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Isolation by environmental distance in mobile marine species: molecular ecology of franciscana dolphins at their southern range

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Abstract

The assessment of population structure is a valuable tool for studying the ecology of endangered species and drafting conservation strategies. As we enhance our understanding about the structuring of natural populations, it becomes important that we also understand the processes behind these patterns. However, there are few rigorous assessments of the influence of environmental factors on genetic patterns in mobile marine species. Given their dispersal capabilities and localized habitat preferences, coastal cetaceans are adequate study species for evaluating environmental effects on marine population structure. The franciscana dolphin, a rare coastal cetacean endemic to the Western South Atlantic, was studied to examine these issues. We analysed genetic data from the mitochondrial DNA and 12 microsatellite markers for 275 franciscana samples utilizing frequency-based, maximum-likelihood and Bayesian algorithms to assess population structure and migration patterns. This information was combined with 10 years of remote sensing environmental data (chlorophyll concentration, water turbidity and surface temperature). Our analyses show the occurrence of genetically isolated populations within Argentina, in areas that are environmentally distinct. Combined evidence of genetic and environmental structure suggests that isolation by distance and a process here termed isolation by environmental distance can explain the observed correlations. Our approach elucidated important ecological and conservation aspects of franciscana dolphins, and has the potential to increase our understanding of ecological processes influencing genetic patterns in other marine species.

Keywords: cetaceans, conservation genetics, isolation by environmental distance, oceanography, population structure, remote sensing

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Introduction

The last two decades have seen an unprecedented increase in our understanding about the structure of natural populations, due in part to high-resolution molecular techniques and analytical frameworks (DeSalle & Amato 2004; Waples & Gaggiotti 2006). As we are able to characterize patterns of genetic diversity, the most relevant question becomes the one about the *processes* causing such patterns. One of the most commonly cited mechanisms for the observed genetic structure

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patterns both in the terrestrial and marine realms is known as 'isolation by distance' (IBD), which postulates that the genetic distance between populations is correlated to the geographic distance between them (Wright 1943). While the IBD mechanism is probably unable to represent the complexities of most natural systems, it has served as a useful hypothesis for testing scenarios.

Recent evidence for terrestrial species suggests that habitat fragmentation and patchiness, disturbance, and vicariance events also influence population structure (Paulo *et al.* 2008; Pimenta *et al.* 2008; Pavlacky *et al.* 2009). Similar evidence linking environmental discontinuities to population structure of mobile marine species is rare, perhaps due to the difficulty to gather broad-coverage oceanographic information. As remote sensing technology has helped overcome this difficulty, providing high-resolution and high-coverage oceanographic data, it is now possible to integrate environmental and genetic information for marine species. Good candidate species to evaluate environmental influences to dispersal in the marine environment would have wide distribution ranges and yet present localized habitat preferences. If these habitat preferences cause population structure, one could then evaluate which environmental variables partitioning habitats are responsible for the observed genetic structure patterns. Some cetacean species present such combination of ecological traits and habitat preferences, making them suitable for these evaluations (Hoelzel 1994).

In an attempt to shed light on the mechanisms for the interaction between genetic structure and environmental factors, we test an initial set of hypotheses or simple mechanisms, which can then be modified and combined to better reflect reality. We evaluate the extent to which habitat heterogeneity can explain the genetic patterns of population structure. Specifically, we will use environmental data to assess heterogeneity in the study area and evaluate if such environmental discontinuities coincide with the observed genetic breaks between populations. Second, we will evaluate if IBD-like models, which have been documented in cetaceans (Rosel *et al.* 1999; Krützen *et al.* 2004), can be extended to describe adequately the type of relationships between environmental and genetic structure. Specifically, we test for IBD and also evaluate if the genetic differences between populations are correlated with environmental differences in their habitats. The last concept is similar to IBD, although it is the *environmental* distance between populations that correlates with their genetic separation, so we call it 'isolation by environmental distance' (IBED). We anticipate the possibility that these three mechanisms (environmental brakes, IBD and IBED) could interact, and we seek to demonstrate that by using appropriate testing tools, at least some of them can be decoupled.

Our choice of the environmental variables for this evaluation is related to previous environmental assessments involving cetaceans (Forney 2000; Baumgartner *et al.* 2001; Hamazaki 2002). Variables such as chlorophyll concentration, zooplankton biomass, upwelling areas, marine fronts (all related to productivity), surface temperature, marine currents (related to water masses) and depth have been identified as important factors influencing the distribution and abundance of cetaceans (Reilly 1990; Baumgartner *et al.* 2001; Davis *et al.* 2002; Jaquet & Gendron 2002). Building upon this knowledge, we propose to evaluate the influence of some of the abovementioned oceanographic variables on cetacean population structure.

Our study species is the rare franciscana dolphin (*Pontoporia blainvillei*), a coastal cetacean endemic to the Southwestern Atlantic Ocean (Crespo *et al.* 1998). In a previous assessment using franciscana dolphin mitochondrial DNA (mtDNA) data, we saw evidence of population structure and mixed signs of IBD as a potential driver of the observed genetic structure. Based on qualitative data, we suggested that some heterogeneous environmental variables in the area could also be influencing such patterns (Mendez *et al.* 2008). These preliminary data make our study species and area suitable for the topic of this analysis.

Furthermore, franciscanas may be the most threatened cetacean in South America (Bordino *et al.* 2002; Secchi *et al.* 2003), and it has been suggested that information on population structure is needed to complement the scarce data on abundance and incidental mortality of this species (Bordino *et al.* 2002; Secchi *et al.* 2003). This information can be used for explicit evaluations of conservation priorities such as stocks or management units (Palumbi & Cipriano 1998; Natoli *et al.* 2008). While marine protected areas are recognized as important elements of marine conservation strategies (Norse & Crowder 2005), they have also been regarded as frameworks lacking explicit information on the species of interest (Allison *et al.* 1998; Palumbi 2003). The combination of genetic and environmental data could provide a more informative framework to better understand the interactions between populations and their environment, which could then help identify more appropriate areas for protection.

In this study, we combine mtDNA and microsatellite data with environmental information to investigate the influence of a suite of oceanographic variables on cetacean population genetic structure. Through an integrated genetic approach to ecology, we seek to increase our understanding of marine dispersal and contribute

new elements to consider for conservation strategies involving mobile marine species.

Methods

Sample collection and DNA extraction

Tissue samples of 228 individuals were obtained from incidentally entangled franciscana dolphins in coastal fishery gillnets, and stranded animals, in Argentina between 2000 and 2009. In addition, 16 skin biopsies were collected between 2005 and 2008 as part of four capture-tag-release operations in the study area (Bordino *et al.* 2008). All samples were preserved in ethanol (96% v/v). Our data set was completed with the inclusion of GenBank mtDNA control region sequences from 31 individuals collected in southern Buenos Aires, Argentina (Lazaro *et al.* 2004). Location data for the 275 samples and sequences included in this study are presented in Fig. 1.

Total genomic DNA was extracted from tissue samples following the procedures in the QIAamp Tissue Kit (QiaGen). A fragment of 560 bp of the mtDNA control region was amplified (primers L159256 and H00651) (Kocher *et al.* 1989), and sequenced in both directions using a 3730xl DNA Analyzer [Applied Biosystems, Inc. (ABI)] (Mendez *et al.* 2008).

