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Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (*Orcinus orca*)

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Abstract

In social species, breeding system and gregarious behavior are key factors influencing the evolution of large-scale population genetic structure. The killer whale is a highly social apex predator showing genetic differentiation in sympatry between populations of foraging specialists (ecotypes), and low levels of genetic diversity overall. Our comparative assessments of kinship, parentage and dispersal reveal high levels of kinship within local populations and ongoing male-mediated gene flow among them, including among ecotypes that are maximally divergent within the mtDNA phylogeny. Dispersal from natal populations was rare, implying that gene flow occurs without dispersal, as a result of reproduction during temporary interactions. Discordance between nuclear and mitochondrial phylogenies was consistent with earlier studies suggesting a stochastic basis for the magnitude of mtDNA differentiation between matriline. Taken together our results show how the killer whale breeding system, coupled with social, dispersal and foraging behaviour, contributes to the evolution of population genetic structure.

Introduction

Social structure and breeding behaviour have an important effect on population genetic structure. Breeding system properties, including the number of breeders in a social group, their genetic relatedness, and skew in their parentage, determine group composition and the distribution of genetic variation within and between social units (Ross, 2001). This in turn may have a substantial effect on large-scale population genetic structure (Sugg *et al.*, 1996). Studying individual dispersal and mating decisions underlying gene flow may improve our understanding of the factors underlying the formation and maintenance of population genetic structure, though it only offers a contemporary window into this long-term process.

In various highly mobile mammals, cryptic population differentiation in the absence of physical barriers to

dispersal has been revealed and it has been explained by nonrandom dispersal, resulting from dependence on local habitat and prey (e.g. Rueness *et al.*, 2003; Geffen *et al.*, 2004; Natoli *et al.*, 2005). The killer whale *Orcinus orca* provides an extreme example of such cryptic population differentiation (Hoelzel *et al.*, 1998, 2007). In the North Pacific, sympatric populations of foraging specialists (ecotypes), fish-eating 'residents', marine mammal-eating 'transients' and 'offshores' feeding on marine fish and possibly other offshore prey (Ford *et al.*, 2000; Krahn *et al.*, 2007; Dahlheim *et al.*, 2008) each have different mtDNA haplotypes, implying no female-mediated gene flow. F_{ST} values between populations belonging to different ecotypes (ranging from 0.10 to 0.23) at nuclear DNA revealed differentiation in sympatry, but could not rule out male-mediated gene flow (Hoelzel *et al.*, 2007). While dietary differentiation between ecotypes is well documented (e.g. Ford *et al.*, 1998; Krahn *et al.*, 2007), as well as social organization of residents (Bigg *et al.*, 1990; Dahlheim *et al.*, 1997; Ford *et al.*, 2000) and transients (Ford & Ellis, 1999; Baird & Whitehead, 2000), much less is known about mate

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choice and its potential influence on gene flow between social groups. A genetic study of mating patterns in killer whales from the western North Pacific showed that 14 of 19 likely paternal matches occurred between individuals from different acoustic clans, and 18 of them occurred between individuals from different pods (Barrett-Lennard, 2000). Hoelzel *et al.* (2007) suggested that the exploitation of patchy, seasonal food resources by killer whales of each ecotype promotes social philopatry in both sexes, and that inbreeding is avoided through reproduction outside a social group (pod) during temporary interactions (though the data presented there could not prove this). Such interactions could be expected to occur mainly between groups exploring the same resources, but coalescent methods suggest that they also occur between groups specialized on different prey types (Hoelzel *et al.*, 2007). Fitting an isolation with migration (IM) model (Hey & Nielsen, 2004) to the data on genetic variability of North Pacific killer whales suggested ongoing, low-level gene flow between populations, at a similar rate within and between ecotypes (Hoelzel *et al.*, 2007). However, the IM model can only confirm migration following some point of division, which for these populations was estimated at 10 000–20 000 years ago (Hoelzel *et al.*, 2007), and assumes a constant post-isolation migration rate. In this study, we analyse breeding, gene flow and kinship patterns in killer whales using data from recent generations. Based on these data and our knowledge of killer whale social behaviour (e.g. Bigg *et al.*, 1990), we test the hypothesis that gene flow among social groups occurs without permanent dispersal, during temporary associations. Given evidence suggesting isolation by distance within an ecotype (Hoelzel *et al.*, 2007), we further test the hypothesis that genetic dispersal will be more common among social groups more likely to share the same spatial and temporal pattern of habitat use.

Materials and methods

Materials

This study provides individual-based analyses using 16 microsatellite loci and complete mtDNA control region sequences previously assessed at the population level by Hoelzel *et al.* (2007). We studied 213 killer whales from the North Pacific and North Atlantic: 30 from Washington State southern residents (SR), 41 from Southeast Alaskan residents (AR), 14 from a resident population off Kamchatka in Russia (RU), 20 from a resident population off the Aleutians and in the Bering Sea (BS), 14 from the North Pacific offshore population (OS), 33 from transient pods sampled in Southeast Alaska (AT), 22 from transient pods sampled in California (CT), and 40 from the southeast region of Iceland (IC). Sampled individuals from AR belonged to two pods, and sampled individuals from SR belonged to three pods. Samples from AT were

