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GENETIC RELATIONSHIPS OF CORY'S SHEARWATER: PARENTAGE, MATING ASSORTMENT, AND GEOGRAPHIC DIFFERENTIATION REVEALED BY DNA FINGERPRINTING

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ABSTRACT.—We used DNA fingerprinting to assess genetic structure of populations in Cory's Shearwater (*Calonectris diomedea*). We analyzed mates and parent-offspring relationships, as well as the amount and distribution of genetic variation within and among populations, from the level of subcolony to subspecies. We found no evidence of extrapair fertilization, confirming that the genetic breeding system matches the social system that has been observed in the species. Mates were closely related, and the level of genetic relatedness within populations was within the range usually found in inbred populations. In contrast to previous studies based on allozymes and mtDNA polymorphism, DNA fingerprinting using microsatellites revealed consistent levels of genetic differentiation among populations. However, analyzing the two subspecies separately revealed that the pattern of genetic variation among populations did not support the model of isolation by distance. Natal dispersal, as well as historic and / or demographic events, probably contributed to shape the genetic structure of populations in the species. *Received 14 January 1999, accepted 24 November 1999.*

THE STUDY of genetic relationships within and among populations is important for understanding mating systems, dispersal, and population structure (Avise 1996). These population characteristics traditionally have been studied using direct mark-recapture techniques (e.g. Perrins et al. 1991). However, this approach has several limitations. For example, it is relatively time consuming, unsuitable where the probability of recapture is low, and provides a strictly contemporaneous picture of dispersal patterns and gene flow. In contrast, molecular comparisons allow reconstruction of the evolutionary events and historical demographic processes that resulted in the population structure observed at the present time (Avise 1994).

A prerequisite to the study of population genetics is the availability of genetic markers that are sufficiently variable to detect changes. This may represent a problem in species that have inherently low levels of genetic variation. In birds, most previous studies have used allozymes or mtDNA restriction fragment length polymorphism as molecular markers to trace genetic structure of populations, but these markers have shown low intraspecific geographic variation (e.g. Barrowclough 1983, Kessler and Avise 1985). Two alternative methods have appeared recently. First, DNA fingerprinting using minisatellite sequences (Jeffreys et al. 1985a, b) has been shown to be a powerful molecular tool for assessing parentage and mating structure within populations (Burke 1989, Burke et al. 1991). These markers often are not suitable for distinguishing between populations because their high degree of variability yields too much variation within populations. However, small or bottlenecked populations, where effective population size is reduced and inbreeding occurs, may exhibit low genetic variation. In such situations, minisatellites have been useful for revealing intra- and interpopulation variation that other molecular tools may have failed to detect (e.g. Triggs et al. 1992, Degnan 1993, Fleischer et al. 1994, Freeman-Gallant 1996). Second, microsatellites are another class of hypervariable and randomly dispersed repetitive elements that are widespread in eukaryote genomes (e.g. Tautz 1989, Ellegren 1992). These short sequences have only recently received much attention for their application to comparisons at the population level (Bruford and Wayne 1993). Their usefulness in detecting variation in genetically de-