Twelve microsatellite loci previously developed for other cetacean species were optimized and successfully amplified for most samples (Buchanan *et al.* 1996; Valsecchi & Amos 1996; Shinohara *et al.* 1997; Hoelzel 1998; Krützen *et al.* 2004). Each forward primer was

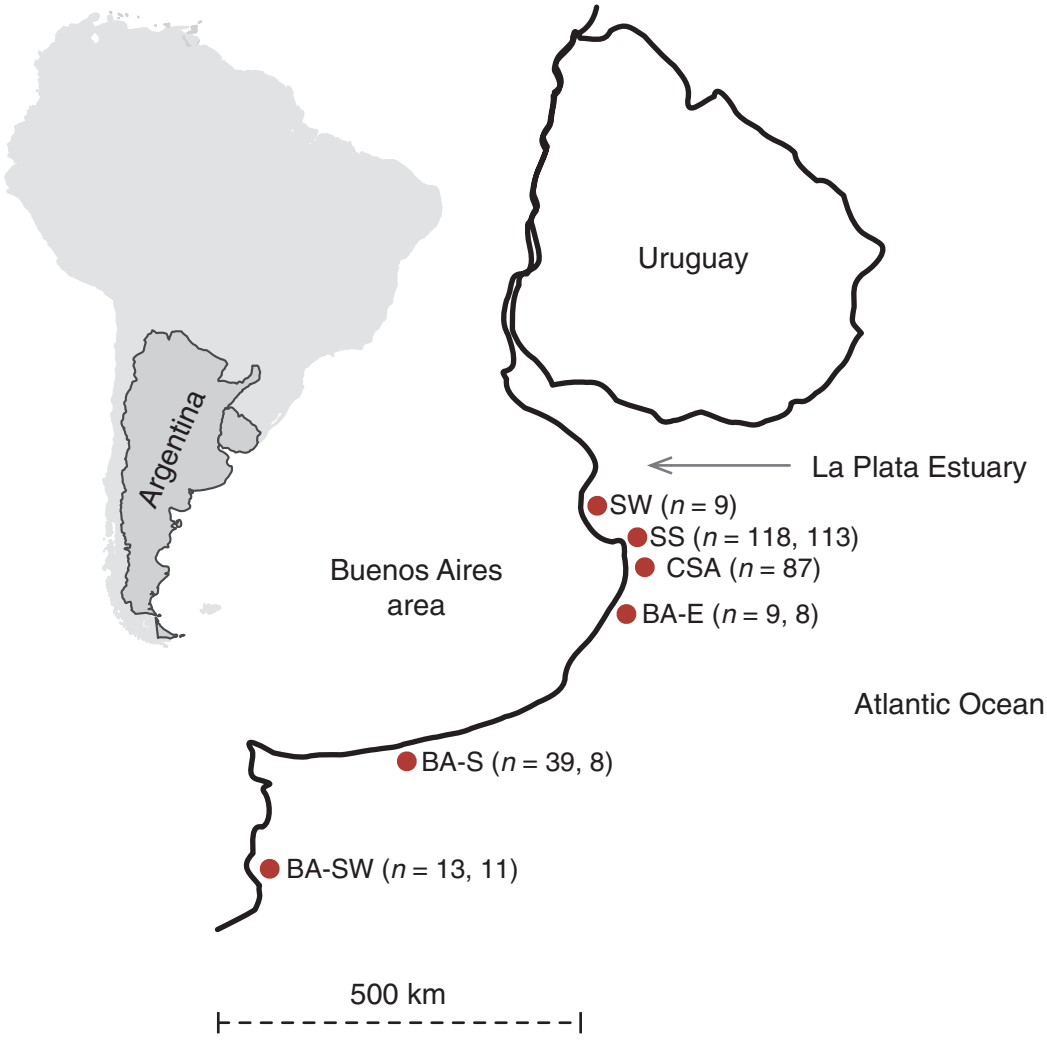

Fig. 1 Area map depicting the sampling localities included in the genetic and environmental analyses, with sampling sizes for the mtDNA and microsatellite data sets, respectively. SW, Samborombón West; SS, Samborombón South; CSA, Cabo San Antonio; BA-E, Buenos Aires East; BA-S, Buenos Aires South; BA-SW, Buenos Aires Southwest.

modified adding an M13 sequence tail to its 5′ end for fluorescent labelling (labelled M13 primer) (Schuelke 2000). PCRs were performed in a 25 μL reaction volume, consisting of 0.25 mM Tris–HCl, 1.25 mM KCl, 0.0375 mM $MgCl_2$, 0.03 mM each dNTP, 0.04 μM forward primer, 0.4 μM reverse primer, 0.18 μM labelled M13 primer, 1 U of AmpliTaq DNA Polymerase (ABI), and ~5–10 ng of genomic DNA. Thermal profiles for the different loci were adapted from the original amplification conditions and are reported in Table S1 (Supporting Information). PCR products were separated electrophoretically using a 3730xl DNA Analyzer (ABI), and allelic sizes were scored against the size standard GS600 LIZ (ABI) and analysed using the GeneMapper v4.0 software (ABI).

mtDNA—haplotyping and diversity estimates

DNA sequence variation was characterized into mtDNA haplotype definitions following the nomenclature developed sequentially in Secchi *et al.* (1998), Lazaro *et al.* (2004) and Mendez *et al.* (2008). The 560-bp mtDNA fragment was truncated to a 407-bp region containing about 95% of the variation, in order to integrate the shorter sequences obtained from GenBank into our data set. Matching of sequences to haplotypes was done using COLLAPSE v1.2 (available from http://darwin.uvigo.es) and DnaSP v5.0 (Rozas *et al.* 2003). Haplotype diversity, H_d, (Nei 1987), the mean number of pairwise differences among sequences, k (Kimura 1980; Tajima 1983), and the nucleotide diversity, π (Nei 1987) in our sample were assessed using Arlequin v3.1 (Excoffier *et al.* 2005) and DnaSP.

Microsatellite data—genotyping and diversity estimates

Degraded samples and biopsies were amplified and genotyped in duplicates to minimize typing error. Genotyping error was checked for the remaining samples by re-amplifying and re-typing 10% of the total, chosen at random. Overall, 11 cases of allele dropout were detected in our samples, which were solved by triplicate genotyping. GENEPOP v4.0 (Rousset 2008) was used to evaluate linkage disequilibrium (LD) between all pairs of loci for each population (1000 dememorization iterations, 1000 batches, 10 000 iterations per batch) and Hardy–Weinberg equilibrium (HWE). Significance levels ($P = 0.05$) for departure from HWE and for LD were corrected for multiple comparisons with the sequential Bonferroni correction (Rice 1989). Number of alleles, observed (H_O) and expected heterozygosities (H_E) were estimated in Arlequin.

Analysis of population structure

We used mtDNA sequence and microsatellite data to test for population structure between the six locations with the biggest artisanal fisheries operating along the Buenos Aires coast, in Argentina, and where franciscanas have been systematically reported as by-catch: Bahia Samborombon West (SW), Bahia Samborombon South (SS), Cabo San Antonio (CSA), Buenos Aires East (BA-E), Buenos Aires South (BA-S) and Buenos Aires Southwest (BA-SW) (Fig. 1). These fisheries operate independently and typically seek to minimize overlap, providing additional justification for genetic testing.

Spatial structure of the mtDNA data set among the putative populations was evaluated utilizing the samples we collected and the 31 sequences from GenBank, through the analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) implemented in Arlequin. Pairwise F_{ST} (haplotype frequencies only) and Φ_{ST} statistics (pairwise differences between haplotypes) were computed in Arlequin. The significance of the observed Φ- or F-statistics was tested using the null distribution generated from 10 000 nonparametric random permutations of the data. Spatial structure was evaluated with a smaller data set for microsatellite markers, as the GenBank samples were only mtDNA sequences and some samples failed to provide microsatellite data (see Results). Here, we also assessed spatial structure among the putative populations though the AMOVA analysis, and estimated pairwise F_{ST} statistics using Wier and Cockerham's θ, which assumes an infinite allele model of mutation (Weir & Cockerham 1984), with Arlequin. No corrections for multiple tests were made (Perneger 1998; Narum 2006); we present significance values for each pairwise comparison in the Results section.