taken from six pods and two solitary males. Nine individuals excluded from the earlier study because they were known offspring of sampled females were included here. Six mtDNA control region haplotypes were found among these eight populations, and each population had one, fixed haplotype, except for the IC population, where two haplotypes occurred (Hoelzel *et al.*, 2007). The analysis of population genetic structure showed that these groups constitute distinct populations, with the exception of BS group, being a mixture of individuals from RU and AR populations (Hoelzel *et al.*, 2007). However, because BS and RU had different mtDNA haplotypes, which implies that they belong to different matrilineal lines and have different population dynamics (Avice, 1995), we kept the subdivision into eight populations in the subsequent analyses. The populations from the North Pacific belong to three ecotypes: resident, transient and offshore, which were defined based on differences in diet, morphology and habitat preferences established during long-term field studies (e.g. Bigg *et al.*, 1990; Dahlheim, 1997, 2008; Ford *et al.*, 1998, 2000). The Iceland population is fish-eating, but it is unclear whether it can be considered as the same ecotype as the North Pacific residents, and therefore it was classified as a distinct ecotype. Hoelzel *et al.* (2007) showed that estimates of splitting time and long-term gene flow between the North Pacific residents and the Icelandic population were within the range of estimates for the foraging specialists from the North Pacific, which may reflect both historical and contemporary connectivity between populations. Therefore, we included the Icelandic population in the current study to test whether this pattern is reflected in the rates of contemporary gene flow.

Parentage and kinship analysis

The limitations to parental and kin assignments based solely on genetic data are well known (see review in Van Horn *et al.*, 2008), and therefore we compared results from several assessment methods, and where possible, incorporated observational data on genealogies and demographics. In three well-studied populations, SR, AR and AT, mother–offspring pairs were identified based on field observations of adult female–calf associations (nine pairs), and adult female–subadult individual associations (which implies lower level of confidence in these assignments; 17 pairs). Parentage analysis was performed using a maximum-likelihood approach implemented in the program CERVUS 3.0 (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007). We considered parentages assigned at strict (95%) and relaxed (80%) confidence levels, determined by simulating parent–offspring pairs and trios based on the allele frequencies in the study population. At first, we compared genotypes of the nine known mother–calf pairs against genotypes of males to identify fathers. In this case, we performed a preliminary test to confirm maternity, and then included information about the

mothers into the input data of the paternity test. We simulated 10 000 offspring and 100 candidate fathers, allowing for 70% of fathers to be unsampled.

Next, we performed parentage tests (maternity, paternity and parent pair analyses) for all other individuals, including the individuals with mothers assigned based on behavioural observations of adult female – subadult individual associations. As a precaution, we did not include *a priori* information about the mothers assigned based on field observations. We simulated 10 000 offspring and 100 candidate parents of each sex, allowing for 50% of mothers and 70% of fathers to be unsampled. The assumed percentage of unsampled parents influences the confidence assigned by CERVUS to parentage assignments. Because these values could not be assessed from the field data, they were likely underestimated. Underestimating these values allowed us to reduce the number of false negatives (rejections of true parentage assignments), while we used external criteria (mtDNA data, results of other analyses – see below) to reduce the number of false parentage assignments. We allowed for less mothers than fathers to be unsampled due to a matrilineal social structure of the killer whale, documented during long-term field studies (e.g. Dahlheim *et al.*, 1997; Ford *et al.*, 2000). Because age of some individuals was unknown, all sampled individuals were considered as both candidate parents and offspring. Parentage assignments were then compared with age data where available (for the SR, AR and AT populations), and in the case of mother–offspring pairs with mtDNA haplotypes, and the assignments inconsistent with these data were excluded. Parents assigned to individuals based on maternity and paternity analyses were compared with parent pairs assigned based on the parent pair analysis. A parent pair was accepted if at least one of the following conditions was fulfilled:

1. the parent pair assignment was significant at least at the relaxed confidence level, and had no more than two mismatching loci, or
2. each parent was assigned at least at the relaxed confidence level (in maternity and paternity assignments), and the parent pair assignment had no more than two mismatching loci.

The threshold of two mismatching loci was decided as a trade-off between two contradictory goals: minimizing the number of rejections of true parents due to genotyping errors, and minimizing the number of false assignments of close relatives as parents.

The results of CERVUS analyses were further assessed by comparison with the results of kinship analysis performed using the programs KINGROUP (Konovalov *et al.*, 2004) and COLONY (Wang, 2004). Both programs implement a maximum-likelihood approach to reconstruct kin groups, accounting for genotyping errors. In KINGROUP, we reconstructed four kinds of kin groups: parent–offspring, full-sibs, half-sibs, and cousins, while in COLONY we reconstructed full-sib groups nested within

half-sib groups. All the analyses were run twice to check for consistency of results. KINGROUP and COLONY have limited possibilities of distinguishing between parent–offspring and full sibs. Therefore, we accepted CERVUS parent–offspring assignments if they were identified as full sibs by either KINGROUP or COLONY, and were consistent with age data, as well as mtDNA data in the case of mother–offspring pairs.

KINGROUP was also applied to estimate pair-wise relatedness between individuals using the maximum-likelihood estimator of Konovalov & Heg (2008) and a group bias correction procedure (Queller & Goodnight, 1989). Pair-wise relatedness estimates were used as an alternative method to identify pairs of closely related individuals within and between populations.

Relatedness within and among pods, populations and ecotypes

Relatedness between groups of individuals was estimated using Queller & Goodnight's (1989) relatedness coefficient *R* and the program RELATEDNESS 5.0.8. Relatedness was estimated within and among pods (when known), populations, and ecotypes, as well as within each sex and between sexes in each population. We also estimated mean relatedness of individuals grouped according to mtDNA haplotypes. As advised by the authors of the program, we applied the group bias correction procedure (Queller & Goodnight, 1989). We compared relatedness levels between groups by calculating *R*-values difference and its 95% CI to test whether the difference is significantly different from zero.

