Population structure in a highly pelagic seabird, the Cory's shearwater *Calonectris diomedea*: an examination of genetics, morphology and ecology

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ABSTRACT: Increasing evidence suggests oceanic traits may play a key role in the genetic structuring of marine organisms. Whereas genetic breaks in the open ocean are well known in fishes and marine invertebrates, the importance of marine habitat characteristics in seabirds remains less certain. We investigated the role of oceanic transitions versus population genetic processes in driving population differentiation in a highly vagile seabird, the Cory's shearwater, combining molecular, morphological and ecological data from 27 breeding colonies distributed across the Mediterranean (*Calonectris diomedea diomedea*) and the Atlantic (*C. d. borealis*). Genetic and biometric analyses showed a clear differentiation between Atlantic and Mediterranean Cory's shearwaters. Ringing-recovery data indicated high site fidelity of the species, but we found some cases of dispersal among neighbouring breeding sites (<300 km) and a few long distance movements (>1000 km) within and between each basin. In agreement with this, comparison of phenotypic and genetic data revealed both current and historical dispersal events. Within each region, we did not detect any genetic substructure among archipelagos in the Atlantic, but we found a slight genetic differentiation between western and eastern breeding colonies in the Mediterranean. Accordingly, gene flow estimates suggested substantial dispersal among colonies within basins. Overall, genetic structure of the Cory's shearwater matches main oceanographic breaks (Almería-Oran Oceanic Front and Siculo-Tunisian Strait), but spatial analyses suggest that patterns of genetic differentiation are better explained by geographic rather than oceanographic distances. In line with previous studies, genetic, phenotypic and ecological evidence supported the separation of Atlantic and Mediterranean forms, suggesting the 2 taxa should be regarded as different species.

KEY WORDS: Almería-Oran Oceanic Front · Atlantic Ocean · Biometrics · Genetic differentiation · Mediterranean Sea · Marine transition · Oceanic habitat · Procellariforms · Ringing-resight

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INTRODUCTION

The open ocean offers a vast landscape with few physical barriers to dispersal compared to terrestrial environments. This is particularly true for seabirds, which have been shown to travel vast transoceanic distances (Schreiber & Burger 2002), yet many species have very restricted breeding distributions and conspecific populations often show geographic differences in vocalizations, body size and plumage (see Brooke 2004). In marine organisms, geographical distance, *per se*, can be responsible for generating patterns of isolation by distance (Palumbi 1994). However, oceanic transitions can also act as effective barriers to gene flow (Nielsen et al. 2004, Bekkevold et al. 2005, Natoli et al. 2005). In this case, selection pressures imposed by differences in the oceanic habitat may lead to local habitat adaptation. Indeed, several marine species dis-

playing phylogeographic structure show sharp genetic breaks in the marine environment (for review see Patarnello et al. 2007), including seabirds (Friesen et al. 2007). However, little is known about the origin as well as the maintenance of these oceanic barriers in seabirds.

The Atlantic–Mediterranean transition offers an interesting case study to investigate the impact of oceanographic breaks on marine biota. Even though the Mediterranean Sea and the Atlantic Ocean are contiguous basins and exchange water, salt and heat, their physical and oceanographic characteristics are clearly distinct (Longhurst 1998). The Atlantic and the Mediterranean are connected through the Gibraltar Strait, a 14 km wide channel which is characterized by a 2-layer flow regime. Atlantic waters inflow in the upper layer and Mediterranean waters outflow in the lower layer (Özgökmen et al. 2001). The Strait of Gibraltar has been shown to represent a barrier for some fish species and invertebrates (e.g. review by Borsa et al. 1997), but also for marine mammals (e.g. *Balaenoptera physalus*, Bérubé et al. 1998) and seabirds (e.g. European storm petrel *Hydrobates pelagicus*, Cagnon et al. 2004). For other species, a more likely boundary to gene flow was identified as an oceanic front some 350 km to the east of the Strait of Gibraltar in the Alboran Sea, where the colder water from the Atlantic enters into the warmer Mediterranean, creating a quasi-permanent anticyclonic gyre that generates an oceanographic break known as the Almería-Oran jet (Millot 1999) (see Fig. 1). Genetic differentiation across this oceanographic break has been observed in the European hake *Merluccius merluccius* (Cimmaruta et al. 2005), the common cuttlefish *Sepia officinalis* (Pérez-Losada et al. 2007), Mediterranean mussels *Mytilus galloprovincialis* (Quesada et al. 1995) and bottlenose dolphins *Tursiops truncatus* (Natoli et al. 2005). Recent evidence also suggests a third transition zone between the eastern and western Mediterranean around the Siculo-Tunisian Strait (examples of genetic breaks at this transition are common sole *Solea vulgaris*, Guarniero et al. 2002, and sea bass *Dicentrarchus labrax*, Bahri-Sfar et al. 2000). Previous studies suggest a role of oceanographic boundaries as effective barriers in some pelagic seabirds (Gómez-Díaz et al. 2006, Friesen et al. 2007), yet more detailed and integrative studies can help provide further insights into the underlying processes. In this context, new disciplines such as landscape genetics that integrate population genetics, landscape ecology and spatial statistics provide the framework to test the role of environmental features on gene flow, genetic discontinuities and population structure (Manel et al. 2003, Storfer et al. 2007).

Understanding the dynamics of gene flow between closely related taxa across a transition zone may pro-

vide insight into the process of genetic differentiation and, ultimately, speciation. Hybridization with or without introgression (i.e. the backcrossing of individuals between populations with the subsequent fixation of new genes) is relatively common in transition zones where ecological shifts between habitats occur, so the gene flow barrier is reinforced by different habitat preferences of the populations on either side (Arnold 1992, Harrison 1993). But dispersal in the absence of effective gene flow (i.e. movement of individuals without genetic exchange) can be misinterpreted as hybridization and introgression events at the transition zones. Contrary to the expected pattern for migration, we will find a mismatch between individual genetic and morphologic traits only if hybridization occurs. Thus, disentangling introgression from immigration requires an assessment of geographic variation in phenotypic and genetic patterns, combined with contemporary records regarding movements. This is particularly important in the case of seabirds because dispersal among breeding localities is common in many species (Goostrey et al. 1998, Pons et al. 2004), but does not necessarily lead to successful breeding and thus effective gene flow (Dearborn et al. 2003). Indeed, most studies are based on a single data source (e.g. genetic), which may have masked the potential role that introgression plays among seabirds, and only a few studies have compared phenotypic and genotypic traits to assess introgression (Gay et al. 2007, Genovart et al. 2007).