To assess the degree of partitioning in our total sample without a priori definition of putative populations, a Bayesian clustering algorithm was utilized with the microsatellite data as implemented in Structure v2.3.1 (Pritchard *et al.* 2000; Falush *et al.* 2003; Hubisz *et al.* 2009). Given the number of genetic clusters (K) as a prior hypothesis, and under the assumption of Hardy–Weinberg and linkage equilibrium within clusters, the algorithm estimates the log-likelihood of the data for the pre-defined K values, and cluster memberships for all individuals in the total sample. We used the admixture model, which assumes that individuals have mixed ancestry. Although Structure v2.3.1 allows for the incorporation of sampling location priors, we did not include such information in our model, making it more stringent. We followed a heuristic method to evaluate $1 \leq K \leq 10$, performing between 30 and 45 independent long runs (10^6 burn-in steps, 10^7 total steps) for *each*

value of K, for a total of 375 runs. The maximum log-likelihood values from all runs corresponding to each given K were checked for consistency and averaged. The most likely number of clusters that better explains our microsatellite data set is derived from the K with highest averaged maximum log-likelihood.

Population structure between contiguous localities

In addition to the general patterns of population structure, we were interested in the genetic patterns between contiguous putative populations. A near linear coastal distribution pattern of franciscanas means animals most probably move through adjacent (i.e. contiguous) strata as they move along the coast. Therefore, comparisons of adjacent strata would be biologically most plausible (i.e. SW/SS, SS/CSA, CSA/BA-E, BA-E/BA-S and BA-S/BA-SW). We use these comparisons to investigate the extent to which any heterogeneity in the coastal habitat influences such movements. To complement our genetic distance estimations between contiguous populations, we estimated migration rates from the mtDNA data using a maximum-likelihood procedure implemented in the MDIV software (Nielsen & Wakeley 2001). MDIV simultaneously estimates the migration rate per gene per generation between populations scaled by the effective population size ($M = 2N_em$), the divergence time scaled by the effective population size ($T = t/2N_e$), and the parameter theta ($\theta = 4N_e\mu$).

Markov chains of 10^7 cycles were run with 10^5 cycles of burn-in to minimize dependence on initial conditions. The model values for θ and T_{max} (maximum value for the scaled divergence time) were $\theta = 0$ and $T_{max} = 5$. The choice of $\theta = 0$ provided the model with a flat prior hypothesis, which had the least influence on the parameter estimation. A $T_{max} = 5$ and a comparison-dependent M_{max} (maximum value for the scaled migration rate), were well above the estimated T and M in our sensitivity analysis, ensuring that our model contained all possible T and M values. Ten converged runs for each population comparison were used to identify M, T and θ values corresponding to the maximum likelihood.

Environmental structure analysis

Among the oceanographic variables previously related to habitat heterogeneity (Bost *et al.* 2009), we gathered data on chlorophyll concentration (CHL or chlorophyll, from now on), surface temperature (SST or temperature, from now on) and water turbidity (K490 or turbidity, from now on), as these are available with wide-coverage through remote sensing. Satellite-derived data were obtained from the NASA OceanColor Website (oceancolor.gsfc.nasa.gov). To assess regional environmental heterogeneity, we constructed chlorophyll, temperature and turbidity maps for the entire region between 1997 and 2007, at 8-day resolution (each pixel in the maps corresponds to an 8-day average of the given variable) using the SeaDAS software (oceancolor.gsfc.nasa.gov). To evaluate whether the franciscana sampling areas are environmentally distinct, we defined coastal square polygons of 50 km by 50 km at the centre of each franciscana sampling locality, based focal habitat inferences (Bordino *et al.* 2008), to gather environmental data. Chlorophyll, temperature, and turbidity time-series data were obtained for each of these polygons at a 1-month resolution (each point in these time series is a 30-day average), from September 1997 to August 2007 for chlorophyll and turbidity (120 monthly data points each series), and from September 2002 to August 2007 for temperature (58 monthly data points).

We evaluated environmental differences between the franciscana areas as follows. First, we inspected the regional maps of each of the three variables to identify spatial structure in the data, and geographical areas of high environmental heterogeneity. Second, to compare contiguous franciscana sampling areas (polygons), we averaged the monthly time series and assessed differences between the means with a paired t-test, accounting for the spatial dependence between contiguous sampling sites. Third, to assess the spatial structure of the monthly time-series data among all polygons and to take into account the sequential order of the monthly data points (which an analysis of means or an ANOVA-type test fail to account for) we used a modelling approach. Each of the three environmental variables was modelled using a mixed effects linear regression model, which included sampling sites as fixed effects, and the month and year of each sample as random effects to account for seasonal and multi-year trends that might affect summary values (Gelman & Hill 2007). Fourth, to assess seasonal patterns between the areas, we used the monthly time-series data to build climatologies for each of the three variables at the sampling polygons, and qualitatively compared these climatologies. Here, the maxima and minima, and the overall shape of the climatology were compared to assess differences among localities.

Joint genetic and environmental analyses

To evaluate our hypothesis that the genetic discontinuities (or brakes) between populations along the Buenos Aires coast could be influenced by environmental heterogeneity, we assessed if there were contiguous populations that were genetically differentiated, and for which the environmental data showed significant discontinuities.

To evaluate our hypothesis that IBD can explain the genetic patterns in the study area, we tested for potential correlations between the pairwise genetic (sequence and microsatellite-derived F_{ST}) and geographical distances, using Mantel tests in IBD v3.16 (Bohonak 2002). The significance of these tests was assessed through 10 000 random permutations of the variables. Geographical distances were calculated as the linear distance between sampling sites, measured along the coastline using Arc GIS (Environmental Systems Research Institute, Inc.) (Table S2, Supporting Information). Rejection of the null hypothesis of a negative or flat slope for the correlation between variables is used as evidence of IBD.

To evaluate our hypothesis that IBED can explain the genetic patterns in the study area, we tested for potential correlations between the pairwise genetic and environmental distances, again using Mantel tests. Environmental distances were calculated as the pairwise difference in mean chlorophyll, temperature and turbidity between sites (Table S2, Supporting Information). In this case, rejection of the null hypothesis of a negative or flat slope for the correlation between variables could be used as a suggestion of IBED. However, because an apparent IBED pattern may actually be driven, or at least influenced, by the geographical distance between the localities, it is necessary to filter such effects. We used Partial Mantel tests, as implemented in IBD v3.16, to assess the potential correlation between genetic and environmental distances (IBED) while controlling the effect of geographical distances (IBD).

Results

Genetic diversity

Our mtDNA sequences collapsed into 34 haplotypes (GenBank Accession nos EF394099–EF394117), 14 of which are private to unique sampling locations, and the remaining 20 common to more than one location. Most private haplotypes were identified for CSA ($n = 9$), followed by SS ($n = 2$) and BA-E ($n = 1$). Populations SS, BA-E and BA-SW exhibit the lowest genetic diversity indices overall (Table S3, Supporting Information), whereas the haplotype diversity is significantly correlated to sample size among localities (Pearson's Correlation Test, $P < 0.05$).

The microsatellite data provided no evidence for LD and allowed rejection of a deviation from HWE in all pairwise tests ($P < 0.05$). No significant differences were observed between the expected heterozygosity under HW and that observed in the data, for any of the putative populations (Table S4, Supporting Information). The mean number of alleles per putative population is significantly correlated with population size (Pearson's Correlation Test, $P < 0.01$), the loci with highest allele number are FB17 and MK6, and the ones with lowest number MK8, D14 and EV104.