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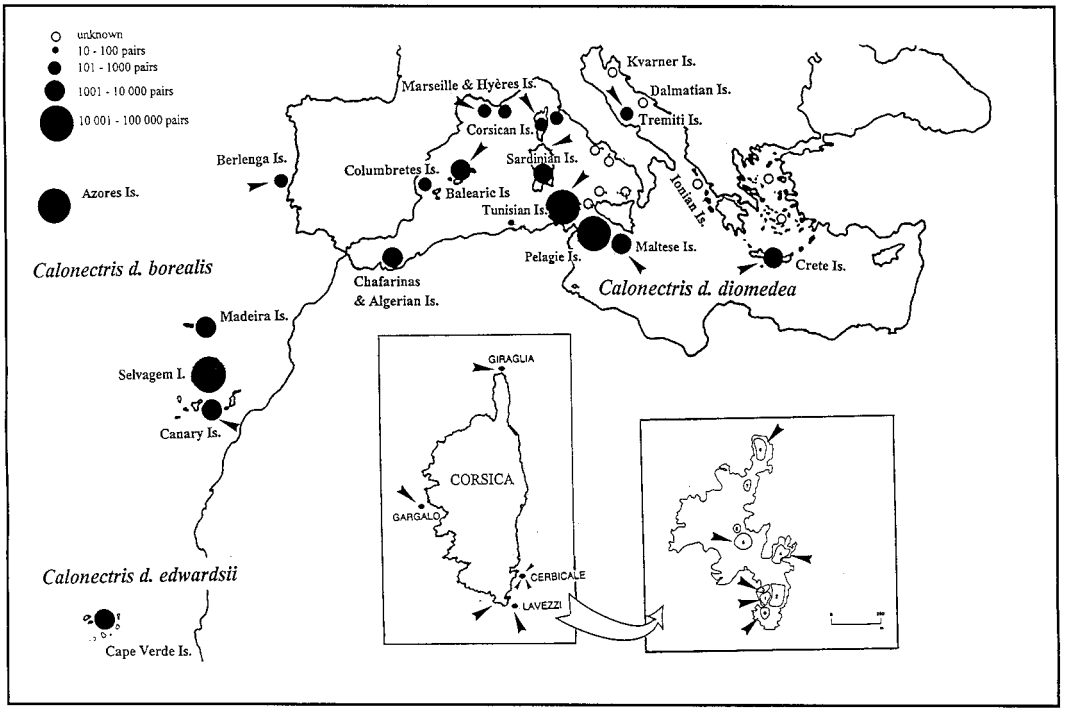

FIG. 1. Distribution of the Cory's Shearwater and size of breeding colonies. Arrows indicate sites where blood samples were taken for this study.

pauperate populations has already been demonstrated with fingerprints (e.g. Hughes and Queller 1993, Miller and Kapuscinski 1996, Barker et al. 1997).

Within species, procellariiforms typically show low levels of genetic variation (Barrowclough et al. 1983, Sibley and Ahlquist 1990). Cory's Shearwater (*Calonectris diomedea*) nests in colonies on islands (Fig. 1), and geographically discrete breeding populations are established across the Mediterranean sea (*C. d. diomedea*) and along the coast of northwestern Africa from the Cape Verde (*C. d. edwardsii*; possibly a full species) to the Macaronesian (Azores, Madeira, Canaries, and Selvagem islands) and Berlenga (*C. d. borealis*) islands. Long-term studies have shown strong philopatry to natal (Ristow et al. 1990, Thibault 1993, Borg and Cachia-Zammit 1997, Rabouam et al. 1998) and breeding (Mougin et al. 1987, Ristow et al. 1990, Thibault 1994) sites, which should promote genetic isolation and differentiation among populations. Populations are distinguishable morphologically (Granadeiro 1993) and by their vocalizations (Bretagnolle and Lequette 1990, Thibault and Bretagnolle 1997). Surprisingly, however, tests of whether these geographic subdivisions are reflected in the genotype have revealed a lack of spatial structuring of populations based on allozymes (Randi et al. 1989), mtDNA (Wink et al. 1993, Heidrich et al. 1996), and minisatellite DNA (Da Silva and Granadeiro 1999).

In the present study, we performed DNA fingerprinting using a microsatellite probe to assess genetic relationships within and among populations of *C. d. borealis* and *C. d. diomedea.* In contrast to previous genetic studies, our analysis covered a large geographic scale, from subcolony to subspecies. This is also the first study to analyze parentage, mating assortment, and population differentiation (i.e. different levels of genetic relationships) using the same genetic marker. First, we analyzed parentage in Cory's Shearwater to assess whether genetic relatedness between parents and offspring was consistent with social monogamy observed in the species (Mougin et al. 1987, Ristow et al. 1990, Thibault 1994). We also analyzed the mating structure within populations

to assess whether birds pair with relatives, as predicted from limited natal dispersal and the observation of incestuous breeding in the species (Rabouam et al. 1998). These two aspects of genetic relationships directly affect the genetic structure of populations and therefore are important for understanding population differentiation. Second, we examined the amount and distribution of genetic variation within and between populations at different geographic scales across the breeding area. Given the high level of natal philopatry observed in the species, we expected to find inbreeding as well as low genetic variation within populations and high genetic variation between populations.