Patterns of gene flow among populations

Genetic distances between populations and individuals were assessed using Principal Coordinate Analysis (PCA) implemented in the package GENALEX 6 (Peakall & Smouse, 2006). PCA was performed based on pair-wise F_{ST} between populations and pair-wise distances between individuals calculated using the method of Smouse & Peakall (1999). We also constructed a Neighbour Joining phylogram of pair-wise F_{ST} between populations, and a Neighbour Joining tree of mtDNA control region haplotypes of the respective populations constructed in PAUP (Swofford, 1998) using K81uf+I+ Γ model of nucleotide substitution selected with Modeltest (Posada & Crandall, 1998).

The program GENECLASS2 (Piry *et al.*, 2004) was used to assign each individual to the most probable population of its origin based on its microsatellite genotype, and to detect first generation migrants (i.e. individuals that have moved from one population to another). Both tests were performed using Rannala & Mountain's (1997) Bayesian method and Monte-Carlo resampling algorithm of Paetkau *et al.* (2004) with 10 000 simulated individuals and type I error of 0.01.

The program *BAYESAss* 1.3 (Wilson & Rannala, 2003) was used to estimate recent, asymmetric gene flow rates between populations from microsatellite data using a Bayesian approach. This program also estimates the posterior probability distributions of individual immigrant ancestries, which allows identifying migrants (i.e. individuals that physically moved between populations). The analysis was run three times to check for consistency of results. The comparison of gene flow rates (obtained from *BAYESAss*) with the number of migrants between populations (obtained from *BAYESAss* and *GENECLASS*) allowed us to distinguish gene flow from dispersal.

Results

Parentage analysis

Out of nine mother–offspring pairs known from observations of adult female–calf associations, *CERVUS* identified seven with strict confidence. In one case, no confidence was assigned to a mother–offspring pair by *CERVUS* despite a lack of mismatches between the genotypes because another closely related female was also assigned as a mother (pair AF15/AF49 – field observed mother indicated in Appendix S1). In another case, no confidence was due to a mismatch that most probably resulted from a genotyping error. Because of the strong evidence from the field observations, as well as from other genetic tests (see Appendix S1), all nine of these mother–offspring pairs were accepted as true relationships. *CERVUS* assigned fathers to four of these nine mother–offspring pairs: one father was from the same pod as the offspring, one from different pod within the same population, and two from different populations within the same ecotype (see Appendix S1).

In the parentage analysis performed for all individuals without mothers assigned *a priori*, *CERVUS* assigned a single parent to 95 individuals and both parents to 22 individuals. However, 57 of these assignments were rejected based on age, mtDNA data, or because they were not confirmed by either *KINGROUP* or *COLONY* results.

Among the accepted assignments, there were 50 individuals with a single parent assigned and 19 individuals with both parents assigned (including the nine individuals with mothers assigned *a priori*; see Appendix S1). Four mother–offspring pairs established based on field observations of associations between adult females and subadults were confirmed by *CERVUS* and six such pairs were rejected: although the putative mother was present among sampled individuals, another female was assigned as a mother instead (see Appendix S1). There were four other cases of inconsistency between behavioural and genetic maternity assignments. However, because in these cases the putative behaviourally-assigned mothers were not sampled, behavioural maternity assignments could not be reliably rejected.

Out of 69 individuals with assigned parentage, 37 individuals shared the same mother and 15 the same father with at least one other individual. Among 19 individuals with both parents assigned, there were two pairs of individuals that had the same mother but different fathers, one pair and one group of five individuals that had the same father but different mothers, and two pairs of individuals that shared both parents. There were also eight groups of individuals that shared the same mother, but only some of them had fathers assigned – these individuals might be half-siblings. The average number of offspring assigned to putative mothers and fathers was 1.78 (SD \pm 1.01; n = 32) and 1.41 (\pm 0.91; n = 22), respectively (Appendix S1).

In seven Pacific populations, in all but one case offspring were sampled in the same population as their mothers. In one case, a mother and an offspring were sampled in two neighbouring populations (BS and AR), which have been suggested by Hoelzel *et al.* (2007) to admix. In 55% cases, offspring were sampled in the same population as their fathers, and in 45% cases from a different population within the same (resident) ecotype (Table 1). In six out of these 10 father–offspring pairs, mothers were assigned as well, and each was sampled in the same population as the offspring. In the IC population, all assigned parents were sampled in the same

Table 1 Number and percentage of offspring sampled in: (A) the same or different populations than each of their parents, calculated for the seven North Pacific populations, (B) the same or different pods than each of their parents, calculated for SR, AR and AT populations, where pod membership of individuals was known.

Parent	(A) N offspring sampled in a population:		(B) N offspring sampled in a pod:		
	The same as a parent	Other than a parent	The same as a parent	Other than a parent, within the same population	Other than a parent, in another population within ecotype
Mother	43 (98%)	1 (2%) [†]	25 (93%)	2 (7%) [‡]	0
Father	12 (55%)	10 (45%)	3 (17%)	6 (33%)	9 (50%)

[†]This individual was sampled in the BS population, which has been suggested by Hoelzel *et al.* (2007) to be an admixture of the neighbouring AR and RU populations, and the assigned mother came from the AR population.

[‡]In these two cases, females assigned as mothers based on field observations (belonging to the same pods as the offspring) were absent from the sample.

population as their offspring, but this could be due to the absence of geographically proximate populations in the sample.

