Genetic Evidence of Multiple Matrilines and Spatial Disruption of Kinship Bonds in Mass Strandings of Long-finned Pilot Whales, *Globicephala melas*

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**Abstract**

Mass strandings of whales and dolphins have puzzled biologists since Aristotle. Although environmental factors are often assumed to initiate strandings, social forces must also influence the dynamics of many of these events, particularly for the primary species involved in mass strandings, the long-finned pilot whales (*Globicephala melas*). Here, we test two hypotheses derived from common assumptions about the social dynamics of long-finned pilot whales by identifying maternal lineages from mtDNA haplotypes and inferring kinship from microsatellite genotypes of 490 individuals from 12 stranding events. Contrary to the “extended matriline” hypothesis, we found that multiple maternal lineages were present in at least 9 of the 12 mass strandings. Contrary to the “kinship cohesion” hypothesis, we found no correlation between spatial distribution and kinship along the stranding beach. Most notably, we documented the spatial disruption of the expected proximity between mothers and their dependent calves. These results challenge the common assumption that kinship-based behavior, such as care-giving, are a primary factor in these mass strandings. We suggest instead that disruption of kinship bonds could result from interactions among unrelated social groups during feeding or mating aggregations, perhaps playing a causal role in these events. Our finding that dependent calves were often spatially separated or absent from their mothers has important implications for humane management of rescue efforts. To improve our understanding of the social causes and consequences of mass strandings, future documentation of strandings should include exhaustive DNA sampling, with accompanying spatial and temporal records.

**Key words:** care-giving behavior, kinship, long-finned pilot whale, mass stranding, matrilineal

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“It is not known for what reason they run themselves aground on dry land; at all events it is said they do so at times and for no obvious reason.”—A description of cetacean stranding by Aristotle, 350 BCE (*Historia Animalia*, Book IX, Ch. 48)

Mass strandings of whales and dolphins, involving apparent intentional beaching and subsequent death of up to several hundreds of individuals in one event, remain an enigma. To date, efforts to understand mass strandings have largely focused on the role of presumably causal environmental factors, such as climatic events (*Evans et al. 2005*), bathymetric features (*Brabyn and McLean 1992*), geomagnetic topography (*Klinowska 1986*) or anthropogenic sound, such as navy SONAR (*Jepson et al. 2003*). These studies provide a better understanding of the timing and distribution of mass strandings, but they give little insight into the unique group dynamics of these events (*Bradshaw et al. 2006*). In particular, they do not explain the important contribution of social behavior, which has long been assumed as a proximate cause in the mass strandings of healthy whales (*Norris and Schill 1988*; *Perrin and Geraci 2002*).

The large majority of mass strandings involve only a few species thought to form strong social bonds within a matrilineal social structure, such as the pilot whales (*Globicephala* sp.), the false killer whale (*Pseudorca crassidens*), the sperm...
whale (*Physeter macrocephalus*), the rough-toothed dolphin (*Steno bredanensis*), the melon-headed whale (*Peponocephala electra*), and the pygmy killer whale (*Feresa attenuata*) (*Norris and Schilt* 1988). Such a social structure among long-lived species points to kinship as an underlying force in mass strandings, with instincts for group cohesion having evolved through kin selection. However, despite this common assumption, no study has yet demonstrated that kinship-based interactions are involved in mass strandings. As such, the proximate causes of mass stranding events, and in particular the social component, remain poorly understood.

The long-finned pilot whale (*G. melas*) is, by far, the species most commonly involved in mass stranding worldwide (*Baker* 1981; *Sergeant* 1982; *Evans et al.* 2005). Compelling evidence for a matrilineal social structure in long-finned pilot whales has come from kinship analyses using microsatellite genotyping on large groups taken in “drive-kills”” (see *Fehring and Wells* 2004). Parentage and relatedness analyses indicate that neither males nor females disperse from their natal group after reaching sexual maturity, a pattern also reported in killer whale, *Orcinus Orca* (*Baker et al.* 1990), but otherwise thought to be rare in mammals (*Greenwood* 1980). Whether or not the large aggregations observed in the wild represent “extended matriline” (i.e., all whales descend from a single ancestral female) remains in question. Although previous analyses of kinship in the drive-kills support this scenario (*Amos et al.* 1993; *Fullard* 2000), these studies did not include mtDNA as a direct marker of matrilineal kinship. As an alternate hypothesis, behavioral studies suggest a model of “multiple matriline” in which large groups represent temporary associations of small and stable social units (10–15 individuals), potentially representing the primary matrilineal groups (*Bloch et al.* 2003; *Ottensoos* and *Whitehead* 2003; *de Stephanis et al.* 2008).

