

The Influence of Maternal Lineages on Social Affiliations among Humpback Whales (*Megaptera novaeangliae*) on Their Feeding Grounds in the Southern Gulf of Maine

MASON T. WEINRICH, HOWARD ROSENBAUM, C. SCOTT BAKER, ALEXIS L. BLACKMER, AND HAL WHITEHEAD

From the Whale Center of New England, PO Box 159, Gloucester, MA 01930 (Weinrich and Blackmer); WCS/AMNH Cetacean Conservation and Research Program, International Conservation-Marine, c/o Wildlife Conservation Society, 2300 Southern Blvd, Bronx, NY 10460, and the Conservation Genetics Program and Center for Biodiversity and Conservation, American Museum of Natural History, 79th Street and Central Park West, New York, NY 10024 (Rosenbaum); the School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand (Baker); the Department of Neurobiology, Physiology, and Behavior, University of California, Davis, Davis, CA (Blackmer); and the Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada (Whitehead).

Address correspondence to M. T. Weinrich at the address above, or e-mail: mason@whalecenter.org.

Abstract

Humpback whales on their feeding grounds in the Gulf of Maine typically form fluid fission/fusion groups of two to three individuals characterized by noncompetitive and, at times, cooperative behavior. Here we test the hypothesis that, despite the apparent absence of close kinship bonds, the fluid associations between feeding whales are influenced by “maternal lineages” as represented by mtDNA haplotypes. Using skin samples collected with a biopsy dart, variation in the hypervariable segment of the mtDNA control region identified 17 unique haplotypes among 159 individually identified whales from the southern Gulf of Maine. The haplotypes of a further 143 individuals were inferred from known direct maternal (cow-calf) relationships. The frequencies of associations among these 302 individuals were calculated from 21,617 sighting records collected from 1980 to 1995, excluding associations between a cow and her dependent calf. For groups of two where the haplotypes of both individuals were known ($n = 3,151$), individuals with the same haplotype were together significantly more often (26%) than expected by random association (20%). To account for different group sizes and associations with individuals of unknown haplotype and sex, we used Monte Carlo simulations to test for nonrandom associations in the full data set, as well as known female-only ($n = 1,512$), male-only ($n = 730$), and mixed-sex ($n = 2,745$) groups. Within-haplotype associations were significantly more frequent than expected at random for all groups ($P = .002$) and female-only groups ($P = .011$) but not male-only groups, while mixed-sex groups approached significance ($P = .062$). A Mantel test of individual pairwise association indices and haplotype identity confirmed that within-haplotype associations were more frequent than expected for all sex combinations except male-male associations, with females forming within-haplotype associations 1.7 times more often than expected by random assortment. Partial matrix correlations and permutation analyses indicated that the skew toward within-haplotype associations could not be accounted for by short-term temporal co-occurrence or fine-scale spatial distributions of individuals with shared haplotypes. While the mechanism by which individuals with a common mtDNA haplotype assort remains unknown, our results strongly suggest an influence of maternal lineages on the social organization of humpback whales within a regional feeding ground.

Cooperative foraging, especially among kin, is well documented in many mammals and other vertebrate species (Bertram 1983; Packer et al. 1990). In many social carnivores, group foraging has evolved because it allows the capture of

prey that is normally difficult, if not impossible, to catch by a single animal [e.g., lions (*Panther leo*; Stander 1992); cheetahs (*Acinonyx jubatus*; Caro 1994)]. Many cetaceans also forage in groups, sometimes numbering hundreds of individuals

(Connor 2000). Pelagic dolphins are thought to form pods to aid both in finding schooling fish and, once located, to aid in capture by surrounding and capturing them (Wursig 1987). Killer whales (*Orcinus orca*) also have been known to cooperate for the capture of marine mammal prey in a number of ways (Baird and Dill 1996; Jefferson et al. 1991). While baleen whales do not usually form large or stable groups, cooperative foraging has been suggested in humpback whales (*Megaptera novaeangliae*; Baker 1985; Clapham 1993, 2000; D'Vincent et al. 1985; Perry et al. 1990; Sharpe 2001; Weinrich 1991; Weinrich and Kuhlberg 1991; Whitehead 1983), bowhead whales (*Balaena mysticetus*; Wursig et al. 1985), and fin whales (*Balaenoptera physalus*; Whitehead and Carlson 1988). The tendency of whales to congregate in large numbers within an area could also result from less direct cooperative interactions.

In most baleen whales, the year is divided into two distinct phases: winter breeding in low latitude waters followed by migration to summer feeding grounds in higher latitudes (Mackintosh 1965). The lowered productivity of tropical areas forces prolonged winter fasts, during which whales live off stored reserves of lipids (Chittleborough 1965; Dawbin 1966; Mackintosh 1965; Slijper 1962). During summer, breeding does not take place and the primary focus of the whales' activities is thought to be feeding. In the North Atlantic Ocean, long-term studies of naturally marked individuals demonstrate that whales feeding during spring, summer, and autumn in waters off New England, Canada, Greenland, Iceland, and Norway migrate to the West Indies for the winter breeding season (Katona and Beard 1990; Matilla et al. 1989; Palsbøll et al. 1997; Stevick et al. 1998). Migratory fidelity to feeding grounds appears to be the result of early maternal experience (Weinrich 1998). Offspring seem to learn the migration route to a particular feeding region during the first year of life (Clapham and Mayo 1987, 1990), most or all of which is spent with the mother (Baraff and Weinrich 1993; Chittleborough 1965).

