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Inferred Paternity and Male Reproductive Success in a Killer Whale (*Orcinus orca*) Population

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Abstract

We used data from 78 individuals at 26 microsatellite loci to infer parental and sibling relationships within a community of fish-eating ("resident") eastern North Pacific killer whales (*Orcinus orca*). Paternity analysis involving 15 mother/calf pairs and 8 potential fathers and whole-pedigree analysis of the entire sample produced consistent results. The variance in male reproductive success was greater than expected by chance and similar to that of other aquatic mammals. Although the number of confirmed paternities was small, reproductive success appeared to increase with male age and size. We found no evidence that males from outside this small population sired any of the sampled individuals. In contrast to previous results in a different population, many offspring were the result of matings within the same "pod" (long-term social group). Despite this pattern of breeding within social groups, we found no evidence of offspring produced by matings between close relatives, and the average internal relatedness of individuals was significantly less than expected if mating were random. The population's estimated effective size was <30 or about 1/3 of the current census size. Patterns of allele frequency variation were consistent with a population bottleneck.

Key words: *effective population size, microsatellite, parentage, pedigree, relatedness, reproductive success*

A population's mating system influences a variety of important evolutionary processes, including the population's effective size, the distribution of diversity within the population, the level of inbreeding, and the degree of differentiation from other populations. The use of genetic data to evaluate patterns of mating and kinship has become a useful tool, particularly when direct observation of mating patterns and parentage is difficult (reviewed by DeWoody and Avise 2001; Jones and Ardren 2003; Jones and Wang 2010). Marine mammals, for example, are often difficult to observe over long periods in the wild due to their aquatic habitat and wide-ranging distribution. Parentage analysis has therefore been useful for studying the mating systems of these species (e.g., Amos et al. 1991; Clapham and Palsboll 1997; Krützen et al. 2004; Frasier et al. 2007).

Genetic parentage analysis has been particularly important for understanding male reproductive success, which can be difficult to observe directly even in terrestrial organisms (Clutton-Brock 1989; Garant and Kruuk 2005). Male mating strategies and the variance in male reproductive success are strongly influenced by a species' ecological situation (Clutton-Brock 1989; Gowans et al. 2008). Environments in which females are clustered into groups due to patchy feeding areas or other patchy resources promote polygenous mating systems with high variance in male reproductive success. In contrast, environments where female groups either range widely or are not defensible by a single male tend to promote less variable male reproductive success. Species who require cooperative behavior to raise their young also tend to have skewed male (and often female) reproductive success in which dominant pairs produce most offspring (Emlen 1991; Griffin et al. 2003).

Some delphinid species, including killer whales (*Orcinus orcas*), are characterized by an unusual social structure in

which neither males nor females disperse from their natal group (reviewed by Berta and Sumich 1999). Cooperative behavior has also been observed in killer whales, including coordinated predation (Frost et al. 1992; Baird and Dill 1995) and prey sharing (Hoelzel 1993; Baird and Dill 1995; Baird 2000; Ford and Ellis 2006). The effects of non-dispersal and cooperation on delphinid mating systems are not known in detail, and the variance of male mating success has been estimated for only one delphinid species: bottlenose dolphins (*Tursiops* sp.; Krützen et al. 2004), although some information on male mating patterns is also available for long-finned pilot whales (*Globicephala melas*—Amos et al. 1993) and killer whales (Barrett-Lennard 2000; Pilot et al. 2010).

Another important factor influencing a species' mating system is inbreeding avoidance. Mammalian species employ a variety of strategies to limit inbreeding, including dispersal from natal groups, aversion to mating with natal associates, kin recognition through olfactory, acoustic or morphological cues, and suppression of subordinates' mating by dominant pairs in cooperative societies (reviewed by Pusey and Wolf 1996). In at least some bottlenose dolphin populations, groups of related males form subgroups, called coalitions, who work cooperatively to herd females and obtain copulations, sometimes resulting in inbreeding (Connor et al. 1992; Krützen et al. 2004; Frère et al. 2010). Killer whales and long-finned pilot whales, in contrast, have been shown in at least some cases to breed primarily with individuals from a different social group during brief periods of multigroup aggregation, which has been hypothesized as a mechanism to avoid inbreeding (Amos et al. 1993; Barrett-Lennard 2000; Pilot et al. 2010). The conclusions regarding delphinid mating systems, however, are based on observations from a relatively small number of confirmed paternities, and it is not clear how general the results are across populations within species.

The killer whales inhabiting the eastern North Pacific have been the subject of nearly 40 years of study (Bigg 1982; Ford et al. 2000), which has illuminated the existence of sympatric communities with differing vocalizations (Ford 1989, 1991), diets and behavior (Bigg 1982; Morton 1990; Ford et al. 1998), and social organization (Bigg et al. 1990; Baird and Whitehead 2000). The fish-eating or "resident" killer whales that seasonally inhabit the inland marine waters of British Columbia and Washington are characterized by a matrifocal social structure in which offspring of both sexes remain with their mother and do not disperse to other populations. Stable groups of matrilines, known as pods, tend to be found together, and pods are further grouped in several distinct communities (Ford et al. 2000; http://www.whaleresearch.com/; Parsons et al. 2009). The southern community, which is the focus of this study, has been observed to range from central California to northern British Columbia but spends about 40–50% of its time within the inland marine waters in Washington and southern British Columbia (Hanson MB, Emmons CK, and Balcomb KC, unpublished data).

The maternal pedigrees, date of birth (within 6 months), and patterns of association of both sexes within several of the eastern North Pacific killer whale populations are well described through field observations (Ford et al. 2000; http://www.whaleresearch.com/; Parsons et al. 2009). For example, the northern community (ranging from Washington to Southeast Alaska) consists of >200 individuals in 16 pods that are grouped into 3 vocal clans (Ford 1991). Another well-studied community in southern Alaska consists of ~500 individuals in at least 22 pods divided into at least 2 vocal clans (Matkin et al. 1999; Allen and Angliss 2009). In contrast, the southern community consists of only 85 individuals in 3 generally recognized pods (designated J, K, and L) in a single vocal clan (Ford 1991; Carretta et al. 2009).

In a previous study of killer whale paternity in the northern community, Barrett-Lennard (2000) found that nearly all (24/25) inferred matings were between individuals from different pods within the same community (the single exception involved a within-pod mating). Matings also tended to involve individuals from different acoustic clans, and Barrett-Lennard hypothesized that acoustic cues were used to avoid mating with related individuals. In contrast to this pattern of between pod/within population mating, another study of 213 killer whales, including 30 from the southern community, found that nearly half of the most likely paternity assignments involved males from outside the mother's population (Pilot et al. 2010).

Here, we report on the results of a study using microsatellite variation to infer mating patterns in the southern community. This group is considerably smaller than the northern community and has a lower average population growth rate (Olesiuk et al. 1990; Ward et al. 2009). Unlike the northern community, the southern community was exploited extensively in the late 1960s and early 1970s due to captures for the aquaria trade. The group is also subjected to a more urbanized environment and therefore may be more impacted by disturbance and environmental contaminants (Baird 2001; Krahn et al. 2002). In Canada, this population was listed as threatened in 1999 and then endangered in 2001 (Baird 2001). In the United States, it was listed as endangered in 2005 (http://www.nwr.noaa.gov/). The goals of our study were to 1) use parentage and kinship analysis to infer patterns of mating and male reproductive success in the population, 2) evaluate whether any of the sampled offspring had fathers from other killer whale populations, and 3) estimate the current effective population size of the population and evaluate evidence for a recent population bottleneck.

Materials and Methods

Field Work/Sample Collection

We obtained samples from remotely collected epidermal/blubber biopsies, feces and mucous from live whales, and samples from dead stranded animals. Field activities were based out of the San Juan Islands during the summer

months and Puget Sound during the fall and winter months from 2005 to 2009. Identities of individual whales were recorded whenever possible. Individuals are designated alphanumerically with the letter representing the pod (J, K, or L) and the number the individual within the pod (e.g., J1). For all biopsied animals and for many fecal/mucous samples, we obtained a photograph of the dorsal fin and saddle patch areas to confirm field identification. For more information on the individuals sampled for this study, see Supplementary Table S1.