In the present study we explored population structure within and between the Atlantic and Mediterranean basins in the Cory's shearwater *Calonectris diomedea*, a pelagic seabird species. Cory's shearwater comprises Atlantic (*C. d. borealis*), and Mediterranean (*C. d. diomedea*) subspecies, although their subspecific status is still a subject of taxonomic debate (Heidrich et al. 1998, Camphuysen & Van Der Meer 2001). Basically, *C. d. borealis* breeds on Atlantic islands, whereas *C. d. diomedea* breeds in the Mediterranean. Both ecological and genetic data suggest a substantial differentiation between the 2 subspecies reported as marked differences in genetics, morphology and behaviour (e.g. colour, size and vocalizations) (Randi et al. 1989, Bretagnolle & Lequette 1990, Granadeiro 1993, Wink et al. 1993, Heidrich et al. 1996, Thibault et al. 1997, Heidrich et al. 1998, da Silva & Granadeiro 1999, Gómez-Díaz et al. 2006), and only one genetic study on nuclear DNA found genetic structuring of populations within each subspecies, although no spatial pattern was apparent (Rabouam et al. 2000). In line with these results, mark-recapture data indicate high philopatry and site fidelity (see review by Thibault et al. 1997). However, short- and long-range dispersal movements are well documented

(Thibault et al. 1997), some cases of hybridization have been identified (Martínez-Abraín et al. 2002) and small breeding colonies of both Atlantic and Mediterranean forms have been reported in the opposite basin (Thibault & Bretagnolle 1998, Gómez-Díaz et al. 2006, Mays et al. 2006). In this context, the extent of exchange, the effective divide and spatial determinants of their current distributions remain uncertain. The integration of different sources of data (i.e. landscape genetics) together with extensive geographic coverage can help understand patterns of population structure in Cory's shearwaters.

Here we present a comprehensive approach combining genetic, morphologic, spatial and ecological data from Cory's shearwaters to: (1) investigate how mitochondrial genetic and body size variation is distributed between and within subspecies; (2) evaluate levels of genetic differentiation and the importance of introgression and immigration processes between basins; and (3) assess the influence of geographic distances and marine habitat characteristics on the population structure of the species.

MATERIALS AND METHODS

Study species and sampling. Cory's shearwaters breed on islands across the Mediterranean from the Iberian coast to the Adriatic and Aegean Seas (mainly *Calonectris diomedea diomedea*), and in the northeast Atlantic from the Canary to the Azores Islands (mainly *C. d. borealis*) (Thibault & Bretagnolle 1998, Gómez-Díaz et al. 2006). From 2001 to 2006 we collected blood samples from adult birds from 27 breeding colonies of Cory's shearwaters across the Mediterranean and the Atlantic regions (Fig. 1, see Table 1). Because of previous genetic studies on the species (Gómez-Díaz et al. 2006), in subsequent analyses we considered Almería as an Atlantic Cory's shearwater colony within the Mediterranean (Fig. 1).

Oceanographic data. Three oceanographic variables were analysed: sea surface salinity (Sal), chlorophyll production (chl) and sea surface temperature (SST). Data on salinity was obtained from the National Oceanographic Data Centre (NODC, www.nodc.noaa. gov/), while chl and SST data were obtained from the NASA Sea-viewing Wide Field-of-view Sensor database (SeaWiFS, http://seawifs.gsfc.nasa.gov/) and the NASA Aqua MODIS satellite database (http://modis. gsfc.nasa.gov/). Satellite tracking data on adult Cory's shearwaters breeding in Gran Canaria (Canary Islands) suggested they mostly feed within a foraging range of 500 km from the colony (14 tracked birds from 1 colony during chick-rearing: mean distance covered = 875.16 ± 364.31 km, mean trip duration = 4.34 ± 1.46 d) (Navarro & González-Solís 2009). This data is consistent with previous studies on Cory's shearwaters tracked in the Azores Islands during the same breed-
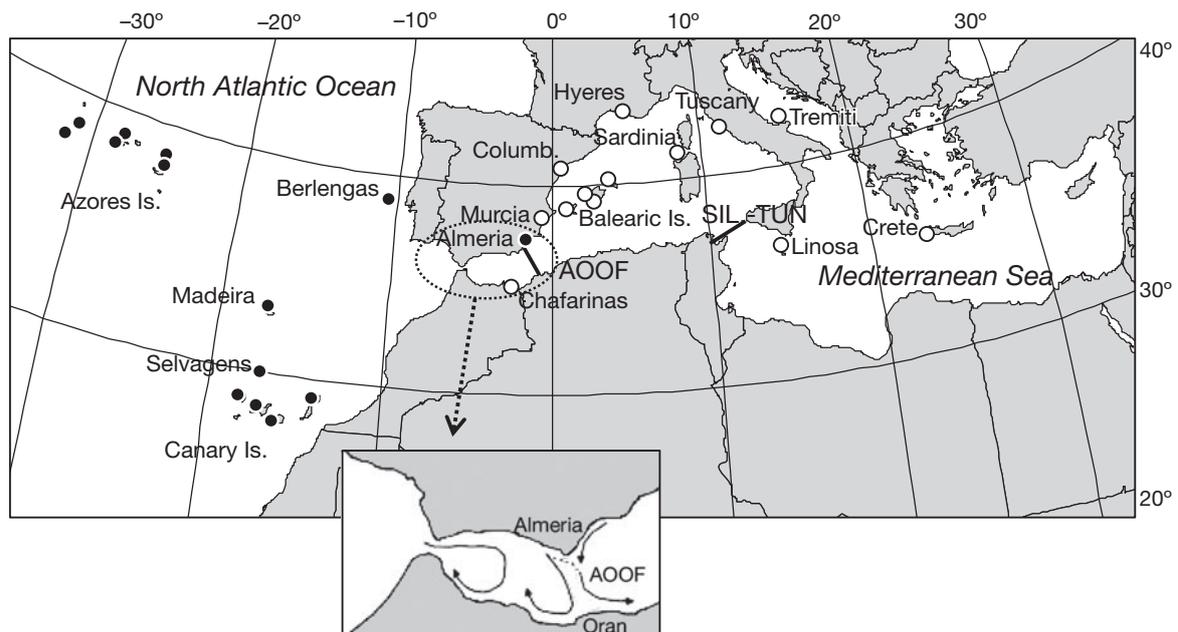

Fig. 1. Breeding colonies of Mediterranean (*Calonectris diomedea diomedea*, ○) and Atlantic (*C. d. borealis*, ●) Cory's shearwaters sampled across their geographic distribution. The Almería-Oran Oceanic Front (AOOF) and the Siculo-Tunisian Strait (SILTUN) are shown. The Balearic Islands include Mallorca, Cabrera, Menorca and Ibiza; the Azores Islands include Flores, São Miguel, Faial, Graciosa, Corvo and St. Maria; and the Canary Islands include Gran Canaria, Tenerife and Lanzarote. Columb.: Columbretes Islands. Inset shows circulation pattern in the Almeria region

ing period (82 birds from 3 colonies) (Magalhães et al. 2008), and in the Canary Islands during the entire breeding season (6 birds from 1 colony) (Navarro et al. 2007). To define the oceanographic characteristics for each breeding colony we estimated mean values of the 3 oceanographic variables during the breeding season of the species from April to September 2003 to 2006 within a radius of 100, 300 and 500 km of the colony.