The maternal basis of seasonal population structure in humpback whales has been confirmed using molecular genetics, especially maternally inherited mitochondrial DNA (mtDNA) (Baker et al. 1990, 1993, 1994a; Larsen et al. 1996; Palsbøll et al. 1995, 1997; Palumbi and Baker 1994). There is a clinal differentiation in frequencies of mtDNA haplotypes among feeding grounds extending from the Gulf of Maine to Norway, although adjacent feeding grounds are not necessarily genetically distinct (Baker and Medrano-Gonzalez 2001; Baker et al. 1990, 1994a; Larsen et al. 1996; Palsbøll et al. 1995).

While on their feeding grounds, humpback whales are often spatially aggregated but typically found alone or in groups of two or three individuals, although larger groups have been reported periodically (Baker 1985; Clapham 1993; Perry et al. 1990; Sharpe 2001; Weinrich 1991; Whitehead 1983). Group composition generally is fluid with most groups lasting from 1 h to a few days, although stable, longer-term associations have been recorded with low frequency (Baker 1985; Clapham 1993; Sharpe 2001; Weinrich 1991; Weinrich and Kuhlberg 1991). Feeding ground associations are thought to form for cooperative foraging, either to minimize prey disturbance (Whitehead 1983) or to increase the chance of cap-

turing highly mobile schooling fish, including herring (*Clupea harengus*), capelin (*Mallotus villosus*), or sand lance (*Ammodytes* spp.). On breeding grounds, and rarely on feeding grounds, males form groups and compete for proximity to a female for possible mating opportunities (Baker and Herman 1984a; Clapham et al. 1992; Mattila et al. 1989; Tyack and Whitehead 1983; Weinrich 1995).

Based on the inferred influence of maternally directed experience in the first year of life and the lack of paternal influence on offspring development, we suggest that any influence of relatedness on social structure is likely to occur within matriline. However, there is little or no evidence of a relationship between social organization and first-order relatedness on the whale's feeding grounds. Although Weinrich (1991), Weinrich and Kuhlberg (1991), and Baker et al. (1994b) hypothesized that foraging associations might be influenced by kin selection, this is not apparent in the extensive observations of individually identified whales in the Gulf of Maine or southeastern Alaska. In the Gulf of Maine, known weaned offspring are seldom found in association with their mother or with their maternal siblings despite continued annual return to the region (Clapham 1993, 2000; Sardi et al., 2005). In southeastern Alaska, estimates of kinship from microsatellite genotypes failed to identify first-order relatives among groups of individuals involved in closely coordinated, and apparently cooperative, cohesive feeding associations including group "bubblenet" feeding (Sharpe 2001). Hence, there seems to be an apparent contradiction between the strong maternal fidelity to a feeding ground, and the corresponding lack of the influence of first-order maternal relatedness on group associations.

Here we investigate this contradiction by documenting group associations and identifying the mtDNA haplotype of individual humpback whales in the Gulf of Maine over nearly 15 summers of seasonal residency. In a population with numerous mtDNA haplotypes, such as the Gulf of Maine (Baker et al. 1994a,b; Rosenbaum et al. 2002), individuals with different haplotypes cannot be maternal relatives, while individuals with a shared haplotype will represent a common maternal lineage, including both close and distant maternal relatives. Under the hypothesis of a "maternal lineage effect," we therefore predicted that individuals sharing the same haplotype would be found in association more often than expected under the null hypothesis that associations are random in regards to haplotype. We were also able to discount the possibility that the observed maternal associations were primarily an artifact of short-term temporal or fine-scale spatial effects. Our results provide further evidence for the influence of maternal lineages on the social organization and demography of humpback whales (e.g., Rosenbaum et al. 2002), although the mechanism by which this effect extends beyond first-order kin is unknown.

Methods

Whale Identification and Associations

Humpback whales were observed and sampled on the feeding grounds of the Gulf of Maine, primarily around Jeffreys

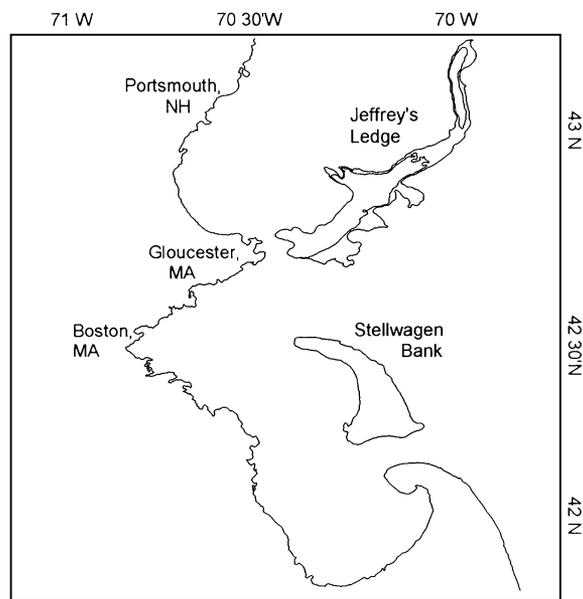


Figure 1. The primary study area, showing the geological features Stellwagen Bank and Jeffrey's Ledge off the coast of Southern New England, USA.

Ledge and Stellwagen Bank (Figure 1). The methods for collection of association information are detailed in Weinrich (1991), Weinrich and Kuhlberg (1991), and Weinrich et al. (1997). Briefly, humpback whales were observed from commercial whale-watch boats, resulting in between 60 and 120 min of whale observation time, and from small research vessels taking daylong trips. Fieldwork was conducted annually from April to November in 1980–1995. Whales' dorsal fins and fluke pigmentation patterns were photographed for individual identification (Katona and Whitehead 1981), and individuals were identified by comparing these photographs with a catalog of Gulf of Maine humpback whales housed at the office of the Whale Center of New England in Gloucester, Massachusetts. The sex of each whale was determined by one or more of several methods: (1) photographing the genital region (Glockner 1983) when a whale was ventral-side up at the surface (both males and females); (2) photographing an adult accompanied by a calf assumed to be its offspring in at least one year of the study (females only); and/or (3) through sex-specific DNA markers using biopsy samples (both sexes; Baker et al. 1991; Palsbøll et al. 1992).