Population structure

All global tests of differentiation were significant for the mtDNA ($F_{ST} = 0.088$ $P < 0.001$, $\Phi_{ST} = 0.078$, $P < 0.001$) and for the microsatellite data ($F_{ST} = 0.018$, $P = 0.003$). Out of the five possible mtDNA pairwise comparisons for each putative population, SS is the most differentiated from other populations (different from three or four populations, depending on the fixation index), and SW the least differentiated from other populations (different from one population) (Table 1). For the microsatellite data set, the putative populations most differentiated are SW, SS and BA-SW (different from four populations), whereas BA-E is the least differentiated (different from one population) (Table 2).

Table 1 Pairwise genetic distances between sampling locations (putative populations) for the mtDNA data set. Sample size (n) is shown for each putative population in the top row. F_{ST} and Φ_{ST} values are below and above the diagonal, respectively. Significance from 10 000 permutations of the data matrix (P-value) is shown below each F_{ST} or Φ_{ST} values

	SW ($n = 9$)	SS ($n = 118$)	CSA ($n = 87$)	BA-E ($n = 9$)	BA-S ($n = 39$)	BA-SW ($n = 13$)
SW		0.08	0	0.268	0	0.028
P-value		0.115	0.681	**0.034**	0.379	0.281
SS	0.173		0.068	0.005	0.162	0.114
P-value	**0.015**		**0.001**	0.309	**0.001**	**0.019**
CSA	0.082	0.054		0.14	0.021	0.026
P-value	0.075	**0**		**0.014**	0.093	0.156
BA-E	0.177	0	0.031		0.214	0.178
P-value	0.051	0.709	0.132		**0.009**	0.066
BA-S	0.059	0.128	0.053	0.073		0
P-value	0.194	**0**	**0.001**	**0.041**		0.511
BA-SW	0.196	0.241	0.139	0.183	0.014	
P-value	0.088	**0**	**0.001**	**0.022**	0.257	

Table 2 Pairwise genetic distances between sampling locations (putative populations) for the microsatellite data set. Sample size (n) is shown for each putative population in the top row. Significance from 10 000 permutations of the data matrix (P-value) is shown below each F_{ST} value

	SW (n = 9)	SS (n = 113)	CSA (n = 87)	BA-E (n = 8)	BA-S (n = 8)	BA-SW (n = 11)
SW						
SS	0.056					
P-value	**0.009**					
CSA	0.041	0.013				
P-value	**0.036**	**0**				
BA-E	0.046	0.014	0.005			
P-value	0.18	0.081	0.405			
BA-S	0.066	0.022	0.001	0.009		
P-value	**0.045**	**0.027**	0.639	0.541		
BA-SW	0.09	0.067	0.035	0.037	0.011	
P-value	**0**	**0**	**0**	**0.027**	0.387	

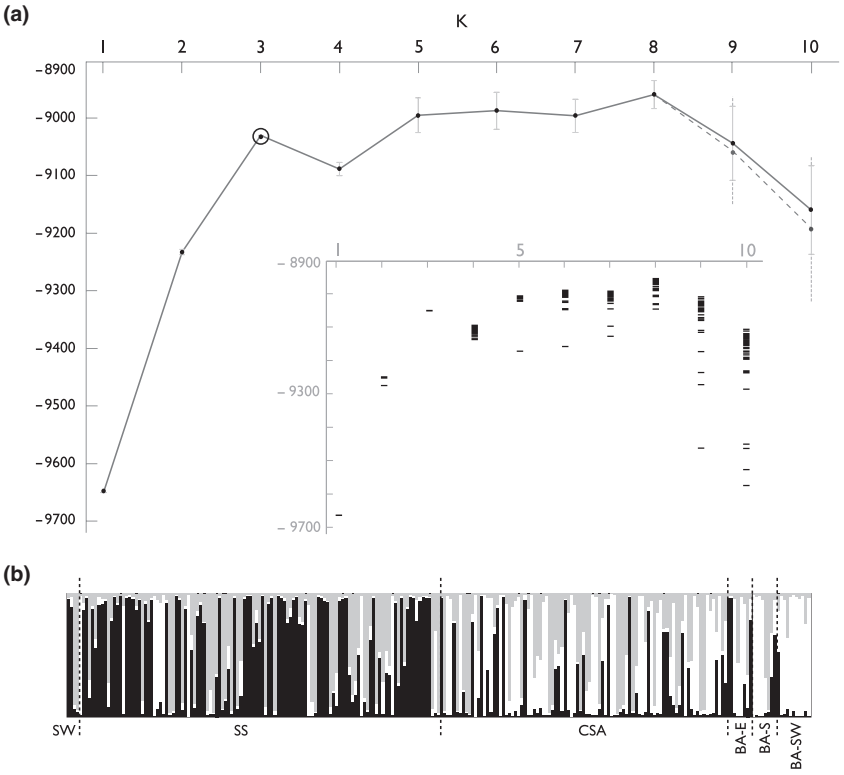

Fig. 2 (a) Log-likelihood of the number of population clusters explaining the genetic data. The larger graph shows the average values of ln $P(K)$ resulting from a minimum of 30 runs, with their corresponding standard deviation bars. The dotted line between $K = 8$ and $K = 10$ shows the effect of the five outlier runs in our analysis. The inset graph shows all the individual runs (notice the close agreement for ln P values between $K = 1$ and $K = 4$, and the resulting narrow standard deviation bars). (b) Individual assignment values for $K = 3$ [the first maximum in the ln $P(K)$ distribution]. Each colour depicts the relative contribution of each of the three clusters to the genetic makeup of every individual. Sampling locations were included in this graph a posteriori, and therefore did not influence the assignment process.

The Bayesian approach shows consistent results for the group of 30–45 runs performed for *each* of the 10 K values. Figure 2 shows a first local maximum of ln $P(K)$ at $K = 3$, and a second one at $K = 5$. Samples collected in different localities show uneven assignment values to alternative inferred clusters, indicating structure in the data (Fig. 2). Five outlier runs did not affect the general patterns observed with the entire data set (Fig. 2; Table S5, Supporting Information).

The only two contiguous putative populations that were not significantly different from each other were BA-S and BA-SW. All other contiguous putative populations were significantly different from each other by one or both genetic markers (Table 3). Likelihood-based migration rates for contiguous putative populations were consistent between runs (Table 3; Table S6, Supporting Information). The only exception to high agreement between runs was for the BA-S/BA-SW comparisons, for which six runs agreed on $M = 21.7$, and the other four runs agreed on $M = 26.6$. Overall, the migration estimates showed consistence with the genetic distances, with lower migration rates for those

Table 3 Genetic distance and migration rates between contiguous putative populations. Significant F_{ST} and Φ_{ST} values ($P < 0.05$) are highlighted in bold. Migration rate, time since divergence and theta values were selected from 10 converging runs for each comparison. The number of concordant runs is shown (see Supporting Information for an inspection of individual runs)

	Genetic distance			Migration rates			
	mtDNA (F_{ST})	mtDNA (Φ_{ST})	nuDNA (F_{ST})	No. equal runs	$M = 2N_em$	$T = t/2N_e$	$\theta = 4N_e\mu$
SW/SS	**0.173**	0.08	**0.056**	9 of 10	2.4	0.05	3.26
SS/CSA	**0.054**	**0.068**	**0.013**	9 of 10	10.36	0.04	4.4
CSA/BA-E	0.031	**0.14**	0.005	10 of 10	30.2	0.01	4.7
BA-E/BA-S	**0.073**	**0.214**	0.009	10 of 10	1.38	3.22	0.11
BA-S/BA-SW	0.014	0	0.011	6 of 10	21.7	0.01	2.80

population comparisons with higher genetic distances (SW/SS, BA-E and BA-S), and vice versa. The estimated scaled divergence times are inversely related to the migration rates as expected.