Similar to previous field studies of killer whales (e.g., Baird and Dill 1995; Ford and Ellis 2006), we recorded pod(s) present and the focal animal or group for each encounter (sensu Altmann 1974). All biopsy samples were obtained using documented sampling techniques (Hoelzel et al. 1998; Ylitalo et al. 2001) under ESA Permit # 781-1824-01. Dart tips measured 0.6 cm by 3.5 cm. After obtaining a biopsy, the dart tip was removed from the shaft and wrapped in aluminum foil and stored on ice packs prior to being transferred to −80 °C until analysis.

Fecal and mucous samples were collected using one of 2 techniques: 1) a modification of a method developed by Ford and Ellis (2006) for prey sampling which involved following a focal animal's "fluke prints" until a sample was observed (for additional details, see Hanson et al. 2010) or 2) using scent detection dogs to locate samples floating on the water's surface (Rolland et al. 2006). During focal follows, all fluke prints and the areas between them were examined for evidence of fecal material or mucous. Feces were visually identified as semicohesive brownish to greenish material in the water column or floating at the surface. Mucous was identified as small cohesive whitish material floating on the surface. Samples were stored in plastic bags on ice packs and later stored at −20 or −80 °C prior to analyses.

DNA Extraction and Species and Sex Identification

Total genomic DNA was extracted from skin biopsies and mucous samples using a silca-membrane kit following the manufacturer's (DNeasy Blood and Tissue Kit, Qiagen, Valencia, CA) protocols. DNA extraction from fecal and mucus samples was performed using Qiagen QiaCube and QIAamp DNA Stool Mini Kit. To confirm the presence of killer whale DNA in each fecal/mucus sample, the 16s ribosomal RNA region of the mitochondrial genome was amplified by PCR, sequenced and compared with known killer whale sequence. Samples were genetically sexed by PCR amplification of the SRY and ZFX genes, following the method described by Rosel (2003).

Microsatellite Discovery, Selection, and Genotyping

Biopsy and fecal samples were genotyped at 26 polymorphic microsatellite loci (Table 1). Thirty-six published cetacean microsatellite loci were screened for use and 23 were selected based on the presence of 2 or more alleles in the southern population, reliable amplification, and ease of scoring. We isolated 3 additional loci following the methods described in Hamilton et al. (1999). All reactions were assembled using aerosol-resistant filtered pipette tips and DNA extraction and PCR-setup were performed in a PCR free laboratory. Negative controls were performed during each step of the procedure including DNA extraction, PCR, sequencing, and genotyping. PCR products were visualized using an ABI PRISM 3100 Genetic Analyzer, using ABI LIZ500 as the internal size standard. Fragments were analyzed using ABI GeneScan and Genotyper v3.7 software.

To minimize genotyping errors, template DNA from a known southern killer whale skin biopsy served as a positive control and was PCR amplified in every plate of reactions. In addition, several of the same samples were duplicated within each plate of samples as positive controls. Genotyping accuracy was evaluated by comparing multiple sample types known or suspected to be from the same individual killer whales.

Initial Analysis, Effective Size, and Relatedness Estimation

Microsatellite data were initially analyzed using the MSToolkit (Park 2001) and DROPOUT (McKelvey and Schwartz 2005) computer programs to check for potential errors and identify matching genotypes. The probability of 2 unrelated or full-sib individuals having identical multilocus genotypes by chance was estimated using the methods of Paetkau and Strobeck (1994) and Evett and Weir (1998) as implemented in the DROPOUT and GENECAP (Wilberg and Dreher 2005) computer programs. Based on these results, distinct individuals were expected to differ at multiple loci. Genotypes were therefore considered to be provisionally from the same individual if they mismatched at up to 2 loci. Electropherograms for all pairs of genotypes that mismatched at up to 2 loci were rechecked for potential errors, and ambiguous genotypes were rerun. Genotypes that could not be resolved with confidence were scored as missing data. In nearly all cases, genotypes that initially differed at 1 or 2 loci were in fact inferred to be from the same individual following reexamination of the electropherograms.

After removal of duplicate genotypes, genotypic counts were tested against Hardy–Weinberg equilibrium expectations using the GENEPOP v4 software package (Raymond and Rousset 1995). The GENEPOP package was also used to estimate *F*-statistics (following Weir and Cockerham 1984) and to conduct exact tests of allele frequency differences among pods. The probability of an individual belonging to a particular pod or population given observed allele frequencies was estimated using the method of Rannala and Mountain (1997) as implemented in the GENECLASS v2.0h program (Piry et al. 2004). Effective population size was estimated using the disequilibrium method (Hill 1981; Waples 2006) as implemented in the program LDNE (Waples and Do 2008) and using a relatedness method (Wang 2009) implemented in the COLONY program (Jones and Wang 2009). We also conducted tests for a recent reduction in effective

Table 1 Summary of diversity measures, by locus, for 78 unique genotypes sampled from southern resident killer whales

Locus	H_e	H_o	F_{IS}	F_{ST}	F_{IT}	Alleles	Citation	GenBank accession no.	T_a (°C)
415/416	0.66	0.80	−0.22*	0.00	−0.22	4	Schlötterer et al. (1991)	X68821	48
464/465	0.32	0.30	0.03	0.10**	0.13	2	Schlötterer et al. (1991)	X68823	49
Dde65	0.41	0.47	−0.12	0.00	−0.12	2	Coughlan et al. (2006)	AM087096	58
Dde66	0.66	0.79	−0.21*	0.00	−0.22	3	Coughlan et al. (2006)	AM087097	64
Dde70	0.49	0.51	0.00	−0.02	−0.02	2	Coughlan et al. (2006)	AM087099	61
EV1	0.38	0.36	0.10	−0.01	0.10	2	Valsecchi and Amos (1996)	G09074	50
EV37	0.56	0.60	−0.09	0.03	−0.06	3	Valsecchi and Amos (1996)	G09081	54
EV5	0.49	0.60	−0.27	0.02	−0.24	2	Valsecchi and Amos (1996)	G09078	65
Fcb11	0.53	0.54	−0.06	0.02	−0.04	4	Buchanon et al. (1996)	G02104	50
Fcb12	0.58	0.61	−0.02	0.02	−0.01	3	Buchanon et al. (1996)	G02105	50
Fcb17	0.34	0.35	−0.03	0.04	0.01	2	Buchanon et al. (1996)	G02108	55
Fcb5	0.64	0.59	0.04	0.03*	0.07	3	Buchanon et al. (1996)	G02111	54
Ttru Gt_{142}	0.49	0.52	−0.07	0.02	−0.05	3	Caldwell et al. (2002)	AF416507	60
Ttru Gt_{39}	0.58	0.63	−0.14	0.05	−0.08	3	Caldwell et al. (2002)	AF416504	49
Ttru Gt_{48}	0.50	0.45	0.17	0.01	0.17	2	Caldwell et al. (2002)	AF416505	55
Kw199	0.50	0.61	−0.24	0.00	−0.24	2	Present study	HM450674	54
Kw207	0.43	0.33	0.26	0.00	0.26	2	Present study	HM450675	61
Kw4	0.50	0.55	−0.13	−0.01	−0.14	2	Present study	HM450673	65
KWM12A	0.69	0.73	−0.10	0.09**	0.00	4	Hoelzel et al. (1998)	—	60
KWM2A	0.52	0.45	0.10	−0.01	0.10	3	Hoelzel et al. (1998)	—	56
MK5	0.66	0.71	−0.05	0.00	−0.05	3	Krützen et al. (2001)	AF237890	65
MK9	0.45	0.61	−0.31*	−0.01	−0.33	2	Krützen et al. (2001)	AF237893	65
Ttr04	0.50	0.47	−0.01	0.06*	0.05	2	Rosel et al. (2005)	DQ018982	55
Ttr11	0.51	0.50	0.00	0.03*	0.02	3	Rosel et al. (2005)	GQ504046	49
Ttr34	0.50	0.62	−0.24	−0.01	−0.26	2	Rosel et al. (2005)	DQ018984	49
Ttr48	0.53	0.64	−0.24*	0.06	−0.17	3	Rosel et al. (2005)	DQ018983	55
Population	0.52	0.55	−0.08**	0.02**	−0.06	2.62			
J pod	0.50	0.58	−0.16	—	—	2.58			
K pod	0.50	0.54	−0.09	—	—	2.58			
L pod	0.53	0.53	0.00	—	—	2.58			

H_e is expected heterozygosity assuming random combination of alleles, H_o is the observed proportion of heterozygotes in the sample. F_{IS}, F_{ST} and F_{IT} are Weir and Cockerham's estimators of Wright's *F*-statistics, treating each pod as a "population" (samples sizes for known individuals from the 3 pods were 25, 19, and 28 for J, K, and L pods, respectively); and alleles refers to the number of alleles in the sample. Locus name, reference, GenBank accession number (if any), and annealing temperature (T_a) are also provided.