METHODS

Study areas and blood sampling.—We obtained blood from two Atlantic populations of *C. d. borealis* and from eight Mediterranean populations of *C. d. diomedea* (Fig. 1). In one of the Mediterranean populations (Corsica), blood was sampled from seven different breeding sites. In one of these sites (Lavezzi Island), six subcolonies were also sampled (Fig. 1). The parentage study was conducted on the Corsican colonies during the 1994 to 1996 breeding seasons. We obtained blood from 22 full families (i.e. both adults and 1, 2, or 3 nestlings from successive years [brood size is always 1], for a total of 34 nestlings for which both putative parents were sampled) and 17 partial families (i.e. male adult and nestling).

We obtained blood samples (0.1 to 0.5 mL) from the tarsus vein. Samples were suspended in 0.5 mL of a buffered solution of guanidium-thiocyanate (Laulier et al. 1995) and preserved at 4°C or at room temperature before storage at −20°C. Before purification of DNA, samples were incubated for 30 min at 65°C and then cooled for 5 min at room temperature. Proteins were removed by successive extraction, twice with phenol/chloroform-isoamylalcohol and once with chloroform-isoamylalcohol, at 20 to 25°C. DNA was precipitated by adding 0.1 volume 3 M sodium-acetate and 2.5 volumes ethanol, incubated for 1 h at −80°C, washed twice with 70% ethanol, and dried. The pellet was resuspended in 40 μL of sterile water and stored at −20°C.

DNA fingerprinting.—About 3 to 5 μg of total cellular DNA were digested with 10 units of *Taq* I for at least 2 h at 65°C, and DNA fragments were subsequently electrophoresed through a 22-cm 1.5% agarose gel in TAE running buffer (Tris-OH 40 mM, acetic acid 20 mM, 2 mM Na_2 EDTA, pH 8.1) with ethidium bromide for 15 to 20 h at 50 V (until the bromophenol blue dye was within 3 cm of the end of the gel). To increase the transfer of long fragments, DNA was depurinated in 0.2 M HCl for 5 min and denaturated in 0.4 M NaOH and 1.5 M NaCl for 30 to 45 min. DNA was transferred from the gel by Southern blotting onto a nylon membrane (Hybond N^+, Amersham) in 0.4M NaOH for 4 h. After blotting, membranes were washed briefly in 3X SSC and dried at 37°C. A synthetic oligonucleotide sequence consisting of a tandemly repeated tetranucleotide motif ($(GGAT)_4$) was used as the microsatellite DNA probe. The probe was 5′ end-labeled with $\gamma^{32}P$ using T4 polynucleotide kinase. Prehybridization and overnight hybridization were performed at 42°C in 0.5M Na_2HPO_4-NaH_2PO_4 and 7% SDS. Hybridized filters were washed twice for 30 min in 5X SSC at 42°C and exposed to X-ray films (Fuji RX) at −80°C for 1 to 4 days in a cassette with two intensifying screens.

Analysis of DNA profile.—Digested DNA samples from nestlings and their putative parents were placed in adjacent gel lanes. On each gel, an assortment of families from the different subcolonies of Lavezzi Island was represented, allowing analysis of genetic relatedness between birds within as well as between subcolonies. The scorer was not aware of individual identities, so scoring was done blind. At larger geographic scales, we analyzed genetic structure of populations from selections of five birds per sex and population, using separate gels for males and females. Individuals from a given population were placed in a block of adjacent lanes, and different populations were represented in adjacent blocks. Pairwise comparisons between individuals were made only from the same gel by marking bands on tracing paper and then moving each DNA profile on the autoradiogram to allow side-by-side comparisons.

