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Social Structure of Pilot Whales Revealed by Analytical DNA Profiling

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Long-finned pilot whales swim in large, extremely cohesive social groups known as pods. Molecular typing revealed that pod members form a single extended family. Mature males neither disperse from nor mate within their natal pods, a situation unusual for mammals. Such behavior could be explained in terms of inclusive fitness benefits gained by adult males helping the large number of female relatives with which they swim.

The inaccessibility of whales makes their social organization difficult to elucidate, yet such knowledge has important consequences for conservation and management. Cetacean mating systems have been inferred from comparisons with other mammals [see (*1*), for example]. However, given the unique marine ecology of whales, such extrapolations need to be substantiated by rigorous paternity testing.

A review of mammalian mating systems suggests that female reproductive behavior is highly constrained by the demands of gestation and lactation (*2*). Males can maximize their fitness in two ways: by mating with many females and by improving offspring number or quality through paternal care. In cases where females live in groups, competition between males tends to lead to polygyny (*2*). In virtually all cases, inbreeding is avoided by the dispersal of one or both sexes, usually the males (*3*). Our study shows that the pilot whale (*Globicephala melas*, Delphinidae) is unusual in its social organization: neither sex disperses from its natal group, and males show no evidence of reproductive dominance. Such a system raises interesting possibilities for the role of inclusive fitness in its evolution.

The long-finned pilot whale swims in large groups, or pods, often containing over 100 individuals. All age classes and both sexes are found in a pod, although there is a female sex bias among adults (*4*). Mating is broadly seasonal, with a diffuse peak in early summer (*4*). Pods are very cohesive, which can result in natural mass strandings and which allows them to be herded with boats. For centuries, this behavior has been exploited by coastal peoples to catch pilot whales for food. Today, only in the Faeroe Islands does this tradition continue, with a mean of some 1700 whales caught annually, mostly as entire pods (*5*). Molecular analysis of samples from this fishery suggests that pod members are related and that males seldom mate within the pods in which they are caught (*6*).

To clarify pilot whale structure and breeding behavior it is necessary to establish the following: (i) the degree of relatedness between pod members, (ii) whether adult males are related to the rest of the pod, and (iii) whether individual males mate with a few or with several females in any one pod. For this analysis we used a panel of highly variable microsatellite sequences (*7*).

Between 1986 and 1988, tissue samples were collected from many (presumed) complete pods from the Faeroese pilot whale drive fishery. Two pods were selected for detailed analysis on the basis of size and completeness of sampling (Miðvágur 240787, $n = 103$, and Leynar 220787, $n = 90$). Each sample was typed for one minisatellite locus [the *HMW* locus (*6*)] and six microsatellite loci. Of the microsatellite loci, five have between three and ten alleles per locus (Table 1). The sixth, however, is extremely polymorphic, with 54 alleles scored in the two study pods, 46% of which are unique to one or the other pod (Fig. 1).

The great variability of locus 468/469 allowed us to reassess male mating behavior. For 33 of 34 fetuses we could exclude all accompanying adult males as fathers, strengthening previous assertions that adult males rarely, if ever, mate within their home pods. Further, we compared paternal alleles among seven fetal cohorts (that is, fetuses conceived in the same pod in the same year; $n = 6, 6, 12, 3, 10, 3$, and 6 fetuses) sampled from four different pods. We found that 89% of paternal alleles were unique within a cohort, an observation incompatible with the idea that one or two males dominate mating (*8*). Given that a cohort's paternal alleles at the less variable minisatellite locus are nonrandom (*6*), our findings imply that groups of related males are the fathers.

To estimate the number of mother-offspring relationships in a pod, we designed a special analytical approach (*9*). The ob-

Table 1. Pilot whale microsatellite loci analyzed in this study. All primers flank a simple sequence stretch consisting either of GT or GA dinucleotide repeats.

