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Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*)

LUCIANO O. VALENZUELA,*† MARIANO SIRONI,†‡ VICTORIA J. ROWNTREE*§ and JON SEGER*
Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA, †Instituto de Conservación de Ballenas, Miñones 1986, CP 1428, Ciudad de Buenos Aires, Argentina, ‡Cátedra de Diversidad Animal II, Universidad Nacional de Córdoba, Av. Vélez Sársfield 299, 5000 Córdoba, Argentina, §Ocean Alliance/Whale Conservation Institute, 191 Weston Rd, Lincoln, MA 01773, USA

Abstract

Ocean warming will undoubtedly affect the migratory patterns of many marine species, but specific changes can be predicted only where behavioural mechanisms guiding migration are understood. Southern right whales show maternally inherited site fidelity to near-shore winter nursery grounds, but exactly where they feed in summer (collectively and individually) remains mysterious. They consume huge quantities of copepods and krill, and their reproductive rates respond to fluctuations in krill abundance linked to El Niño Southern Oscillation (ENSO). Here we show that genetic and isotopic signatures, analysed together, indicate maternally directed site fidelity to diverse summer feeding grounds for female right whales calving at Península Valdés, Argentina. Isotopic values from 131 skin samples span a broad range (–23.1 to –17.2‰ δ^{13}C, 6.0 to 13.8‰ δ^{15}N) and are more similar than expected among individuals sharing the same mitochondrial haplotype. This pattern indicates that calves learn summer feeding locations from their mothers, and that the timescale of culturally inherited site fidelity to feeding grounds is at least several generations. Such conservatism would be expected to limit the exploration of new feeding opportunities, and may explain why this population shows increased rates of reproductive failure in years following elevated sea-surface temperature anomalies off South Georgia, the richest known feeding ground for baleen whales in the South Atlantic.

Keywords: baleen whales, genetic structure, mitochondrial DNA, philopatry, stable isotopes

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Introduction

Might an animal population fail to use all of its available food resources for many generations because cultural traditions direct its foraging to a subset of the suitable locations? Southern right whales (*Eubalaena australis*) had six known feeding grounds in the South Atlantic, based on the locations of catches recorded by 19th and 20th century whalers (IWC 2001). Today, the only known feeding ground used in the western South Atlantic is South Georgia (Moore *et al*. 1999; IWC 2001), despite the species' sustained recovery from near extinction in the early 20th century to a population that probably exceeds 19 000 in 2008 (by extrapolation from population size and growth rate estimates for all Southern Ocean breeding grounds in 1990; IWC 2001). Right whales make long annual migrations between mid-latitude coastal winter nursery grounds and mostly high-latitude offshore summer feeding grounds (IWC 2001). If calves learn these routes from their mothers and then follow them faithfully for life, matrilines will continue to use the same feeding grounds for many generations, despite the availability of better foraging opportunities elsewhere. Here we combine genetic and stable-isotopic analyses of the population calving at Península Valdés, Argentina, to show that such cultural conservatism may help to explain why southern right whales, although recovering numerically, have appeared slow to return to many parts of their historic range throughout the southern hemisphere.

Correspondence: Luciano O. Valenzuela, Fax: (801) 581-4668; E-mail: valenzuela@biology.utah.edu

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In baleen whales, site fidelity is thought to be maternally transmitted with calves learning the location of nursery and feeding grounds during their first annual migration (Hoelzel 1998). Over many generations, maternally directed site fidelity can result in genetic differentiation among seasonal subpopulations (Hoelzel 1998). In the most intensely studied species, humpback whales (*Megaptera novaeangliae*, Palsbøll *et al*. 1997; Baker *et al*. 1998a, b) and North Atlantic right whales (*Eubalaena glacialis*, Schaeff *et al*. 1993; Malik *et al*. 1999), genetic differentiation of mitochondrial DNA (mtDNA) markers has been found among feeding grounds and among nursery grounds (the latter only in humpback whales in the North Pacific and Southern Hemisphere), consistent with female directed fidelity. Southern right whales show site fidelity to nursery grounds off the coasts of South America, South Africa, Australia and New Zealand (IWC 2001). Patenaude *et al*. (2007) detected mtDNA differentiation among these four nursery grounds and between feeding grounds off South Georgia and off south-western Australia. However, within ocean basins, mtDNA haplotypes from the feeding grounds were shared with two different nursery grounds (for example, haplotypes from South Georgia were shared with Península Valdés and South Africa). Thus, Patenaude *et al*. (2007) confirmed southern right whale site fidelity to nursery grounds and suggested that whales from different breeding populations within an ocean basin mix on common feeding grounds. The difficulty of obtaining samples representing the whales' entire feeding range has prevented a thorough intra-oceanic feeding ground comparison. Despite genetic evidence linking southern right whale nursery grounds to shared feeding grounds, the population genetic structure (if any) on the feeding grounds remains unknown.