In three populations where pod membership of individuals was known from field studies (SR, AR and AT), all individuals for which females assigned as their mothers based on field observations were available were assigned to the same pod as their mothers. 50% of offspring were sampled in another population than their fathers, and 83% in another pod (Table 1).

CERVUS, KINGROUP and COLONY results were not entirely consistent in assigning individuals to full-sibling and half-sibling groups (see Appendix S2), probably due to the presence of both maternal and paternal half-siblings in the sample. 112 groups of individuals were recognized as being related at the level of half-siblings by at least one of the methods. 103 groups (92%) included individuals from the same population, six groups included individuals from different resident populations and three groups included individuals from different transient populations. 10 pairs of individuals (9% of all groups) were consistently identified by KINGROUP, COLONY and CERVUS as full-siblings.

Relatedness within and among pods, populations and ecotypes

The average relatedness of individuals within populations (0.245, SE 0.024) was significantly higher than the average relatedness within groups sharing the same mtDNA haplotypes (0.217, SE: 0.025; $R-R' = 0.028$, 95% CI = 0.013, $P < 0.01$), and within ecotypes (0.201, SE: 0.023; $R-R' = 0.044$, 95% CI = 0.019, $P < 0.001$). The average relatedness within pods in three populations with known pod assignment of individuals (0.261, SE: 0.026) was significantly higher than average relatedness within these populations (0.231, SE: 0.023; $R-R' = 0.030$, CI = 0.028, $P < 0.01$). The average relatedness within the transient ecotype, as well as the average relatedness within populations of transients, were significantly lower than the respective measure for residents, offshores and IC (see Table 2). Consistently, the average relatedness within pods in the transient population AT (0.127, SE: 0.058) was substantially lower than the average relatedness within pods in the resident population AR (0.363, SE 0.047, $R-R' = 0.236$, CI = 0.188, $P < 0.0001$) and SR (0.239, SE: 0.050, $R-R' = 0.113$, CI = 0.140, $P < 0.0001$).

In transients, offshores and RU residents, the average relatedness of males within populations was significantly higher than the average relatedness of females within populations, while the reverse situation was observed in SR and AR populations (Table 3). Relatedness between pairs of resident populations ranged from 0.126 to 0.274, while relatedness between two transient populations was close to zero (0.031, SE: 0.036). Relatedness values between pairs of populations from different ecotypes were close to zero or negative.

For 476 pairs of individuals, the maximum likelihood relatedness estimate was above 0.4. These pairs included all but two identified parent-offspring pairs and all but three full-siblings pairs. 66% of pairs consisted of individuals from the same population, 31% from different populations within an ecotype, and 3% from different ecotypes.

Based on the results of KINGROUP and COLONY we identified groups of individuals linked by a network of kinship relationships. These groups corresponded to the four ecotypes, except for the fact that some offshore individuals (one individual in KINGROUP analysis and five individuals in COLONY) were indicated as having closer kinship relationships with transients than with other offshores. Populations within each ecotype were linked by extensive networks of kinship bonds.

Patterns of gene flow among populations

The PCA plot based on pair-wise F_{ST} values among populations showed that transient and offshore populations (AT, CT and OS) group together, and resident populations (AR, BS, RU, and SR) form another group (Fig. 1a). The Iceland population was distant from all other populations. The phylogenetic relationships among populations revealed based on microsatellite loci were inconsistent with those based on mtDNA (Fig. 2).

The individual-based PCA plot showed three main clusters consisting of (i) transients and offshores, (ii) residents, and (iii) Iceland individuals (Fig. 1b). However, these three clusters overlapped, and some individuals from each group were placed closer to individuals from another group than to their own. Within the transients/offshores and residents clusters, individuals representing different populations did not form distinct subclusters.

Ecotype	Relatedness within ecotype (SE)		Mean relatedness within populations (SE)	
		R-R' (CI)		R-R' (CI)
Transients	0.073 (0.038)	–	0.102 (0.029)	–
Residents	0.233 (0.031)	0.160* (0.118)	0.305 (0.038)	0.203* (0.086)
Offshore	0.291 (0.051)	0.217* (0.116)	0.291 (0.051)	0.189* (0.101)
Iceland	0.282 (0.065)	0.198* (0.111)	0.282 (0.065)	0.180* (0.112)

* $P < 0.00001$.

Table 2 Relatedness within ecotypes and mean relatedness within populations calculated for each ecotype studied. R-R' denotes the difference in mean relatedness between a given ecotype and transient ecotype. SEs and CIs are reported in parentheses.

Table 3 Mean within-population relatedness calculated for all individuals, males, females, and between sexes. SEs are reported in parentheses.

Population	Ecotype	Average relatedness			
		All	Males	Females	Between sexes
IC	IC	0.282 (0.065)	0.284 (0.076)	0.286 (0.062)	0.281 (0.064)
AT	T	0.105 (0.046)	0.156*** (0.051)	0.082 (0.048)	0.093 (0.055)
CT	T	0.097 (0.029)	0.108**** (0.034)	0.036 (0.039)	0.107 (0.033)
OS	O	0.291 (0.051)	0.350** (0.085)	0.257 (0.061)	0.288 (0.052)
AR	R	0.318 (0.049)	0.300 (0.080)	0.347* (0.044)	0.300 (0.055)
SR	R	0.253 (0.045)	0.223 (0.057)	0.266* (0.049)	0.254 (0.046)
BS	R	0.289 (0.063)	0.268 (0.060)	0.284 (0.102)	0.315 (0.077)
RU	R	0.361 (0.061)	0.393**** (0.067)	0.154 (0.142)	0.331 (0.068)
Average		0.245 (0.024)	0.254 (0.028)	0.236 (0.027)	0.240 (0.025)

Ecotype symbols are as follows: IC: Icelandic ecotype, T: North Pacific transients, O: North Pacific offshores, R: North Pacific residents. The average relatedness between individuals of one sex that is significantly higher than the average relatedness between individuals of another sex is marked as: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.00001$.