A primary role for sociality in mass stranding of long-finned pilot whales is suggested by observations of group cohesion behavior, such as intentional restranding of whales after being refloated during rescue attempts (*Fehring and Wells* 1976; *Sergeant* 1982; *Robson* 1984; *Dawson et al.* 1985). This is not unexpected if individuals that restrand are injured or disoriented by their initial stranding (*Geraci and Lounsbury* 1993). However, available descriptions of human-managed mass strandings often describe restranding behavior as the apparently deliberate return toward the shore, despite having been escorted by rescuers into deeper and safer water and despite showing no obvious sign of injury or disorientation (*e.g.,* *Fehring and Wells* 1976). The social component of mass strandings is also suggested by observations of group dynamics prior to the actual stranding, particularly in New Zealand where these events are frequent and where human intervention (i.e., rescue by refloating) is managed by a national agency (the Department of Conservation, DoC). Here, prestranding behavior has been characterized by a chain reaction in which successive subgroups detach from the larger herd and strand, one after another (*Robson* 1984). The first individual (or group of individuals) to strand has been referred to as the “key whale,” and its initial stranding is thought to trigger the chain reaction that follows (*Robson* 1984). Together, these behavioral observations suggest that pilot whales try to maintain group cohesion, even during mass stranding, presumably influenced by care-giving among close maternal kin (*Porter* 1977; *Norris and Schilt* 1988; *Whitehead et al.* 2004).

Here, we use a unique collection of samples from mass strandings of long-finned pilot whale from New Zealand and Tasmania, Australia, to test two predictions on the group dynamics of these events, in relation to matrilineal social structure and kinship. First, the “extended matriline” hypothesis predicts that all individuals found in a single mass stranding will be maternally related. As mitochondrial genome is maternally inherited, we can test this prediction by looking at the pattern of mtDNA diversity within and among mass strandings, with individuals involved in a similar event expected to share the same mtDNA haplotype (within the limits of diversity; *e.g.,* Oremus et al. 2009). We also test a prediction for the alternate hypothesis that mass strandings involve multiple matriline, rather than the null hypothesis that strandings are random with respect to maternal lineages (i.e., random in respect to mtDNA haplotypes). Second, the “kinship cohesion” hypothesis predicts that close relatives will remain in proximity to their kin when they strand, in particular the mother/calf pairs, between whom social bonds are expected to be the strongest. As mass strandings often occur in linear formation along gently sloping beaches (*Robson* 1984), this second prediction can be tested using a correlation of spatial proximity and kinship inferred from microsatellite genotypes. For these, we take advantage of the frequent strandings around New Zealand and Tasmania and the potential to collect genetic samples and detailed individual records, including total length, sex, and relative spatial position along the beach.

### Material and Methods

#### Stranding Collection and Stranding Records

Small samples of skin were collected from carcasses involved in 12 mass stranding events from North and South Islands of New Zealand (*n* = 7 events) and Tasmania, Australia (*n* = 5 event), between 1992 and 2006 (Table 1, Figure 1). Samples were stored in 70% ethanol and transferred at the University of Auckland Cetacean Tissue Archive. Measurements of total length (from tip of upper jaw to deepest part of fluke notch) and sex identification (from descriptions of mammary slits) were collected. Field data were collected by staff of the New Zealand DoC, University of Auckland, and Australian Department of Primary Industries, Parks, Water and Environment.