Unless otherwise noted, two or more whales were considered a "group" if they were within two body lengths of each other and behaving in a coordinated manner (surfacing together, diving together, and engaging in similar behavioral modes). This definition is consistent with previous studies of humpback whale social association (Baker 1985; Clapham 1993; Weinrich 1991; Weinrich and Kuhlberg 1991). All sightings of calves still with their mothers were excluded from this database as we assumed that both the social affiliation of the calf and its mtDNA haplotype were not independent of its mother. The assumption of social dependence

has also been used in other analyses of humpback whale social associations (Clapham 1993; Weinrich and Kuhlberg 1991). For some analyses, alternate definitions of association were used; these are clearly noted below.

Molecular Genetic Analysis

Between 1988 and 1993, epidermal samples were obtained from 159 individual whales using now-standard biopsy techniques (Lambertsen 1987; Weinrich et al. 1991). Total genomic DNA was isolated from each sample using standard Proteinase K/phenol/chloroform procedures for extraction (Sambrook et al. 1989). Specifically, two oligonucleotide primers were developed to amplify the first 550-bp fragment within the mtDNA control region of the humpback whale (Baker et al. 1993, 1998) via the polymerase chain reaction (PCR). The first 450 bp of this region contain the majority of variable nucleotide positions in the mtDNA control region (Baker and Medrano-Gonzalez 2001; Rosenbaum et al. 2002) and corresponds to the hypervariable region I of the human control region (Vigilant et al. 1991). PCR products were purified using Bio 101's Gene Clean or Centricon 100 columns according to the manufacturer's protocol. Manual sequencing of double-stranded PCR products was conducted with United States Biochemical Sequenase Version 2.0 DNA Sequencing Kit according to the manufacturer's protocol. Automated DNA sequencing of PCR products was conducted by cycle sequencing with fluorescently labeled dideoxy terminators (ABI part #401113) and run on an Applied Biosystems (ABI) 373A DNA Sequencer (Foster City, CA). We organized sequences for this portion of the mtDNA control region by individual using MACCLADE version 3.4 (Maddison WP and Maddison DR 1992). Haplotype diversity was calculated with the computer program ARLEQUIN following Nei (1987).

Data Storage and Analysis

Sightings data for each social group were stored in database and spreadsheet files on PC-based computers. A separate but linked data file contained information on each identified individual whale, including name, sex, and year of birth, whether it had been biopsy sampled, and other pertinent information. If a whale was assigned an mtDNA haplotype through PCR sequencing, the haplotype was also recorded in the latter data file. Any other whales known to be part of the same matriline through photo-identification studies (e.g., calves of a sampled female or a mother who has had offspring sampled) were assigned the same mtDNA haplotype (Fernando and Lande 2000). As our database includes no more than three generations within a matriline, we feel that the assumption of no mutations in the mtDNA within matrilines is justified for this study.

Monte Carlo simulations and Mantel tests of matrix correlations (Zar 1998) were used to test for a nonrandom relationship between group membership and mtDNA haplotype identity. We calculated pairwise association indices for individual associations and conducted Mantel tests using

Table 1. The number of individually identified humpback whales with known mtDNA haplotypes determined by amplification and direct sequencing from biopsy samples (“biopsied”) and assigned through maternal first-order relationships (“assigned”), with haplotype diversity (b) and the frequencies of sightings in the Gulf of Maine

mtDNA haplotype	No. whales biopsied	No. whales assigned	No. sightings
I	67	56	11,636
C1	6	5	1,751
C	16	14	4,705
D1	3	5	651
D	15	25	2,645
I2	2	0	101
I1	6	4	1,090
K1	6	1	672
K	2	4	347
K1A	6	6	1,237
K2	4	2	820
K3	2	1	348
J	9	7	1,986
J1	5	4	835
J2	1	0	184
J3	5	6	971
J4	4	3	384
b (SE)	0.795 (0.0291)	0.800 (0.0265)	

the computer program SOCPROG, written for the analysis of animal social structure data (documentation and software available from <http://myweb.dal.ca/~hwhitehe/social.htm>). In such cases, the permutation procedure used to test for nonrandom associations was run using both the simple ratio and the mean half-weight index of association (as described by Cairns and Schwager 1987) to help control for biases inherent in the calculation of each index.

Table 2. Observed and expected number of within- and between-haplotype associations for groups of two humpback whales in the Gulf of Maine ($n = 3,151$)^a

Within haplotypes	Observed association frequency	Observed proportion of associations	Expected association frequency	Expected proportion of associations
I	609	0.193	529	0.168
C1	10	0.003	3	0.001
C	92	0.029	28	0.009
D1	1	0.000	3	0.001
D	77	0.024	54	0.017
I2	0	0.000	0	0.000
I1	0	0.000	3	0.001
K1	4	0.002	3	0.001
K	0	0.000	3	0.001
K1A	3	0.001	6	0.002
K2	5	0.002	3	0.001
K3	0	0.000	0	0.000
J	16	0.005	9	0.003
J1	1	0.000	9	0.001
J2	N/A	N/A	1	N/A
J3	2	0.001	3	0.001
J4	0	0.000	3	0.001
Between	2,331	0.740	2,491	0.791

^a One haplotype (J2) was not included in this analysis, as only one sampled individual was found to have that haplotype, making within-haplotype pairing impossible.

