.* (2016) found that prey sharing among the northern resident (coastal British Columbia) fish-eating killer whales was strongly biased toward maternal kin, and concluded that the benefits of prey sharing could explain bisexual philopatry. Kin recognition, possibly through distinct call types, was invoked as a mechanism for inbreeding avoidance.

Our finding of closely inbred individuals in an ecologically similar population indicates that such mechanisms for inbreeding avoidance are not entirely effective, even though many matings were between members of different pods. Inclusive fitness theory suggests that there are benefits to inbreeding by helping relatives increase their fitness (Puurtinen, 2011; Smith, 1979), and that inbreeding will therefore be tolerated if inbreeding depression is not severe. The fact that all four of the inbred offspring we observed in our study survived to date and the equivocal evidence of a MLH-fitness relationship suggest the possibility that the negative effects of inbreeding may not be large enough to offset the benefits of remaining with a natal group. Mammal-eating populations of killer whales have a different social system in which both sexes may disperse from their natal group (Baird & Whitehead, 2000; Ford *et al.*, 2000). This high degree of plasticity among con-specific populations suggests diet and predation behaviors have a stronger influence on killer whale social structure than inbreeding avoidance.

Killer whale populations are believed to form by matrilineal fission (Ford *et al.*, 2000), in some cases into new niches that facilitate ecological divergence (Foote, Vijay, Avila-Arcos *et al.*, 2016). This process likely involves population bottlenecks and inbreeding (Hoelzel, Hey, Dahlheim *et al.*, 2007; Moura, van Rensburg, Pilot *et al.*, 2014). Low levels of gene flow from other populations may be an important source of genetic variation, particular as new populations may experience reduced population growth due to inbreeding after the original founders have died (cryptic inbreeding depression; Taylor *et al.*, 2017). The southern resident population is particularly isolated from other populations (Ford *et al.*, 2011; Parsons *et al.*, 2013; Pilot *et al.*, 2010), and it is possible that this isolation is contributing to inbreeding and relatively low population growth rate compared to other similar populations (Allen & Angliss, 2014).

EFFECTIVE NUMBER OF BREEDERS

The estimated effective number of breeders ranged over time from 10 to 53 and averaged 22, similar to the value of 26 reported previously for the population as a whole (Ford *et al.* 2011). In the sib-ship method of estimating N_b (Wang, 2009), the estimate is the N_b of the parents of the sample. The estimated N_b of the 14 individuals in our sample born prior to 1970 was similar to current N_b (24 and 26, respectively; Table S8; Figure 3), suggesting the population has had a small effective breeding size since at least the mid-to-early 1900's. The historical estimates of

N_b depend critically on the assumption that these older individuals are a random sample of the population as it existed at the time of their birth. This assumption will be violated if survival is non-random with respect to family structure, so the estimates of historical N_b should be viewed cautiously.

CHOICE OF GENETIC MARKER

The initial pedigree for this population was estimated using 26 microsatellite loci (Ford *et al.* 2011), compared to 68 SNP loci genotyped for the current study. Both studies were conducted in the same laboratory, and the decision to switch marker types was based on cost and labor-savings considerations rather than the biological characteristics of the different markers. The parentage results based on the subset of samples genotyped for both locus sets were very similar (Table S3), similar to what has been observed in other studies (e.g., Hauser, Baird, Hilborn *et al.*, 2011). Although either marker set appears to be sufficient parentage analysis when combined with extensive field observations (Tables S3 and S7), neither data set alone nor the combined data were sufficient to accurately characterize variation in genomic heterozygosity among individuals. The high variance in estimated relatedness among unknown individuals (Figure S4) also suggests some half-sibling or more distant relationships may not have been detected. Collecting data at additional genetic loci using genomic methods is therefore a high priority for fully characterizing the effects of inbreeding in this population.

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