Only well-resolved bands were considered, corresponding to bands clearly scored within the size range of 1.3 to 21.8 kb. The degree of genetic similarity between individuals was measured as the proportion of bands shared between their DNA profiles. This proportion was calculated following Wetton et al. (1987):

$$S_{xy} = 2N_{xy}/N_x + N_y, \quad (1)$$

where N_{xy} is the number of fragments shared between individuals x and y, and N_x and N_y are the total numbers of fragments scored in each individual. We treated bands from different individuals as identical if they had similar electrophoretic mobility (center of bands less than 0.5 mm apart) and the same approximate intensity. We calculated band-sharing coefficients for pairwise combinations of individuals corresponding to different classes of relatedness, including putative parents and nestlings versus unrelated adults and nestlings for the study of parentage, and mates versus unmated birds. We used ''unrelated'' birds (i.e. birds taken at random, but excluding mates and close neighbors) for comparisons of within versus between populations at different geographic scales (Lavezzi subcolonies, Corsican populations,

populations within subspecies, and populations between subspecies) for the analysis of genetic structure. For the paternity analysis, we further evaluated parentage based on the number of novel bands in the offspring fingerprint.

For each population, we calculated the within-population mean similarity value by averaging all pairwise comparisons of individuals from the same population. For each pair of populations compared, we calculated the between-population mean similarity value by averaging all of the pairwise comparisons of individuals from different populations. These values were used to calculate between-population genetic similarity corrected for within-population similarity (S_{ij}; Lynch 1990: equation 11). Genetic differentiation between populations in a specific geographic scale was measured using an estimate of Wright's (1951) F_{ST} statistic (Lynch 1990: equation 14).

Statistical analysis.—For the paternity analysis, each chick was compared with its putative parents, and / or with one unrelated adult of each sex from the same subcolony. For comparisons of mates versus unmated birds, each adult was compared with its mate and with another bird from the same subcolony (excluding immediate neighbors). Comparisons of intrapopulation versus interpopulation genetic similarity were based on a subsample of individuals, each being compared with one individual from the same population and with one from another population. No individual was used in more than one of the comparisons. In these three analyses, we used paired *t*-tests or Wilcoxon signed-rank tests to test for differences in band-sharing coefficients between the different groups being compared.

To test for differences in the amount of genetic variation among different geographic scales, we used average band-sharing coefficients calculated from all possible pairwise comparisons between individuals on a gel. The use of individuals in more than two pairwise comparisons resulted in high covariation because of the interdependence of S_{xy} values (Lynch 1990). To correct for this bias, we followed the approach of Danforth and Freeman-Gallant (1996), which is based on iterative sampling (1,000 times) of independent comparisons that are randomly extracted from the overall data set. Differences in mean band sharing between groups can be evaluated using a corrected *t*-test (t_s) that is based on the standard error of the mean derived from the 1,000 subsamples corrected for interdependence (Danforth and Freeman-Gallant 1996). This approach was used when the sample size and the organization of individuals on gels allowed subsampling of at least 10 independent pairwise comparisons. We present both uncorrected and corrected values, but we conducted statistical tests only on the corrected values.