*$P < 0.05$, **$P < 0.01$.

population size based on allele frequency distribution using the BOTTLENECK program (Cornuet and Luikart 1996).

Maximum likelihood estimates of pairwise coefficients of relatedness (*r*) were obtained using the computer program ML-Relate (Kalinowski et al. 2006) and the relatedness coefficient between the 2 parental genotype contributions within an individual (internal relatedness—IR) was estimated using the method of Amos et al. (2001). The statistical significance of the population mean IR was tested by comparing the observed value against the expected distribution under the assumption of random mating using a Monte Carlo approach implemented in the Mathematica computer program. Observed male and female genotypes were randomly paired to create simulated population samples the same size as the observed sample. The distribution of the means of a large number (1000) of such simulated samples were compared with the observed sample mean, and the *P* value of the test was estimated as the proportion of the simulated sample means that were more extreme than the observed sample mean.

Parentage Analysis

Maternity and paternity analysis utilizing sampled mother–offspring pairs and potential fathers was conducted using the maximum likelihood method implemented in the CERVUS 3.03 program (Kalinowski et al. 2007). In the case of mother–offspring pairs that were confidently identified based on field observations, the CERVUS program was first used to confirm that the pairs had genotypes compatible with a parent–offspring relationship. The simulation function of the CERVUS program was then used to determine delta LOD thresholds (log likelihood of the most likely father minus the log likelihood of the second most likely father) at the 80% and 95% confidence levels, assuming that between 15% and 50% of potential fathers were sampled. Male killer whales reach sexual maturity between 11 and 15 years of age (Olesiuk et al. 1990) so all males in the sample that were born at least 12 years prior to the tested offspring were considered potential fathers (taking into account an ~18 month gestation period).

Extended Pedigree Estimation

The maximum likelihood configuration of an extended pedigree that took into account both parental and sibling relationships was evaluated using the method of Wang and Santure (2009) as implemented in the COLONY program. This method uses simulated annealing to find the most likely pedigree of a sample, taking into account any prior known pedigree relationships. The COLONY method has several advantages over pairwise parentage or pairwise relatedness approaches. In particular, it allows for a priori identification of known and excluded relationships, and it takes into account the pedigree configuration of the entire sample simultaneously, including both parental and sibling relationships, thus making greater use of all available information.

We ran the COLONY program with the following prior constraints from the known histories of individuals in the population (Supplementary Table S1): all known mother–offspring pairs were identified as such; a male was considered a potential father of an individual only if he was born 12 or more years prior to the potential offspring and was alive in the year of conception (based on earliest age at maturity—Olesiuk et al. 1990); a female y was excluded as a potential mother of an individual x if the mother of x was known and was not y, if the mother of x was unknown and y was less than 11 years older than x (Olesiuk et al. 1990) or if the mother of x was unknown and y died prior to the birth year of x; 2 individuals were excluded from being potential maternal half-sibs if both mothers were known and were not the same individual or if only 1 mother was known and that mother reached sexual maturity (assumed to be age 11) after the start of the field study in 1973. All fecal samples not associated with a known individual were assumed to be potential parents unless a known mother was already sampled, a mother was known to be dead at the time of sampling or an individual was too old to have a living sire in the population (the oldest living male in the population was estimated to have been born in ~1950). We initially ran several "medium" length COLONY runs to check for convergence and to help confirm the identity of ambiguously identified fecal samples, followed by 2 "long" runs. The pedigree drawing function in the kinship package for the R statistical program language was used to visualize the pedigrees.

Variance in Male Reproductive Success

We estimated the standardized variance in male reproductive success (variance/mean—Coltman et al. 1998) by weighting the observed numbers of offspring assigned to each male in the population by the number of years the male was reproductive (>age 11 and alive) times the proportion of the sampled animals that were born during the period the male was potentially reproductively active. We tested the observed variance against the expected variance assuming random mating success using the chi-square test simulation function in R with the weights described above (R statistical package; 10 000 iterations).

We evaluated the effects of male age on reproductive success following the methods described by Hollister-Smith et al. (2007). Briefly, for each known-id offspring assigned to a father, we calculated the age-specific opportunities for paternity by counting all of the males in the population in the year of conception for each 1-year age class ranging from 11 (start of sexual maturity) to 54 (oldest known paternity). Average reproductive success at age was then estimated by dividing the total number of offspring produced by a particular age class by the total number of opportunities that age class had to produce offspring. In addition, because sample effort varied among age classes, we calculated an effort term as the proportion of males in an age class in our sample divided by the total number of males in that age class known from census data. The relationship between offspring per opportunity per effort was examined visually and tested statistically using logistic regression as follows:

$$\log\left(\frac{p_i}{1-p_i}\right) = \beta + \beta_1 \cdot \text{age} + \beta_2 \cdot \text{age}^2 + \beta_3 \cdot \text{pod} + \beta_4 \cdot \text{id},$$

where p_i is the expected offspring/opportunity for age class i, the β's are the coefficient terms of the model, and the predictor variables are age, pod, and individual. We evaluated alternative models with different combinations of predictor variables using Akaike's information criterion (AIC). Model fits were conducted using the R statistical package (glm function, family = binomial, link = logit, offset = 1/effort).

Results

Genetic Variation among Samples

We analyzed samples from 33 biopsies, 3 necropsies, and 206 fecal/mucus samples, for a total of 242 samples. The date of sampling ranged from 1990 to 2009, but most samples were collected between 2005 and 2008. Of the samples, 214 (including all of the biopsy and necropsy samples) were successfully genotyped for at least 25 of the 26 loci, and all but 10 samples were successfully genotyped for at least 20 loci. Based on photoidentification, all 36 of the biopsy and necropsy samples were from unique individuals. Each of these samples also had a unique multilocus genotype, consistent with the photoidentification.

Of the 206 fecal or mucus samples, 102 had a matching multilocus genotype to one of the biopsy or necropsy samples and an additional 76 had a matching multilocus genotype to at least one other fecal sample. Of the 42 unique fecal/mucus samples, 8 were identified a priori with confidence to a specific individual based on field identification and 34 were either only tentatively identified, identified to pod only, or had no field identification. In addition, one necropsy sample was obtained from a neonate individual whose mother was not known. In total, after

accounting for genotypes sampled from the same individual, there were 78 unique genotypes in our data set. The estimated probability of 2 individuals having identical genotypes ranged from 4.9×10^{-7} to 9.3×10^{-14}, assuming the 2 individuals were full sibs or were unrelated, respectively. The total number of comparisons among samples was 2.9×10^{4}, so matching samples were very unlikely to belong to different individuals. Based on this, we concluded that the 78 unique genotypes belonged to 78 individuals, 43 of which were associated with a known whale from the photoidentification database (Supplementary Table S1). These 78 individuals account for 43% of the animals identified in the population since 1971 (including animals that died prior to this study) and 82% of the 95 animals alive during at least a portion of the period between 2006 and 2008 when most of the samples for this study were collected (Supplementary Table S1).

Based on comparisons between genotypes from resampled individuals (Hoffman and Amos 2005), the overall genotyping error rate in the study was 0.6%/allele (49 mismatches/8114 comparisons). The actual rate of errors in the final analyzed data set is likely to be considerably lower, however, because most individuals were sampled (and hence genotyped), multiple times and suspected errors were resolved, including the 49 that went into the error rate calculation.

Among the sample of 78 unique genotypes, the number of alleles ranged from 2 to 4 per locus and expected heterozygosity assuming random combination of alleles (unbiased heterozygosity—Nei 1987) ranged from 0.33 to 0.69 (Table 1). Observed heterozygosity was generally higher than expected, both for the entire sample and when the 3 pods were analyzed individually, resulting in negative F_{IS} values for most loci (Table 1). Despite the overall trend for an excess of heterozygotes, however, no single locus deviated statistically from Hardy–Weinberg equilibrium proportions when the samples were analyzed by pod, although several did when analyzed at the whole population level (Table 1).