Leaper *et al*. (2006) recently showed that the reproductive success of southern right whales breeding at Península Valdés, Argentina is affected by sea surface temperature (SST) anomalies off South Georgia. High-SST anomalies at South Georgia have been correlated with periods of low krill abundance (Trathan *et al*. 2003). Although southern right whale populations are recovering well from their former exploitation (IWC 2001), reproductive failures resulting from food stress are cause for concern (Leaper *et al*. 2006). The correlation between breeding failures, SST anomalies and low krill abundance also suggests that a large proportion of whales that use the Península Valdés nursery ground may feed near South Georgia, which is only one of six major historic feeding grounds for right whales in the South Atlantic (IWC 2001). Furthermore, whaling records show that southern right whales killed south of 50°S had stomachs filled with krill, north of 40°S had stomachs filled with copepods, and between these latitudes had stomachs filled with a mix of krill and copepods (Tormosov *et al*. 1998). The exact number and location of current feeding grounds, and the proportion of whales associated with each has not been documented (IWC 2001).

Understanding a species' migratory connections and genetic structure is critical to understanding the impact that fluctuations in food availability may have on it. If a species shows site fidelity to feeding areas, then the effects of changes in food abundance in one feeding ground may not spread throughout the entire breeding population, but be focused instead on particular genetic lineages. Intrinsic markers such as stable isotopes and genetic variability have been used with varying degrees of success to study migratory biology (Webster *et al*. 2002; Rubenstein & Hobson 2004). Stable carbon and nitrogen isotope ratios in animal tissues are good indicators of food sources and have been used to study animal movements in a broad range of species including butterflies, birds, fish and mammals (Hobson 1999; Rubenstein & Hobson 2004). Predictable patterns of isotope ratios across landscapes provide the basis for their use as tracers of migration (Hobson 1999; Rubenstein & Hobson 2004; West *et al*. 2006). In marine ecosystems, carbon isotope ratios ($^{13}C/^{12}C$) decline with increasing latitude, and coastal waters have higher ratios than pelagic waters (Rau *et al*. 1982; Hobson 1999; Kelly 2000; Rubenstein & Hobson 2004).

Population-specific genetic markers have been widely used to study animal movements, particularly bird migration (Bensch & Hasselquist 1999; Wennerberg 2001). However, few studies have combined genetic and isotopic markers to study animal migration (Clegg *et al*. 2003; Kelly *et al*. 2005). In one such study, Clegg *et al*. (2003) used a combination of hydrogen isotope ratios and genetic markers to study the migratory patterns and connectivity of Wilson's warbler (*Wilsonia pusilla*). These authors found a north–south structure of hydrogen isotope values and an east–west structure of genetic markers in breeding areas, confirming a previously suspected migratory pattern and population structure across North America (Clegg *et al*. 2003).

Here we describe the population genetic structure of southern right whales on their feeding grounds by using a novel combination of genetic and stable-isotopic analyses of skin samples collected from live whales at Península Valdés, Argentina. Each skin sample provides the maternal lineage of the whale and information on its feeding location several months before sampling. We find that individuals from a given maternal lineage tend to have similar carbon and nitrogen isotopic values. These associations suggest that, throughout life, individuals tend to follow the migratory routes that they learned from their mothers during their first year.

Materials and methods

Fieldwork and sample collection

Skin samples were collected by biopsy darting adult female southern right whales on their nursery ground off Península

Valdés (42°30′S, 64°10′W), Argentina. Sample collection took place over four consecutive years (2003–2006) at the time of peak whale abundance (September and October, Payne 1986). Whales sampled in 2003 and 2006 correspond to the same calving cohort (Payne 1986). Adult females were recognized by the presence of an accompanying calf. To avoid including resampled whales, individuals were photographed for later identification based on callosity patterns (Payne *et al*. 1983). Each skin sample was divided into two subsamples in the field. One subsample was dried in preparation for stable carbon and nitrogen isotope analysis and the other was preserved in saturated sodium chloride with 20% dimethyl sulphoxide for genetic analysis (Amos & Hoelzel 1991). Samples were frozen on return to the laboratory at the University of Utah.

Stable carbon and nitrogen isotope analysis

Dried samples were ground to a fine powder and lipid extracted following Todd *et al*. (1997). Approximately 1 mg of material per sample was analysed in a Carlo Erba 1108 elemental analyser coupled to a Thermo Finnigan Delta S Isotope Ratio Mass Spectrometer at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah. Isotope ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ (‰) $= [(R_{sample}/R_{standard}) - 1] \times 1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, for $\delta^{13}C$ or $\delta^{15}N$, respectively. Standards were referenced to Pee Dee Belemnite for carbon and to atmospheric air for nitrogen. The reproducibility of these measurements was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ after repeated analyses of an internal laboratory standard.