Environmental structure

The chlorophyll, temperature and turbidity maps exhibit obvious regional discontinuities (Fig. 3). Chlorophyll concentration and turbidity are higher in-shore, and particularly high in the La Plata Estuary (SW and SS) and south to the CSA area. The southern Buenos Aires area (BA-S and BA-SW) shows relatively high chlorophyll and turbidity values, although not as high as those in the La Plata Estuary. The surface temperature seems similarly structured, with the La Plata Estuary and southern Buenos Aires areas displaying higher average temperatures to those in the rest of the Buenos Aires coastline. The mouth of the La Plata Estuary is the area of highest variability in chlorophyll and turbidity, and also high variability in the temperature patterns. In addition, temperature is also highly variable in the southern Buenos Aires area (BA-S and BA-SW).

Mean values of chlorophyll, turbidity and temperature are significantly different in all comparisons between contiguous polygons representing the franciscana areas [two-sided $P_{(t\text{-test})} < 0.005$ in all cases, Fig. 4]. Range values for chlorophyll and turbidity also vary between sampling most sampling locations, whereas the temperature range values seem to vary less (Fig. 4).

The mixed effects linear regression model satisfactorily explained the variance in temperature (96% of variance explained), chlorophyll (74%) and turbidity (72%) amongst all polygons representing the sampling locations. Regional variation in chlorophyll and turbidity is mostly related to sampling polygons (84.9% and 82.5% of explained variance respectively), with yearly trends explaining some variation (15.1% and 16.9%, respectively), and month of the year explaining a small fraction of variance in turbidity (0.6%) and being not significant to the chlorophyll model ($P = 0.99$). Most of the regional variation in temperature is related to seasonal trends (94.2%), with little variation between sampling polygons (5.8%). Modelled mean and standard deviation values are displayed for illustrative purposes, together with the empirical values in Fig. 4.

The chlorophyll and turbidity climatologic series show analogous patterns, which differ between localities. Specifically, for instance, the extreme values and their location in the series (i.e. month) differ between localities (Fig. 5a). The temperature climatologic data show analogous patterns between localities, although with different extreme values, and in some cases different month of maximum temperature (Fig. 5a). The chlorophyll, turbidity and temperature time-series data are shown in for illustrative purposes (Fig. 5b–d), as their differences were assessed statistically with the mixed effects linear regression model. For all three variables, the extreme values and their location in the series (i.e. month/year) differ between localities, as does the mean trend. The overall shape of the time series also varies between localities.

Genetic and environmental patterns of structure

All evaluated environmental variables were significantly different between any two contiguous areas. Therefore, all contiguous putative populations occur in areas that are environmentally distinguishable. A positive trend (slope > 0) was detected between the genetic and geographical distances of the putative populations for both markers. However, the Mantel test of autocorrelation between genetic and geographical distances was significant only for the mtDNA sequence data and not significant for the microsatellite data set (nuDNA) (Table 4). Positive trends were also detected between genetic distance and chlorophyll, turbidity and temperature. All three correlations between the microsatellite and environmental data sets were significant, and two of these (chlorophyll and turbidity) remained significant after controlling the effect of geographical distance with

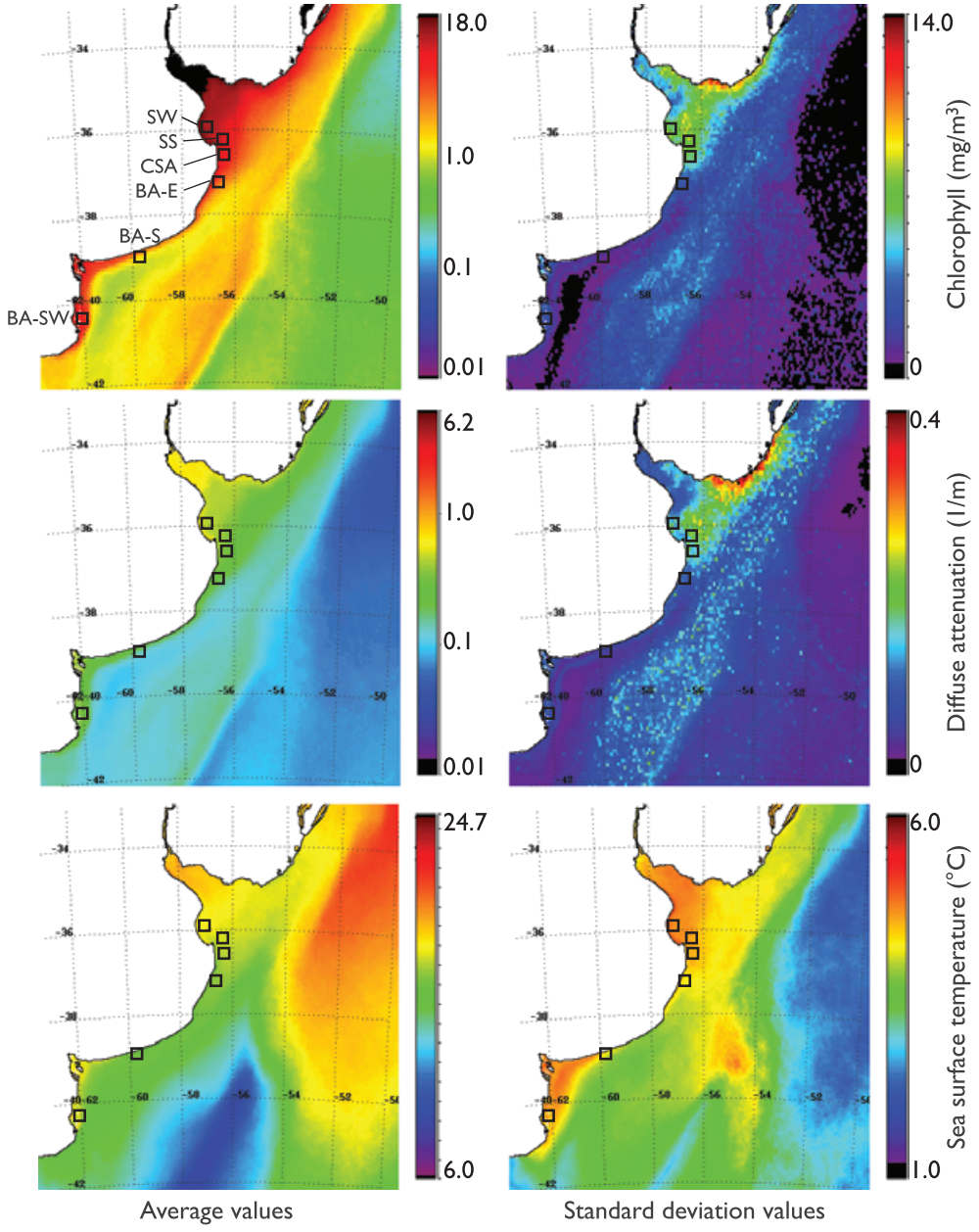

Fig. 3 Average and standard deviation regional maps of chlorophyll, turbidity and temperature in the study area, at 8-day resolution. Sampling polygons for the monthly time-series data extraction and analyses measure 50 km by 50 km and are scaled to the maps. Both data sets were collected from 1997 to 2007.

a Partial Mantel test. For the mtDNA data set, the only significant correlation was with temperature; this correlation was not significant after controlling the effect of geographical distance (Table 4).