Locus	Primer (5′ to 3′)	Number of alleles	Size range (base pairs)
199/200	TGAAATTCTTCATCAGT GTTAATGTAGGCAGACT	5	110 to 134
409/470	GTTTTGGTTGCTTGA TAAAAGACAGTGGCA	8	174 to 188
415/416	GTTCCTTTCCTTACA ATCAATGTTTGTCAA	6	222 to 234
417/418	GTGATATCATACAGTA ATCTGTTTGTCACATA	3	181 to 187
464/465	GGGGTTTCTCCTCTA TGATCTGCCAATAAGA	8	138 to 154
468/469	ACCCCAGAGAAAACA CAAGGTATTTCAGAA	54	87 to 185

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served number (O) of genetically compatible females was compared with the number expected (E) on the basis of random assortment of alleles among mature females in the pod. It can be shown that the statistic {O − E} will be distributed about 0 when the true mother is absent and about some value V when she is present. In a highly variable genetic system, or when the number of possible mothers is large, V tends toward 1 (*10*). Thus, in a sample of individuals, some accompanied by their mothers and some not, {O − E} will be distributed bimodally, with peaks at 0 and near 1. In such samples, the mean value of {O − E} is an estimate of the proportion of individuals that are with their true mothers.

Applied to the two study pods, this analysis indicated that many mother-offspring pairs were present. Mean {O − E} values were calculated for each of four age classes (Fig. 2). As an internal control, the same procedure was also used to look for fathers. The estimated proportion of individuals accompanied by their mothers declines from about 95% among younger whales to 35% among adults. In contrast, in the equivalent paternity analysis, {O − E} values remained close to 0, consistent with results from the paternity analysis.

To eliminate the possibility that a pod comprises many unrelated mother-offspring pairs, we used a second type of analysis. In the absence of dispersal, a group of animals will become enriched for those alleles carried by successful parents. Greatest enrichment will usually be associated with the oldest females, that is, those with the most descendants. In such a group, the age of an animal should correlate with the probability of observing its genotype, on the basis of random assortment of the pod's alleles. This correlation will be strongest in highly variable systems where allelic identity within a pod is approximated by the probability of identity through descent.

Pod-specific genotype frequency indices (*11*) were calculated for each individual, for both locus *468/469* and the *HMW* minisatellite locus, and the values obtained were plotted against age (Fig. 3). A positive correlation ($P < 0.001$) was found for both pods, the significance of which was investigated by computer simulations (*8*). Two simulations failed to yield correlations as strong as those observed. In the first, alleles were assigned randomly back to the original pod members (*12*). This confirms a relation between age and genotype. In the second simulation, alleles were redistributed to create small unrelated "families" (*13*). Failure to equal the empirical value here suggests an overall relatedness between pod members. In a third set of simulations, model pods generated either from a single founder or from a group of related females gave r values similar to the real pods (*14*).

Our data also suggest that even as adults, male pilot whales still swim with their mothers. Animals unrelated to a pod should have very low genotype indices. The adult males have indices similar to females in the same age range (Fig. 3), suggesting that they belong to the pod. We investigated this possibility more rigorously by calculating likelihood ratios for the fit of adult males to the two study pods. In both cases, the hypothesis that the male's alleles are derived from their "home" pod is strongly favored over the alternative hypothesis that they come from the "away" pod (*15*). For these two pods at least, we conclude that the mature males are in their natal pods.

The picture that emerges from our molecular studies shows that pilot whales are strongly matrifocal, with both sexes usually staying with their natal pod. This is reminiscent of the situation with killer whales,

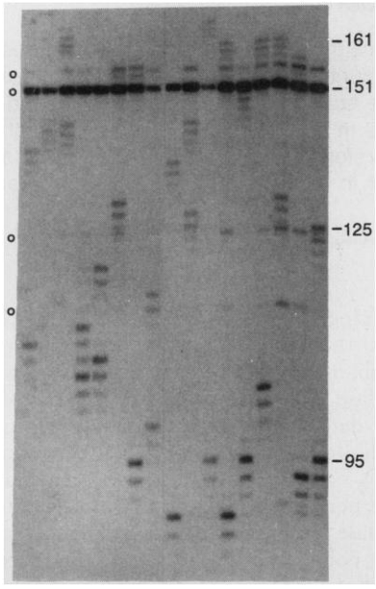

Fig. 1. Allelic diversity at locus *468/469*. DNA samples prepared from either the skin or kidney of 16 pilot whales were subjected to polymerase chain reaction (PCR) for 30 cycles of 1 min at 95°C, 2 min at 45°C, and 1.5 min at 72°C. Radioactivity was incorporated by end-labeling of one primer with ^{32}P (*7*). Portions were fractionated on a 6% denaturing polyacrylamide gel. The sizes of some of the DNA products (in base pairs) are indicated on the right. Consistency of interpretation was confirmed by repeated blind scoring. Four PCR artifact bands are seen (open circles). One of these is strong enough to mask the difference between alleles 149 and 151, but its position varies. Ambiguous genotypes were therefore rescreened. Some alleles differ by only a single base pair (lanes 2 and 3 from the left).