Genetic analysis

DNA was extracted using standard protocols for cetacean skin described in Amos & Hoelzel (1991). Six hundred and thirty base pairs of the mitochondrial control region were amplified by polymerase chain reaction (PCR) using primers AB6617 and H00034 (Malik *et al*. 1999). The purified PCR product was then directly sequenced in both directions either at the DNA Sequencing Core Facility at the University of Utah Health Science Center or at the High-Throughput Genomics Unit, University of Washington. Sequences were assembled using Sequencher 4.5 software (Gene Codes Corp.). Haplotype (h) and nucleotide (π) diversity (Nei 1987) were estimated using Arlequin 2.0 (Schneider *et al*. 2000). The degree of differentiation among years was estimated by analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) as implemented in Arlequin 2.0.

Statistical analysis

The $\delta^{13}C$ and $\delta^{15}N$ distributions were significantly non-normal (Shapiro–Wilk W-test: $N = 131$; $P < 0.001$; Fig. 1).

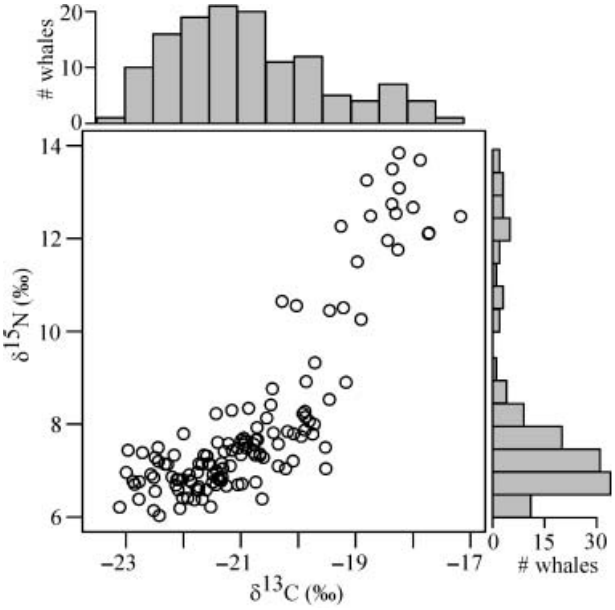

Fig. 1 Scatter plot and histograms of stable carbon and nitrogen isotope values for 131 adult female southern right whales sampled at Península Valdés, Argentina from 2003 to 2006. $\delta^{13}C$ and $\delta^{15}N$ values are not normally distributed (Shapiro–Wilk W test; $P < 0.001$).

Nonparametric statistics (Kruskal–Wallis analysis of variance by ranks and Dunn's multiple comparisons test) were used to test for differences of isotopic values among years and among haplotypes (Dunn 1964; Sokal & Rohlf 1981). α was set at 5% for all tests, which were conducted in R (R Development Core Team 2005) and JMP (SAS Institute Inc. 2005).

Under the null hypothesis of no site fidelity, it is expected that the isotopic variation within matrilines will be as great as the isotopic variation between matrilines. The standard way to test such a hypothesis would be by ANOVA with haplotypes as factors. This straightforward approach is inappropriate here, however, owing to the lack of normality and the small sample sizes within some haplotypes. Instead, we used a randomization test (Manly 1997) to ask whether the isotopic variation within matrilines is as great as the isotopic variation between matrilines. As in a traditional one-factor ANOVA, we estimated F as the ratio of between-group to within-group mean squares, but we compared this observed F-ratio to a sampling distribution constructed by randomization of the data, rather than to tabled values of the standard theoretical F-distribution (Manly 1997). This procedure involved scrambling the isotopic values and randomly assigning them to haplotypes, while maintaining the original distributions of isotopic values and haplotype frequencies. For each such randomized data set, we estimated the F-ratio exactly as for the real, nonrandomized data. We generated test-specific F-distributions by conducting 10 000 randomizations of the data under consideration. The significance

level for any given randomization test was the proportion of randomly generated *F*-ratios that were greater than or equal to the *F*-ratio for the real, nonrandomized data (Manly 1997).

We also conducted a variation of this test that is directly suggested by the working hypothesis. If site fidelity is maternally directed, then we would predict that the isotopic difference between two whales from the same matriline should be smaller, on average, than the distance between two whales from different matrilines. We used the unsigned magnitudes of the isotopic differences between whales as a metric referred to as pairwise difference. We calculated the pairwise difference for each possible combination of individual whales and then separated the values into two groups: a group where both members of the pair had the same haplotype (difference within haplotypes, ΔWH) and a group where members had different haplotypes (difference between haplotypes, ΔBH). Under the null hypothesis, the mean values of ΔWH and ΔBH are equal. Under the alternative hypothesis, the mean difference within haplotypes (ΔWH) should be smaller than that between haplotypes (ΔBH). Because each sample appears in many pairwise comparisons, the assumption of independence for a two-way comparison (e.g. a *t*-test of ΔWH vs. ΔBH) is violated (Sokal & Rohlf 1981). For this reason, we used randomization, as described above, to generate sampling distributions of the test statistic $D = \Delta WH - \Delta BH$ under the null hypothesis. We randomly associated observed isotopic values with observed haplotypes and then calculated values of *D* for the randomized data, repeating the randomization 1000 times for any given data set. The significance level was the proportion of randomly generated values of *D* that were smaller than or equal to the value of *D* for the original, nonrandomized data (Manly 1997).