Genetic analyses. *DNA isolation, amplification and sequencing:* We amplified a 293 bp fragment of Domain I of the mitochondrial control region of *Calonectris* spp. (see Gómez-Díaz et al. 2006 for protocols). To control for possible sequencing errors, for most birds both DNA strands were sequenced. A tandem duplication of part of the mitochondrial control region has been previously reported in other Procellariiform species by Abbott et al. (2005). In their study, the 2 control region copies were not identical in sequence or in length, whereas in the present study, all control region sequences were of similar length and appeared homologous with a number of conserved sequence blocks. In addition, to control for single site heteroplasmy (Moum & Bakke 2001, Genovart et al. 2007), we compared control region sequences to previously published sequences of sister *Puffinus* spp., various individuals were re-sequenced twice and we checked for ambiguity between forward and reverse sequence strands. All sequences are available in Gen-Bank (accession nos. FJ755483 to FJ755616). A total of 241 samples were analysed (Fig. 1, see Table 1). Of these, 197 samples were sequenced in the present study for the first time and combined with 44 previously sequenced Cory's shearwater samples (Gómez-Díaz et al. 2006) for the same loci.

Population genetic structure: To test whether genetic variation deviated from neutral expectations, we performed Ewens-Watterson and Chakraborty's tests (Ewens 1972, Watterson 1978, Chakraborty 1990) using Arlequin 3.0 (Excoffier 2005). We calculated Φ_{ST} statistics (Kimura 2-parameter mutation model, Kimura 1980) among all pairs of breeding colonies. We calculated haplotypic diversity (H_s, Nei 1987) and nucleotide diversity (π, Nei 1987) at the interspecific (between Atlantic and Mediterranean Cory's shearwaters) and intraspecific levels (among colonies within each subspecies) using DnaSP version 4.0 (Rozas et al. 2003). Net sequence divergence between the 2 Cory's colony groups (average no. differences) were calculated using MEGA version 4.1 (Tamura et al. 2007).

We evaluated population genetic structure based on the Φ_{ST} estimates or Wright's fixation index of population differentiation (Φ_{IS}: among individuals within populations, Φ_{ST}: among populations within groups, and Φ_{CT}: among groups of populations) using the nested analysis of molecular variance (AMOVA) (Excoffier et al. 1992) included in Arlequin 3.0 (Excoffier 2005). We conducted 2 AMOVAs. First, to test the existence of a Mediterranean–Atlantic genetic transition, we defined 2 groups of breeding colonies corresponding to each Cory's shearwater subspecies (the Almería colony close to the Almería-Oran Oceanic Front was placed within the Atlantic subspecies group, see Fig. 1). Within each subspecies group we pooled together colonies corresponding to archipelagos (Azores, Canary and Balearic Islands). Second, to test the presence of a genetic break around the Siculo-Tunisian Strait, we conducted an AMOVA grouping breeding colonies in the Eastern and Western Mediterranean. Φ_{ST} estimates were tested for significance with 10 000 randomizations of the data. In both cases, data were pooled, treating each breeding colony as a separate sample within a particular group (i.e. Atlantic and Mediterranean or Eastern and Western Mediterranean, respectively).

To visualize the genetic relationships among individuals we generated an haplotype tree using neighbournet algorithm in SplitsTree version 4.6 (Huson & Bryant 2006). In addition, a population tree was constructed on Slatkin's linearized Φ_{ST} (Kimura 2-parameter mutation model) using SplitsTree.

Dispersal pattern and gene flow: We estimated gene flow in Cory's shearwaters between the Atlantic and the Mediterranean colonies and among colonies within each region using a non-equilibrium method based on coalescent theory and Bayesian statistics implemented in the program MDIV (Nielsen & Wakeley 2001). Within each region we also estimated gene flow between Eastern and Western breeding colonies within the Mediterranean (see AMOVA results). We ran MDIV under the finite sites mutation model (Hasegawa et al. 1985) using the default set parameters of one chain (Markov chain length = 2 000 000 cycles, burn-in time = 500 000 cycles), M_{max} and T_{max} set at 10 and 5 respectively, and 3 different random seeds. To test whether M (number of migrants in females per generation) or T (time of divergence) were significantly different from zero, we used a maximum likelihood test as described in Nielsen & Wakeley (2001). Only those colony pair comparisons for which T differed from zero provide reliable estimates of M_i for all others (T not different from zero) we re-ran MDIV. In this case, M_{max} was set between 1 and 200 (depending on what value approximated a Poisson-shaped posterior probability distribution), and T_{max} was set between 1 and 20 (depending on what value yielded an asymptotic posterior probability distribution).

Spatial patterns of genetic variation: To investigate the existence of spatial patterns in the genetic structure of the species, we first examined the isolation by distance model by measuring the correlation between

genetic and geographic distances among colony pairs (Rousset 1997). Second, we examined habitat mediated differentiation by correlating genetic distances with measures of oceanographic features, as indicated by differences in the 3 oceanographic variables analyzed. Spatial analyses were first carried out including all breeding colonies. In a second stage, we analyzed Atlantic and Mediterranean Cory's shearwater breeding colonies separately.

Genetic relationships among colonies were calculated as Slatkin's linearized Φ_{ST} ($\Phi_{ST}/1 - \Phi_{ST}$) pairwise genetic distances using Arlequin. Geographic distances were calculated as the log of by-sea geographic distances between colony pairs. Oceanographic variables (chl, Sal and SST) were transformed into a matrix of pairwise Euclidean distances using SPSS version 15.0; i.e. the larger the Euclidean distance the more dissimilar the oceanographic value between localities. We first applied a sim-

ple Mantel test analysis using 'zt' software (Bonnet & Van de Peer 2002) to test the correlation of these distance matrices to each other. Significance was tested by a randomization procedure permuting the *n* objects of one of the distance matrices (10 000 randomizations). Environmental data frequently exhibit some degree of spatial autocorrelation; in our case similarities in oceanographic traits among colonies may be strongly influenced by their geographic distances. Thus, to control for the effect of the spatial structure of our data, we then applied a partial Mantel test (Smouse et al. 1986) using the 'zt' software. This test computes the degree of correlation between 2 distance matrices, *A* (genetic distances) and *B* (oceanographic or geographic distances), while controlling for the effect of a third matrix, *C* (geographic or oceanographic distances). This is achieved by regressing each matrix by the third one and then computing a Mantel test with the residuals of the regression. In this case,

