# Are the southern feeding group of Eastern Pacific gray whales a maternal genetic isolate?

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## ABSTRACT:

The management of eastern Pacific gray whales that feed in southern waters depends on how individuals are recruited into this feeding group, which remains uncertain. Separate management is needed if recruitment is internal with mothers bringing their calves to their life-long feeding grounds. Such exclusively internal recruitment would make the southern feeding group a maternal genetic isolate. We simulate mitochondrial haplotypic diversity for the southern feeding group under the assumption that it is a maternal genetic isolate and compare our results to empirical genetic data from the southern feeding group. We find that the haplotypic diversity and number of haplotypes are higher than predicted for a maternal isolate by the simulations. Other empirical data are also inconsistent with maternal genetic isolation. Comparisons with the genetics of other small populations of large whales show that both the number of haplotypes and haplotypic diversity are much higher for the southern feeding group. Also, genetic analysis shows that the sex ratio is significantly male biased, which may not be consistent with a closed population. Thus, we can reject the hypothesis that the southern feeding group is a maternal isolate. Other hypotheses remain and we discuss future directions.

## INTRODUCTION:

Although all eastern North Pacific gray whales breed in the lagoons off Baja California and most feed north of the Aleutian peninsula, some feed in more southern waters between California and southeast Alaska (Braham, 1984, Darling, 1984, Sumich, 1984, Weitkamp *et al.*, 1992, Calambokidis, 1991). Long-term photo-identification has revealed that many of the whales using the southern feeding area return each year, raising the possibility that they form a distinct group requiring separate management for maintenance of their feeding range. Management of the southern feeding group has become particularly important recently, as subsistence whaling has been resumed off the coast of Washington by the Native American Makah tribe.

Successful management of the southern feeding group requires understanding recruitment into this population. Humpback whales, another migratory species that feed in coastal waters, have strong matrilineal site fidelity to feeding areas (Palsbøll *et al.*, 1995). If this were the case for the southern feeding group of gray whales, recruitment would be from calves following their mothers to the feeding grounds. Such internal recruitment would require separate management for the southern feeding group, as local extirpation would cause the loss of part of the natural range. Because a calf inherits its mothers' mitochondrial haplotype, matrilineal site fidelity would eventually result in genetic drift such that the southern feeding area would be characterized by different frequencies (provided only internal recruitment) of mitochondrial haploytypes than the rest of the gray whales, making this population a maternal genetic isolate. Ramakrishnan and Taylor (in press) showed through simulations that such a maternal genetic isolate would be easy to detect using mitochondrial DNA sequences. This paper takes the next step by using the simulation approach together with the actual distribution of mtDNA haplotypes from 45 individuals in the southern feeding group to address the hypothesis that these animals are completely isolated (no external recruitment) from the northern feeding animals. The samples are all from whales that were biopsied in peak summer when only southern feeding group members should be in the southern waters.

In addition, we consider other empirical evidence relevant to whether southern feeding whales could be completely separate. We compare the number of haplotypes with numbers found in other small populations known to be isolated. Steeves (in press) also noted a male biased sex ratio though the effect was not significant, which might be inconsistent with a closed population. We use the greater sample size available in this study to expand Steeves' conclusions and address whether the sex ratio is consistent with an isolated population.

This paper only considers the hypothesis of maternal genetic isolation. Data that are not consistent with this hypothesis may still be consistent with hypotheses that would require separate management (low recruitment from the northern feeding group) and also hypotheses that would not require separate management (high recruitment from the northern feeding group). We return to these other hypotheses in the discussion.

Because this study uses a model similar to Ramakrishnan and Taylor (in press) we will review the logic of the model and summarize the results. The model simulated population dynamics and genetics of both northern and southern feeding groups assuming that the southern feeding group was colonized by a single founding event in the last century. Results showed that if the southern feeding group was founded by a single founding event after which there was no external recruitment, the statistical power to distinguish between the feeding groups was high.

Results were robust to uncertainty in four model parameters: current population size of the southern feeding group, growth rate of the southern feeding group, initial haplotypic distribution for the northern feeding group and the size of the post-commercial harvest bottleneck for eastern North Pacific gray whales. In summary, the study showed that genetic data from the southern feeding group could be used to conclusively eliminate the extreme hypothesis that the southern feeding group was founded by a single founding event in the past.