Results

Stable carbon and nitrogen isotopes

Skin samples collected in September and October from 131 adult female southern right whales from 2003 to 2006 show a wide range of stable carbon and nitrogen isotope values (Fig. 1, Table 1), with $\delta^{13}C$ ranging from –23.1 to –17.2‰ (mean = –20.8‰, SD = 1.3‰) and $\delta^{15}N$ ranging from 6.0 to 13.8‰ (mean = 8.0‰, SD = 1.9‰). $\delta^{13}C$ values differ among years (Kruskal–Wallis $\chi^2 = 13.4$; $P = 0.004$), with values in 2006 (median = –20.2‰) being significantly higher than those in 2004 and 2005 (median = –21.4 and –21.4‰ respectively; Dunn's multiple comparisons tests; $P < 0.05$ for both comparisons). $\delta^{15}N$ values also differ among years (Kruskal–Wallis $\chi^2 = 8.2$; $P = 0.041$), with values in 2006 (median = 7.8‰) being higher than those in 2005 (median = 7.1‰; Dunn's multiple comparisons test; $P < 0.05$).

Table 1 Mean (SD), range and median $\delta^{13}C$ and $\delta^{15}N$ of southern right whale skin samples by year of collection and for all years combined

Year	Statistic	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
2003	Mean ± SD	–20.9 ± 1.0	7.7 ± 1.5
(*N* = 12)	Range	–22.3 to –19.2	6.7 to 12.3
	Median	–21.1[a,b]	7.2[a,b]
2004	Mean ± SD	–21.1 ± 1.3	8.0 ± 1.8
(*N* = 39)	Range	–23.1 to –17.9	6.1 to 13.7
	Median	–21.4[a]	7.3[a,b]
2005	Mean ± SD	–21.1 ± 1.3	7.8 ± 1.9
(*N* = 49)	Range	–23.0 to –18.3	6.0 to 13.5
	Median	–21.4[a]	7.1[a]
2006	Mean ± SD	–20.1 ± 1.3	8.7 ± 2.2
(*N* = 31)	Range	–22.0 to –17.2	6.4 to 13.8
	Median	–20.2[b]	7.8[b]
All years	Mean ± SD	–20.8 ± 1.3	8.0 ± 1.9
(*N* = 131)	Range	–23.1 to –17.2	6.0 to 13.8
	Median	–21.1	7.3

Nonsignificant differences (Dunn's multiple comparisons test; $P < 0.05$) between years are indicated by the same letter next to the medians. *N* is sample size.

Mitochondrial DNA sequence data

Sequence analysis of a 630 bp region of the mitochondrial control region revealed 49 polymorphic sites defining 31 unique sequences or haplotypes (Table 2). The haplotypes are not equally represented in the sample. Six haplotypes account for 53% of the sample while ten occur only once (singletons, Table 2). Levels of haplotype and nucleotide diversity are similar across all four years (overall haplotype diversity $h = 0.94$ and nucleotide diversity $\pi = 1.6\%$, Table 2). A modest but almost significant differentiation is detected by AMOVA among years at the haplotype level (overall $F_{ST} = 0.01$; $P = 0.06$), and a similarly slight differentiation is significant at the nucleotide level (overall $\Phi_{ST} = 0.01$; $P = 0.02$).

Isotopic values of individual haplotypes

Isotopic values are not independent of haplotypes (Figs 2 and 3), and this effect is significant under all three of the tests that we used to examine it statistically. The most conservative is the nonparametric Kruskal–Wallis test, which rejects the null hypothesis both for $\delta^{13}C$ ($X^2 = 41.1$; $P = 0.004$) and for $\delta^{15}N$ ($X^2 = 34.3$; $P = 0.024$). The most liberal is the analogous one-way ANOVA with significance estimated by randomization ($\delta^{13}C$: $F = 3.02$, $P = 0.0004$; $\delta^{15}N$: $F = 3.64$, $P = 0.0002$). The mean pairwise difference test yields intermediate significance levels (Table 3).