When the samples were grouped by pod (based on individual identity when known or pod identity for fecal samples not identified to a specific individual), allele frequencies differed significantly among pods when all loci were considered simultaneously (J vs. K: $X^2 = 93.10$, $P = 0.0004$; J vs. L: $X^2 = 74.82$, $P = 0.02$; K vs. L: $X^2 = 102.61$, $P = 0.00004$), and several individual loci had F_{ST} values significantly greater than 0 among the pods (Table 1).

Parentage Analysis

Among the photoidentified samples, there were 14 known and 1 tentative mother–calf pairs (Table2). All of the known mother–calf pairs had genotypes consistent with maternity at every locus. In 3 cases, another female had a genotype with a slightly higher likelihood of maternity than the known mother, but because in each of these cases the field identification was considered definitive and the field observed mother had a genotype that was also consistent with maternity (no mismatches), we concluded that the field identified mother was the true mother. The tentative pair (K7/K11) had incompatible genotypes at 4 of the 26 loci and was therefore not treated as a known mother–calf pair in any subsequent analysis.

One of the sampled males was identified as a likely father for 4 of the 14 confirmed mother–offspring pairs (Table2). In 3 of the cases, 1 sampled father (J1) had a genotype consistent with paternity at all loci and all other potential fathers mismatched at multiple loci. In the fourth case, the inferred father (L41) mismatched at one locus but had a delta LOD score consistent with paternity using the relaxed criteria.

Extended Pedigree Estimation

Pairwise parentage analysis of the 34 unidentified fecal samples proved to be difficult due to the inability to determine the polarity of relationships. In theory, one could use the known maternal pedigree of the population and other information to exclude impossible relationships and attempt to narrow down the potential identities of the unknown individuals based upon their pairwise relationships with known samples. However, the very large number of such pairwise comparisons made this task essentially impossible to perform by hand. It is also a less powerful approach than considering the entire sample simultaneously.

We therefore used the COLONY program to infer additional relationships in the sample using a 2-step approach. First, we analyzed the 78 unique genotypes treating individuals without a known field identification as unknowns. This first round of analysis allowed us to assign identities to several of the initially unidentified individuals based upon their relationships to the confidently identified individuals. We than ran a second COLONY analysis with the updated individuals (including updated maternity, paternity, and maternal sib constraints) to achieve a final "best" configuration.

The most likely 1000 configurations from the second round analysis ranged in log likelihood values from −4549.0 to −4551.5, indicating that the data are compatible with numerous potential configurations, most of which differed from each other in only minor ways. We therefore focused only on those groupings that appeared in >90% of the best 1000 configurations because these groupings are most robustly supported by the data.

The families inferred from the COLONY analysis were consistent with, and extended, the paternity analysis results (Appendix). In particular, the same 2 males—J1 and L41—were the only 2 sampled males to be identified as fathers of individuals sampled for the study. Because the 2-step analysis allowed for the identification of additional fecal/mucus samples based on their relationships to known samples, the number of offspring with identified paternities increased compared with the initial

Table 2 Summary of paternity analysis of known mother–offspring pairs

Ind	Sex	Born	Died	Observed mother	Maternity confirmed?	Inferred paternity	Potential fathers (sampled individuals in bold)
K11	Female	1933	—	K7[a]	No[d]	NA	
L60	Female	1971	2002	L26	Yes[c]	None	**J1**, J3, J6, K1, K2, K5, K19, L1, L8, L10, L13, L16, L20
K13	Female	1972	—	K11	Yes[b]	None	**J1**, J3, J6, K1, K2, K5, K19, L1, L8, L10, L13, L16, L20
L67	Female	1985	—	L2	Yes[b]	None	**J1**, J3, J6, K1, K5, K17, K19, L1, L6, L10, L14, L33, L38, L42, L50, L61
L78	Male	1989	—	L2	Yes[c]	None	**J1**, J3, J6, K1, K5, K17, L1, L10, L14, L33, L38, L39, **L41**, L42, L44, L50, **L57**, L61
K25	Male	1991	—	K13	Yes[b]	None	**J1**, J3, J6, J18, K1, K5, K17, L1, L10, L14, L33, L38, L39, **L41**, L42, L044, L50, **L57**, L61
J27	Male	1992	—	J11	Yes[b]	J1[c]	**J1**, J3, J6, J18, K1, K5, K17, L1, L10, L33, L38, L39, **L41**, L42, L44, **L57**, L58, L61, L62
L88	Male	1993	—	L2	Yes[c]	None	**J1**, J3, J6, J18, K1, K5, K17, L1, L10, L33, L38, L39, **L41**, L42, L44, **L57**, L58, L61, L62
K27	Female	1994	—	K13	Yes[b]	J1[c]	**J1**, J3, J6, J18, K1, K17, L1, L10, L33, L38, L39, **L41**, L42, L44, **L57**, L58, L61, L62
J31	Female	1995	—	J11	Yes[c]	None	**J1**, J3, J6, J18, K1, K17, L1, L10, L33, L38, L39, **L41**, L42, L44, **L57**, L58, L61, L62
L98	Male	2000	2006	L67	Yes[e]	None	**J1**, J6, J18, **K21**, L1, L38, L39, **L41**, L44, **L57**, L58, L62, L71, L73, L74
K34	Male	2002	—	K13	Yes[b]	L41[b]	**J1**, **K21**, L1, L39, **L41**, **L57**, L58, L62, L71, **L73**, L74, **L78**, L79
J38	Male	2003	—	J22	Yes[e]	None	**J1**, **K21**, **K25**, **L41**, **L57**, L58, L71, **L73**, L74, **L78**, L79, L84, **L85**
J39	Male	2003	—	J11	Yes[e]	J1[c]	**J1**, **K21**, **K25**, **L41**, **L57**, L58, L71, **L73**, L74, **L78**, L79, L84, **L85**
L101	Male	2003	—	L67	Yes[c]	None	**J1**, **K21**, **K25**, **L41**, **L57**, L58, L71, **L73**, L74, **L78**, L79, L84, **L85**

Samples include biopsies/necropsies and positively identified fecal/mucus samples only.

[a] Observational maternity considered tentative.

[b] 80% confidence.

[c] 95% confidence.

[d] Tentative mother's genotype was inconsistent with maternity at 4/26 loci.

[e] Observed mother's genotype was consistent with maternity but observed mother not most likely mother in sample.

paternity analysis. In particular, J1 was inferred to be the father of 8 individuals (5 from J pod, 2 from K pod, and 1 unidentified) and L41 was the inferred father of 4 individuals (3 from J pod and 1 from K pod) (Appendix, Figure 1, Supplementary Figure S1).

Among the families that consistently appeared in the best 1000 configurations, there were inferred to be 7 unidentified (and unsampled) fathers. Based on the ages of their inferred offspring, all but 2 of these unknown males corresponded to 2 or more potential males who were alive during the time period necessary to have sired the inferred offspring (Table 3). Of the 2 inferred males who did not correspond to any known southern resident male based on offspring age, one (Uns-M1) was the inferred father of a very old individual and there were simply no identified males in the population old enough to be his father. The other (Uns-M6) was inferred to be the father of one old individual (J2) and one young individual (K38) and there were no males in the population old enough to have sired both of these individuals, suggesting that this inferred paternal sibling relationship was spurious or perhaps caused by some other relationship between the 2 individuals.

Most of the inferred matings, including those within pods, involved pairs of individuals with coefficients of relatedness < 0.15, and none were between inferred siblings or parents and offspring (Appendix, Figure 1). IR for individuals in the population was on average less than the value of 0.0 expected if matings were between random individuals in the population (mean $= -0.07$, standard deviation [SD] $= 0.18$, $P < 0.001$), further indicating a tendency for matings between individuals less related than expected by chance. The 2 pods differed significantly ($F = 3.983$, degrees of freedom [df] $= 2,70$, $P < 0.05$) in IR values, with J pod having lower average IR than K or L pod (mean (SD) $= -0.15$ (0.15), -0.06 (0.19), and -0.01 (0.20), for J, K, and L pods, respectively).