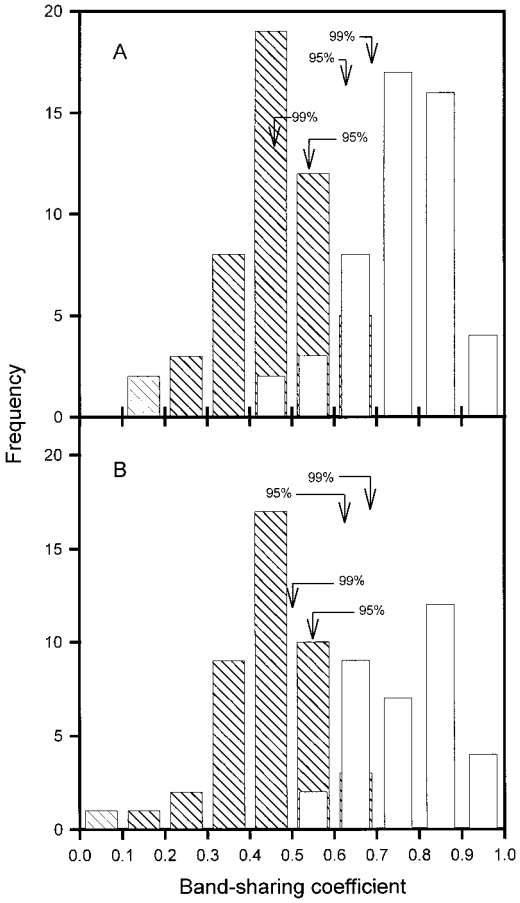

FIG. 2. Frequency distribution of the proportion of bands shared between each fledgling Cory's Shearwater and its (A) putative father (open bars; $n = 51$) and presumably unrelated males (hatched bars; $n = 49$) and its (B) putative mother (open bars; $n = 34$) and presumably unrelated females (hatched bars; $n = 42$). Arrows denote confidence limits of the two distributions.

RESULTS

Genetic similarity between close relatives and mates.—The mean number of bands scored per bird was 26.7 ± SD of 7.2, which was adequate for comparisons between individuals (Jeffreys et al. 1985b). The frequency distributions of band-sharing coefficients for pairwise comparisons of unrelated adults and nestlings versus putative parents and nestlings appeared to be distinctive but overlapped at the one-tailed 95% and 99% confidence limits (Fig. 2). Mean band sharing between nestlings and their putative parents was 0.753 ± 0.111 ($n = 51$) with

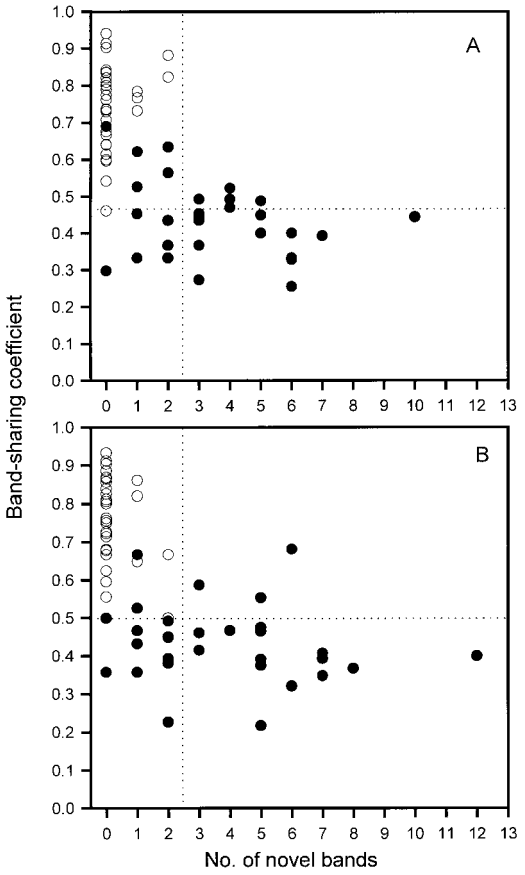

FIG. 3. Relationship between band-sharing coefficients and number of novel bands for Cory's Shearwater chicks and (A) adult males and (B) adult females. Open circles denote attending adults of the nestling's nest, and closed circles denote unrelated adults. Dashed lines indicate thresholds for distinguishing parentage from attending versus nonattending adults.

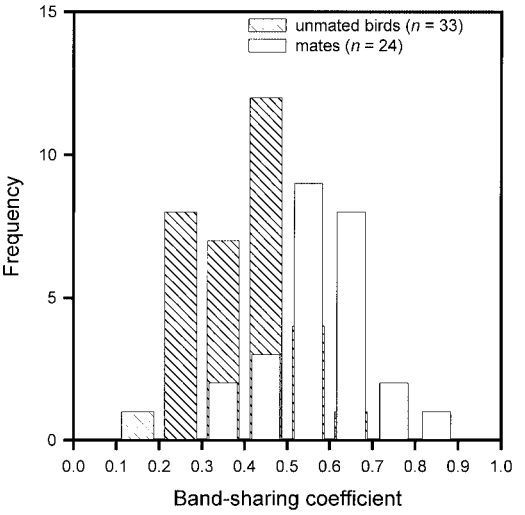

FIG. 4. Frequency distribution of the proportion of bands shared between mates versus unmated adult Cory's Shearwaters.