The data for $\delta^{13}C$ are shown graphically in Fig. 3a, which organizes them by haplotype and year. Some haplotypes show mostly low $\delta^{13}C$ values (e.g. haplotype X), some show

Table 2 Frequency of mtDNA haplotypes and diversity indices by year of collection and for all years combined. Singletons are haplotypes observed in only one whale

Haplotype	2003	2004	2005	2006	All years
M	3	3	9	2	17
F	3	2	4	3	12
K		5	6	1	12
E	1	4	3	3	11
J	1	1	7		9
B		1	2	5	8
I		6		1	7
A	1	2	1	2	6
Q		2	1	3	6
O	1	1	1	1	4
W	1		1	2	4
P		2	2		4
H		1	2	1	4
D		1	1	1	3
C	1	1			2
N		2			2
L		1		1	2
X			2		2
Y			2		2
Z			1	1	2
BB			1	1	2
Singletons		4	3	3	10
h	0.91	0.95	0.93	0.95	0.94
(SD)	(0.06)	(0.02)	(0.02)	(0.02)	(0.01)
π (%)	1.6	1.7	1.6	1.4	1.6
(SD)	(0.97)	(0.88)	(0.84)	(0.72)	(0.82)
N	12	39	49	31	131

Haplotype (*h*) and nucleotide diversity (π) indices were indistinguishable across years. *N* is sample size.

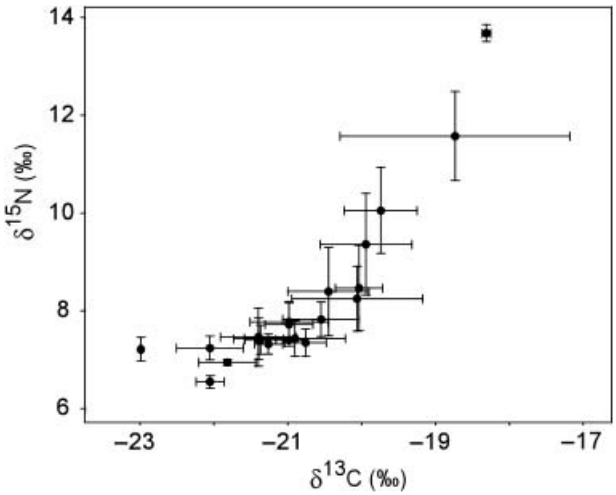

Fig. 2 Scatter plot of mean (± SE) carbon and nitrogen isotopic values for the 21 haplotypes with two or more individuals (nonsingletons). Haplotypes are not randomly distributed over the observed isotopic ranges (see text for statistical analyses).

relatively average values (e.g. haplotypes N and B), and some show mainly high values (e.g. haplotype BB). Not surprisingly, the more abundant haplotypes tend to show broader ranges of $\delta^{13}C$, but over all of the 21 haplotypes with two or more individuals, the correlation between sample size and variance of $\delta^{13}C$ is weak ($r = 0.10$, $P = 0.37$ by randomization). The data for nitrogen show similar patterns (Fig. 3b), but the correlation between sample size and within-haplotype variance appears to be stronger ($r = 0.40$, $P = 0.06$). Substantial fractions of the overall variance are explained by haplotype both for $\delta^{13}C$ (intraclass correlation coefficient $r_I = 0.27$) and for $\delta^{15}N$ ($r_I = 0.32$). The effect does not arise from just a few extreme haplotypes. Most, including the common ones, are less variable than expected. Nor does the effect arise from just one year. The mean pairwise difference in isotope values within haplotypes (ΔWH) is smaller than the mean pairwise difference between haplotypes (ΔBH), both for carbon and for nitrogen, in all years except 2004, and these yearly differences are individually significant in several cases (Table 3).

Table 3 Mean pairwise difference in isotopic values within haplotypes (ΔWH) and between haplotypes (ΔBH) for $\delta^{13}C$ and $\delta^{15}N$, for all samples combined (total) and for each sampling year. Singletons have been removed in each analysis

		$\delta^{13}C$				$\delta^{15}N$			
Year	*N*	ΔWH (‰)	ΔBH (‰)	W < B?	*P*	ΔWH (‰)	ΔBH (‰)	W < B?	*P*
Total	121	1.19	1.47	Y	0.002**	1.45	1.80	Y	0.001**
2003	6	0.88	1.65	Y	0.10	1.85	1.93	Y	0.19
2004	32	1.66	1.59	N	0.81	2.51	1.865	N	0.987
2005	43	0.85	1.43	Y	0.002**	0.82	1.71	Y	0.005**
2006	25	0.91	1.42	Y	0.023*	1.28	1.80	Y	0.16

W < B? indicates whether (Y, N) ΔWH is smaller than ΔBH. *P* is the significance level of the randomization test (**$P < 0.01$, *$P < 0.05$) and indicates the proportion of randomly generated values of the difference (*D*) between ΔWH and ΔBH smaller than or equal to the observed *D*. *N* is sample size.