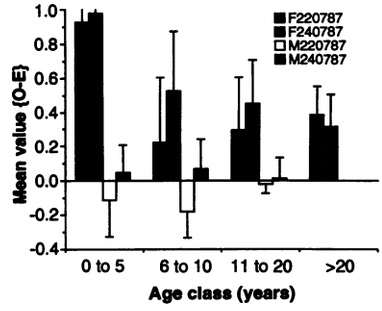

Fig. 2. Estimated proportion of individuals in two pilot whale pods (Miðvágur 240787 and Leynar 220787) accompanied by their mothers and fathers, based on mean {O − E} values [see (*8*) and text]. All females older by at least x years are considered potential mothers. Five years is the absolute minimum breeding age (*4*), but x was varied to allow for less accurate aging of older animals: age < 10, $x = 5$; 11 to 15, $x = 4$; 16 to 20, $x = 3$; 21 to 25, $x = 2$; >25, $x = 0$. When testing was done for fathers, x was modified appropriately: age < 10, $x = 13$; 11 to 15, $x = 12$; 16 to 20, $x = 11$; 21 to 25, $x = 10$; >25, $x = 8$. Error bars show 95% confidence intervals (C.I.). Mean {O − E} values, calculated over all individuals in both pods, are 0.46 ± 0.1 (95% C.I.) and −0.03 ± 0.07 (95% C.I.) for females and males, respectively. F, female; M, male.

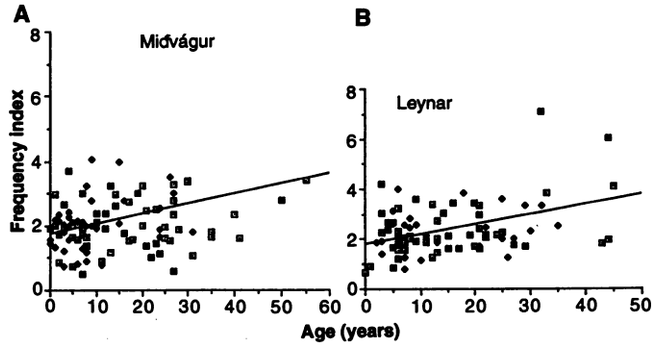

Fig. 3. Regression of age against pod-specific genotype frequency index for pilot whale pods (**A**) Miðvágur ($n = 94$, $r = 0.33$) and (**B**) Leynar ($n = 87$, $r = 0.41$). Males are indicated by solid diamonds and females by open squares. Age was determined from dental growth rings (*4*). Indices of genotype frequency (*9*) were calculated for the two most variable loci, *468/469* and *HMW*. Because these loci have different allele frequency profiles, we normalized data before combining by expressing each index as a locus percentage. For each animal, the value plotted was the sum over both loci. A few individuals (<5%, all subadult) were not typed for all loci because the DNA was degraded or because there were PCR amplification problems.

where long-term observations have shown that neither sex disperses (*16*). However, our data further show that adult males never father offspring within their home pods (whether this is also true of killer whales must await parentage testing). By implication, pilot whales must mate when two or more pods meet or when adult males pay short visits to other pods. Both possibilities are supported by field observations. Large aggregations of whales have been reported, sometimes numbering well over 1000 individuals, and probably result from the temporary merging of several pods (*4*). On the other hand, there are also rare, seasonal sightings of small all-male groups (*4*). In either case, the transitory nature of these events is emphasized by the general failure of paternity testing to reveal fathers.

This behavior pattern is unusual for mammals. Normally, adult males living in social groups are expected to maximize their reproductive success by competing for access to females. This behavior may lead to harem polygyny, with one or a few dominant breeding males who either force subordinate males to disperse or prevent them from breeding (*2*). It appears that pilot whales neither show strong reproductive dominance nor disperse from their natal groups.

The ecology of the pilot whale provides a possible explanation for this behavior. If opportunities to mate with females in other pods are not limited, the optimal male strategy need not involve caring for his direct offspring. He might do better by helping the large number of known relatives in his natal pod (*17*). However, it is unclear what benefits a male could provide. Both defense and assistance in a communal feeding strategy are possibilities, but they lack observational support. Whatever the selective forces involved, it seems clear that the high degree of relatedness between pod members can explain to a large extent the extraordinary cohesion of pilot whale pods.