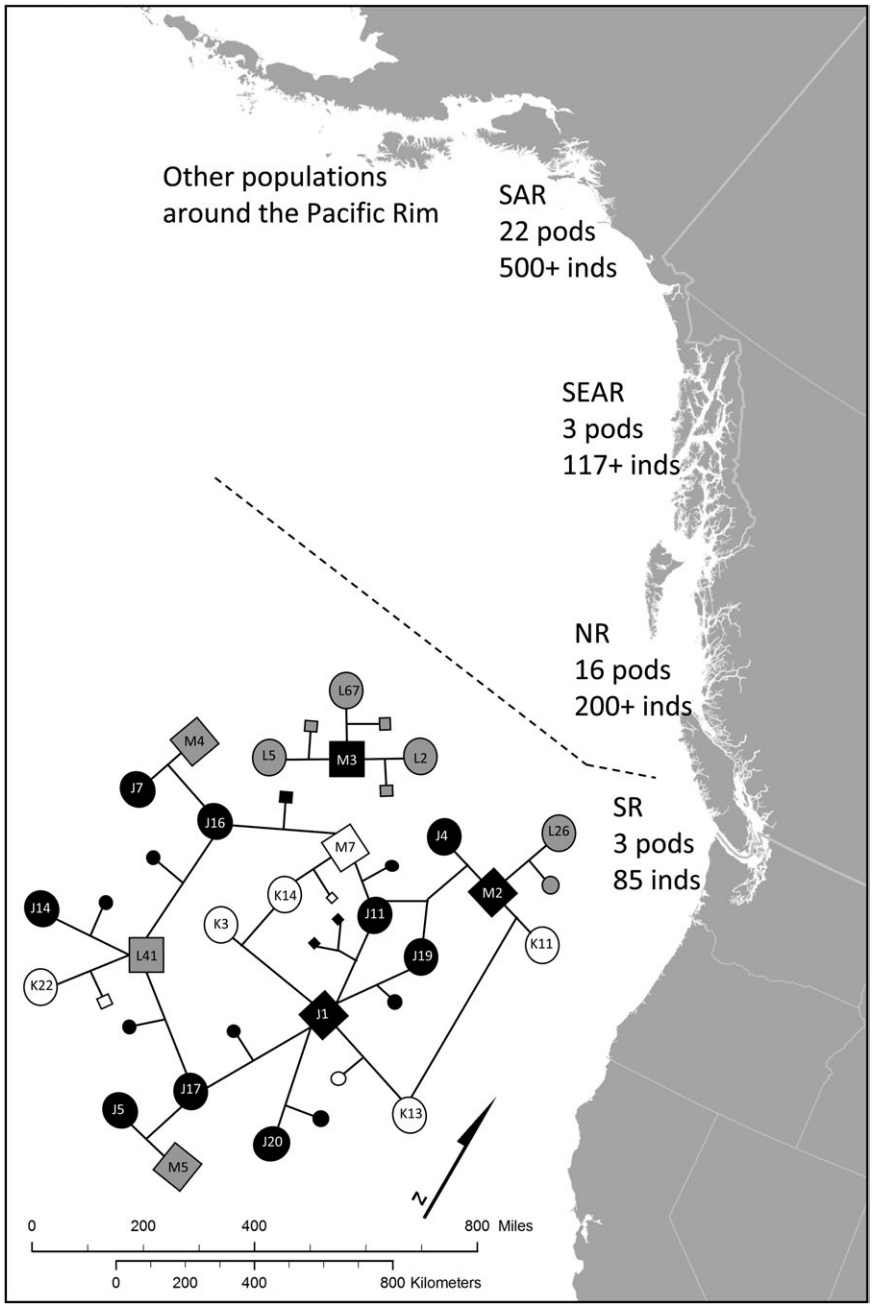

Figure 1. Pedigree network illustrating patterns of paternity within and between the 3 southern community pods based on the most likely pedigree configuration from the COLONY analysis (Appendix). Symbols for J, K, and L pods are black, white, and gray, respectively. Males are symbolized by squares, females by circles. The pod identities of inferred but unsampled males (designated M) are based on the most likely pod of origin and are not known with confidence (Table 3). For a more detailed version of the pedigree that includes offspring identify, see Supplementary Figure S1. Information on other resident killer whale populations is provided for context (SAR refers to Southern Alaska Residents—Matkin et al. 1999; Allen and Angliss 2009; SEAR refers to Southeastern Alaska Residents—Dahlheim et al. 1997; Allen and Angliss 2009; NR refers to Northern Residents—Ford 1991; Allen and Angliss 2009). The dashed line indicates the inferred lack of first generation immigrants from other populations based on the GENECLASS analysis (note that not all populations were sampled; for details, see text).

Gene Flow from Other Populations

When compared with samples of killer whales representing 11 different ecotype/regions across the northern North Pacific (Parsons KM, unpublished data; Supplementary Table S2), none of the southern resident samples or the inferred but unsampled fathers were likely to be first

Table 3 Potential identities of unsampled fathers based on inferred genotype from the pedigree analysis

Unknown father	Potential identities based on offspring age[a]	Relative % probability of pod membership		
		J	K	L
Uns-M1	None	14	58	28
Uns-M2	~~J1~~, J3, K1, K19, K5, L13, L16, L20	69	22	9
Uns-M3	~~J1~~, J6, L1	76	0	24
Uns-M4	~~J1~~, J3, K1, K19, K2, K5, L13, L16, L20	6	30	64
Uns-M5	~~J1~~, J3, J6, K1, K19, K5, L1, L10, L13, L16, L20, L33, L6, L8	7	27	66
Uns-M6	None	43	6	52
Uns-M7	~~J1~~, J18, J3, J6, K1, K17, L1, L10, L33, L38, L39, ~~L41~~, L42, L44, ~~L57~~, L61	9	76	15

The most likely pod of origin based on pod assignment using GENECLASS is underlined.

[a] Genetically excluded individuals (J1, L41, and L57) shown with strike through.

generation immigrants based on the criteria of Rannala and Mountain (1997) using the GENECLASS computer program. In particular, every southern resident sample and all of the inferred paternal genotypes were more likely to belong to the southern resident population than any of the other region/ecotype sample groups, and none of the samples had a significantly lower ($P < 0.05$) than expected likelihood of belonging to the southern population.

Within the southern population, the ability to self-assign individuals to their known pod using GENEGLASS was limited. The average rate of self-assignment of known southern individuals to their known pod was 59%. Correct self-assignment rates to J and K pods were somewhat higher than to L pod (60% and 68% for J and K pods vs. 54% for L pod). The lower self-assignment rate to L pod is consistent with the more fluid social connections in this pod compared with J and K pods (Parsons et al. 2009) and a previous suggestion that L pod may represent multiple pods (Hoelzel 1993; Baird 2000). Consistent with the modest rates of self-assignment, the unsampled paternal genotypes inferred from the COLONY analysis are generally consistent with fathers from more than one pod (Table 3). However, in some cases membership in a pod could be ruled out. In particular, male Uns-M3 was inferred to be either J6 or L1 based on progeny age (because there were no potential reproductive age males in K pod during that period), and the assignment results from his inferred genotype were consistent with this inference (Table 3).

Assuming that unsampled males Uns-M2, Uns-M3, Uns-M4, Uns-M5, and Uns-M7 were the oldest member of the most likely pod based on genetic assignment (J3, J6, L16, L1, and K1, respectively), the standardized variance in male reproductive success was estimated to be 0.38. Essentially the same value was obtained under alternative assumptions about the identities of the unsampled fathers. This variance is significantly greater than expected if reproductive success were random (Monte Carlo chi-square test, $\chi^2 = 228$, $P < 0.001$).

Graphically, there appears to be a positive relationship between reproductive success (offspring per mating opportunity) and age. Similar results were obtained assuming that unsampled males were either the oldest or the youngest members of the most likely pod based on genetic assignment (Figure 2). Under the assumption that unsampled fathers were the oldest member of their most likely pod (Table 3), a model that included only age as a predictor variable was the most informative according to its AIC value and the effect of age was highly significant in this model (Table 4). A model that included pod and age was nearly as well supported, but models that did not include age were not well supported. Under the assumption that unsampled fathers were the youngest member of their most likely pod (Table 3), results were similar except that the model that included individual identity had the lowest AIC value (Table 4). The results remained similar if the oldest sampled individual (J1) was removed from the analysis (age term = 0.07, $P = 0.004$, old assumption set; age term = 0.043, $P = 0.10$, young assumption set).