Discussion

Our combined data set and analyses provide explicit evidence suggesting that some environmental discontinuities influence dispersal in mobile marine species. Specifically, we found that (i) both mtDNA and nuDNA genetic markers show population structure in franciscanas, (ii) the study area shows recognizable environmental discontinuities, which overlap with the observed genetic breaks, and (iii) the mtDNA population structure data are best explained by IBD, whereas the microsatellite data are best explained by IBED.

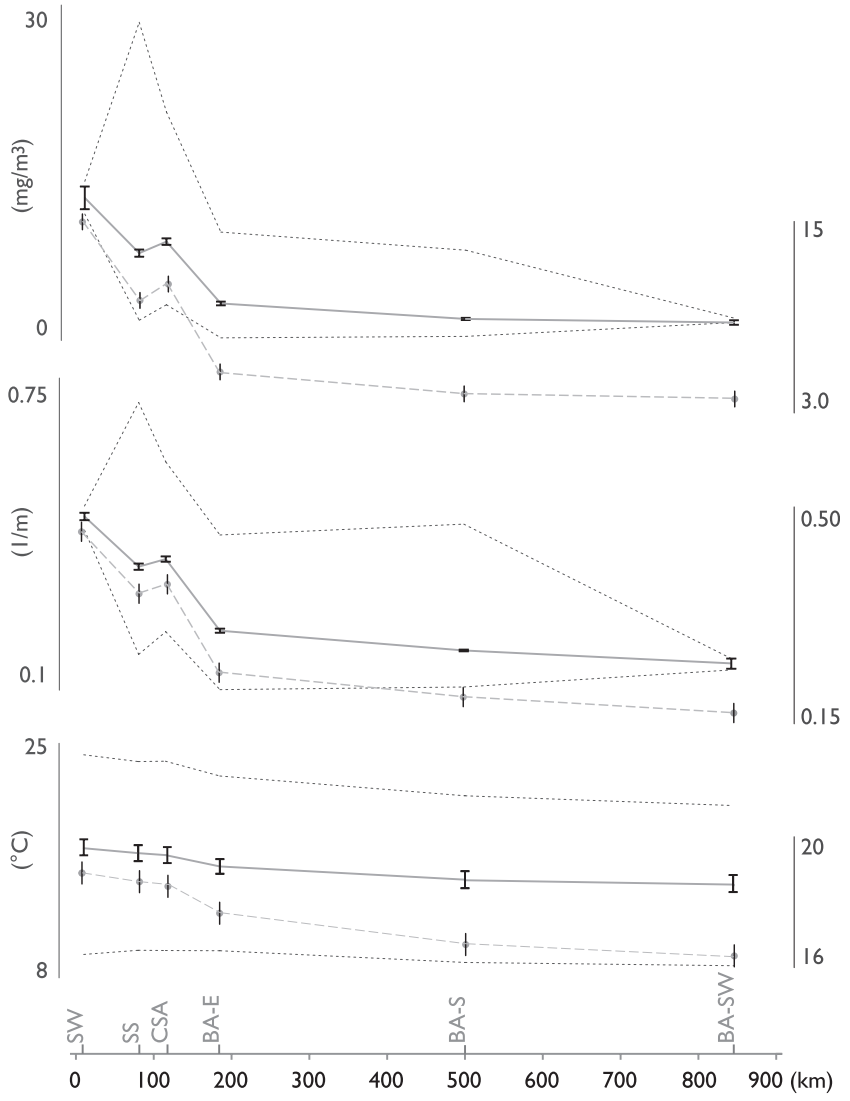

Fig. 4 Mean and standard deviation values (bars) for each of the three oceanographic variables at the each sampling polygon. The horizontal axis displays the linear coastal distance between localities, using the northernmost locality (SW) as a reference. Empirical data are the top of each pair of series plotted against the left vertical axis, with mean values connected by a continuous line, and range values connected by a dotted line. All comparisons involving any of the three variables between contiguous localities are significant [two-sided $P_{(t\text{-test})} < 0.005$]. Comparisons between all localities where assessed with a modelling approach described in the text. Modelled data, shown for comparison, are the bottom of each pair of series plotted against the right vertical axis, with mean values connected by a dashed line.

Population structure

All analyses for both genetic markers (mtDNA and microsatellites) show evidence of population structure of franciscana dolphins in coastal Argentina, in the southernmost portion of their distribution range. To understand the biological meaning of the spatial structuring among these franciscana sampling units, we need to consider the species' ecology and behaviour. Because franciscanas are coastal dolphins rarely found beyond 5 km from the shore, they are most likely to move between neighbouring sampling areas in a stepwise fashion; therefore comparisons between contiguous populations are of particular interest. The significant genetic structure and relatively small migration rates between most contiguous populations (with the exception of BA-S\BA-SW) point to biologically meaningful population structure. Satellite tagging data of two male and two female franciscanas in SS in 2006, consisting of over 260 continuous tracking days, show strong fidelity to this area and supports our genetic assessment (Bordino *et al.* 2008). The comparison between BA-S and