Results

Variation in the mtDNA sequences of the 159 sampled individuals revealed 17 unique haplotypes, organized following the nomenclature of Baker et al. (1994a) as modified by Rosenbaum et al. (2002; Table 1). The haplotypes of an additional 143 individuals were inferred from sighting records indicating direct maternal relationships (e.g., calves of cows with known haplotypes; Table 1). The 302 whales included 137 females, 106 males, and 59 whales of unknown sex. Haplotype diversity for the total of 302 individuals was 0.795 (95% CI = 0.757–0.835) and the number of individuals within each haplotype ranged from 1 (haplotype J2) to 123 (haplotype I; Table 1). We found no evidence of a downward bias in the estimation of haplotype diversity from the inclusion of individuals with haplotypes “assigned” through direct maternal descendents (Table 1).

From the sighting database of associations collected from 1980 to 1995, we extracted all records of groups that included one or more of the 302 individuals with known or inferred mtDNA haplotype. This “population database” consisted of 21,617 groups, of which 8,254 (38.2%) were solitary whales, 7,901 (36.6%) were groups of two, 3,056 (14.1%) were trios, and 2,406 (11.1%) were groups of more than three whales. The largest group observed was 12 whales. The mean number of sightings per individual of known haplotypes was 100 (SD = 63.62), and for all individuals of a given haplotype combined the number of sightings ranged from 101 ($n = 2$ individuals of haplotype I2) to 11,636 ($n = 123$ individuals of haplotype I; Table 1).

We first considered the null hypothesis of random association using only groups of two in which the haplotype of both members was known ($n = 3,151$; Table 2). Following the logic of the gene diversity index, we expected that the

Table 3. The proportion of observed within-haplotype associations of individual humpback whales in the Gulf of Maine compared to the expected proportions (with 95% CI) derived from Monte Carlo simulations

Group type	Observed (%)	Expected (%)	Upper 95% CI (%)	P value
Overall	24.27	16.06	20.32	0.002
Male/male	21.37	16.10	23.56	0.127
Female/female	27.05	16.10	24.14	0.011
Male/female	22.95	16.14	23.32	0.062

frequency of association among pairs of individuals with shared haplotypes should approximately equal the square of the frequency of that haplotype in the population if there was no preference for within-haplotype associations. Contrary to this expectation, however, the frequencies of within-haplotype associations were higher than expected for most commonly found haplotypes (Table 2). Overall, 820 (26.0%) of the observed pairs consisted of individuals with the same haplotype, significantly greater than the expected frequency of 20.4% ($\chi^2_{df=1} = 49.1, P < .001$) based on the sum of the squared haplotype frequencies (i.e., 1 minus gene diversity, referred to as the gene identity index, Nei 1987).

Because the comparison of gene identity and associations only applied to groups of two with known haplotypes, we then used a Monte Carlo simulation to generate random permutations of the larger data sets. This approach accounted for differing group sizes, individuals with unknown haplotypes, and unequal sighting frequencies of individuals. In this analysis, individuals were selected at random and assigned to groups according to the frequency in which the group sizes were found in the population database. This simulation was repeated 1,000 times to generate an expectation under the null hypothesis of random associations. In the overall population database, 5,326 (24.3%) of the groups involved pairings of whales with the same mtDNA haplotype. By comparison, the mean expected value based on Monte Carlo sim-

ulations was 16.06%, with an upper 95% CI of 20.32%. The observed number of associations within haplotypes was greater than all but two of the 1,000 runs for the permuted data, indicating significantly more within-haplotype associations than expected by chance ($P = .002$; Table 3).

To evaluate the influence of sex on matrilineal associations, we reran the Monte Carlo simulations using three subsets of the overall data: (1) groups where more than one female of known haplotype were associated and no males were present ($n = 1,512$); (2) groups where more than one male of known haplotype were associated and no females were present ($n = 730$); and (3) groups of both sexes where the haplotype of more than one individual was known ($n = 2,745$). For these groups, both inter- and intrasexual associations among individuals of the same haplotype exceeded those expected by chance. However, the difference was significant only for female groups ($P = .011$), although male/female groups approached significance ($P = 0.062$; Table 3).

Finally, we considered the relationship between haplotype identity and pairwise association indices of individual whales of known haplotypes in all groups (Table 4). The Mantel tests (10,000 permutations to determine significance) showed a significant effect for all pairwise associations except males. The strongest effect was observed for females, where within-haplotype associations were more than 1.7 times greater than between-haplotype associations. Results were similar for both the simple and half-weighted association indices.

We sought to exclude the possibility that the observed excess of within-haplotype associations was due primarily to temporal co-occurrence of individuals with shared haplotypes or highly localized site fidelity within the study area. To investigate temporal effects, we extended the Mantel test of haplotype identity and pairwise association indices using the partial matrix correlation technique described by Smouse et al. (1986) to control for the co-occurrence of individual whales in a moving window of 5-day intervals. For this, individuals found to co-occur in a 5-day window were considered “temporally associated” and this variable was included in the analysis along with the group association (Table 4). The

Table 4. The mean and standard deviation (SD) of pairwise association indices (simple and half-weighted) for individually identified humpback whales in the Gulf of Maine showing differences in within- and between-haplotype associations overall and by intra- and intersex groupings. The significance of the correlation between association indices and haplotype identity, with and without inclusion of the partial correlation for temporal co-occurrence (by 5-day periods), was calculated with a Mantel test using 10,000 permutations of the data matrices