Current and Historical Effective Population Size

The linkage disequilibrium (LDNE) and variance in family size (COLONY) based estimators of current effective population size were very similar: 26 (95% confidence interval: 21–32) for the LDNE estimate and 30 (19–50) for the family sized based estimate. The LDNE estimates for the individual pods were 16 (11–26), 14 (9–16) and 10 (8–15) for J, K, and L pods, respectively. Both of the statistical tests for a population bottleneck described by Cornuet and Luikart (1996) as performed by the BOTTLENECK program were highly significant, suggesting that the population has experienced a recent reduction in population size. In particular, under the stepwise mutation model, the expected number of loci with excess variation was 13.45, compared with 24 loci with observed variation excess (binomial $P = 0.00001$), and the standardized difference between expected and observed

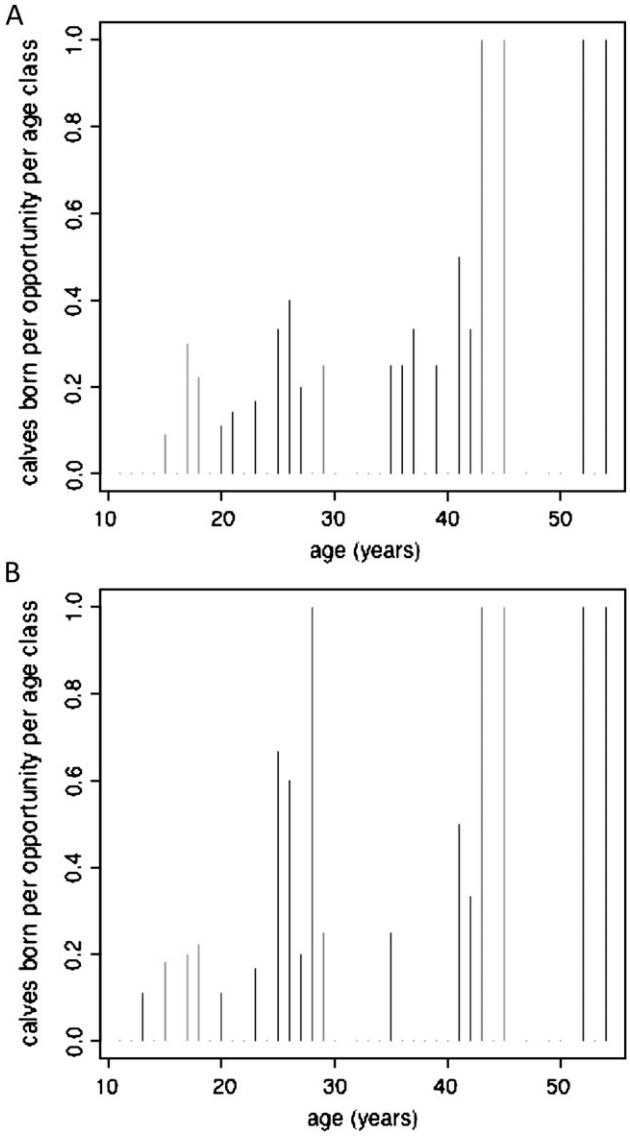

Figure 2. Number of sampled calves per mating opportunity for males age 11–55. A mating opportunity was defined as a potential father being alive during the year prior to the birth year of an offspring with an inferred paternity in this study, corrected for sampling effort. (**A**) Results under the assumption that unsampled, inferred fathers in Table 3 were the oldest member of the most likely pod. (**B**) Results under the assumption that the unsampled, inferred fathers in Table 3 were the youngest member of the most likely pod. For details, see text.

levels of variation across loci was highly significant ($T_2 = 5.408$, $P < 0.00001$).

Discussion

The inferred paternal pedigree of the southern resident killer whales indicated that the variance in male reproductive success in this population was greater than random expectations. The standardized variance in male reproductive success we estimated (0.38) is similar to that observed in North Atlantic right whales (*Eubalaena glacialis*), humpback whales (*Megaptera novaeangliae*), and harbor seals (*Phoca vitulina*) (Frasier et al. 2007 and references therein) and is lower than the within-coalition variance in bottlenose dolphins (2.4—Krützen et al. 2004). Frasier et al. (2007) hypothesized that the moderate variance in male reproductive success observed in these aquatic mammals was a reflection of the difficulty associated with males controlling access to females in an aquatic habitat, a situation that clearly also applies to killer whales.

We found a positive, significant relationship between reproductive success and male age (Figure 2; Table 4). This relationship should be viewed with some caution because it is based on a fairly small number of males, some of whose identities (and hence ages) are not known with confidence (Table 3). Results were similar, but not identical, under alternative assumptions about the identities of inferred but unsampled fathers (Figure 2; Table 4). Despite these uncertainties, however, the data clearly suggest male reproductive success increased with age in this population. Of the 8 mature males included in our sample, only 2 were identified as likely fathers of any of the observed offspring. One of these fathers, J1, was born in ~1950 and was the oldest male in the population at the time of conception of each of the offspring analyzed for this study and ~55 at the time of conception of the youngest of his offspring in the sample. The other, L41, was born in 1977 and (with L57) was the second oldest male in the population at the time of conception of most of the offspring in the study. The youngest paternity involving a sampled male was L41, who was 21 when he sired J35. Depending on the true identities of the unsampled but inferred fathers (Appendix), these fathers generally ranged in age from their late teens to their early 30s. Overall, our results are therefore consistent with the previous observation that killer whale males probably become sexually mature in their teens and physically mature at ~21 (Olesiuk et al. 1990).

In many mammalian species, male reproductive success initially increases with age as animals mature and reach their prime condition and then decreases again at the onset of old age, although the pattern of male reproductive senescence varies greatly among species (e.g., Hollister-Smith et al. 2007; Nussey et al. 2009; Wroblewski et al. 2009). We found no evidence for male reproductive senescence in killer whales, but our sample size (particularly of old males) was small. At 725 cm and 676 cm in length respectively, L41 and J1 are also estimated to be the largest and third largest living males in the population (Fearnbach et al. 2011; L78, born in 1989, is second largest at 698 cm), suggesting that large size could also contribute to male mating success. As more data are collected on the lifetime patterns of reproductive success for males born subsequent to the initiation of the study, it will be possible to gain a better understanding of the relationship between male age and size and reproductive success.

Table 4 Estimated model coefficients and AIC values for 4 alternative models describing the relationship between age and male reproductive success (offspring/mating opportunity at age)

	Old assumption set[a]					Young assumption set				
Model[b]	Age	Age^2	L pod	K pod	AIC	Age	Age^2	L pod	K pod	AIC
Age	0.09***	—			**137.46**	0.07***	—	—	—	139.74
Age, pod	0.07***	—	−0.99	−0.78	137.84	0.06**	—	−1.17*	−1.10	137.99
Age, age^2	0.15	−0.0011	—	—	139.06	0.10	−0.00037	—	—	141.69
Age, Age^2, pod	0.16	−0.0015	−1.04	−0.80	139.09	0.11	−0.00088	−1.20*	−1.13	139.71
Pod	—	—	−1.62**	−1.26	149.02	—	—	−1.65**	−1.43	143.49
Age, id	0.06**	—	—	—	153.36	0.06*	—	—	—	**128.45**
Age, age^2, id	0.08	−0.00023	—	—	155.35	0.06	−0.000063	—	—	130.45
Null	—	—	—	—	156.85	—	—	—	—	152.25
id	—	—	—	—	158.35	—	—	—	—	131.25

[a] Results are presented under 2 different assumptions regarding the identities of inferred but unsampled males (Table 3). The "old" assumption set assumes that each inferred male is the oldest member of the most likely pod; the "young" assumption set assumes that each inferred male is the youngest member of the most likely pod. For details, see text.