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8. In four cohorts (n = 6, 3, 3, and 10), all paternal alleles were different. Pairs of alleles occurred in two cohorts of n = 6 (one pair each) and one cohort of n = 12 (two pairs). Of these four pairs, two were ambiguous because a mother-fetus pair shared both alleles. These data support only one possible instance of a male fathering four offspring. The estimated mean, assuming both ambiguities favored shared paternity, is 1.2 offspring per male per cohort.
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10. The empirical mean value of V was 0.83.
11. Genotype frequency indices were calculated for an individual of genotype *ab* as the square root of $pf_a pf_b$, where pf_a is the pod-specific frequency of allele *a*.
12. In 1000 runs, the mean r value (± SD) was 0.0009 ± 0.112.
13. We reassigned alleles randomly to "mothers" (females of sufficient age) with up to nine offspring, using segregation ratios determined by sampling a binomial distribution. In the most extreme case (two-thirds of the pod in nine-offspring families) slight positive correlations were generated (mean ± SD = 0.082 ± 0.11).
14. A female reproduces first at age 5 and thereafter every 3 years with probability P. Founding and paternal alleles are selected, with full replacement, from a alleles each at equal frequency. Simulations were stopped at target pod size t or when the oldest female reached age 45. We simulated related founders by allowing the founder to reach age 60 and then deleting all individuals >45 years old. Mean r values varied with P, t, and a (default values 0.7, 100, and 100, respectively), but all lay in a range consistent with the empirical value.
15. Following Edwards's method of Support [A. W. F. Edwards, *Likelihood* (Cambridge Univ. Press, Cambridge, 1972)], Support = 13.3 (Leynar) and 9.7 (Midvágur), approximately 6×10^5 and 1.6×10^4 more likely.
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A Laccase Associated with Lignification in Loblolly Pine Xylem

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Peroxidase has been thought to be the only enzyme that oxidizes monolignol precursors to initiate lignin formation in plants. A laccase was purified from cell walls of differentiating xylem of loblolly pine and shown to coincide in time and place with lignin formation and to oxidize monolignols to dehydrogenation products in vitro. These results suggest that laccase participates in lignin biosynthesis and therefore could be an important target for genetic engineering to modify wood properties or to improve the digestibility of forage crops.

In 1933, Erdtman proposed that the final step in lignin biosynthesis was enzymatic oxidation of *p*-hydroxyphenylpropanoid compounds followed by a free radical coupling reaction (*1*). Peroxidase (E.C. 1.11.1.7) and laccase (E.C. 1.10.3.2) were postulated to carry out this oxidation because both enzymes produce dehydrogenation polymers (DHP), a lignin-like material, from monolignol precursors (*2*).

Laccase, in contrast to peroxidase, has rarely been studied in plants. A role for laccase in lignification was suggested by early studies with a fungal enzyme (*2*) but was later discounted because a purified plant laccase from the Japanese lacquer tree (*Rhus vernicifera* Stokes) was shown not to oxidize monolignols (*3*). Many researchers have associated peroxidases with lignification (*3–7*). In studies of green ash (*Fraxinus pennsylvanica* Marsh.) sapling stems, Harkin and Obst showed by histochemical staining with syringaldazine or furoguaiacin in the presence of H_2O_2 that the xylem tissue adjacent to the cambium contained large amounts of peroxidase activity (*4*). These researchers did not detect laccase activity when syringaldazine was used as the substrate without H_2O_2, and on this basis they concluded that peroxidase was the exclusive phenol oxidase responsible for the dehydrogenative polymerization of lignin precursors. Further evidence of peroxidase involvement in lignification was provided by the demonstration of peroxidase activity in differentiating poplar (*Populus x euramericana*) xylem (*7*) and in lignifying cell walls of differentiating tobacco (*Nicotiana tabacum* L.) xylem (*6*), although some phenol oxidase activity was detected in the absence of added H_2O_2 in tobacco xylem.

The recent characterization of a laccase purified from the cell culture medium of sycamore maple (*Acer pseudoplatanus* L.) has prompted a reevaluation of the role of this enzyme in lignification (*8–10*). We undertook a study of oxidative enzymes in differentiating xylem of loblolly pine (*Pi-*

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