Table 1. *Calonectris diomedea diomedea* and *C. d. borealis*. Number of birds sampled, localities and geographic coordinates from breeding colonies of Cory's shearwaters. Mean colony values of chlorophyll concentration (chl), sea surface temperature (SST) and salinity (Sal) within 500 km radius of the breeding colony are indicated (see 'Materials and methods' for calculations). Genetic statistics for each breeding colony as mean nucleotide (P_i) and haplotypic (H_d) diversities are also shown. Population size estimates (breeding pairs) are taken from Thibault (1997), Bolton (2001), Paracuellos et al. (2003), Carboneras & Lorenzo (2003), BirdLife International IUCN (2004), and Granadeiro et al. (2006)

Locality	Geographic region	Pop. size	n	Coordinates (°) Latitude	Longitude	Oceanographic features Chl	SST	Sal	Genetic statistics P_i	H_d
C. d. diomedea										
Mallorca	Balearic Islands		11	39.5833	2.3667	83.73	32.590	37.42	0.029 ± 0.005	1.000 ± 0.039
Menorca	Balearic Islands		10	39.8020	4.2878	83.66	32.601	37.46	0.024 ± 0.004	1.000 ± 0.045
Ibiza	Balearic Islands		12	38.9618	1.1983	83.65	32.714	37.31	0.024 ± 0.003	0.985 ± 0.040
Cabrera	Balearic Islands	11 000	6	39.2024	2.97514	83.52	32.607	37.42	0.019 ± 0.004	1.000 ± 0.096
Columbretes	Spain	70	10	39.8500	0.6500	85.36	32.542	21.46	0.021 ± 0.003	0.956 ± 0.059
Murcia	Spain	30	10	37.5833	−0.9833	87.40	32.789	37.14	0.019 ± 0.002	0.844 ± 0.103
Tremiti	Italy		10	42.1256	15.4939	85.11	33.392	37.93	0.020 ± 0.003	0.933 ± 0.077
Tuscany	Italy		6	42.4000	11.8667	85.66	32.971	37.80	0.026 ± 0.004	0.933 ± 0.122
Sardinia	Italy		4	41.0756	8.2606	82.63	32.680	37.68	0.029 ± 0.007	1.000 ± 0.177
Linosa	Italy	15 000–18 000	9	35.8667	12.8667	76.80	33.900	37.74	0.017 ± 0.005	0.917 ± 0.092
Hyeres	France	970–1200	10	43.0089	6.2106	86.91	32.189	37.79	0.030 ± 0.004	1.000 ± 0.045
Chafarinas	Morocco coast	1000	10	35.1833	−2.4167	91.98	32.126	36.76	0.032 ± 0.005	1.000 ± 0.045
Crete	Greece	5000	9	36.4423	25.2272	66.14	33.812	38.63	0.024 ± 0.005	0.917 ± 0.092
Total n			117							
C. d. borealis										
St. Maria	Azores Islands		9	36.9420	−25.1710	71.62	31.617	36.29	0.022 ± 0.003	1.000 ± 0.052
Graciosa	Azores Islands		10	39.0557	−27.9549	78.64	30.538	36.08	0.020 ± 0.004	0.978 ± 0.054
Corvo	Azores Islands		10	39.6745	−31.1060	81.71	30.431	36.10	0.019 ± 0.002	0.889 ± 0.075
Faial	Azores Islands		7	38.5244	−28.7468	77.61	30.947	36.15	0.016 ± 0.002	0.905 ± 0.103
Flores	Azores Islands		9	39.3749	−31.1974	80.96	30.626	36.10	0.022 ± 0.004	0.972 ± 0.064
São Miguel	Azores Islands	188 000	10	37.7064	−25.4433	73.83	31.190	36.21	0.023 ± 0.003	0.978 ± 0.054
Madeira	Portugal	2000	10	32.3445	−16.4857	65.85	32.410	36.55	0.023 ± 0.003	0.956 ± 0.059
Selvagens	Portugal	30 000	7	30.1333	−15.8667	70.34	32.607	36.59	0.023 ± 0.003	1.000 ± 0.076
Berlengas	Portugal	200	10	39.4089	−9.4939	93.59	28.844	35.97	0.023 ± 0.003	0.978 ± 0.054
Gran Canaria	Canary Islands		5	27.8456	−15.7887	75.42	32.804	36.64	0.027 ± 0.006	0.900 ± 0.161
Lanzarote	Canary Islands		9	29.2930	−13.5372	77.87	32.137	36.56	0.021 ± 0.006	0.944 ± 0.070
Tenerife	Canary Islands		10	28.4460	−16.2333	72.74	32.875	36.67	0.024 ± 0.005	0.844 ± 0.103
La Palma	Canary Islands	30 000	8	28.7800	−17.7965	67.12	33.274	36.71	0.023 ± 0.003	0.893 ± 0.111
Almería	Spain	30	10	37.3489	−1.6507	89.56	32.650	37.11	0.023 ± 0.002	1.000 ± 0.045
Total n			124							

the significance of the r-statistic was determined by applying the permutation approach developed by Anderson & Legendre (1999).

Biometric data. We employed principal component analysis (SPSS version 15.0) to assess variation in morphometrics over the species' range. Two observers (E.G.D. and J.G.S.) measured morphometrics. In addition, based on the geographic distribution of the breeding colonies, we tested significance of the biometric differences between subspecies using a discriminant function analysis. In both analyses we included 4 biometrical measures for 159 sexed individuals included in the genetic analyses: (1) tarsus length, metatarsus length from the depression in the angle of the intertarsal joint to the base of the last complete scale before the toe diverges; (2) wing length, maximum flattened chord from carpal joint to the tip of the longest primary; (3) bill length, exposed culmen from the tip of the hook to the edge of the feather implantation; and (4) bill depth at nostril, thickness of upper and lower mandibles at the nostril. Tarsus length, bill length and bill depth measurements were taken with a dial caliper (±0.01 mm), and the wing was measured with a ruler (±0.5 mm). To avoid the effect of sexual size dimorphism, morphometric variables of males and females were standardized by subtracting the mean value of each sex.

Mark-recovery data. To assess movements of birds and dispersal rates, we compiled ringing and recovery data for Cory's shearwaters from 1050 birds ringed from 1972 to 2002 in Spain (data provided by the Migratory Species Office from the Ministerio de Medio Ambiente of Spain). In addition, we included recovery data from 8 dispersing birds ringed in Italy (data provided by the Italian Ringing Center from the Instituto Nazionale per la Fauna Selvatica). For the ringing-recovery dataset, only birds recovered alive in the breeding colony (trapped *in situ*) during the breeding period (April to October) were considered. We used SPSS version 15.0 to calculate the distance between the ringing and the corresponding recovery latitude and longitude geographic positions. Distance and direction of movements were considered to measure the connectivity among Cory's shearwater populations. We represented ringing and re-sighting records as well as the corresponding distances of dispersing birds obtained with SPSS using ArcView.