[b] Each model is described by the terms included—age (in years), age squared, individual, or pod. Estimated model coefficients for age, age^2, and pod (in contrast to J pod) are provided in the table. No coefficients for "individual" were statistically significant. The lowest (most supported) AIC value for each assumption set is in bold. For details, see text.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

There are several plausible biological mechanisms that could lead to a relationship between male age and reproductive success, including female choice for older or larger males, competitive dominance of younger males by older males, or sperm competition. Consistent with a mating system involving either female choice or male competition, killer whales are sexually dimorphic: Adult males are both much larger than adult females and they have greatly enlarged appendages (dorsal fins, flippers, and flukes). Baird (2002) suggested that the sexual dimorphism in appendage size may be a sexually selected trait, with females attracted to males with large appendages. The relationship between size and male reproductive success we observed is consistent with this hypothesis, although more detailed morphological information on individual males will be necessary to test this relationship with rigor.

Overt male–male aggression has been observed only rarely in killer whales, perhaps due in part to limitations associated with surface observations (Jacobsen 1986). There is little evidence of conspecific scarring on southern population males (Center for Whale Research, unpublished data), which suggests that males in this population are not routinely fighting each other. There are also frequent periods when large males forage on their own, so females would appear to have ample opportunities to mate with smaller, younger males if they were inclined to do so. Indeed, all females with more than one offspring in our sample were inferred to have mated with more than one male. These observations suggests that variance in male reproductive success in this population is unlikely to be driven by aggressive male–male competition, although more subtle forms of competition via sperm competition or nonaggressive dominance hierarchies cannot be ruled out. Indeed, Frasier et al. (2007) concluded that sperm competition was a likely mechanism for a similar level of variance in male mating success in North Atlantic right whales. Compared with right whales, however, killer whales (and all other cetaceans) have a much lower residual testes size for their body weight (MacLeod 2010), suggesting that although sperm competition cannot be ruled out it may not be a dominant factor driving variance in reproductive success in killer whales.

We found no evidence that any of the sampled offspring were fathered by nonsouthern community males, consistent with previous model-based estimates of <1 migrant/generation into the southern community (Hoelzel et al. 2007). This differs from another recent pedigree-based study of North Pacific killer whale mating patterns that suggested 45% of inferred paternities were from outside the maternal population, including 3 of the 4 paternity assignments for the southern group (Pilot et al. 2010). While our results do not conflict with any of the specific paternity assignments in the Pilot et al. (2010) study, we think the weight of evidence suggests that females in the southern community mate rarely, if at all, with males from other populations. In particular, the degree of differentiation among North Pacific resident populations (F_{ST} ranges from ~0.1 to ~0.2; Barrett-Lennard 2000; Hoelzel et al. 2007) and associated estimates of gene flow (Hoelzel et al. 2007) are not consistent with nearly half of the successful matings occurring between populations. In addition, both our study and the Pilot et al. (2010) study are consistent in finding a lack of putative immigrants into the southern community based on population assignment tests. Our results therefore reinforce the demographically isolated nature of this small population.

Matings within and among Pods

Of the 12 identified paternities, 5 involved matings between J1 and a J pod female (Appendix, Figure 1). By

process of elimination, the mating that produced L101 also appears likely to be between individuals of the same pod (father is inferred to be one of L58, L71, L74, L79, or L84—Table 2). These results suggest that southern community individuals do not entirely avoid mating with members of their own pod, in contrast to what has been found in the northern community (Barrett-Lennard 2000) and long-finned pilot whales (Amos et al. 1993). Pilot et al. (2010) also found evidence of intrapod mating in killer whales, in both the southern population and an Alaskan population. There are several possible explanations why the level of interpod mating may vary among populations. First, most of the inferred intrapod matings involved a single individual, J1. It is possible that the pattern is largely due to the idiosyncrasies of a single individual. Second, the northern community pods are associated with distinct acoustic clans, unlike the southern community, which consist of only a single acoustic clan (Ford 1991). Barrett-Lennard hypothesized that females used acoustic cues to avoid mating within their clan, a strategy that might be less effective in the single-clan southern community. However, the 3 southern resident pods differ substantially in their use of distinct call types (albeit in the same acoustic "clan"; Foote et al. 2008), so this explanation appears unlikely. Finally, the small size of the southern community may also limit available mate choice. In all of the mating events inferred in our study, however, at least one and usually several mature males were available from each pod, suggesting at least the opportunity for female choice based on pod membership.

Despite the evidence for within-pod matings, there was also evidence of inbreeding avoidance in the population. None of the offspring sampled were the result of matings between members of the same matriline (Supplementary Table S1) and the negative within-pod F_{IS} statistics and lower than expected IR values also suggest ongoing avoidance of inbreeding, similar to what has been observed in other killer whale populations (e.g., Barrett-Lennard 2000). Interestingly, there was no evidence of offspring from father–daughter matings, suggesting that males may be able to recognize their offspring, as apparently occurs in other mammal species (e.g., Archie et al. 2007; Widdig 2007). In contrast, in a wild population of bottlenose dolphins in East Shark Bay, Australia, Frère et al. (2010) found higher levels of IR than expected under random mating and in the same population, Krützen et al. (2004) found evidence of father–daughter mating. Frère et al. (2010) suggested that a combination of male and female philopatry, overlapping generations and male sexual coercion were contributing factors to inbreeding in the East Shark Bay dolphin population. The first 2 factors also clearly apply to the temperate Eastern Pacific coastal killer whale populations. Relatively little is known about killer whale sexual behavior but neither obvious male sexual coercion or male mating coalitions have been reported (e.g., Jacobsen 1986; Osborne 1986). The lack of sexual coercion may therefore at least partly explain the difference in the degree of inbreeding between the 2 groups, despite many other similarities in their social systems and life-history patterns.

Both of the tests for a recent population bottleneck described by Cornuet and Luikart (1996) were highly significant, suggesting that the southern community has experienced a reduction in effective population size. Cornuet and Luikart's method does not estimate either the time of the bottleneck or the prebottleneck population size, but the power analysis they describe indicates that greatest power to detect a bottleneck occurs from ~0.1 to 2.5 × $2N_e$ generation after a population size reduction, depending on the severity of the bottleneck. Based on their simulations, the tests have little or no power to detect very recent (<1 generation) bottlenecks, so the population size reduction that has influenced patterns of variation in the southern population likely predates the 20th century decline that ultimately led to the listing of the population both in Canada and in the United States. A signal of a more distant bottleneck would be consistent with the findings of Hoelzel et al. (2007) who estimated that the eastern North Pacific fish-eating killer whale populations are currently ~1000× smaller than the ancestral population from which they diverged several thousand or more (see also Morin et al. 2010) years ago.

The estimated effective population size of ~26 is roughly 1/3 of the average census size of 85 since 1971, a ratio typical for many mammalian populations (Frankham 1995). The estimated effective size is much smaller than is generally considered to be optimal for the viability of an isolated population (Gilpin and Soule 1986), suggesting that in the absence of gene flow from other populations, the southern population may be at significant risk of genetic deterioration from inbreeding, accumulation of deleterious mutations, and lack of adaptive variation (Lande 1995; Lande and Shannon 1996). The southern population's rate of population growth is slightly lower than the ecologically similar but larger sized northern population (Olesiuk et al. 1990; Ward et al. 2009), and it is possible that the very small effective size of the southern population contributes to this difference.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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Appendix Most likely sample family configuration estimated by the method of Wang and Santure (2009) using the Colony program. Only families that appeared in >90% of the most likely 1000 configurations are shown. Unsampled fathers are designated "Uns-M," unsampled mothers "Uns-F." Identified offspring and parents are designated using their standard names (Supplementary Table S1). Unidentified individuals are designated by their pod and gender when known and "Ukn" when not. r_{fm} is maximum likelihood estimate of the coefficient of relatedness between the father and mother. NA, not applicable.