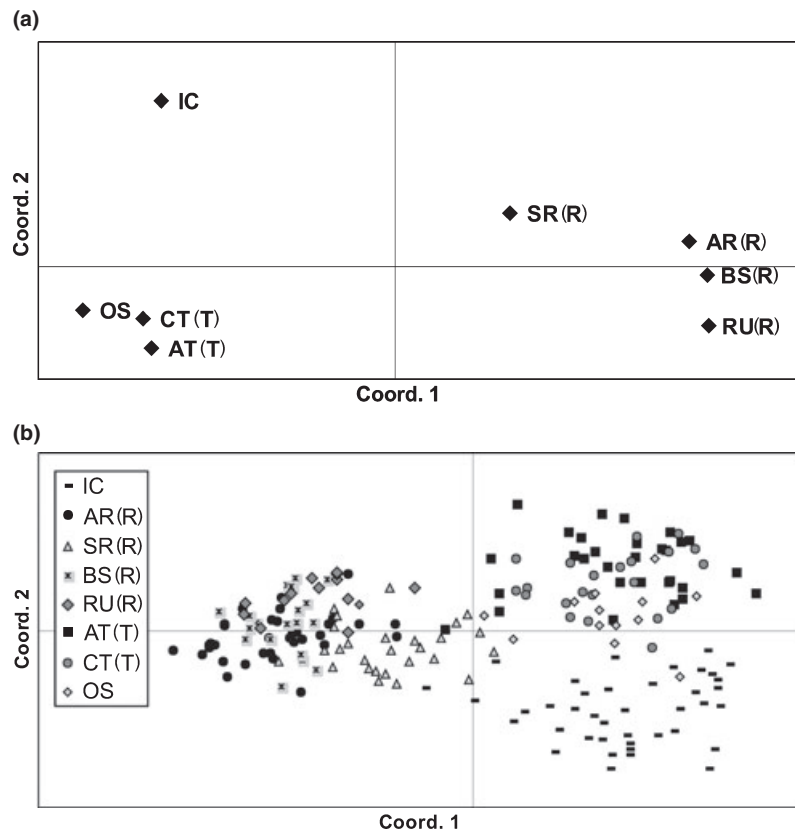


Fig. 1 (a) Genetic distances between populations of killer whales assessed using PCA and pair-wise F_{ST} . The first and second axes explain 46% and 19% of genetic variation, respectively. Names of populations are as in the text. Ecotype symbols are given in parentheses (IC: Icelandic ecotype, T: North Pacific transients, O: North Pacific offshores, R: North Pacific residents). (b) PCA plot of pair-wise distances between individuals from different populations calculated using the method of Smouse & Peakall (1999). The first and second axes explain 33% and 22% of genetic variation, respectively.

GENECLASS indicated that mean assignment value of an individual to its population varied between 0.72 in RU to 0.89 in SR (Table 4). Mean cross-assignment values between populations within an ecotype were high, with maximal values of 0.61 in residents and 0.34 in transients. The mean cross-assignment values of resident individuals to transient populations (0.05–0.29) were substantially higher than the mean cross-assignment values of transient individuals to resident populations

(0.00–0.03). Offshores had higher cross-assignment values to transient populations (0.25–0.27) than to resident populations (0.00–0.12). Out of 24 individuals assigned to another population with higher probability than to their own, 17 were assigned to another population within an ecotype, and seven to another ecotype.

When the BAYESASS analysis was run assuming subdivision into eight populations, all individuals from a given ecotype were assigned to one group. Therefore, to

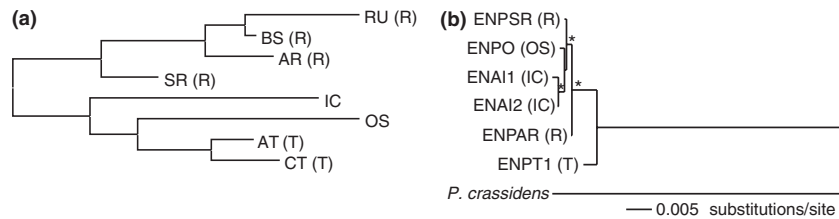


Fig. 2 Phylogenetic relationships between the studied populations based on nuclear and mtDNA data. (a) Neighbour Joining phylogram of pair-wise F_{ST} calculated based on microsatellite genotypes. (b) Neighbour Joining tree constructed using 915 bp of mtDNA control region and K81uf+I+ Γ model of nucleotide substitution, rooted with the false killer whale *Pseudorca cradissens* (GenBank accession number: EF601206) sequence. Stars denote support for branches if found in more than 50% of 1000 bootstrap replicates. Ecotype symbols are given in parentheses (IC: Icelandic ecotype, T: North Pacific transients, O: North Pacific offshores, R: North Pacific residents). For a mtDNA phylogeny of worldwide killer whales, see Hoelzel *et al.* (2002).