RESULTS

Sequence data

The results of the Ewens-Watterson's and Chakraborty's tests were not significant (all p > 0.10), providing no indications of departures from neutrality.

Mitochondrial sequences were highly variable: among the 241 sequences from 27 colonies (Table 1), 57 of 293 sites were polymorphic, including 53 with transitions and 4 with transversions (Appendix 1, available at www.int-res.com/articles/suppl/m382p197_app.pdf). We found 134 different haplotypes (Appendix 1). There was no fixed mutation between the 2 subspecies, while the number of shared variable sites was 31. Net sequence divergence between Atlantic and Mediterranean Cory's shearwaters (Kimura two-parameter model; Kimura 1980) was 3.1% (SE = 0.8; bootstrap 1000 replicates). Haplotypic and nucleotide diversities were high, ranging from 0.84 to 1.00 and from 1.8 to 3.4% for individual colonies, respectively. Private haplotypes were found in all breeding colonies. Most haplotypes appeared in only 1 or 2 populations, with few haplotypes occurring at high frequencies. Indeed, of 134 haplotypes only 32 were shared between 2 or more colonies, while 102 where unique to single colonies. The most representative and geographically widespread haplotypes were H70, H75, H12, H16 and H21, which occurred in 10 (H70), 8 (H75) and 6 colonies (H12, H16 and H21) (Appendix 1).

Population genetic structure and gene flow

Φ_{ST} estimates between breeding colony pairs ranged from 0 to 0.70 and were significantly greater than zero (p < 0.05) in most pairwise comparisons (between some Mediterranean and Atlantic breeding colonies) (Appendix 2, at www.int-res.com/articles/suppl/m382p197_app.pdf). In the hierarchical AMOVA, results suggest strong genetic structuring between Atlantic and Mediterranean breeding colonies (Φ_{CT} = 0.56, p < 0.001, Φ_{ST} = 0.58, p < 0.001, Φ_{IS} = 0.04, p < 0.05). Indeed, the largest variance component was due to differences among groups (56.0%). The global estimate of Φ_{ST} was high (0.58) and significantly greater than zero (p < 0.001). Within each region, estimates of population differentiation were very low but still revealed some genetic structuring within the Atlantic (Φ_{ST} = 0.05, p < 0.05). However, we did not find any significant genetic differentiation among archipelagos (Φ_{CT} = 0.003, p = 0.35). In contrast, within the Mediterranean there was a low but significant genetic differentiation around the Siculo-Tunisian Strait between eastern and western Mediterranean populations (Φ_{CT} = 0.06, p < 0.05).

The haplotype tree grouped most haplotypes into 2 groups according to subspecies (Fig. 2). However, haplotypes corresponding to 3 Mediterranean breeding individuals were placed within the Atlantic cluster: H3 from Chafarinas, H13 from Mallorca and H61 from Hyeres. Conversely, H63, corresponding to one Mediter-

ranean (Crete) and one Atlantic (Tenerife) individual, was placed within the Mediterranean group.

Gene flow between the Mediterranean and Atlantic Cory's shearwater subspecies was estimated as less than 1 female per generation (M = 0.48), and the likelihood ratio (LR) test of MDIV indicated that divergence time (T) is significantly greater than zero (LR = 4.7, p < 0.01), supporting the reliability of the migration rate estimate. In the case of the Siculo-Tunisian Strait, the estimated migration rate was high (M = 10.70), but T was not significantly different from zero (LR = 1.60, p > 0.05). Similarly, within each basin, divergence times among all pairwise colony comparisons from MDIV were not significantly different from zero, and migration rates estimates were inaccurate (data not shown).

Biometrics

Principal component analysis revealed 2 morphologically distinct groups: the larger (in size) *Calonec-*

tris diomedea borealis and the smaller *C. d. diomedea* subspecies, showing a clear segregation among individuals belonging to each taxon. The first 2 factors explained 86.99% of the total variance (Fig. 3, Appendix 3, at www.int-res.com/articles/suppl/m382 p197_app.pdf). Thereafter, discriminate function analysis confirmed that significant morphological divergence exists between Mediterranean (except Almería) and Atlantic colonies (including Almería) sampled in the present study (Wilks' lambda = 0.304, df = 3, p < 0.0001). However, group assignment based on discriminant functions revealed that a slight overlap exists and a few individuals corresponding to Mediterranean colonies were placed within the Atlantic group (7 of 159), while some Atlantic breeders were placed within the Mediterranean group (4 of 159). Of those, there were 4 birds (2 males and 2 females) for which the phenotype also disagrees with their Control Region sequence haplotype (H3, H13, H61 and H63, Fig. 3). More specifically, there are 2 genetically Atlantic birds breeding in the Mediterranean that show an Atlantic
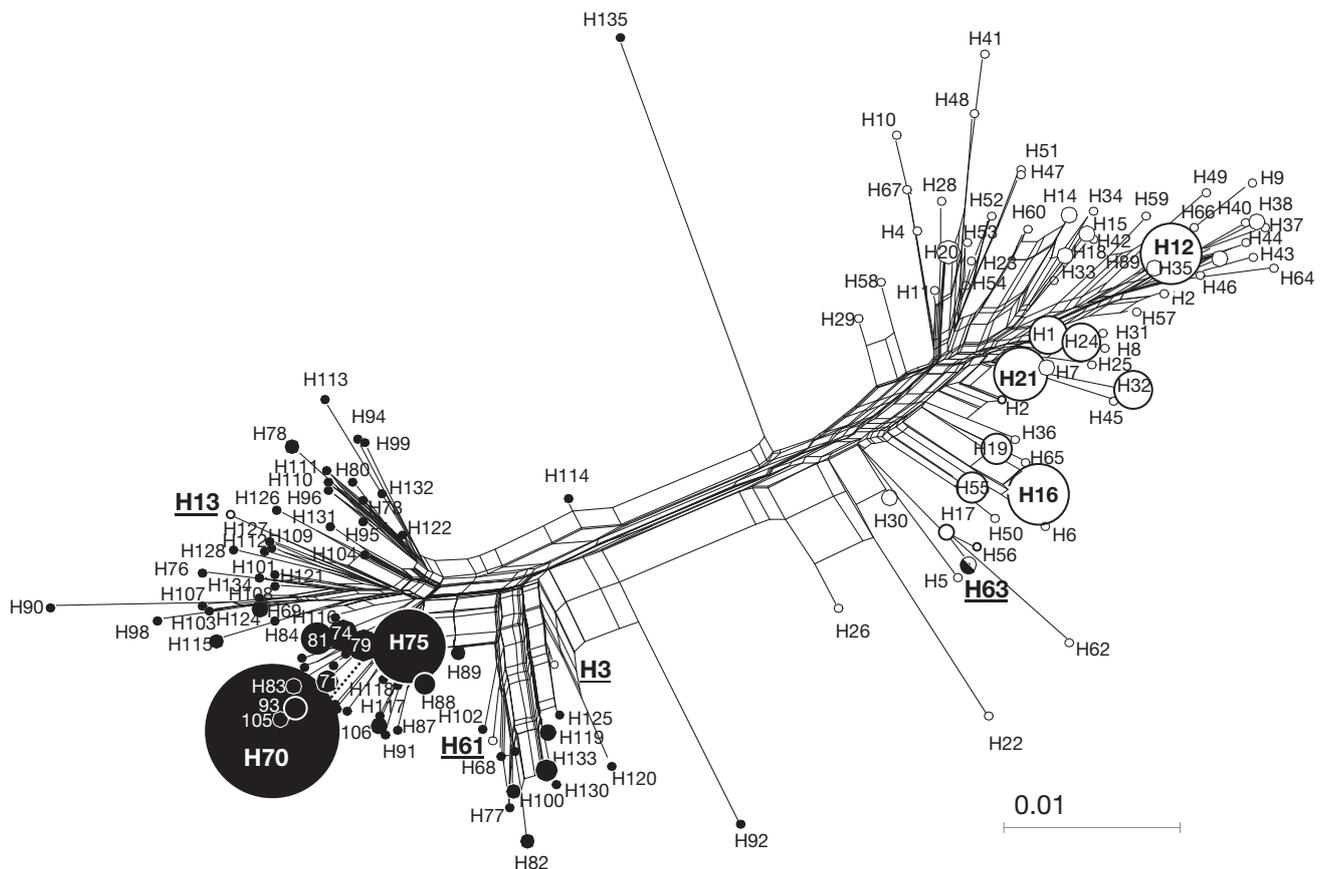