Offspring	Father	Mother	r_{fm}	Offspring birth year	Potential fathers (age at offspring birth year, relatedness of potential father to mother [values > 0.25 in bold])
Ukn-1	J1	Uns-F4	0.00	NA	NA
K14	J1	Uns-F6	0.11	1977	J1 (27) J3 (24) J6 (19) K1 (22) K5 (24) K19 (24) L1 (18) L6 (15) L8 (19) L10 (18) L13 (27) L16 (28) L20 (22) L33 (14) L38 (12)
J27	J1	J11	0.07	1992	J1 (42, 0.07) J3 (39) J6 (34) J18 (14) K1 (37) K5 (39) K17 (26) L1 (33) L10 (33) L33 (29) L38 (27) L39 (17) L41 (15, 0.00) L42 (19) L44 (18) L57 (15, 0.00) L58 (12) L61 (19) L62 (12)
J28	J1	J17	0.00	1993	J1 (43, 0.00) J3 (40) J6 (35) J18 (15) K1 (38) K5 (40) K17 (27) L1 (34) L10 (34) L33 (30) L38 (28) L39 (18) L41 (16, 0.06) L42 (20) L44 (19) L57 (16, 0.00) L58 (13) L61 (20) L62 (13)
K27	J1	K13	0.02	1994	J1 (44, 0.02) J3 (41) J6 (36) J18 (16) K1 (39) K17 (28) L1 (35) L10 (35) L33 (31) L38 (29) L39 (19) L41 (17, 0.00) L42 (21) L44 (20) L57 (17, 0.00) L58 (14) L61 (21) L62 (14)
J32	J1	Uns-F8	—	1996	J1 (46) J3 (43) J6 (38) J18 (18) K1 (41) K17 (30) L1 (37) L10 (37) L33 (33) L38 (31) L39 (21) L41 (19) L42 (23) L44 (22) L57 (19) L58 (16) L61 (23) L62 (16) L63 (12)
J39	J1	J11	0.07	2003	J1 (53, 0.07) K21 (17, 0.01) K25 (12) L41 (26, 0.00) L57 (26, 0.00) L58 (23) L71 (17) L73 (17, 0.00) L74 (17) L78 (14, 0.04) L79 (14) L84 (12) L85 (12, 0.15)
J41	J1	J19	0.00	2005	J1 (55) J26 (13) J27 (13) K21 (19) K25 (14) K26 (12) L41 (28) L57 (28) L71 (19) L73 (19) L74 (19) L78 (16) L79 (16) L84 (14) L85 (14) L87 (13) L88 (12) L89 (12)
J35	L41	J17	0.06	1998	J1 (48, 0.00) J6 (40) J18 (20) K1 (43) K21 (12, 0.00) L1 (39) L10 (39) L38 (33) L39 (23) L41 (21, 0.06) L44 (24) L57 (21, 0.00) L58 (18) L61 (25) L62 (18) L71 (12) L73 (12, 0.12) L74 (12)
K33	L41	K22	0.00	2001	J1 (51, 0.00) J18 (23) K21 (15, 0.11) L1 (42) L39 (26) L41 (24, 0.00) L57 (24, 0.04) L58 (21) L62 (21) L71 (15) L73 (15, 0.00) L74 (15) L78 (12, **0.28**) L79 (12)
J40	L41	J14	0.00	2004	J1 (54, **0.54**) J26 (12, 0.00) J27 (12, 0.12) K21 (18, 0.00) K25 (13, 0.00) L41 (27, 0.00) L57 (27, 0.02) L58 (24) L71 (18) L73 (18, **0.50**) L74 (18) L78 (15, **0.21**) L79 (15) L84 (13) L85 (13, 0.00) L87 (12, **0.37**)
J42	L41	J16	0.11	2007	J1 (57, 0.00) J26 (15, **0.50**) J27 (15, 0.13) K21 (21, 0.22) K25 (16, 0.01) K26 (14, 0.00) L41 (30, 0.11) L57 (30, 0.07) L71 (21) L73 (21, 0.00) L74 (21) L78 (18, 0.00) L79 (18) L84 (16) L85 (16, **0.25**) L87 (15, 0.06) L88 (14, 0.00) L89 (14) L92 (12)
J1	Uns-M1	Uns-F1	0.37	1950	NA
UKn-m2	Uns-M2	Uns-F6	0.00	NA	

L60	Uns-M2	L26	0.09	1971	J1 (21) J3 (18) J6 (13) K1 (16) K2 (21) K5 (18) K19 (18) L1 (12) L8 (13) L10 (12) L13 (21) L16 (22) L20 (16)
K12	Uns-M2	Uns-F10	—	1971	J1 (21) J3 (18) J6 (13) K1 (16) K2 (21) K5 (18) K19 (18) L1 (12) L8 (13) L10 (12) L13 (21) L16 (22) L20 (16)
J11	Uns-M2	Uns-F2	0.5	1972	J1 (22, 0.00) J3 (19) J6 (14) K1 (17) K2 (22) K5 (19) K19 (19) L1 (13) L8 (14) L10 (13) L13 (22) L16 (23) L20 (17)
K13	Uns-M2	K11	0.00	1972	J1 (22, 0.00) J3 (19) J6 (14) K1 (17) K2 (22) K5 (19) K19 (19) L1 (13) L8 (14) L10 (13) L13 (22) L16 (23) L20 (17)
J19	Uns-M2	Uns-F2	0.5	1979	J1 (29, 0.00) J3 (26) J6 (21) K1 (24) K5 (26) K17 (13) K19 (26) L1 (20) L6 (17) L8 (21) L10 (20) L13 (29) L16 (30) L20 (24) L33 (16) L38 (14)
Ukn-Jf1	Uns-M3	Uns-F2	0.00	NA	NA
J14	Uns-M3	Uns-F3	0.10	1974	J1 (24, **0.30**) J3 (21) J6 (16) K1 (19) K2 (24) K5 (21) K19 (21) L1 (15) L6 (12) L8 (16) L10 (15) L13 (24) L16 (25) L20 (19)
L73	Uns-M3	L5	0.05	1986	J1 (36, 0.00) J3 (33) J6 (28) K1 (31) K5 (33) K17 (20) K19 (33) L1 (27) L10 (27) L14 (14) L33 (23) L38 (21) L42 (13) L44 (12) L50 (13) L61 (13)
L78	Uns-M3	L2	0.08	1989	J1 (39, 0.08) J3 (36) J6 (31) K1 (34) K5 (36) K17 (23) L1 (30) L10 (30) L14 (17) L33 (26) L38 (24) L39 (14) L41 (12, 0.00) L42 (16) L44 (15) L50 (16) L57 (12) L61 (16)
L98	Uns-M3	L67	0.00	2000	J1 (50, 0.00) J6 (42) J18 (22) K21 (14) L1 (41) L38 (35) L39 (25) L41 (23, 0.04) L44 (26) L57 (23) L58 (20) L62 (20) L71 (14) L73 (14, 0.00) L74 (14)
J16	Uns-M4	Uns-F4	0.00	1971	J1 (21, 0.00) J3 (18) J6 (13) K1 (16) K2 (21) K5 (18) K19 (18) L1 (12) L8 (13) L10 (12) L13 (21) L16 (22) L20 (16)
J17	Uns-M5	Uns-F5	—	1977	J1 (27) J3 (24) J6 (19) K1 (22) K5 (24) K19 (24) L1 (18) L6 (15) L8 (19) L10 (18) L13 (27) L16 (28) L20 (22) L33 (14) L38 (12)
J2	Uns-M6	Uns-F6	0.00	1916	NA
K38	Uns-M6	K20	0.00	2004	J1 (54, 0.00) J26 (12, 0.02) J27 (12, 0.00) K21 (18, 0.01) K25 (13, **0.35**) L41 (27, 0.22) L57 (27, 0.00) L58 (24) L71 (18) L73 (18, 0.00, 0.00) L74 (18) L78 (15) L79 (15) L84 (13) L85 (13, **0.50**) L87 (12, 0.00)
UKm1	Uns-M7	Uns-F6	0.52	NA	
J26	Uns-M7	J16	0.00	1992	J1 (42, 0.00) J3 (39) J6 (34) J18 (14) K1 (37) K5 (39) K17 (26) L1 (33) L10 (33) L33 (29) L38 (27) L39 (17) L41 (15, 0.11) L42 (19) L44 (18) L57 (15, 0.07) L58 (12) L61 (19) L62 (12)
K26	Uns-M7	K14	0.36	1993	J1 (43, **0.61**) J3 (40) J6 (35) J18 (15) K1 (38) K5 (40) K17 (27) L1 (34) L10 (34) L33 (30) L38 (28) L39 (18) L41 (16, 0.00) L42 (20) L44 (19) L57 (16) L58 (13) L61 (20) L62 (13)
J31	Uns-M7	J11	0.00	1995	J1 (45, 0.09) J3 (42) J6 (37) J18 (17) K1 (40) K17 (29) L1 (36) L10 (36) L33 (32) L38 (30) L39 (20) L41 (18, 0.00) L42 (22) L44 (21) L57 (18, 0.00) L58 (15) L61 (22) L62 (15)

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