	IC	AT (T)	CT (T)	OS (O)	AR (R)	SR (R)	BS (R)	RU (R)
IC	0.789	0.090	0.181	0.026	0.003	0.117	0.001	0.000
AT (T)	0.020	0.754	0.341	0.028	0.001	0.030	0.000	0.000
CT (T)	0.013	0.224	0.811	0.016	0.000	0.012	0.000	0.000
OS (O)	0.082	0.268	0.250	0.790	0.013	0.118	0.008	0.000
AR (R)	0.050	0.146	0.205	0.062	0.755	0.516	0.358	0.028
SR (R)	0.122	0.206	0.288	0.079	0.280	0.887	0.134	0.020
BS (R)	0.052	0.122	0.166	0.057	0.534	0.510	0.774	0.137
RU (R)	0.012	0.047	0.144	0.044	0.400	0.547	0.613	0.717

Mean assignment values of individuals to the populations where they were sampled are marked in bold. Within-ecotype assignment values are marked by rectangles. Ecotype symbols are given in parentheses (IC: Icelandic ecotype, T: North Pacific transients, O: North Pacific offshores, R: North Pacific residents).

Recipient populations	Source populations			
	IC	Residents	Offshores	Transients
IC	0.972/0.792	0.009/0.049	0.004/0.026	0.015/0.181
Residents	0.003/0.066	0.992/0.781	0.003/0.062	0.003/0.265
Offshores	0.013/0.083	0.022/0.121	0.930/0.790	0.036/0.418
Transients	0.003/0.017	0.003/0.030	0.004/0.024	0.991/0.773

The values on the left denote immigration rates from ecotypes in columns to ecotypes in rows. The values on the right denote mean assignment values of individuals from ecotypes in rows to ecotypes in columns. Nonmigration rates and mean assignment values of individuals to their own ecotype are marked in bold.

assess gene flow between ecotypes, four groups were considered: residents, transients, offshores and the Iceland population. The highest gene flow rates were observed from transients and residents to offshores (Table 5). Gene flow rates between the Iceland population and North Pacific ecotypes were within the range of gene flow rates between North Pacific ecotypes. We identified one first generation (F1) immigrant, i.e. an individual that moved between populations (from transients to offshores), and four second generation (F2) immigrants, i.e. offspring of F1 immigrants and local individuals.

To estimate levels of gene flow between populations within an ecotype, the additional BAYESASS runs were performed separately for resident and transient populations. High gene flow rates were revealed between the two transient populations (0.08 from CT to AT and 0.12 from AT to CT). One F1 migrant and nine F2 migrants were detected. In residents, very high gene flow rates were revealed from RU to BS (0.28) and from BS to AR (0.14), and much lower rates (ranging from 0.005 to 0.024) between other pairs of resident populations. All individuals from the BS population were assigned either to AR or RU, which is consistent with results of

Table 4 Mean assignment values of individuals from populations in rows to populations in columns, calculated in GENECLASS2.

Table 5 Migration rates between ecotypes calculated in BayesAss, and mean assignment values calculated in GENECLASS2.

STRUCTURE (Pritchard *et al.*, 2000) analysis from the earlier study (Hoelzel *et al.*, 2007). Besides this, two F1 migrants from RU to AR were detected, and one F2 migrant from AR to SR.

The test for F1 migrants performed in GENECLASS identified 14 individuals as immigrants. These results were compared with the results of GENECLASS assignment test, as well as with the assignments from BAYESASS and results of STRUCTURE analysis from the earlier study (Hoelzel *et al.*, 2007). We also checked parentage and kinship assignments of these individuals. After these comparisons, three of these individuals were eliminated from the pool of possible immigrants due to insufficient evidence, as most methods did not confirm their immigrant status. Of the remaining 11 cases, eight individuals were assigned to another population within the same ecotype, and three individuals to another ecotype (Appendix S3). Two of these between-ecotype migrants were individuals from the IC population assigned to transient populations, and the third one was an offshore individual assigned to a transient population. These three individuals were excluded as F1 immigrants based on their mtDNA haplotypes, which were inconsistent with the putative population of their origin, but consistent with the population where they were sampled. They were likely the offspring of a female from the population where they were sampled and a male from the putative 'origin' population assigned by the program. Indeed, for one of the Iceland individuals, the most likely (but nonsignificant) father indicated by CERVUS was from the AT population. For the other two individuals CERVUS did not assign any putative father.

Out of eight assignments between populations within an ecotype, four were within the transient ecotype (between AT and CT) and four within the resident ecotype (between AR, BS and RU). One of the putative immigrants within the resident ecotype was excluded as an F1 immigrant based on its mtDNA haplotype, so it was probably an F2 immigrant. Indeed, this individual had a father from the RU population, assigned by CERVUS with relaxed confidence. Three other putative migrants between resident populations could not be excluded as F1 migrants. The four putative immigrants within the transient ecotype were more likely F2 immigrants, taking into account the results from BAYESASS and GENECLASS (see Appendix S3). One of these individuals, from the CT population, had a father from the AT population, assigned by CERVUS with strict confidence (however, this paternity was not confirmed by KINGROUP and COLONY analyses).

Discussion

Mating patterns

Our results suggest promiscuous mating in the killer whale, given the low frequency of detected full-sibs as

compared with half-sibs (a consequence of promiscuous mating when multi-foetal pregnancies are rare). If as our data suggests, mating takes place outside the social group during temporary associations, this would prevent the controlling of an individual's mating opportunities by the opposite sex, excluding monogamy, polygyny or polyandry. This contrasts with the 'fission-fusion' groups of bottlenose dolphins (*Tursiops aduncus*), where adult males are thought to form alliances to monopolize breeding females (Connor *et al.*, 2001).