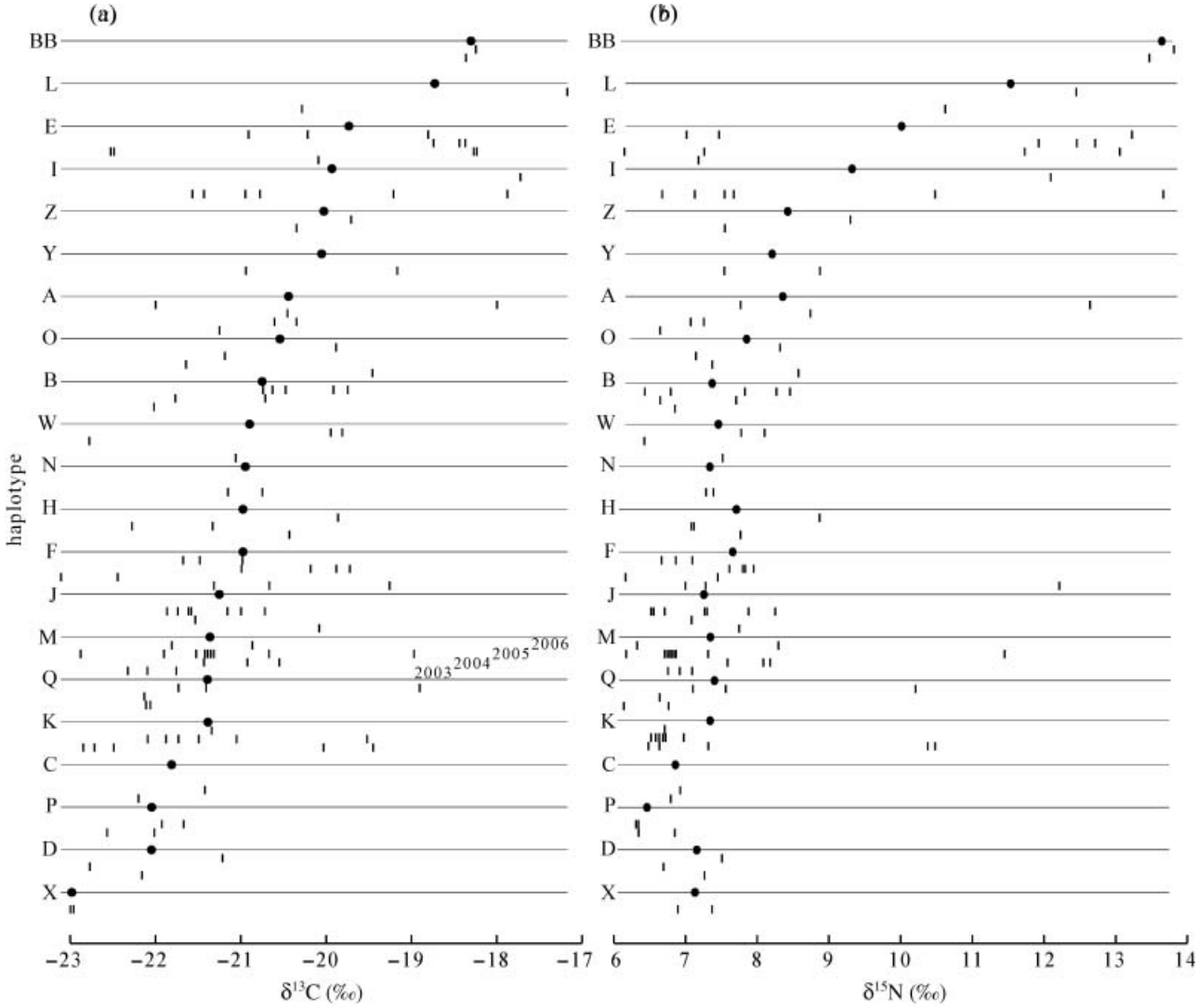

Fig. 3 $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values of individual haplotypes. For each haplotype, the filled circle represents the mean isotopic value and the vertical lines (below haplotype means) represent the raw values. Raw isotopic values are organized vertically by year, with 2003 at the bottom and 2006 at the top (see haplotype M). Haplotypes are ordered from low (bottom of figure) to high (top) mean $\delta^{13}C$ values. Only haplotypes sampled more than once are presented.

Discussion

Southern right whale mitochondrial haplotype diversity is structured with respect to stable isotope values. Three independent statistical tests show that the isotope values are influenced by the haplotypes. The non-normal distributions of the isotope ratios (both carbon and nitrogen) prevented us from using parametric tests with greater statistical power (Sokal & Rohlf 1981). However, the results from our three alternative tests indicate a significant influence of maternal lineages on isotope ratios. This pattern is most simply explained by maternally directed site fidelity to feeding areas. Individual haplotypes show isotopic values that are more similar than expected, indicating that whales from the same matriline tend to consume isotopically similar food. Some haplotypes show larger ranges and more variation within and between years than others. Haplotypes with broad ranges may represent distinct but closely related matrilines that use different feeding grounds; these might be distinguished by longer mtDNA sequences, and work is under way to test this hypothesis. Nitrogen isotope values show larger variation than carbon, possibly because physiological processes and trophic position have a greater effect on $\delta^{15}N$ than on $\delta^{13}C$ (Hobson & Clark 1992b).