Local positions of each whale on the stranding beach were recorded for two large mass strandings at Stewart Island, New Zealand (hereafter referred to as “Stewart Island 2003”, to distinguish it from another mass stranding in the dataset that occurred at Stewart Island in 2000) and at Marion Bay, Tasmania. Positions were obtained using manual mapping by one of the authors (H.K.) at Stewart Island 2003, and latitude
Table 1  Summary of the 12 mass strandings from around New Zealand and Tasmania, including number of individuals involved in the stranding, number of DNA samples, number of mtDNA haplotypes, and sex information

<table>
<thead>
<tr>
<th>Mass Stranding</th>
<th>Date</th>
<th>No. ind. involved</th>
<th>No. of samples</th>
<th>No. of mtDNA haplotypes</th>
<th>No. of females</th>
<th>No. of males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no. juveniles</td>
<td>no. matures</td>
</tr>
<tr>
<td>New Zealand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no. juveniles</td>
<td>no. matures</td>
</tr>
<tr>
<td>Golden Bay</td>
<td>28/12/1992</td>
<td>63</td>
<td>33</td>
<td>4</td>
<td>5 (1)</td>
<td>16 3</td>
</tr>
<tr>
<td>Long Bay*</td>
<td>07/12/1993</td>
<td>~80</td>
<td>27</td>
<td>2</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Chatham Islands*</td>
<td>24/10/1999</td>
<td>128</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Stewart Island</td>
<td>21/12/2000</td>
<td>67</td>
<td>20</td>
<td>2</td>
<td>1 12</td>
<td>5 2</td>
</tr>
<tr>
<td>Stewart Island</td>
<td>08/01/2003</td>
<td>159</td>
<td>122</td>
<td>1</td>
<td>20 (8)</td>
<td>56 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no. juveniles</td>
<td>no. matures</td>
</tr>
<tr>
<td>Point Hibbs</td>
<td>19/11/2003</td>
<td>117</td>
<td>32</td>
<td>2</td>
<td>2 16</td>
<td>4 6</td>
</tr>
<tr>
<td>King Island</td>
<td>28/11/2004</td>
<td>55</td>
<td>36</td>
<td>4</td>
<td>4 12</td>
<td>6</td>
</tr>
<tr>
<td>Maria Island</td>
<td>29/11/2004</td>
<td>19</td>
<td>16</td>
<td>2</td>
<td>0 10</td>
<td>2 4</td>
</tr>
<tr>
<td>Marion Bay</td>
<td>25/10/2005</td>
<td>145</td>
<td>108</td>
<td>6</td>
<td>13 (8)</td>
<td>57 16</td>
</tr>
<tr>
<td>Ocean Beach</td>
<td>01/12/2006</td>
<td>27</td>
<td>23</td>
<td>1</td>
<td>2 (2)</td>
<td>8 5 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>&gt;1033</td>
<td>490</td>
<td>11</td>
<td>55 (23)</td>
<td>231 64</td>
</tr>
</tbody>
</table>

When possible, individuals were classified as juveniles (with further distinction for the subcategory “unweaned calf,” numbers being shown in brackets) or mature individuals, based on total body length (see Methods).

* No body length available.

Figure 1. Distribution, number of individuals (N), and frequencies of 11 mtDNA control region haplotypes within and among 12 mass strandings of long-finned pilot whales (N_{TOTAL} = 490), in New Zealand (a) and Tasmania (b), with inclusion of the inferred genealogical relationship among mtDNA haplotypes based on median-joining algorithm where numbers give the positions of the mutations across the 345-bp consensus fragment investigated here (c).
and longitude coordinates from a Global Positioning System (GPS) device were recorded at Marion Bay. The map of the Stewart Island 2003 event was used to allocate Y-axis and X-axis coordinates using an arbitrary linear scale.

Definitions of Age–Sex Classes

Each individual was classified as mature or immature based on total length measurements and sex. It has been shown that sexual maturity in long-finned pilot whales from the Faroe Islands is closely related to body size rather than age, with all males less than 480 cm and all females less than 375 cm being immature (Block et al. 1993). Given that long-finned pilot whales from New Zealand appear to have similar parameters of growth and reproductive maturity (Schröder and Castle 1998), these thresholds were considered appropriate for our dataset. Hereafter, immature individuals are referred to as “juveniles.”

A subcategory of juveniles was considered to represent the youngest immature whales thought to be still dependent on their mothers for lactation; these are referred to as “unweaned calves.” Growth and reproduction parameters estimated from studies of long-finned pilot whales in the Faroe Islands were used to confirm the length threshold for this subcategory. The mean duration of lactation in long-finned pilot whale has been estimated at 3.4 years (Martin and Rothery 1993). The average length of female and male calves just before this age (i.e., 3 years old) has been estimated at 309 cm (SE = 1.9) and 317 cm (SE = 2.6), respectively (Block et al. 1993). Therefore, based on these estimates, all the juveniles measuring 300 cm or less were classified as “unweaned calves.” Data were incomplete for some individuals, explaining sample size discrepancy between analyses (Table 1).