This study uses the same simulation with the most recent abundance estimates for the southern feeding group (Calambokidis *et al.*, 2000 a and b). We also incorporate genetic data on sex ratios in the southern feeding group to estimate the current population size of adult females in this population, a parameter used in our model. Rather than testing whether the haplotypic distribution from the southern feeding group is consistent with panmixia with the northern feeding group, which requires a random sample from the northern feeding group, we restrict our analyses to what can be gleaned by looking only at the southern feeding group's haplotypic distribution. We then compare haplotypic diversity and number of haplotypes in our simulated data to empirical genetic data from the southern feeding group.

## METHODS:

**Simulation modeling:**
Because we are interested in whether southern feeding group individuals are a maternal genetic isolate, we model only adult females. We assume a single birth and death rate for adult females. The model recapitulated the history of population dynamics by simulating the bottleneck experienced by the entire eastern North Pacific gray whale population due to commercial whaling. We assume that after both bouts of commercial whaling, the population size is reduced to 13% (one of the possible scenarios in Butterworth *et al.*, 2001) of the present carrying capacity by 1900. The population then grows at a density dependent rate of 2.5% per year to the present. Because the time span is short, we ignore the effects of mutation. Selection is also ignored because we are modeling a neutral genetic marker. Next, we sample the appropriate number of individuals to simulate colonization of the southern feeding area from the northern feeding group. Founding of the southern feeding group is simulated in years 1900, 1920, 1940, 1960 and 1980. Collaborative photo-identification efforts have estimated the population size to be as high as 269 (estimate for 1999) and as low as 169 (estimate for 1996) (Calambokidis *et al.*, 2000 a and b). We model both these estimates of population size. Using the sex ratio estimated in Steeves *et al.* (in press) and the above estimates of current population size, we estimated the current number of females in the southern feeding group. The number of founders is then determined assuming an exponential model of growth ($r = 0.025$) by back calculating from the present female population size. Sensitivity analyses from Ramakrishnan and Taylor (in press) show that results are insensitive to the assumed growth rate (which could be as high as 3.4% per year in the absence of Russian harvest (Wade and Demaster, 1996)), and slightly more sensitive to the assumed current population size of the southern feeding group. After the founding event, the southern feeding group is allowed to grow assuming the same birth death process described above from the year of founding until the year 2000. In our main simulations we assume that there is no significant difference between male and female haplotypic distribution and hence sample 45 individuals (the total number of empirical genetic samples), though our model simulates only the adult female component of the population. Later (see description below) we examine females only to check to see whether at least the female component of the southern feeding group is a maternal genetic isolate. This sample is used to calculate haplotypic diversity and the number of haplotypes. Simulations are carried out for the range of possible current population sizes and are repeated 1,000 times to account for stochastic effects. Further details of the simulation model are described in Ramakrishnan and Taylor (in press).

We do not have the haplotypic distribution for gray whales prior to the commercial harvest. Minke whales in the western North Pacific were not heavily commercially harvested and have approximately the same number of individuals as the eastern North Pacific gray whales do now. Therefore, we use an empirical distribution of d-loop haplotypes from minke whales (Goto and Pastene, 1999) as the initial haplotypic distribution representing pre-harvest eastern North Pacific gray whales. We test whether this assumption is valid by comparing the observed number of haplotypes (33) found in a sample consisting primarily of stranded eastern North Pacific gray whales (n=114) with a distribution of the expected number of haplotypes for the same number of samples taken from the minke whale distribution. The minke whale distribution was sub-sampled for a sample size of 114 using a sampling without replacement algorithm.

*Sensitivity analysis:*
Steeves et al (in press) found a biased sex ratio in 19 southern feeding group genetic samples. This could indicate that the southern feeding group may not be a closed population. In many mammals, males disperse more than females (Greenwood, 1980). Based on this assumption, if the population is not closed, the dispersers are more likely to be male than female. As a result, matrilineal philopatry to the feeding ground would be most strongly represented in the female component of the population especially if the southern feeding group is not a closed. We test the sensitivity of our results to sampling the observed number of females (16 out of 45) instead of the total number of sampled individuals (45).