We detected only a few cases of possible dispersal of individuals between pods and between populations (using individual-based assignment tests), but many more cases of inter-pod and inter-population mating (using paternity tests). The assignment of maternal kinship was typically within a natal population and pod, while the assignment of a father was as often outside as within the population, and in most (83%) cases outside the natal pod. These results suggest male-mediated gene flow occurring without male dispersal.

Three cases of putative mating within a pod were detected, two involving a single father (to which a total of five paternity assignments were made). Since the matrilineal structure of pods results in high kinship within pods, inbreeding avoidance would be expected to preclude intra-pod mating. However, the average kinship within pods was not much higher than within populations (0.261 vs. 0.231), which may mean that the consequences of mating within social groups do not differ greatly from those of mating within populations for this species. There are a number of examples of social mammals that tolerate inbreeding (e.g. the dwarf mongoose (*Helogale parvula*) Keane *et al.*, 1996; naked mole rat (*Heterocephalus glaber*) Reeve *et al.*, 1990; and banded mongoose (*Mungos mungo*) Gilchrist *et al.*, 2004), possibly as a consequence of purging deleterious alleles (but see Reed *et al.*, 2003). However, for our study, evidence for intra-pod mating was uncommon. It is also possible that specific parentage assignments based on genetic methods alone could be wrong, especially given the presence of close relatives within these social groups that could be falsely assigned as parents in the absence of real parents from the sample (e.g. Van Horn *et al.*, 2008).

Eight putative F2 immigrants were detected among the sampled individuals, which may imply inter-population mating (consistent with the CERVUS results), and in three cases between-ecotype mating. One case implied mating between a transient male and an offshore female. Because of the sympatric/parapatric occurrence of all three ecotypes in the North Pacific, mating between ecotypes may take place without the need for mating individuals to leave their natal pods. However, the supposed mating between females from the IC population (the North Atlantic) and transient males (the North Pacific), suggested by detection of F2 immigrants between these populations, must have involved either the long-distance movement of mating individuals or

entire pods, or gene flow through intermediary populations. Killer whale social groups are capable of migration over distances of several thousand kilometers in a season (Sternson & Simila, 2004; Dahlheim *et al.*, 2008), so the possibility of long-distance associations cannot be excluded.

If mating does typically occur without permanent dispersal of individuals from their natal pods, and foraging behaviour is learned during training interactions between a parent and offspring (Hoelzel, 1991; Guinet & Bouvier, 1995), then there should be no 'outcrossing' disadvantage to mating outside a given ecotype. However, contemporary associations of pods belonging to different ecotypes may be rare due to differential temporal and spatial habitat use, and this may limit between-ecotype mating (Hoelzel *et al.*, 2007).

Social groups of vertebrates often exhibit some degree of reproductive skew, the uneven distribution of reproductive effort among group members, though the degree of skew can vary (see Keller & Perrin, 1995). This matters for the evolution of structure at the population level, as high skew reduces diversity within groups and typically influences which sex disperses and the timing of dispersal. For the killer whales in this study, the average number of offspring per female showed relatively low variance, consistent with genealogical studies showing that most females within social groups reproduce at sexual maturity (e.g. Ford *et al.*, 2000). For males identified as fathers, the average number of offspring and its variance were again relatively low, though one male was an exception, apparently fathering five offspring (Appendix S1). Overall, this suggests low skew, which is relatively rare for mammals (but see Gilchrist *et al.*, 2004).

Relatedness and social structure

As for many social mammals, killer whale social groups comprise philopatric maternal kin. Studies on bottlenose (Möller *et al.*, 2006) and striped dolphins (*Stenella coeruleoalba*; Gaspari *et al.*, 2007) also showed bonds among adult female relatives, though social groups in these species are much more fluid, and nonkin associations are also common.

The average relatedness within social groups of killer whales decreased with increasing hierarchical level of spatial organization: from pods through populations to ecotypes. The average relatedness of individuals sharing the same mtDNA haplotype was lower than the average relatedness within populations, but higher than the average relatedness within the entire ecotype. This reflects the fact that multiple, differentiated populations share the same haplotype, but in some cases shared haplotype across an ecotype reflects recent kinship.

The average relatedness within pods and populations of transients, as well as within the entire transient ecotype was substantially lower than in residents. To date

the strongest evidence for altruism (which can be promoted by kin selection; Hamilton, 1964) among killer whales was food provisioning (one whale hunting and providing food for the rest of the pod) when total intake was close to the minimum required (Hoelzel, 1991). This was seen among killer whales that prey on marine mammals, yet here similar groups show the lowest average kinship. The mechanism driving down kinship in these groups may be about optimal group size for efficient hunting (see below), and further research would be required to determine if this creates a conflict with expectations related to kin selection.

For transients and offshores, average within-population relatedness was significantly higher for males than for females. With one exception this was not the case for resident populations. While this may suggest that transient and offshore females have higher dispersal rates than males, it may also be a sampling artefact. Contrary to the residents, some transient individuals of both sexes disperse from their natal pods, but while females join other pods within a population, males may remain solitary after dispersal except for occasional associations for breeding (Baird & Whitehead, 2000). If these males are less likely to be sampled than nondispersing males, it could lead to higher F_{ST} and relatedness estimates for males than for females. In fact, some solitary transient males have been included in this study, and therefore the data most likely indicate a greater propensity for females to move among transient populations, together with some degree of sampling bias.