Fig. 2. *Calonectris diomedea diomedea* and *C. d. borealis*. Haplotype network showing genealogical relationships within Cory's shearwater. Haplotypes corresponding to Atlantic and Mediterranean breeders are indicated by black and white circles, respectively. Circle size is proportional to the number of birds sharing that haplotype. Haplotypes for which genotype and breeding area did not match are underlined. Scale bar represents genetic distance

phenotype (H3: Hyeres and H61: Chafarinas), 1 genetically Atlantic bird breeding in the Mediterranean showing a Mediterranean phenotype (H13), and 1 genetically Mediterranean bird breeding in the Atlantic with an Atlantic phenotype (H63).

Ringing-recovery data

Of 1050 ringed adults that were re-sighted, 97.1 % were recovered in the colony where they were ringed. However, we identified 31 birds that moved away from their ringing site (Appendix 4, at www.int-res.com/articles/suppl/m382p197_app.pdf). Of those, 21 were ringed as chicks, suggesting in this case natal rather
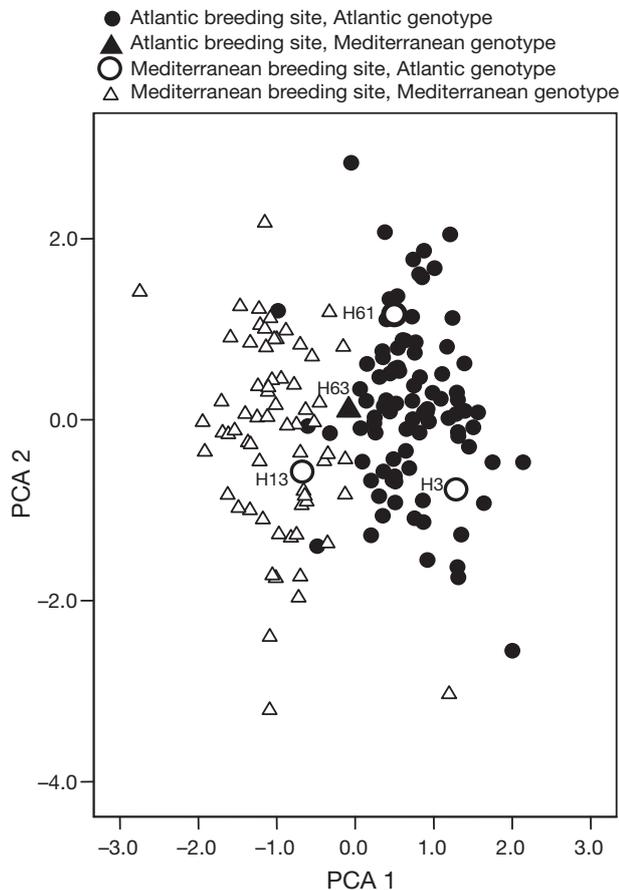

Fig. 3. *Calonectris diomedea diomedea* and *C. d. borealis*. Principle component analysis. Plot of first (PCA 1) vs. second (PCA 2) function scores based on 4 biometric measurements of 159 Cory's shearwater individuals (Appendix 3, at www. int-res.com/articles/suppl/m382p197_app.pdf) included in the genetic analysis. Black and open symbols represent Atlantic and Mediterranean breeding sites, respectively, whereas circles and triangles represent Atlantic and Mediterranean genotypes, respectively. Possible immigrants and introgressed birds are labelled (introgressed birds are those for which genotype and phenotype do not match, while the contrary is considered to be an immigration event)

than adult breeding dispersal. Moreover, most birds (21 of 31) dispersed into neighbouring breeding sites less than 300 km away, while the rest (10 birds) moved distances greater than 1000 km (Appendix 4). Interestingly, among these long-distance movements there were 4 interbasin dispersal events: 3 birds from the Atlantic moved into the Mediterranean — 2 moved between the Selvagens and Columbretes Islands and 1 moved from Gran Canaria into the Columbretes Islands — whereas only 1 bird moved from the Mediterranean (Pelagie Islands, Italy) into the Atlantic (Selvagens Islands) (Fig. 4, Appendix 4).