Pairwise association	Overall		Within haplotypes		Between haplotypes		Ratio within/ between	P value	P value controlling for 5-day periods
	Mean	SD	Mean	SD	Mean	SD			
Simple									
All-all	0.0015	0.0080	0.0020	0.1020	0.0014	0.0074	1.43	.013	.000
F-F	0.0017	0.0087	0.0026	0.0135	0.0015	0.0073	1.73	.002	.001
M-M	0.0012	0.0057	0.0011	0.0041	0.0013	0.0061	0.85	.700	.342
F-M	0.0016	0.0083	0.0020	0.0099	0.0015	0.0078	1.33	.043	.001
Half-weight									
All-all	0.0028	0.0133	0.0035	0.0175	0.0026	0.0120	1.35	.009	.000
F-F	0.0031	0.0147	0.0047	0.0231	0.0027	0.0122	1.74	.001	.000
M-M	0.0022	0.0097	0.0020	0.0075	0.0023	0.0103	0.87	.678	.273
F-M	0.0028	0.0136	0.0036	0.0169	0.0026	0.0125	1.38	.028	.000

partial correlation of these temporal associations did not affect the significance of the relationship between haplotype identity and pairwise associations (Table 4), indicating that short-term patterns of co-occurrence in the study area could not explain the lineage effect. Furthermore, there was no significant relationship ($P > .4$ in all cases) between temporal association indices and haplotype identity overall or for any combination of sexes.

To investigate fine-scale spatial effects, we used a permutation procedure to test for local clumping of individuals with shared haplotypes. For this, the study area was divided into sets of squares at four resolutions: 2, 5, 10, and 20 km². The number of individual whales with an identified haplotype sighted in each square was then counted at each level of resolution. Squares with less than a certain minimum number of individuals were discarded (minimum cutoffs of 5, 10, and 20 individuals per square were tried). A contingency table of squares × haplotypes was calculated for each level of resolution and minimum cutoff, with each cell of the table giving the number of individual whales with a specific haplotype found at least once in that square. The observed χ^2 value of each contingency table was then tested against a null distribution of values generated from 1,000 randomized tables produced by permuting the haplotypes among individuals so that the number of individuals with each haplotype was kept constant (Table 5). We considered that a significant relationship between haplotype identity and fine-scale spatial distribution would be indicated by an observed χ^2 value that was greater than 95% of the randomized values. The results of the permutation procedure do not support such an effect; the observed χ^2 values were not significantly greater than expected at random (at $P < .10$) for any of the 12 contingency tables (four resolutions by three cutoff values of the number of individuals per square; Table 5).

Discussion

Our results strongly suggest that humpback whales with the same mtDNA haplotype, especially females, are more likely to associate on the feeding grounds than expected by chance alone. Further, given the observations that reproductive females do not tend to associate closely with their own mature offspring (Clapham 2000; Sardi et al., 2005), the influence of maternal lineages on social or foraging associations seems to extend beyond first-order kin. Although it is possible for kin recognition to operate in the absence of close kinship bonds, it seems more likely that the observed lineage effect is the result of one or more indirect mechanisms. Having attempted to control for simple spatial and temporal effects in our analyses, the most likely of these indirect mechanisms may be the influence of maternal experience on feeding styles and prey preferences. If feeding styles and prey preference are learned in part through maternal experience, these would be expected to correlate with the mtDNA haplotypes of associated individuals. Although specific feeding “styles” continue to develop well past the period of maternal dependency and much of the learning appears to be cultural in the 2–3 years after weaning (Weinrich et al. 1992), prey

Table 5. The χ^2 values for the contingency tables of observed and randomized distributions of humpback whales with known mtDNA haplotypes in the Gulf of Maine (with P values calculated as proportion of randomized $>$ observed from 1,000 permutations). The study area was divided into squares at different resolutions (2, 5, 10, 20 km²) and with varying minimum numbers of whales in each square (min 5, 10, 20 per square)

	Resolution = 2 km ²			Resolution = 5 km ²			Resolution = 10 km ²			Resolution = 20 km ²		
	Min 5 per square	Min 10 per square	Min 20 per square	Min 5 per square	Min 10 per square	Min 20 per square	Min 5 per square	Min 10 per square	Min 20 per square	Min 5 per square	Min 10 per square	Min 20 per square
No. squares	313	188	105	342	215	134	340	213	127	308	195	122
χ^2 observed	4,560	2,429	1,101	4,887	2,749	1,504	4,898	2,763	1,470	4,093	2,400	1,336
χ^2 randomized (mean)	4,748	2,494	1,147	4,916	2,830	1,577	4,597	2,646	1,414	3,892	2,305	1,337
P value	.670	.592	.675	.537	.681	.737	.132	.194	.265	.186	.226	.497

preference could have a more persistent influence on foraging associations.

The influence of maternal lineages on prey preference was also suggested previously by Baker et al. (1994a,b) to explain the nonrandom frequencies of mtDNA haplotypes among humpback whales killed by dinoflagellate poisoning along Cape Cod, Massachusetts, in 1987. The poisoned whales were observed feeding on an unusual prey (mackerel, *Scomber scombrus*), thought to be contaminated with the toxin, very late in the feeding season (December) in a limited geographic area along the coast of Cape Cod (Geraci et al. 1989). Nine of these same whales were also sighted feeding on Jeffreys Ledge, more than 50 miles north of Cape Cod, during the preceding 2 months (Weinrich MT, unpublished data). These observations suggest that the poisoned whales either moved together as a population unit, perhaps as part of a larger migratory pattern, or congregated in response to the movement of a preferred prey species. Thus, it is possible that the interaction of temporal shifts in the distribution of both whales and their prey favors formation of associations between whales with common mtDNA haplotypes. Such an interaction would be difficult to detect in a simple analysis of temporal or spatial effects, such as those conducted here, but could be explored by more detailed analysis of the prey targeted by individuals of different matrilineages.