DNA Extraction, mtDNA Sequencing, and Molecular Sexing

Total cellular DNA was isolated by Proteinase K digestion followed by phenol:chloroform as described by Baker et al. (1994). An 800-bp fragment of the 5’ end of the mtDNA control region (D-loop) was amplified and sequenced as described in Oremus et al. (2009). Sequences were aligned using SequencherTM v. 4.1.2 (Genes Codes Co.) and edited manually. Variable sites and unique haplotypes were identified using MacClade v. 4.0 (Maddison and Maddison 2000) and confirmed by visual inspection of peak heights with Sequencher. Sex was confirmed by coamplification of the male-specific sry gene and the ZFX positive control gene, as described by Gilson et al. (1998).

Microsatellite Genotyping

DNA samples were genotyped using up to 20 previously published microsatellite loci developed from different cetacean species (Table 2). PCR reactions were performed in 10-μL volumes, with 1× Platinum-Taq buffer, 1.5 mM MgCl₂, 0.4 μM each primer, 0.2 mM dNTPs, 1/8U of Platinum-Taq® DNA polymerase, and cycling profile varying by locus (Table 2). Data were collected by GeneScan v. 3.7, and the fragments’ size was measured using Genotyper v. 3.7 (Applied Biosystems Inc.).

GenAIEx v. 6 (Peakall and Smouse 2005) was used to calculate the probability of identity of individuals—P(ID), the probability that two whales will have the same genotype by chance (Paetkau and Strobeck 1994)—and the more conservative probability of identity for siblings—P(ID)sibs, probability that two siblings will have the same genotype by chance (Waits et al. 2001)—for the overall dataset. Hardy–Weinberg equilibrium and linkage disequilibria were tested for microsatellite loci using the program Genepop v. 3.4 (Raymond and Rousset 1995). Genotyping error was estimated by regenotyping an average of 80 individuals per locus and by calculating the ratio between the observed number of allelic differences and the total number of allelic comparisons (Borin et al. 2004).

Matrilineal Social Structure

Patterns of mtDNA haplotype diversity were described for each stranding to test the prediction of “extended matriline.” We further assessed if level of relatedness within groups of long-finned pilot whales, as represented by mass stranding events, was consistent with a matrilineal social structure. To do so, we tested the hypothesis that mature individuals involved in the same stranding were more likely to share mtDNA haplotype than expected by chance using a Mantel test of matrix correlation over the 12 mass strandings. The first matrix indicated whether whales were from the same mass stranding or not (coded as 1 and 0 in the matrix, respectively), whereas the second matrix indicated whether they shared the same mtDNA haplotype or not (also coded as 1 and 0, respectively). This test was also conducted for each sex separately in order to look for a sex-specific effect. Significant correlation between the two matrices was assessed using Monte-Carlo randomization (10 000 replicates), as implemented in GenAIEx.

Kinship and Spatial Correlation within Mass Stranding

To test the “kinship cohesion” hypothesis, we conducted three types of spatial analyses using the two strandings for which spatial positions of whales on the beach were available (i.e., Stewart Island 2003 and Marion Bay). First, Mantel tests of matrix correlation were used to investigate if individuals sharing the same mtDNA haplotype within a mass stranding were more likely to be found closer to each other on the shore. Significant correlation was assessed using Monte-Carlo randomization (10 000 replicates) with GenAIEx.

Second, an analysis of global spatial autocorrelation was conducted using pairwise geographic and pairwise squared distance matrices for individuals (Anselin 1995; Peay et al. 1995). The method developed by Smouse and Peay (1999), as implemented in GenAIEx, employs a multivariate approach to simultaneously assess the spatial signal generated by multiple genetic loci. This analysis generates an autocorrelation coefficient, r, for each distance class, providing a measure of the genetic similarity between pairs of individuals whose geographic separation falls within the specified distance class. Here, the data were analyzed for several sizes
of distance class, ranging from 2 to 20 m of radius (distances were approximate for Stewart Island mass stranding). By varying distance class size, the presence of spatial genetic structure was investigated using different average number of individuals per distance class. Statistical significance was tested by 10 000 random permutations.