the father and 0.764 ± 0.115 (n = 34) with the mother. Mean band sharing between nestlings and unrelated adults was 0.451 ± 0.115 (n = 49) with males and 0.439 ± 0.118 (n = 42) with females.

Comparisons of nestlings with each of their putative parents resulted in a low number of novel bands (0 to 2) and high band-sharing coefficients (>0.460). Conversely, comparisons involving unrelated adults resulted in a high number of novel bands (0 to 12), whereas band-sharing coefficients were low (Fig. 3). Among the 34 nestlings for which both putative parents were sampled, only 5 exhibited one (n = 3) or two (n = 2) bands that could not be assigned to either parent (Fig. 3). However, each of these five nestlings had high band sharing with its putative father (0.732 to 0.882). These band-sharing values were higher than those between nestlings and unrelated males (0.450 to 0.630) and also were above the 99% confidence limit of the distribution of band-sharing coefficients between nestlings and unrelated males (0.69; Fig. 2). Therefore, these nestlings were considered to be genetic offspring of their putative fathers, and the seven novel fragments were more likely to have resulted from mutations than from extrapair fertilizations (EPFs). Thus, we estimated the mutation rate per band per generation from the mean number of novel fragments per nestling (7/34 = 0.20) and the mean number of fragments scored per individual (26.7) to be 0.20/26.7, or 0.007.

We compared the frequency distributions of band-sharing coefficients between mates and unmated birds from the same breeding sites (Fig. 4). Genetic similarity was significantly higher between mates (0.585 ± 0.121, n = 24) than between unpaired adults (0.389 ± 0.122, n = 33; paired t = 7.86, P = 0.0001).

Genetic similarity within and among populations.—For males and females, genetic similarity was significantly higher within than between populations, except at the subcolony level (Fig. 5). Genetic similarity within populations between ''unrelated'' adults did not vary

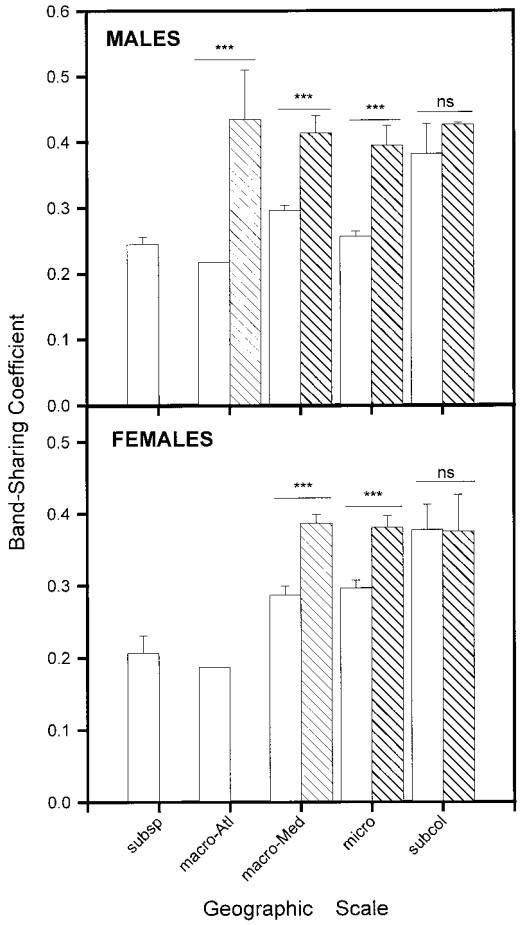

FIG. 5. Comparison of genetic relationships between (open bars) and within (hatched bars) populations of Cory's Shearwaters for males and females at different geographic scales (subsp = subspecies; macro-Atl = Atlantic populations; macro-Med = Mediterranean populations; micro = Corsican populations; subcol = subcolonies on Lavezzi Island). Statistical differences were tested using paired *t*-tests except at the subcolony scale (Wilcoxon signed-rank test); ns, $P > 0.05$; ***, $P = 0.0001$. Sample sizes indicated in Table 1.