Spatial patterns of genetic variation and oceanography

Simple Mantel tests revealed a highly significant correlation between genetic and geographic distances among colonies at a regional scale, considering both the Atlantic and the Mediterranean regions together (r = 0.59, p < 0.001). Within each region, spatial correlation was positive and still significant within the Mediterranean (r = 0.47, p < 0.01), but negative within the Atlantic (r = −0.16, p = 0.05). When controlling by each oceanographic variable, partial Mantel tests results in higher and more significant correlation coefficients at a regional (GeoDist by chl: r = 0.58, by SST: r = 0.57, by Sal: r = 0.59) and a local scale (GeoDist by chl: r = 0.30, by SST: r = 0.34, by Sal: r = 0.47) within the Mediterranean (all p < 0.05), although significance did not hold within the Atlantic (all p > 0.05). Regarding oceanographic differences across the breeding range, there was a significant association between oceanographic and genetic distances among colonies when considering colonies from both taxa (simple: chl: r = 0.13, p < 0.05; SST: r = 0.17, p < 0.01; Sal: r = 0.13, p < 0.001). When controlling by geography we found a significant but negative correlation for chl (partial: r = −0.11, p < 0.05), and significance did not hold for SST (r = −0.11, p = 0.07) or salinity (r = 0.15, p = 0.10). Within the Atlantic we found significant correlations for chl (simple: r = 0.31, p < 0.05) and salinity (r = −0.21, p < 0.05), but significance did not hold for SST (r = −0.15, p = 0.21). When controlling by geographic distances (partial Mantel test), the resulting correlation was negative and significant for chl (r = −0.28, p < 0.05), but non-significant for salinity and SST (p > 0.05). Within the Mediterranean, simple Mantel test correlations were positive and approached significance for chl (r = 0.41, p = 0.06), and were significant for SST (r = 0.35, p < 0.05) but not for salinity (r = −0.05, p = 0.53). After application of the partial Mantel test, correlations were no longer significant for any of the 3 variables (p > 0.05).
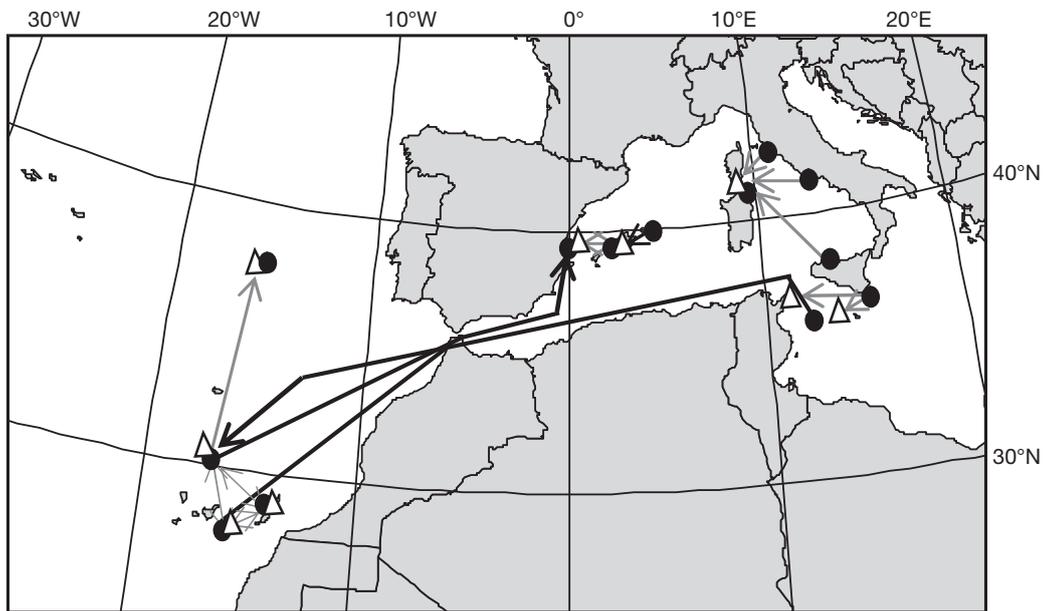
Fig. 4. *Calonectris diomedea diomedea* and *C. d. borealis*. Distances and directions of movement of 78 Cory's shearwaters recovered in a different colony to the colony where they were ringed (as adults or chicks). Circles and triangles represent ringing and recovery records, respectively. Lines represent dispersal movements between colonies and arrows represent direction; black line colour highlights the 3 long-distance movements between the Mediterranean Sea and the Atlantic Ocean

DISCUSSION

Genetic population structure

Phenotypic and genetic data point out the existence of 2 well-differentiated groups representing Atlantic and Mediterranean breeding colonies according to their mostly segregated geographic distribution into 2 oceanic distinct basins: (1) genetically, almost all Mediterranean haplotypes corresponded to individuals breeding at Mediterranean localities and almost all Atlantic haplotypes corresponded to individuals breeding at Atlantic localities (Fig. 2); and (2) phenotypically, individuals clearly segregated into 2 groups: the larger (in size) Atlantic phenotype and the smaller Mediterranean phenotype. Similarly, the phenotype of most birds matched their corresponding breeding area (Fig. 3). However, comparisons of genetic, phenotypic and distribution data revealed both current and historical dispersal events. That is, we identified 4 out of 241 birds in which the haplotype did not match the breeding area. Further examining the phenotype of the 4 birds, there were 2 genetically Atlantic birds breeding in the Mediterranean with an Atlantic phenotype, suggesting 2 migration events rather than introgression, while in the other 2 cases the phenotype and genotype did not match, suggesting in this case introgression rather than migration events. Intro-

gression between the 2 Cory's subspecies agrees with the absence of fixed genetic differences as well as the existence of a slight phenotypic overlap in the Atlantic–Mediterranean transition zone (Gómez-Díaz et al. 2006, present study). Hybridization may occur in the Atlantic– Mediterranean oceanic transition and these 2 introgressed birds may well be hybrid individuals. As previously pointed out, Chafarinas Islands is the Atlantic closest breeding colony and is composed of 78% Mediterranean and 22% Atlantic Cory's shearwaters (Navarro et al. 2009), which would explain frequent immigration and hybridization events at this locality. In the case of Hyeres, the presence of few Atlantic breeders may be related to the influence of the Gulf of Lyon gyre, which shows some similarities with the Atlantic oceanography (Beckers et al. 1997, Longhurst 1998, Millot 1999, present study).

Ecological observations at the breeding colonies further support the existence of some exchange of individuals between the 2 basins, again mainly from Atlantic individuals migrating to Mediterranean colonies, although at a rather low rate. That is, we found at least 4 shearwaters, 1 ringed in the Mediterranean and 3 in the Atlantic, re-sighted in the opposite basin (Appendix 4, Fig. 4). In addition, other studies have reported various cases of Atlantic pairs breeding in the Mediterranean (Lo Valvo & Massa 1988, Sánchez 1997, Thibault & Bretagnolle 1998,

Martínez-Abraín et al. 2002), and the opposite has been also found (Mays et al. 2006). Dispersal could take place when birds return from the wintering areas to the breeding colonies. Since Atlantic and Mediterranean Cory's shearwater populations mix at several wintering areas (i.e. Benguela and Agulhas currents, the Brazilian current and the Canary current) (González-Solís et al. 2007), it is likely that a few *Calonectris diomedea borealis* enter the Mediterranean and a few *C. d. diomedea* remain and breed in the NE Atlantic. Previous work suggests natal dispersal of subadult birds may be an important source of genetic exchange (see review by Thibault et al. 1997). In agreement with this, our results show that more than half of the dispersal events corresponded to individuals ringed as chicks that moved away from their natal colonies (see Appendix 4). Moreover, gene flow estimates also indicate some exchange of individuals between the 2 taxa, but with <1 female per generation, suggesting that part of the dispersal movements between the Atlantic and the Mediterranean basins do not result in effective gene flow (see 'Results: ringing-recovery data').