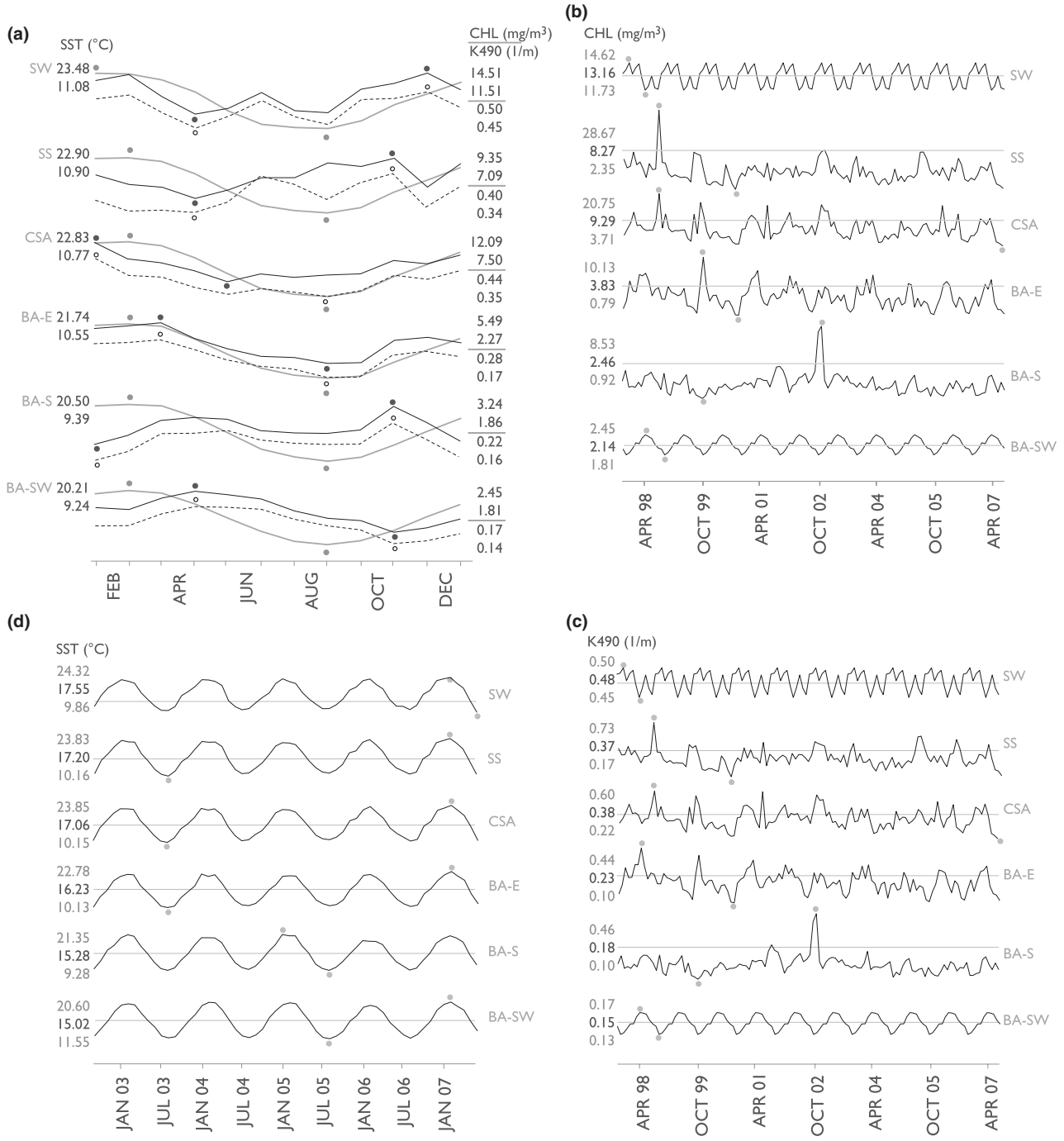

Fig. 5 (a) Climatologies of the three oceanographic values at the sampling localities. Maximum (top) and minimum (bottom) values scale each series, maxima and minima location are displayed with dots above and below the curves. Temperature (SST, °C) is plotted in thick grey against the left vertical axis, with its extremes marked in solid grey. Chlorophyll (CHL, mg/m^3) is plotted in thin black against the right vertical axis, with its extremes marked in solid black. Turbidity (K490, 1/m) is plotted in dotted black, below chlorophyll against the right vertical axis, with its extremes marked as open circles; (b–d) monthly time series of the three oceanographic variables at the sampling localities. Maximum (top, grey), mean (centre, bold) and minimum values (bottom, grey) scale each series; maxima and minima location are displayed with dots above and below the curves.

BA-SW is particularly interesting. Although we see no significant genetic structure and a relatively high migration rate between these two putative populations, BA-SW is significantly different to all other putative populations for one or both genetic markers. In addition, recent satellite tracking of five female and three male

Table 4 Summary results for the IBD and IBED tests. The significance [$P(r \leq 0)$], slope values (r), and correlation coefficients (R^2) of the correlations between genetic (mtDNA and nuDNA), geographical (km) and environmental (CHL, K490, SST) distances are presented. The slope between F_{ST} and km is expressed as F_{ST}/100 km. The last three rows (−km) assess the significance of the correlations between each environmental variable, while controlling for geographical distance, through Partial Mantel tests. Bold values correspond to significant correlations ($P < 0.05$)

	mtDNA			nuDNA		
	$P(r = <0)$	r (slope)	R^2	$P(r = <0)$	r (slope)	R^2
Geographical distance (km)	**0.01**	0.027	0.337	0.1	0.009	0.281
Chlorophyll (CHL)	0.09	0.022	0.128	**0.006**	0.007	0.394
Turbidity (K490)	0.09	0.779	0.156	**0.005**	0.279	0.379
Sea surface temperature (SST)	**0.044**	0.094	0.216	**0.008**	0.034	0.279
CHL (−km)	0.28			**0.042**		
K490 (−km)	0.3			**0.049**		
SST (−km)	0.47			0.24		

franciscanas for 189 days in the BA-SW area shows highly resident movement patterns that do not overlap with the BA-S area (Bordino *et al.* 2008). Considering the genetic data and assuming that the satellite tracking findings are representative for most BA-SW individuals, it could be that these two putative populations are just beginning to diverge and that this presumed divergence is yet too recent to result in genetic differentiation. Another interpretation would be that a few BA-SW individuals, not detected by the satellite tracking work, could reach BA-S (and/or vice versa) and interbreed there, impeding genetic differentiation.

The general genetic structure patterns are supported by the Bayesian analysis, which suggests the presence of three genetic clusters in the area, with a second likelihood peak at $K = 5$. Cases of bimodal ln $P(K)$ can be explained by hierarchical genetic structure patterns (Hubisz *et al.* 2009). We therefore interpret that Structure may mainly be resolving the higher hierarchical structure in our study area, identifying a Northern Buenos Aires assemblage grouping SW and SS (individuals with higher assignment to the black cluster in Fig. 2b), an Eastern Buenos Aires assemblage grouping CSA and BA-E (higher assignment to grey and white clusters, Fig. 2b), and a Southern Buenos Aires assemblage grouping BA-S and BA-SS (higher assignment to white cluster, Fig. 2b).

Environmental structure

We have provided clear evidence of environmental heterogeneity at different spatial and temporal scales in our study area. The satellite-derived maps allowed a qualitative assessment of structure and variability in the environmental data, which was complemented by a quantitative evaluation between contiguous sites and for all pairwise comparisons. In addition, the climatologic assessments, although qualitative, allowed exploring aspects of the data that may be related with biological seasonality, which in turn could be relevant for population structuring.

The fact that productivity, turbidity and temperature mean values were significantly different between all contiguous population areas suggests strong heterogeneity of marine productivity, and highlights these variables as potentially significant for habitat occupancy, dispersal and hence population structure, in accordance to previous findings in other cetaceans (Forney 2000; Baumgartner *et al.* 2001; Hamazaki 2002). The mixed effects model allowed us to evaluate statistically such differences at a broader spatial scale and even account for seasonal trends in the data, showing clear and statistically significant differences between all population areas for chlorophyll and turbidity. Given the strong regional seasonality in temperature, it is not surprising that the mixed effects regression model attributed most of the temperature regional variation to seasonal trends, and little variation to sampling sites. Perhaps, the most illustrative display of comparative environmental data in our study is the unprocessed time-series panel. Although this display does not have statistical value per se, it does allow visualizing the entire data set at its finest resolution, and enhances our understanding of the causes of statistical significance in our other tests.

Seasonal environmental patterns probably have a special biological significance, as most cetaceans show some degree of seasonal variations in most demographic and ecological parameters (Reilly 1990; Chaloupka *et al.* 1999; Martin & da Silva 2004). Our climatologic assessment was intended as a first qualitative approach to this issue, showing noticeable climatologic differences between most of the franciscana population areas. Because chlorophyll concentration accounts for part of the water turbidity, it is not surpris-

ing that these two variables are seemingly correlated for a given sampling area. However, turbidity is also caused by other variables (i.e. dissolved solids) and it is therefore important to measure both these variables. The general similarity between the temperature patterns for different localities probably responds to the regional seasonality of this variable (clearly detected by our linear model), whereas the differences in the warmest month between areas are probably influenced by local oceanographic processes such as currents and water mixing (Guerrero *et al.* 1997).

Integration of genetic and environmental data

Based on our collective analyses, we show that every putative population (excepting BS-SW) is genetically differentiated from its contiguous populations, and that their population areas are environmentally distinct for all three variables. This is an interesting result suggesting that environmental discontinuities can influence marine population structure, irrespective of the particular shape of those discontinuities. The environmental distinctiveness between the northern sites is particularly interesting given the geographical proximity (SW and SS are 70 km apart, SS and CSA are 35 km apart, and CSA and BA-E are 70 km apart). Although dolphins are considered as highly mobile marine animals, franciscanas in this area show restricted movement patters between putative population areas, which are environmentally very different. Although we cannot attribute causality to this concordance, the documented influences of oceanographic variables to cetacean distribution and abundance, and the relationship between distribution patterns, dispersal and population structure, suggest that these variables *may* actually be driving patterns of population structure in mobile marine species. Testing for this causality would be particularly challenging, if not unfeasible given the available data. Presuming we had precise estimations of divergence time between populations (which we do not have and are not possible to estimate without appropriate demographic scaling factors), we would need to show that this genetic divergence was posterior to some particular environmental change causing the observed oceanographic patterns in the area. However, the kind of oceanographic data necessary to evaluate this possibility dates back to only one or two decades at most, making such an evaluation impossible. Therefore, our above-mentioned hypothesis is solely based on the evidence presented here and on what is known and accepted for marine mobile species.