**Empirical analysis:**
*Molecular genetic analysis:*

Samples were collected via biopsy from gray whales summering in British Columbia, Canada (Darling) and Washington, USA (Calambokidis, Gearin and Gosho). Samples from both localities were part of ongoing studies of the southern feeding group (Darling 1984, Calamabokidis 2000a,b, Steeves, 1998). The haplotypic data from the British Columbia samples are already published (Steeves *et al.* in press). Using standard protocols, we extracted DNA and amplified and sequenced a 523 bp region of the 5' end of the mitochondrial control region for each sample. The primers used for amplification and sequencing were 5'-TACCAAATGTATGAAACCTCAG-3' (Rosel *et al.* 1995) and 5'-CCTCCCTAAGACTCAAGGAAG-3' (designed at SWFSC). In addition, each animal was sexed by co-amplification of the Y chromosome-specific SRY gene with segments of the ZFX and ZFY genes. The latter served as positive control for the PCR conditions, while the former served as an indicator for the SRY gene. Single bands of PCR product indicated females and double indicated males.[1] The samples were also genotyped for seven polymorphic microsatellite loci to eliminate duplicate samples (data not shown). After removing the duplicates, there were 45 individuals represented in the data set.

A binomial test ($H_0$: sex ratio=1:1, $H_a$: sex ratio not equal to 1:1) was used to investigate significance of sex ratio bias.

*Literature review:*
We reviewed the literature for other baleen whale populations that were either numerically small now or were known to have been depleted to very low numbers and where comparable genetic studies had been completed. We found five such populations: bowheads in the Sea of Okhotsk, North Atlantic right whales, two populations of Southern right whales: off Australia, and off Auckland Island, and western North Pacific gray whales. Comparisons were restricted to other studies with similar datasets, specifically those involving the 5' end of the mitochondrial control region.

**RESULTS:**

**Simulations:**
The 45 samples from the southern feeding group contained 20 different haplotypes, representing a haplotypic diversity of 0.93. The most common haplotype was represented by 6 individuals. 35 percent (7) of the haplotypes were represented by only one individual.

We found that the number of haplotypes observed in 114 eastern North Pacific gray whales (number of haplotypes=33) is within one standard error of the mean in the distribution of the number of haplotypes observed in a sample of size 114 drawn from the minke whale haplotypic distribution (number of haplotypes=34), validating the use of the minke whale haplotypic distribution.

The observed number of haplotypes (20) was consistently higher than the number of haplotypes in the simulated samples (Table 1) for both lower and upper population sizes. The number of haplotypes increased as founding events became more recent, but in no case reached the number observed. The same results were observed for haplotypic diversity (Table 1), with simulated diversity lower than observed diversity (0.93).

| | Proportion of simulations less than observed |
|---|---|
| Number of haplotypes (16) | 1.0 |
| Haplotypic diversity (0.856) | 1.0 |

*Table 1. Proportion of simulations with values less than the observed for number of haplotypes (observed = 20) and haplotypic diversity (observed = 0.93). The highest values from the simulations are in parentheses.*

Sensitivity analyses showed that results are not significantly affected by sampling only adult females (Table 2). Observed haplotypic diversity (0.904) was higher than simulated haploypic diversity in all but one simulation. The observed number of haplotypes (12) was higher than the number of haplotypes in the simulated samples in all but two simulations.

| | Proportion of simulations less than observed |
|---|---|
| Number of haplotypes (13) | 0.998 |
| Haplotypic diversity (0.906) | 0.999 |

*Table 2 Proportion of simulations with values less than the observed for number of haplotypes (observed = 12) and haplotypic diversity (observed = 0.904) when sample size was reduced to the number of females. The highest values from the simulations are in parentheses.*

**Empirical:**
Molecular sexing showed that the sex ratio in the southern feeding group is significantly different (binomial test, probability = 0.0346) from parity (males:females = 1.8:1). These results are similar to Steeves et al (in press), though the increased sample size of this study provided significant sex ratio bias unlike Steeves *et al.* (in press) where the bias was not significant.

[1] Rosenberg A, Mesnick SL. unpublished. Protocol for the determination of gender. Contact author RGL.