Dispersal

The low rate of dispersal from a natal population in the killer whale may be explained by foraging specialization: given significant investment in learning strategies associated with the exploitation of local resources, individuals may risk a reduction in fitness when they move to a population that forages on a different type of prey using a different hunting strategy. The dispersal rate from the natal pod may also be dependent on the type of prey taken (marine mammal vs. fish), if the energetic value and abundance of the most frequently taken prey sets the upper limit for group size (e.g. Ford *et al.*, 1998). The group size of transients typically ranges from 2 to 10 (Hoelzel, 1991; Baird & Dill, 1996; Ford & Ellis, 1999), while resident pods typically consist of 10–50 individuals (Bigg *et al.*, 1990; Dahlheim *et al.*, 1997; Ford *et al.*, 2000), and offshore groups include up to 75–100 individuals (Dahlheim *et al.*, 2008). Dispersal of young individuals from the natal group may be necessary for social groups preying on marine mammals, if there is a maximum group size that can be sustained using this foraging strategy, or if smaller groups forage more efficiently for this resource (Hoelzel, 1991; Baird & Whitehead, 2000). This is consistent with our comparative assessments of relatedness within groups – low

within transient groups and higher within resident groups.

Two putative F1 migrants (WRA and SEA1, see Appendix S3) were the only individuals from the AR population with unknown pod assignment, which may suggest temporary association. The transient male CA20, identified as a putative F2 immigrant from AT to CT, was reported mostly from the coast of California (eight sightings between 1987 and 1995), but was encountered twice outside this region, in Glacier Bay (Southeast Alaska, 1989), and along the British Columbia/Washington coast (1995; Black *et al.*, 1997). Therefore, the migration cases detected may in fact represent temporary interactions among individuals from different populations, rather than permanent dispersal.

Population differentiation and gene flow

Mitochondrial and nuclear DNA variability indicate contrasting patterns with respect to the relationship between three North Pacific ecotypes of killer whales. While phylogenetic relationships between mtDNA haplotypes show that offshores share more recent maternal ancestry with residents than with transients (and are in fact at opposite ends of the mtDNA phylogeny), microsatellite loci group offshores with transients (see Figs 1 and 2). Our detection of an F2 immigrant from a transient to an offshore population and relatively high estimated migration rates are consistent with ongoing gene flow between these ecotypes, perhaps during encounters in offshore habitat. The contrasting patterns of mitochondrial and nuclear DNA variability between the ecotypes may result from the stochastic distribution of mtDNA haplotypes following a post-bottleneck expansion (Hoelzel *et al.*, 2002), and rare female-mediated gene flow. These inconsistent inferences based on markers with different modes of inheritance may be problematic for attempts to delimit subspecies of the killer whale. In such cases, using markers experiencing higher levels of gene flow is recommended (Petit & Excoffier, 2009).

While there is evidence for ongoing gene flow between offshores and transients, there is much less indication of ongoing gene flow between transients and residents. However, low estimates of gene flow between ecotypes are consistent with the low rate of between-population migration (both within and between ecotypes) suggested by nonequilibrium models in Hoelzel *et al.* (2007). At the same time, the estimates of contemporary gene flow within ecotypes are much higher than expected, especially among resident populations. This could suggest a relatively recent increase in gene flow between populations of the same ecotype, possibly resulting from range expansion. Earlier studies suggest that small founder groups established local coastal populations that would have expanded over the last ~10 000 years (Hoelzel *et al.*, 2002, 2007), which could have resulted in an increasing probability of encounters between pods from

different populations within an ecotype. This hypothesis should be tested further, ideally including data from ancient populations.

The geographically distant Icelandic population did not have a basal position in either phylogeny (Fig. 2), which may reflect the complex post-glacial history of the species (see above). The possibility of ongoing gene flow among oceans can also not be excluded, especially through unsampled intermediate populations.

Conclusions

Although the assessment of parentage and kinship based on genotypes may lead to some erroneous associations, the patterns we report are consistently supported by multiple analyses at the individual and population level. These data suggest that the mating system of killer whales is promiscuous, but highly selective. Most matings occur outside natal pods, during temporary associations of pods or as a result of the temporary dispersal of males. Contemporary mating between populations within the resident ecotype appears to be at a higher rate than predicted from the long-term estimates based on population-level analyses, which suggests increasing contact among pods, possibly due to range expansion of resident populations. Genetic structure among populations is enhanced by kin associations within social groups, as suggested for various social mammals, e.g. striped dolphins (Gaspari *et al.*, 2007), black-tailed prairie dogs (*Cynomys ludovicianus*; Sugg *et al.*, 1996), and Asian elephants (*Elephas maximus*; Vidya *et al.*, 2005). Only a few matings among ecotypes were detected, but these include possible interactions over an unexpectedly large geographic range, possibly through intermediary populations. Individual-based genotypes confirmed earlier expectations about the association of different populations, and reinforced the proposed relationship between transients and offshores. The latter is possibly a consequence of shared habitat, though we know little about the ranges of these pods. Taken together, these data emphasize the importance of social cohesion in this species, probably driven by the requirements of specialist foraging strategies, for the evolution of genetic differentiation among parapatric and sympatric populations, despite a capacity for long-distance dispersal (e.g. Dahlheim *et al.*, 2008).

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 The results of parentage assignment in CERVUS.

Appendix S2 Comparison of full and half-sib relationships between killer whales determined by different methods.

Appendix S3 Putative immigrants in the killer whale populations.

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