The most parsimonious mechanism that accounts for the observed genetic structure, IBD, has been commonly proposed for cetaceans (Rosel *et al.* 1999; Krützen *et al.* 2004) and other mobile marine species like sea lions (Chivers *et al.* 2002; Gonzalez-Suarez *et al.* 2009). However, the franciscana genetic patterns are consistent with IBD only when assessed with the mtDNA data, whereas the microsatellite data show no evidence of IBD. Female philopatry, which has been documented in other cetaceans (Greenwood 1980; Gladden *et al.* 1999) could drive IBD and explain the lack of significance of IBED for the mtDNA data. Conversely, male-biased dispersal has been shown to be associated with lack of genetic structure in nuclear markers (Greenwood 1980; Möller & Beheregaray 2004) and can produce a lack of IBD patterns (Hoelzel 1994). The lack of significance of IBD in the microsatellite data set points to other potentially relevant mechanisms causing the genetic patterns. Interestingly, IBED is highly consistent with our microsatellite data, even after controlling for the effect of geographical distance. This finding suggests that environmental distance, but not geographical distance, could be influencing dispersal patterns in mobile marine organisms with no strong behavioural ties to their natal sites (i.e. males in some cetacean species). We should highlight, however, that we are proposing these mechanisms of female philopatry and male-biased dispersal based on what is known about cetacean ecology, and that such hypotheses should be explicitly tested when a representative sample of females and males becomes available for all putative populations. Moreover, and irrespective of the mechanisms accounting for the presence/absence of IBD/IBED in mtDNA/nuDNA, we recognize that adding new data sources into the analysis results in a more complex final picture than that resulting from the genetic data alone, and that the overall interpretation of our results will necessarily be equally complex. This complexity is a better reflection of reality, and therefore a step forward in our general understanding of dispersal and structure among marine populations.

Although ecological and population genetic theory indicate that environmental factors are plausible determinants of population structure, this is an expectation that remained largely untested for mobile marine species, with very few exceptions (i.e. Fullard *et al.* 2000; Gaggiotti *et al.* 2009). Our evaluation explicitly tested the interaction between environmental and population structure patterns in cetaceans, showing that environmental discontinuities can influence population structure, and that such influence can take a pattern of IBED. These results could enhance our understanding of other marine taxa with similar ecological niches, dispersal abilities and behavioural features.

By providing evidence supporting the influence of environmental variables on population structure patterns, we do not discount the importance of behavioural processes driving population structure. Moreover,

population structure is the resulting signature of the suite demographic, ecological, environmental and behavioural processes (Gaggiotti *et al.* 2009). Cetaceans are highly social species, and it is therefore possible that the movement patterns and observed population structure respond also, or at least in part, to sociality, group interactions and philopatry (Hoelzel 1998). Foraging specializations, for instance, have been shown to have the potential to influence population structure patterns in bottlenose dolphins (*Tursiops truncatus*) and Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in Australia (Chilvers & Corkeron 2001; Chilvers *et al.* 2003). To better understand the role of each type of mechanism on population structure, we suggest that behavioural studies and environmental data be integrated to genetic assessments of population structure.

Conservation and management implications

Genetic evidence (mtDNA) suggesting the existence of at least two franciscana populations in Brazil (Secchi *et al.* 1998), a third one in Uruguay and a fourth one in Southern Argentina, BA-S in our current study (Lazaro *et al.* 2004), prompted proposals of franciscana Management Areas (FMAs) I to IV respectively (Secchi *et al.* 2003). A posterior analysis incorporating over 100 franciscana specimens from previously unsampled areas in Argentina uncovered finer-scale population structure within FMA IV (Mendez *et al.* 2008). This analysis documented a new, genetically differentiated population in northern Buenos Aires (BSS in our current study), suggesting the existence of at least two franciscana populations in Argentinean waters.

Our current analysis of fixation indices supports all previous coarse findings and provides greater resolution by doubling the sample size in Argentina, and incorporating microsatellite markers in the assessment. Because the Structure results are not as clear-cut as those from the fixation indices, especially given the ambiguity of some of the assignment values, our considerations of population structure implications are drawn from the collective evidence provided by the fixation indices and the Bayesian framework. Considering all the available data, we suggest that they could be explained by the existence of three biologically meaningful franciscana populations: Northern Buenos Aires (BSW and BSS), Eastern Buenos Aires (CSA and BAE) and Southern Buenos Aires (BAS and BASS). More fine-scale structure, particularly in Northern Buenos Aires, seems likely but should be further investigated with larger sample sizes before any strong statements can be made. In its present state, our genetic data simply question the validity of a single FMA in Argentina, if such units are meant to conserve distinct franciscana populations. We therefore propose that FMA IV be updated to display areas where distinct franciscana populations are found and correspond to previously unknown population structure.

Finally, multidisciplinary evaluations are important to complement efforts aimed at pinpointing biologically and ecologically relevant areas for protection and management. Our detection of concordant biological (i.e. genetic data and movement patterns) and oceanographic boundaries provides a clear example for the Northern Buenos Aires area. This area houses a differentiated franciscana population (possibly two) and is characterized by unique oceanographic regimes. Additional evidence of residency patterns of franciscanas in this area (Bordino *et al.* 2008), frequent sightings of adult females, calves and juveniles (Bordino, personal communication), and high fish and invertebrate biomass (Guerrero *et al.* 1997; Jaureguizar *et al.* 2004) could indicate that Northern Buenos Aires is used by franciscanas for breeding or calving. Considering all evidence, we suggest that this area should be protected to advance ongoing conservation efforts for this species.

In summary, we provide the most comprehensive genetic structure assessment in the southern portion of the franciscana dolphin distribution, and the first assessment combining mtDNA with nuclear markers for the species. Moreover, this work is one if the very few cetacean genetic assessments combining mitochondrial and nuclear markers with a suite of relevant spatially explicit oceanographic data sets, to account quantitatively for environmental drivers of population structure. Although this analysis is far from encompassing all potentially relevant aspects of cetacean or other mobile marine species' population structure, it is an important step combining typically disconnected data sets for understanding the molecular ecology of highly mobile marine species.

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This work is part of M.M.'s thesis research on environmental influences to cetacean dispersal. His research focuses on the use of molecular tools for ecology and conservation, particularly on gaining a better understanding of the environmental forces influencing marine dispersal and spatial population structure. H.R. directs the Wildlife Conservation Society's Ocean Giants Program, which aims to conserve significant populations of whales, dolphins, sea turtles and sharks. A significant focus of his research program involves the use of genetic approaches to understand important biological parameters, relationships, trends and ecological requirements of cetaceans to better promote their conservation. A.S. is a biological oceanographer at the Lamont Doherty Earth Observatory at Colum-

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1 Optimized PCR settings within the nested reaction for each microsatellite locus

Table S2 Geographical and environmental distances between putative populations' areas

Table S3 Genetic diversity indices of the mtDNA sequence data

Table S4 Genetic diversity indices of the microsatellite data, for each locus

Table S5 Individual runs for the Bayesian population structure analysis

Table S6 Likelihood-based migration rate runs. Individual run results are displayed

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