The marked differentiation between Atlantic and Mediterranean populations contrasts with the weak genetic structure within each basin. Accordingly, gene flow among colonies within the Mediterranean as well as within the Atlantic was high, suggesting there is no restriction to bird movements at this spatial scale. In agreement and despite the overall highly philopatric behaviour of the species (97%, present study, see review by Thibault et al. 1997), our analysis on recovery data suggests short-range dispersal among neighbouring islands within each basin is frequent compared to movements between basins (Fig. 4, Appendix 4). This result is also supported by previous genetic analyses of dispersal movements among Cory's shearwaters from neighbouring breeding colonies in Sicily and Sardinia (Randi et al. 1989). Nevertheless, ringing-recovery and genetic data are not directly comparable mainly due to their different time scales. Ecological observations provide information on contemporary dispersal, whereas genetic estimates can reflect both current and historical gene flow (Hedrick 1999). Therefore, results and conclusions regarding dispersal rates should be taken with caution. Finally, our results disagree with previous microsatellite analyses on the species showing high levels of genetic structuring among and within populations (Rabouam et al. 2000). Similar patterns (lower population structure with mtDNA data than microsatellites) have been found in other seabirds (Burg & Croxall 2001, Abbott & Double 2003). These differences may also arise from differences in resolution among molecular markers.

Spatial patterns of genetic variation

Previous studies have suggested that environmental features such as water temperature and productivity may influence population dynamics and distribution in Atlantic and Mediterranean marine birds (Zotier et al. 1999) including the Cory's shearwater (Bretagnolle & Zotier 1998). In this species, the importance of oceanographic boundaries as gene flow barriers has been suggested by the fact that the Almería-Oran Oceanic front, rather than the Gibraltar Strait, seems to act as the effective divide between the 2 Cory's shearwater forms (Gómez-Díaz et al. 2006, present study). Interestingly, apart from Almería colony, which is geographically Mediterranean but all individuals are genetically and biometrically Atlantic (Gómez-Díaz et al. 2006, present study), a small Mediterranean Cory's shearwater colony has been reported on the Atlantic French coast (Mays et al. 2006), and a similar case may exist in a Mediterranean colony in Corsica (Thibault & Bretagnolle 1998). Although in the latter 2 cases genetic evidence is needed to further confirm their identities, all available evidence suggests a more complex scenario regarding oceanic boundaries in Cory's shearwaters. In this context, in the present study we further tested the role of oceanic features (chl concentration, salinity and SST) in shaping gene flow and patterns of genetic differentiation in the species. Simple correlations between genetic and oceanographic distances among populations revealed significant and positive associations between them. However, we also found a strong positive correlation between genetic and geographic distances. That is, these correlations may result from the association between geographic and oceanographic distances; i.e. closer colonies usually experience more similar oceanographic conditions and oceanography which, *per se*, may not explain genetic variability beyond that already explained by the isolation by distance model. Indeed, when controlling for the effect of geographic distances on oceanographic distances, previously positive and significant relationships between genetic and oceanographic distances were no longer significant or became negative. Such a change may be indicative of a spurious correlation between oceanographic and genetic distances (Legendre 1993). Conversely, when controlling for the effect of oceanographic distances on geographic distances, the relationship between genetic and geographic distances became even more significant. Consequently, Atlantic and Mediterranean basins show clear differences in the marine habitat which match with phenotypic and genetic differences between the birds breeding in each basin; however, these differences can even better be explained by the isolation by distance model.

Within each basin, neither distance nor oceanographic traits explained the observed patterns of genetic variation for the Atlantic. On the contrary, within the Mediterranean, results supported the isolation by distance model. In agreement, previous studies have identified morphometric gradients through the Mediterranean, but found very weak or absent gradients in the Atlantic (Massa & Lo Valvo 1986, Granadeiro 1993, Gómez-Díaz & González-Solís 2007). Likewise, results from the present study also indicated a slight genetic divergence between Eastern and Western Mediterranean populations. This may be related to the complex oceanography of the Mediterranean (Zotier et al. 1999). Indeed, compared with the Western Mediterranean, the east shows lower productivity and relatively warmer waters and higher salinity (SeaWiFS, MODIS and NODC data). However, similar to the spatial analysis between basins, results of partial Mantels tests relating genetic, oceanographic and geographic distances suggest that geographic rather than oceanographic distances better explained patterns of genetic structuring in the Mediterranean.

Implications for taxonomy and concluding remarks

In the present study we combined genetic, biometric, ecological and spatial data to investigate genetic discontinuities, gene flow patterns and population structure in Cory's shearwaters. Our study highlights the need for more integrative approaches for a better understanding on mechanisms of population differentiation in seabirds. In addition, our results are of particular significance in the context of the current taxonomic debate on this seabird species. The Atlantic (*Calonectris diomedea borealis*) and Mediterranean (*C. d. diomedea*) forms appeared genetically distinct, with quasi-exclusive haplotypes within each taxon, which in the majority of the cases match with the mostly segregated distributions and the phenotypic traits and agree with previous work on the species using both mitochondrial and nuclear markers (Randi et al. 1989, Wink et al. 1993, Heidrich et al. 1998, Rabouam et al. 2000). Immigration and introgression events were rare, gene flow between Atlantic and Mediterranean populations was <1 female per generation and there were significant levels of net sequence divergence (3.1%, present study; 5.4%, Gómez-Díaz et al. 2006). Biometric differences provided in the present and other studies agree with differences in plumage colour and vocalizations (Bretagnolle & Lequette 1990, Granadeiro 1993, Thibault et al. 1997, Gutiérrez 1998, Gómez-Díaz et al. 2006). In addition, at present the taxonomic classification of the group is paraphyletic (Gómez-Díaz et al. 2006). In conclusion, genetic, phenotypic and ecological data on Cory's shearwaters from the present study supported a clear differentiation of the Atlantic and Mediterranean forms of this species, and we therefore recommend that the 2 taxa be regarded as different species, the Cory's shearwater *Calonectris borealis* and the Scopoli's shearwater *Calonectris diomedea*, respectively. Nevertheless, and despite the strength of such a combined analysis, the present study lacks nuclear data, and therefore population structure of the species needs to be further confirmed.

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