An indirect influence of prey preference would explain the fluidity of association and the relatively weak influence of maternal lineages on associations of humpback whales compared to cetaceans that form stable matrilineal associations (e.g., killer whales). Because both the geographic location of the prey and the size and dynamics of the fish school are changeable, associations formed among whales for cooperative foraging should also vary in response (Baker and Herman 1984a,b; Clapham et al. 1993, 1996), possibly with rapid changes in group size related to disruption of prey patches (Weinrich and Kuhlberg 1991; Whitehead 1983). The influence of maternal lineages on group associations formed for cooperative foraging is probably also limited by other conflicting social strategies of each sex. Both Clapham et al. (1993) and Weinrich (1993) suggest that males may use feeding ground associations to create bonds with females that might extend to the wintering/breeding grounds. Such a strategy may be facilitated by staggering the presence of whales from different feeding populations on their common breeding ground (Stevick et al. 1999). However, on the feeding grounds the reproductive benefits of forming nonrelated mixed-sex pairs (i.e., inbreeding avoidance) might be balanced against the reproductive benefits of forming cooperatively foraging and feeding pairs of maternal relatives, regardless of their sexes (i.e., kin selection). There is currently no way of distinguishing between these types of pairs in the field, making this hypothesis untestable with the data at hand.

We acknowledge that the inclusion of individuals with haplotypes assigned through direct maternal descent could, in theory, bias downward the estimation of haplotype frequencies (an "inbreeding effect") and thus bias upward the observed frequency of within-haplotype association. However, we consider this unlikely for the following reasons:

(1) the inclusion of the assigned haplotypes did not affect the estimated haplotype diversity, discounting the potential inbreeding effect (Table 1); (2) the observations used to assign haplotypes (i.e., observations of the dependent calf with the mother in the calf's first year of life) were excluded from the analysis and the potential effect of nonindependence in later associations is essentially randomized by the repeated sightings and fluid associations among the individuals; and (3) such an effect, by itself, would not be sex specific, as haplotypes were assigned to both male and female offspring of sampled mothers.

It remains unclear, however, if the influence of maternal lineages shown here through association of mtDNA can be considered evidence for kin selection. This will require better estimates of kinship using more variable markers, such as microsatellites, and some plausible mechanism for kin recognition, beyond the first-order relationships of reproductive females and their offspring. To date, however, microsatellite analyses have failed to detect any patterns of close kinship within groups of humpback whales on the migratory corridor of eastern Australia (Valsecchi et al. 2002) or feeding grounds of southeastern Alaska (Sharpe 2001). An increased knowledge of the proximate function of associations (e.g., cooperative feeding, pre-mating exploration), along with estimates of kinship and identification of matrilineages together, are required to understand the social structure humpback whales and other baleen whales on the feeding grounds.

Acknowledgments

We thank the staff and interns of the Whale Center of New England (formerly the Cetacean Research Unit) who have helped gather much of the field data and biopsy samples, especially Cindy Belt, Mark Cappellino, Rachel Griffiths, Malcolm Martin, David Morin, Mark Schilling, and many others over the years. We also thank the staff and crew of Captain Bill and Sons Whale Watch, Boston Harbor Cruises, and the Daunt Fleet, from whose whale-watch vessels many of the observations were made. Neil Willits (University of California at Davis Statistical Consulting Laboratory) generously gave statistical advice. A portion of the fieldwork was conducted under U.S. Marine Mammal Research Permit Nos. 681 and 959, issued to the Cetacean Research Unit. Biopsy sampling was conducted under U.S. Marine Mammal Research Permit No. 675, issued to C.S.B. The experiments reported in this study comply with the current laws of the country in which they were performed. We thank Brian Bowen and anonymous reviewers for critical comments on previous drafts of the manuscript.

References

- Baird RW and Dill LM, 1996. Ecological and social determinants of group size in transient killer whales (*Orcinus orca*). *Behav Ecol* 7:408–416.
- Baker CS, 1985. The population structure and social organization of humpback whales (*Megaptera novaeangliae*) in the central and eastern North Pacific (PhD dissertation). Honolulu, HI: University of Hawaii.
- Baker CS and Herman LM, 1984a. Aggressive behavior between humpback whales wintering in Hawaiian waters. *Can J Zool* 62:1922–1937.
- Baker CS and Herman LM, 1984b. Seasonal contrasts in the social behavior of humpback whales. *Cetus* 5:14–16.
- Baker CS, Lambertsen RH, Weinrich MT, Calambokidis J, Early G, and O'Brien SJ, 1991. Molecular genetic identification of the sex of humpback