Finally, maternity of juveniles was inferred independently for the Stewart Islands 2003 and Marion Bay strandings. We employed the likelihood-based approach implemented in Cervus v. 3.0.3, which was more conservative than exclusion method to assigned mother and juvenile pairs (Marshall et al. 1998; Kalinowski et al. 2007). Nevertheless, we also performed an exclusion analysis (i.e., mismatches between candidate mother and offspring genotypes are used to exclude parentage) in order to provide information on the number of calves with no “potential mother” in the sampled stranded whales. Allele frequencies for the likelihood analyses were based on all Tasmanian data for the Marion Bay stranding and on all New Zealand data for the Stewart Island 2003. Simulations were run considering the proportion of the loci typed for the Stewart Island 2003 and Marion Bay datasets, respectively, and the genotyping error estimated over the whole dataset. Because candidate offspring (i.e., juveniles) were immature whales, and thus expected to be in their mother's group, and because more than 75% of individuals were sampled from each of the two mass strandings, we assume that a majority of the true mothers were represented in the dataset. Therefore, we set the proportion of candidate mothers at 50% for the simulation. The average number of candidate mothers per offspring was set up as the total number of mature females available in each stranding multiplied by two in order to account for 50% of candidate mothers that were not sampled.

Close relatives can lead to false positives in maternity inference among individuals in wild populations (Marshall et al. 1998). For pilot whales, we considered the most likely source of false positive to be female half-siblings of the calves. To avoid this bias, the proportion of half-sibling (\( r = 0.25 \)) in each stranding was taken into account by Cervus simulations of maternity. We estimated this proportion using the “average lifetime calf production” estimated for long-finned pilot whale females in the Faeroe Islands of 2.7–3.5 offspring per female (Martin and Rothery 1993). We took an average of three offspring per female, so that each juvenile would have two half-siblings within their group. Of these two, we assume that one was a male and one was a mature female (assuming a 1:1 sex ratio), the later one being represented among the candidate mothers. Therefore, Cervus simulations for maternity inferences were run so that each candidate offspring was related as half-sibling to one of the candidate mothers. For these analyses, we used primarily maternity relationships that were inferred with 95% confidence.

To assess any potential bias from false exclusion, we also explored the results for relationships inferred with 80% confidence.

### Results

#### Stranding Records and Samples

In total, the 12 mass strandings considered in this study involved a minimum of 1,033 long-finned pilot whales.
Skin samples were collected from 490 individuals (i.e., about 50% of all individuals involved). For some strandings, most individuals were documented, whereas for other strandings, only a subset of samples was collected (Table 1). Total body length measurements were collected from 448 individuals at 10 mass strandings. No measurements of length were taken at the mass strandings of Long Bay and Pitt Islands.

Sex was identified for 484 individuals from both field observation and molecular markers. These were in agreement for all but 15 cases. For these disagreements, molecular sexing was replicated and accepted as the correct sex. Based on sex and length measurements, 440 individuals could be categorized as immature (i.e., juvenile) or mature: juvenile female (12.5%), mature female (52.5%), juvenile male (20.5%), or mature male (14.5%; Table 1). Of the 33% classified as juveniles (males and females), about a third could be further classified as unweaned calves, that is, 9.8% (n = 43) of the all individuals in the mass strandings (Table 1).

Spatial Coordinates and Stranding Dynamics for Stewart Islands 2003 and Marion Bay

The most comprehensive description of spatial coordinates and stranding dynamics were available from Stewart Island 2003 and Marion Bay, based on accounts by coauthors (H.K. and R.G.). At Stewart Island 2003, 159 long-finned pilot whales live-stranded on a gently sloping sandy beach the 8 January 2003. During this stranding, 37 of the 159 whales were refloated and herded out to sea. The re-floating effort was apparently a success with no sign of re-stranding on the following days (although two whales, with healing skin damage, live-stranded at a different location of the island on 25 January). The mapping of the stranding is not precise in terms of distances, but it gives an accurate picture of the position of the whales relative to each other. These positions are thought to be representative of the location where the whales initially beached themselves, as the seas were reportedly calm. The carcasses were distributed along 150 m, with no clear cluster of individuals (see Supplementary Material online). Spatial locations, along with genetic samples, length, and sex, were collected from all of the 122 whales that died on the beach, that is, 77% of the whales initially stranded (Figure 2a).