It is well established that isotope values in a consumer's tissues reflect those of its food (Hobson 1999; Kelly 2000; Rubenstein & Hobson 2004). It is also well established that $\delta^{13}C$ (and to some extent $\delta^{15}N$) declines with latitude in

marine plankton (Rau *et al*. 1982; Hobson 1999; Kelly 2000; Schmidt *et al*. 2003). The large isotopic ranges seen in our data therefore appear to indicate a large feeding range. The lowest isotopic values seen in skin samples seem to correspond to isotopic values of krill and copepods around South Georgia and in waters of the Polar Front (Wada *et al*. 1987; Schmidt *et al*. 2003), while the highest values seem to correspond to ratios measured in euphausiids and copepods collected on the Patagonian shelf (Rowntree *et al*. 2001) and off the coast of Uruguay (unpublished data of L.O.V.). However, owing to the currently limited isotopic sampling of zooplankton across the South Atlantic, it is not possible to identify the exact feeding locations of southern right whales using only the stable isotope ratios of their skin. Work is under way to more thoroughly sample zooplankton across the South Atlantic and to use other tracers such as sulphur isotopes and metals in skin, baleen plates, and prey, to more accurately identify right-whale feeding locations.

The isotopic values measured in our study fall within the range previously detected along the lengths of baleen plates of southern right whales that were stranded in South Africa (δ^{13}C range = –26.5 to –16.0‰, δ^{15}N range = 4.0 to 12.5‰; Best & Schell 1996) and in Argentina (δ^{13}C range = –26.1 to –15.7‰; Rowntree *et al*. 2008). Both studies found that the isotopic values of the baleen plates oscillate strongly in what appear to be annual cycles and suggested that these cycles correspond to the whales' annual migrations between isotopically different areas (Best & Schell 1996; Rowntree *et al*. 2008). Furthermore, Rowntree *et al*. (2008) found differences among individuals in both their mean isotopic values and the amplitudes of their annual cycles; the authors hypothesize that these variations reflect distinctive foraging patterns associated with the use of different feeding grounds. For example, one of the baleen plates had a higher mean and range of δ^{13}C values (mean = –18.4‰; range = –19.7 to –15.7‰) than the other plates; three had lower values (mean –22.7‰; range = –26.1 to –19.6‰); and one plate had intermediate values (mean = –20.9‰; range = –23.9 to –16.2‰; Rowntree *et al*. 2008).

Baleen, like hair, is a metabolically inert tissue after formation and provides a continuous record of dietary input (Schell *et al*. 1989; Rubenstein & Hobson 2004). However, skin is metabolically active and replaces its isotopes over time, thereby integrating food consumed through a window of time (Rubenstein & Hobson 2004). Metabolically active tissues have different isotope turnover rates; some turn over within hours or days (e.g. blood plasma and liver) and provide information on recent diets, while others take weeks (e.g. muscles) or years (e.g. bone collagen) and thereby integrate diets on longer timescales (Rubenstein & Hobson 2004). The residence time of carbon and nitrogen isotopes in cetacean skin is unknown but has been proposed to be somewhere between one and several months (Todd 1997; Ruiz *et al*. 2004). Thus, our isotope ratios measured from skin samples probably represent signals from the feeding grounds, as modified to some extent by isotope ratios from more recent feeding events along the whales' migratory routes from the feeding grounds to Península Valdés. On this interpretation, the isotopic variation of skin samples can be attributed largely to variation in the use of feeding grounds and to variation in the migratory paths of individual whales between the feeding grounds and Península Valdés, even though the relative contributions of these factors cannot yet be estimated.

Nondietary factors such as age, nutritional status and reproductive status can influence isotope ratios, especially for nitrogen (Hobson & Clark 1992a, b; Roth & Hobson 2000; Fuller *et al*. 2004). In our study, the whales sampled were all nursing mothers that had been under similar physiological stresses for at least a year, including 12 months of gestation, migration to the nursery ground and lactation while fasting (Payne 1986; Cooke *et al*. 2001). To the degree that these stresses affect isotopic values, the effect should be similar for all individuals in the sample. However, the individuals must have varied in age and may also have varied significantly in time since the last major feeding event or time since the onset of lactation. Uncontrolled factors such as these would be expected to add random variation to the isotopic ratios, thereby increasing the variability within haplotypes and weakening any signal of matrilineal structuring of the isotopic variation. Thus, although we cannot say exactly what fraction of the overall isotopic variation arises from isotopic differences in the prey consumed by individuals, the observed differences among haplotypes clearly indicate that matrilineal relatives tend to experience similar effects of one or more factors that influence their isotopic ratios. The major factor seems likely to be prey isotopic ratios which differ substantially among the known and potential feeding grounds, but other factors such as times of return from feeding to breeding grounds could also be involved.