- whales (*Megaptera novaeangliae*). Rep Int Whaling Comm Spec Issue 13: 105–111.
- Baker CS and Medrano-Gonzalez L, 2001. World-wide distribution and diversity of humpback whale mitochondrial DNA lineages. In: Cell and molecular biology of marine mammals (Pfeiffer C, ed). Melbourne, FL: Kreiger Publishing; 84–99.
- Baker CS, Medrano-Gonzalez L, Calambokidis J, Perry A, Pichler FB, Rosenbaum H, Straley JM, Urban-Ramirez J, Yamaguchi M, and von Ziegeler O, 1998. Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. Mol Ecol 7:695–708.
- Baker CS, Palumbi SR, Lambertsen RH, Weinrich MT, Calambokidis J, and O'Brien SJ, 1990. Influence of seasonal migration on geographic partitioning of mitochondrial DNA haplotypes in humpback whales. Nature 344: 238–240.
- Baker CS, Perry A, Bannister JL, Weinrich MT, Abernathy RB, Calambokidis J, Lien J, Lambertsen RH, Urban J, Vasquez O, Clapham PJ, Alling A, O'Brien SJ, and Palumbi SR, 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. Proc Natl Acad Sci USA 90:8239–8243.
- Baker CS, Slade RW, Bannister JL, Abernathy RB, Weinrich MT, Lien J, Urban J, Corkeron P, Calambokidis J, Vasquez O, and Palumbi SR, 1994a. Hierarchical structure of mitochondrial DNA gene flow among humpback whales, *Megaptera novaeangliae*, world-wide. Mol Ecol 3:313–327.
- Baker CS, Weinrich MT, Early G, and Palumbi S, 1994b. Genetic impact of an unusual group mortality among humpback whales. J Hered 85:52–54.
- Baraff L and Weinrich MT, 1993. Separation of a humpback whale mother and calf on a feeding ground in early autumn. Mar Mamm Sci 9:431–434.
- Bertram BC, 1983. Kin selection and altruism. In: Advances in the study of mammalian behavior (Eisenberg JF and Kleiman DG, eds). Lawrence, Kansas: American Society of Mammologists Special Publication No. 7; 721–735.
- Cairns SJ and Schwager SJ, 1987. A comparison of association indices. An Behav 35:1454–1469.
- Caro TM, 1994. Cheetahs of the Serengeti Plains: group living in an asocial species. Chicago: University of Chicago Press.
- Chittleborough RG, 1965. Dynamics of two populations of the humpback whale, *Megaptera novaeangliae* (Borowski). Aust J Mar Freshw Res 16:33–128.
- Clapham PJ, 1993. Social organization of humpback whales on a North Atlantic feeding ground. Symp Zool Soc Lond 66:131–145.
- Clapham PJ, 1996. The social and reproductive biology of humpback whales: an ecological perspective. Mammal Rev 26:27–49.
- Clapham PJ, 2000. The humpback whale: seasonal feeding and breeding in a baleen whale. In: Cetacean societies: field studies of dolphins and whales (Mann J, Connor RC, Tyack PL, and Whitehead H, eds). Chicago: University of Chicago Press; 173–196.
- Clapham PJ, Baraff LS, Carlson CA, Christian M, Mattila DK, Mayo C, Murphy M, and Pittman S, 1993. Seasonal occurrence and annual return of humpback whales, *Megaptera novaeangliae*, in the southern Gulf of Maine. Can J Zool 71:440–443.
- Clapham PJ and Mayo CA, 1987. Reproduction and recruitment of individually identified humpback whales, *Megaptera novaeangliae*, observed in Massachusetts Bay, 1979–1985. Can J Zool 65:2853–2863.
- Clapham PJ and Mayo CA, 1990. Reproduction of humpback whales (*Megaptera novaeangliae*) observed in the Gulf of Maine. Rep Int Whaling Comm Spec Issue 12:171–175.
- Clapham PJ, Palsbøll PJ, Mattila DK, and Vasquez O, 1992. Composition and dynamics of humpback whale competitive groups in the West Indies. Behaviour 122:182–194.
- Connor RC, 2000. Group living in whales and dolphins. In: Cetacean societies: field studies of dolphins and whales (Mann J, Connor RC, Tyack PL, and Whitehead H, eds). Chicago: University of Chicago Press; 199–218.
- Dawbin WH, 1966. The seasonal migratory cycle of humpback whales. In: Whales, dolphins, and porpoises (Norris KS, ed). Berkeley, CA: University of California Press; 145–170.
- D'Vincent CG, Nilson RM, and Hanna RE, 1985. Vocalization and coordinated feeding behavior of the humpback whale in southeast Alaska. Sci Rep Whales Res Inst Tokyo 36:41–47.
- Fernando P and Lande R, 2000. Molecular genetic and behavioral analysis of social organization in the Asian elephant (*Elephas maximus*). Behav Ecol Sociobiol 48:84–91.
- Geraci JR, Anderson DM, Timperi RJ, Early GA, Prescott JH, and Mayo CA, 1989. Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. J Fish Res Bd Can 46:1895–1898.
- Glockner DA, 1983. Determining the sex of humpback whales in their natural environment. In: Communication and behavior of whales (Payne R, ed). AAAS Selected Symposium 76. Boulder, CO: Westview Press; 447–464.
- Jefferson TA, Stacey PJ, and Baird RW, 1991. A review of killer whale interactions with other marine mammals: predation to co-existence. Mammal Rev 21:151–180.
- Katona SK and Beard JA, 1990. Population size, migrations, and feeding aggregations of the humpback whale (*Megaptera novaeangliae*) in the western North Atlantic Ocean. Rep Int Whaling Comm Spec Issue 12:295–306.
- Katona SK and Whitehead HP, 1981. Identifying humpback whales using their natural markings. Polar Rec 20:439–444.
- Kenney RD, Hyman MAM, and Winn HE, 1985. Calculation of standing stocks and energetic requirements of the cetaceans of the northeast United States Outer Continental Shelf. NOAA Tech Memorandum NMFS-F/NEC-41. Washington, DC: U.S. Department of Commerce.
- Lambertsen RH, 1987. A biopsy system for large whales and its use for cytogenetics. J Mammal 68:443–445.
- Larsen AH, Sigurjonsson J, Oien N, Vikingsson G, and Palsbøll P, 1996. Population genetic analysis of nuclear and mitochondrial loci in skin biopsies collected from central and northeastern North Atlantic humpback whales (*Megaptera novaeangliae*): population identity and migratory destinations. Proc R Soc Lond Ser B 263:1611–1618.
- Mackintosh NA, 1965. The stocks of whales. London: Fishing News (Books), Ltd.
- Maddison WP and Maddison DR, 1992. MacClade: analysis of phylogeny and character evolution, version 3.0. Sunderland, MA: Sinauer Associates.
- Mattila DK, Clapham PJ, Katona SK, and Stone GS, 1989. Humpback whales on Silver Bank, 1984: population composition and habitat use. Can J Zool 67:281–285.
- Nei M, 1987. Molecular evolutionary genetics. New York: Columbia University Press.
- Packer C, Scheel D, and Pusey AE, 1990. Why lions form groups: food is not enough. Am Nat 136:1–19.
- Palsbøll PJ, Allen J, Berube M, Clapham PJ, Feddersen TP, Hammond PS, Hudson RR, Jorgensen H, Katona S, Larsen AH, Larsen F, Lien J, Mattila DK, Sigurjonsson J, Sears R, Smith T, Poner R, Stevick P, and Oien N, 1997. Genetic tagging of humpback whales. Nature 388:767–769.
- Palsbøll PJ, Clapham PJ, Mattila DK, Larsen F, Sears R, Siegmund HR, Sigurjonsson J, Vasquez O, and Arctander P, 1995. Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behavior on population structure. Mar Ecol Prog Ser 116:1–10.
- Palsbøll PJ, Vader A, Bakke I, El-Gewely MR, 1992. Gender determination in cetaceans by the polymerase chain reaction. Can J Zool 70:2166–2170.
- Palumbi SR and Baker CS, 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Mol Biol Evol 11:426–435.
- Perry A, Baker CS, and Herman LM, 1990. Population characteristics of individually identified humpback whales in the central and eastern North