At Marion Bay, 71 long-finned pilot whales were found stranded on 25 October 2005. Of these, 11 were alive and were re-floated, whereas the remaining 60 died on the shore. GPS positions and skin samples were obtained for 44 of the whales that died that day. This event is defined as “phase 1” of

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**Figure 2.** Spatial distribution of the long-finned pilot whales on the shore of (a) Old Sand Neck, Stewart Island 2003, New Zealand (N = 122), and (b) Marion Bay, Tasmania, Australia (N = 107), showing the position of the mother/offspring pairs inferred using likelihood approach (arrows point at the location of the offspring). (b) also shows the distribution of six mitochondrial DNA haplotypes carried by each whales at the Marion Bay stranding; at Stewart Islands (a), all whales shared the same mtDNA haplotype and are represented by black dots.
the stranding (Figure 2b). Later in the evening, more whales started to strand just north of the initial stranding location. In the morning, a total of 79 whales were found stranded, only eight of which were alive (these were re-floating). This second event is defined as “phase 2” of the stranding; GPS positions and skin samples were obtained from 64 of the carcasses (Figure 2b). A few more whales stranded the day after, but no biological information was collected on these individuals. In total, spatial coordinates were obtained for 108 of the 145 whales (75%) that stranded and died at Marion Bay.

MtDNA Haplotypes and Microsatellite Genotypes

A total of 486 samples were sequenced for a consensus mtDNA fragment of 345 bp, revealing 11 haplotypes. All haplotypes were described in a previous survey of the worldwide mtDNA diversity of pilot whales (see Oremus et al. 2009 for Genbank accession numbers). The average number of microsatellite loci successfully genotyped per individual was 15. Overall probability of identity were based on analyses of spatial autocorrelation performed for any of the distance classes used (Figure 3).

Extended Matriline Hypothesis

To test the prediction of “extended matriline,” we investigated the pattern of mtDNA diversity within mass stranding. In contrast to the expectation that all individuals in a stranding will share the same haplotype, we found multiple haplotypes within 9 of the 12 mass strandings (Figure 1). Having rejected the “extended matriline” hypothesis, we next tested the prediction of the “multiple matriline” hypothesis using a Mantel test for nonrandom association of haplotypes within each stranding, relative to the pooled samples from all strandings. Consistent with the expectations of the multiple matrilines, we found that mature individuals from the same stranding were more likely to share the same haplotype than expected by chance within the pooled samples (Mantel tests, \( P < 0.001 \)). This was also the case when tested for each sex separately (Mantel tests, \( P < 0.001 \)). To control for bias introduced by geographical structure between Tasmania and New Zealand (Oremus et al. 2009), we repeated this test for each region separately. Results were again significant for both New Zealand (\( P < 0.001 \)) and Tasmania (\( P < 0.001 \)).

Kinship Cohesion Hypothesis

To test the hypothesis of kinship cohesion, we conducted three analyses for Stewart Island 2003 and Marion Bay mass strandings. None of our analyses supported the prediction of a spatial relationship among kin during mass stranding. First, at Marion Bay, we found six distinct haplotypes, all of which were shared by several individuals from the stranding. Mantel test showed no significant correlation between spatial relationship of beached individuals and sharing of mtDNA haplotype (\( P = 0.339 \)). At Stewart Island, all 122 sampled whales shared the same common mtDNA haplotype, and therefore, no information could be used to distinguish different matrilineal groups. Second, the estimated genetic relatedness of individuals based on microsatellite genotypes showed no correlation with spatial proximity for either stranding based on analyses of spatial autocorrelation performed for any of the distance classes used (Figure 3).