Comparing the southern feeding group of eastern gray whales to other small baleen whale populations reveals that the feeding group has both higher numbers of haplotypes and higher haplotypic diversity than other populations of similar size (Table 3). In fact, this level of diversity (0.93) is similar to the value calculated for the population of eastern gray whales as a whole, 0.95 (LeDuc *et al.* submitted). There are several factors that can influence the rate of genetic drift and the levels of haplotypic diversity, such as current and historical population size, degree, duration and recency of population bottlenecks and levels of maternal gene flow with other populations. Of course, each of the populations in Table 3 have their own unique set of parameters, so one must be careful in interpreting the comparative values.

| POPULATION | POP. SIZE | # HAPLOTYPES (SAMPLE SIZE) | *h* |
|---|---|---|---|
| bowhead (Okhotsk Sea) | Few hundred (Burns *et al.*1993) | 4 (25) | 0.61 (LeDuc *et al.* 1998) |
| right (North Atlantic) | 295 (Knowlton *et al.* 1994) | 5 (180) | 0.698 (Malik *et al.* 1999) |
| Right (southern Australia) | >800 (Bannister 1998) | 5 (20) | 0.737 (Baker *et al.* 1999) |
| right (Auckland Is.) | 740-1140 (Patenaude 2000) | 4 (20) | 0.684 (Baker *et al.* 1999) |
| gray (Okhotsk Sea) | <100 (Weller *et al.* 1999; Weller and Brownell 2000) | 10 (42) | 0.70 (LeDuc *et al.* submitted) |
| gray (southern feeding aggregation) | 169-269 (Calambokidis *et al.* 2000a,b) | 20 (45) | 0.9304 (this study) |

*Table 3. Levels of haplotypic diversity (h) measured in small, isolated populations of baleen whales. It should be emphasized that some of the cited estimated population sizes are rough and/or based on limited data.*

**DISCUSSION:**

Comparison of simulated and empirical results show that both haplotypic diversity and the number of haplotypes observed in the southern feeding group are higher than would be predicted if the southern feeding group was founded in the last century. A comparison of haplotypic diversity in the southern feeding group to other baleen whale populations of comparable size shows that haplotypic diversity is higher in the southern feeding group than for other small populations known to be isolated. We can thus reject the hypothesis that the southern feeding group is a maternal genetic isolate. Genetic sexing of samples in the southern feeding group shows that the sex ratio is significantly biased towards males. Male biased sex ratios have also been reported in fin whales (Bérubé, 2001) and humpback whales (Palsbøll *et al.*, 1997). In these studies, the bias is thought to be due to differences in the way males and females use feeding habitat, for example, if males tend to use certain feeding areas more than females. Biased sex ratio in the southern feeding group could be due to the above hypothesis especially since sampling is opportunistic or confined to parts of the range. Alternatively, the biased sex ratio could indicate that this feeding group may not be a closed group, i.e. experiencing only internal recruitment.

Our results show that genetic data can be used to reject the hypothesis that the southern feeding group is a maternal genetic isolate sustained exclusively by internal recruitment. Thus, we cannot propose separate management based on the above hypothesis. However, low levels of external recruitment would still require separate management of the southern feeding group. On the other hand, high levels of external recruitment could allow joint management of the northern and the southern feeding groups.

Though genetics has been useful so far, further progress using genetics alone to estimate dispersal may be difficult. Gene flow from the large northern feeding group would quickly swamp genetic drift in the small southern feeding group. It is likely that it will be difficult to distinguish between levels of dispersal that would span the range of interest, i.e. low levels requiring separate management and higher levels allowing joint management.

From a scientific perspective of understanding how recruitment occurs, there are several possibilities that are likely to be fruitful. A combination of photo-identification and genetic data could be used to investigate the origin of new recruits. Detailed photo-identification could aid identification of individuals that are recruited into the southern feeding group. Genetic sampling of these individuals, along with samples from the extant resident individuals in the southern feeding group and individuals in the northern feeding group could be used to identify their genetic source and the level of external recruitment, which could be then used to determine the appropriate management strategy. Another potential source of information would be examining chemical signatures of pollutant levels or isotope ratios for known long-term southern feeders and comparing their "signature" to that of recent recruits.

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