- Pacific: a summary and critique. Rep Int Whaling Comm Spec Issue 12: 307–317.
- Rosenbaum HC, Weinrich MT, Soleson SA, Gibbs JP, Baker CS, and DeSalle R, 2002. The effect of differential reproductive success on population genetic structure: correlations of life history with matriline in humpback whales of the Gulf of Maine. *J Hered* 93:389–399.
- Sambrook J, Fritsch EF, and Maniatis T 1989. *Molecular cloning: a laboratory manual*, 2nd ed. New York: Cold Spring Harbor Laboratory Press.
- Sardi KS, Weinrich MT, and Connor RC, 2005. Social interactions of humpback whale (*Megaptera novaeangliae*) mother/calf pairs on a North Atlantic feeding ground. *Behaviour* 142:731–750.
- Sharpe F, 2001. *Social foraging of the Southeast Alaskan humpback whale* (PhD dissertation). Vancouver, BC: Simon Fraser University.
- Slijper EJ, 1962. *Whales*. New York: Basic Books.
- Smouse PE, Long JC, and Sokal RR, 1986. Multiple regression and correlation extensions of the Mantel test of matrix independence. *Syst Zool* 35:627–632.
- Stander PE, 1992. Foraging dynamics of lions in a semi-arid environment. *Can J Zool* 70:8–21.
- Stevick PT, Oien N, and Mattila DK, 1998. Migration of a humpback whale between Norway and the West Indies. *Mar Mamm Sci* 14:162–166.
- Stevick PT, Oien N, and Mattila DK, 1999. Migratory destinations of humpback whales from Norwegian and adjacent waters: evidence for stock identity. *J Cetacean Res Manage* 1:147–152.
- Tyack P and Whitehead H, 1983. Male competition in large groups of wintering humpback whales. *Behaviour* 83:132–154.
- Valsecchi E, Hale P, Corkeron P, and Amos W, 2002. Social structure in migrating humpback whales (*Megaptera novaeangliae*). *Mol Ecol* 11: 507–518.
- Vigilant L, Stoneking M, Hawkes K, and Wilson AC, 1991. African populations and the evolution of human mitochondrial DNA. *Science* 253: 1503–1507.
- Weinrich MT, 1991. Long term stability in grouping patterns of humpback whales (*Megaptera novaeangliae*) in the southern Gulf of Maine. *Can J Zool* 69:3012–3019.
- Weinrich MT, 1995. Humpback whale competitive groups observed on a high-latitude feeding ground. *Mar Mamm Sci* 11:251–254.
- Weinrich M, 1998. Early experience in habitat choice by humpback whales (*Megaptera novaeangliae*). *J Mammal* 79:163–170.
- Weinrich MT and Kuhlberg AK, 1991. Short-term association patterns of humpback whale (*Megaptera novaeangliae*) groups on their southern Gulf of Maine feeding ground. *Can J Zool* 69:3005–3011.
- Weinrich MT, Lambertsen RH, Baker CS, Schilling MR, and Belt CR, 1991. Behavioural reactions of humpback whales (*Megaptera novaeangliae*) in the southern Gulf of Maine to biopsy sampling. Rep Int Whaling Comm Spec Issue 13:91–98.
- Weinrich M, Martin M, Griffiths R, Bove J, and Schilling M, 1997. A shift in distribution of humpback whales, *Megaptera novaeangliae*, in response to prey in the southern Gulf of Maine. *Fish Bull* 95:826–836.
- Weinrich MT, Schilling MR, and Belt CR, 1992. Evidence for acquisition of a novel feeding behavior: lobtail feeding in humpback whales, *Megaptera novaeangliae*. *Anim Behav* 44:1059–1072.
- Whitehead HP, 1983. Structure and stability of humpback whale groups off Newfoundland. *Can J Zool* 61:1391–1397.
- Whitehead H and Carlson C, 1988. Social behavior of feeding finback whales off Newfoundland: comparisons with the sympatric humpback whale. *Can J Zool* 66:217–221.
- Wursig B, 1987. Delphinid foraging strategies. In: *Dolphin cognition and behavior: a comparative approach* (Schusterman RJ, Thomas JA, and Wood FG, eds). Hillsdale, NJ: Lawrence Erlbaum Associates.
- Wursig B, Dorsey EM, Fraker MA, Payne RS, and Richardson WJ, 1985. Behavior of bowhead whales *Balaena mysticetus* summering in the Beaufort Sea: a description. *Fish Bull* 83(3):357–377.
- Zar JH, 1998. *Biostatistical analysis*, 4th ed. Englewood Cliffs, NJ: Prentice-Hall Inc.

Received October 4, 2004

Accepted November 30, 2005

Corresponding Editor: Brian Bowen