Third, and most notable, the inference of maternity between mature females and juveniles showed widespread disruption of mother/offspring bonds during mass strandings at Stewart Island and Marion Bay (Figure 2). A total of 21 mother/offspring pairs were assigned with 95% confidence at Stewart Island 2003. From these, we found no obvious pattern of geographic association (Figure 2a). Similarly, no pattern was found when considering 13 additional mother/offspring pairs identified with 80% confidence (results not shown). When looking specifically at pairs comprised of mother and unweaned calf (i.e., subcategory of juveniles), we also found that they were separated from their mothers on the shore (Figure 2a). Among the 18 unweaned calves sampled at Stewart Islands, four were assigned to a candidate mother with 95% confidence (eight assigned at 80% confidence). At Marion Bay, three mother/offspring pairs were assigned with 95% of confidence. In two cases, the mother/offspring pairs were found in the same large cluster of individuals but not in immediate proximity. In the third case, the juvenile was found at about 150 m from its mother and was surrounded by many other whales.

Because it was difficult to reach a conclusion about disruption of the mother/offspring pairs at Marion Bay based on only three cases, we also considered the pairs identified with 80% confidence, on the condition that candidate mother and juvenile shared the same mtDNA haplotype and that they were compatible with exclusion inference (i.e., eight additional pairs; Figure 2b). Using these criteria, we assigned a total of 11 pairs of mother/offspring, including two with unweaned calves. Similarly to Stewart Island 2003, no obvious pattern of geographic association was found between mothers and offspring. Furthermore, 3 of the 11 mother/offspring pairs (including one with an unweaned calf) were separated by multiple strandings, that is, the juveniles and unweaned calf stranded in a different phase from the mother.

Finally, there was a high proportion of juveniles for which no maternity could be established at Stewart Islands (\( \alpha = 31 \), or 60% of the juveniles) and at Marion Bay (\( \alpha = 27 \), or 90% of the juveniles), when considered at 95% confidence of assignment. Because this result could be an artifact of the maximum likelihood method of maternity inference and use of a “strict” confidence level, we repeated the analyses based on simple exclusion. The results confirmed that a high proportion of juveniles had no potential mothers among the mature females that died (33% at Stewart Island 2003 and 57% at Marion Bay).
Discussion

Multiple Matrilineal Groups in Mass Stranding

Mass strandings are typically described as a complex phenomenon with multiple potential environmental causes (e.g., Perrin and Geraci 2002; Bradshaw et al. 2006). With few exceptions (Mesnick 2001; Viricel et al. 2008; Mirimin et al. 2011), empirical studies have largely failed to explore the role of social organization and kinship in these events. Here, we have provided the first evidence that mass strandings of long-finned pilot whales involve individuals with different mtDNA haplotypes and, thus, individuals who are not maternally related. This finding challenges the hypothesis of an “extended matriline” as a primary social structure model in long-finned pilot whales. Note that given the low levels of worldwide mtDNA diversity in pilot whales (Oremus et al. 2009), our test of the extended matriline is conservative, as individuals sharing a common haplotype are not necessarily close maternal relatives. The detection of multiple matriline also challenges the hypothesis that mass strandings are driven primarily by kinship-based behavior. If care-giving behavior (epimeletic behavior) is a force in the group cohesion of mass strandings, it may have evolved through alternate mechanisms, such as reciprocal altruism (Trivers 1971). Studies of social structure have shown that long-term (maybe lifelong) associations can form between genetically unrelated individuals in other cetacean species, such as sperm whales (Mesnick 2001) and bottlenose dolphins (Möller et al. 2001). However, these relationships have not been shown to involve “care-giving” or other forms of altruistic behavior.

Although challenging the universality of “extended matrilines,” patterns of mtDNA diversity in New Zealand and Tasmania mass strandings do not discount the influence of matrilineal bonds in social structure of long-finned pilot whales (Amos et al. 1993). Rather, our results suggest that long-finned pilot whales from New Zealand and Tasmania form association among multiple matrilineal groups, as suggested by behavioral study in the North West Atlantic (Ottensmeyer and Whitehead 2003).

Spatial Distribution of Kin and Missing Mothers

Detailed analyses of Stewart Island 2003 and Marion Bay mass strandings showed no obvious correlation between kinship and spatial distribution along the stranding beach, even for fundamental social units, that is, mother/offspring pairs. Therefore, contrary to the “kinship cohesion” hypothesis, it appears that close kin are not necessarily in close proximity to each other during these often fatal events. For the maternity analyses, it was important to insure that maternity