The interannual differences in the general distribution of δ^{13}C and δ^{15}N detected in this study are most likely a response to variations in the isotopic composition at the base of the food web produced by changes in ocean circulation or by modification of local biogeochemical processes (Peterson & Fry 1987; Druffel & Griffin 1999; Brix *et al*. 2004). An alternative explanation is that in 2006 the whales migrated to isotopically distinct feeding regions. If this were true, however, the isotopic values of the samples from 2006 should not show any particular pattern. Instead, they tend to be higher than values for the same haplotype in other years (see Fig. 3). For example, 9 of the 15 haplotypes sampled in 2006 showed the highest δ^{13}C values for that haplotype, and 7 of the 15 haplotypes had their highest δ^{15}N values. In addition, the lack of genetic differentiation among years at the haplotype level indicates that the 2006 whales were not a genetically distinct subset of whales.

Our results show a pattern of fine-scale, long-term genetic substructuring within a breeding population that is most simply explained by maternally directed fidelity to different feeding sites. North Atlantic right whales (*Eubalaena glacialis*) and humpback whales (*Megaptera novaeangliae*) show similar site fidelity to feeding grounds but mix on common breeding grounds (Schaeff *et al*. 1993; Palsbøll *et al*. 1995; Larsen *et al*. 1996; Malik *et al*. 1999). In contrast, Patenaude *et al*. (2007) suggest that southern right whales from different breeding populations within an ocean basin mix on common feeding grounds. For whale species that feed in the Southern Ocean, where there are no land barriers to circumpolar migration, Hoelzel (1998) suggested that animals from different breeding grounds should form mixed genetic assemblages on widely dispersed feeding areas. We suggest that the assemblages detected by Patenaude *et al*. (2007) represent mixed subsets of maternal lineages with site fidelity to specific feeding grounds rather than an unstructured mix of animals migrating randomly from different nursery grounds. Therefore, southern right whale population structure may mirror that of humpback whales in the North Pacific, where the whales are genetically segregated on both the nursery and feeding grounds (Baker *et al*. 1998b).

The correlation of SST anomalies off South Georgia and reduced calf output at Península Valdés (Leaper *et al*. 2006) suggests a strong migratory connection between these two areas, but our isotopic data imply that the Valdés whales feed in many different locations distributed over a large geographical range. We see two possible explanations for this apparent disagreement: (i) the SST anomalies detected at South Georgia might affect many other feeding locations, and therefore stress most of the whales that visit Península Valdés; or (ii) the SST anomalies might affect only the whales that visit South Georgia, but so strongly that calf production is reduced detectably for the population as a whole. These alternatives could be distinguished by analysing the individual reproductive histories of females using Península Valdés to see whether particular matrilines fail to reproduce in years following SST anomalies and low krill abundance near South Georgia.

On short ecological timescales, strong site fidelity could restrict a population to a set of culturally inherited areas and migratory patterns that represent only a portion of its potential range (Matthiopoulos *et al*. 2005; Clapham *et al*. 2008). The pattern described here suggests that the timescale of matrilineal site fidelity to feeding areas is at least several generations. The finding of Leaper *et al*. (2006) raises concern about the future of southern right whales if krill fisheries and climate change turn out to have a significant impact on krill abundance (Atkinson *et al*. 2004). If whales follow foraging strategies and migratory patterns learned from their mothers, will they be flexible enough to change their strategies and switch to other prey types with different spatial and temporal distributions? Some mitochondrial lineages show relatively large stable isotope ranges, suggesting that a few members of those lineages experimented with different locations or prey types in the relatively recent past. If we can better understand the causes and consequences of this limited plasticity, then we might be better able to predict the responses of southern right whales and other marine migrants to global climate change.

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Luciano O. Valenzuela is currently a postdoctoral fellow in the Department of Biology and in IsoForensics Inc., where he is working with stable isotopes of human and animal hair. This paper is one of the chapters of his doctoral thesis at the University of Utah. Mariano Sironi is the scientific director of the Instituto de Conservacion de Ballenas, an argentine non-profit organization dedicated to protect the whales and their habitat through research, conservation and education activities. Vicky Rowntree directs the long-term study of the Peninsula Valdez population, which was begun by Roger Payne in 1971. The study was the first to use repeated photo-identification of known individuals to estimate demographic and population parameters for a large whale species. Jon Seger studies the evolutionary genetics of the cyamids (whale lice) that live on right whales. Their simple, well-defined ecologies and large, well-mixed populations make them a good system with which to address a number of basic theoretical questions.