Figure 3. Correlogram plots (solid lines) of the genetic correlation coefficient ($r$) as a function of distance class (in meters, ranging from 2 to 20m) in the mass strandings at Stewart Island 2003 (a to d) and Marion Bay (e to h). The permuted 95% confidence interval (dashed lines) and the bootstrapped 95% confidence interval error bars are shown.
assignments between mature females and juveniles were correct, and therefore, we choose to apply conservative parameters of inference. In particular, we account for the presence of closely related individuals, which are likely considering a multiple matrilineal social structure that we consider most consistent with available evidence. Indeed, parentage analyses using likelihood-based approach assumes, like most parentage inference methods, that the candidate parents are unrelated to one another and to the offspring; violating this assumption may result in an overestimation of confidence in parentage assignments (Marshall et al. 1998). This is particularly true in the presence of individuals related to the offspring as half-sibling or more (\( r \geq 0.25 \)), which can be assumed in long-finned pilot whales.

Despite the lack of support for the “kinship cohesion” hypothesis, it was expected that the mothers of stranded juveniles would be present among the mature females that died during the same event. Contrary to this expectation, we found a high proportion of “missing mothers,” that is, juveniles for which there were no potential mothers among sampled carcasses, even when based on the more relaxed exclusion method. This was particularly apparent at Marion Bay, where no potential mother was found for 57% of the juveniles. Although the incomplete sampling of carcasses in the strandings at Marion Bay could have contributed to the missing mothers, this was not the case for Stewart Island 2003. Here, these missing mothers must have been successfully refloated and left without their calves or could have been absent from the stranding from the outset.

Absence of Kin Spatial Cohesion—Cause or Consequence?

Several scenarios could explain the lack of spatial cohesion among kin in the Stewart Island 2003 and Marion Bay mass strandings. First, the separation of kin within large groups of long-finned pilot whales could simply be a usual pattern of group dynamics, and therefore, the spatial distribution that we observed on stranding beach would be a reflection of a natural pod. However, this seems unlikely, given that the spatial disruption of kin extended to mothers and their dependent offspring. Although alloparental care could explain the spatial separation of some mother/offspring pairs (Whitehead 1996), this could not explain the many missing mothers. A second scenario is that kinship bonds were disrupted before the actual mass strandings, perhaps contributing to the causal factors initiating these events. Indeed, the joining together of multiple matriline groups in long-finned pilot whales, as evident in our results, appears to be similar to the “super pods” reported for free-swimming killer whales (Ford et al. 2000). Such aggregations are likely to form in response to feeding or mating opportunities (de Stephanis et al. 2008) and could disrupt kinship bonds during competition or even aggression between different matrilineal groups. Some field observations of behavior prior to and during mass strandings are consistent with competition or aggression. Indeed, Robson (1984) observed that the “key whale” in mass strandings is usually driven off by two or three adult females. In a third scenario, the disruption of kinship bonds could be the consequence of the initial stranding of key whale(s). In this case, distress calls from the first whales ashore could create confusion among swimming whales in the vicinity, resulting in the separation of kin before they end up stranding themselves.

Implications for Animal Welfare and Future Documentation of Strandings

Regardless of the reason(s) for the lack of spatial cohesion among kin, our results demonstrate that proximity on the beach is not a reliable indicator of kinship or relatedness, in particular for mother/offspring pairs. Yet, the youngest individuals in a mass stranding are usually refloated with the nearest mature females, under the assumption that this is the mother. It is further assumed that refloating a mother/offspring pair will prevent their restranding (Geraci and Lounsbury 1993). In fact, the refloating of a juvenile and an unrelated female could increase the tendency to restrand after refloating “rescue.” Our results suggest that difficult decisions relating to the welfare of dependent young (e.g., to rescue, rehabilitate, or euthanize) should not rely on the presence or absence of a stranded adult female.

A better understanding of the social causes or consequences of mass strandings will require better sampling and documentation, despite the often challenging circumstances of these tragic events. Exhaustive sampling, including living whales involved in a mass stranding, is needed to answer the question of the missing mothers raised by our results. Genotyping of living whales also offers the possibility to re-identify individuals in cases of restranding in the following days, months, or years. This could begin to provide information on the success of refloating efforts, a practice that is still a subject of debate (Ketten 2009). Better documentation of prestranding behavior and the temporal sequence of strandings is needed to understand the disruption of kinship bonds. This information could provide further guidance for improved efficiency of rescue efforts and humane animal welfare practices during stranding events.

Supplementary Material

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

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References


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