significantly among different geographic scales (Fig. 5, Table 1), ranging from 0.378 to 0.384 in females ($t_s = 0.411$, df = 25, $P > 0.50$) and 0.392 to 0.418 in males ($t_s = 0.863$, df = 35, $P > 0.20$). In contrast, a geographic trend appeared in the amount and distribution of genetic variation among populations (Fig. 5; Tables 1, 2). As expected, an overall increasing gradient of genetic relatedness occurred among individuals from subspecies to subcolonies (F_{st} values in Table 2). However, analyzing the two subspecies separately revealed that the pattern of genetic variation among populations did not support the model of isolation by distance (Tables 1, 2). First, the degree of genetic subdivision in Atlantic populations was higher than in their Mediterranean counterparts. Mediterranean populations appeared to be less differentiated than were populations of different subspecies ($t_s = 1.86$, df = 25, $P < 0.1$), but genetic isolation in Atlantic populations was slightly higher than that among subspecies (Table 1). Second, Mediterranean populations had lower levels of genetic differentiation than did colonies from Corsica alone ($t_s = 1.72$, df = 25, $P < 0.1$). The latter two results were not significant due to the subsampling approach that yielded means that were close to uncorrected values but with increased standard errors because of lower sample sizes (Table 1). As a result, the statistical power of the tests was low, and differences that may have been significant based on the uncorrected data showed low or no significance based on the subsampling procedure (see Danforth and Freeman-Gallant 1996, Freeman-Gallant 1996).

DISCUSSION

Analysis of parentage.—Although social monogamy is by far the most common mating system in birds (Lack 1968, Møller 1986), field observations and genetic investigations have demonstrated that extrapair copulations (EPCs) are common in many birds (Black 1996). EPCs sometimes lead to EPFs and may be associated with potential fitness gains (Birkhead and Møller 1992). The high density of individuals in colonial-nesting seabirds such as procellariiforms should provide frequent opportunities for EPCs (Birkhead and Møller 1992). However, procellariiforms form long-term pair bonds that involve coordination of breeding activities and require extended investment by both parents to raise an offspring from the single egg that is laid every one or two years (Warham 1990). Therefore, social and genetic monogamy should be strongly selected for in these birds because biparental care is essential for reproductive success.

Paternity was attributed to the attending male in all families that we sampled, so we found no evidence for EPFs in Cory's Shear-

TABLE 1. Genetic similarity (band-sharing coefficients, $\bar{x} \pm$ SD) within and among populations of Cory's Shearwaters at different geographic scales. Values in parentheses were calculated from subsamples of independent comparisons (see Methods).

Geographic scale	Males	n^a	Females	n^a
	Within populations			
Atlantic	0.453 ± 0.035	16		
Mediterranean	0.418 ± 0.017	41	0.384 ± 0.012	33
	(0.419 ± 0.019)	19	(0.384 ± 0.016)	10
Corsica	0.392 ± 0.016	44	0.378 ± 0.017	41
	(0.397 ± 0.017)	18	(0.375 ± 0.015)	17
Subcolonies	0.427 ± 0.024	16	0.381 ± 0.040	8
	Between populations			
Subspecies	0.249 ± 0.007	161	0.209 ± 0.015	41
	(0.247 ± 0.026)	10		
Atlantic	0.217 ± 0.026	9	0.187 ± 0.000	1
Mediterranean	0.296 ± 0.006	180	0.285 ± 0.007	185
	(0.307 ± 0.019)	17	(0.281 ± 0.027)	11
Corsica	0.254 ± 0.006	192	0.288 ± 0.007	162
	(0.253 ± 0.025)	10	(0.291 ± 0.028)	10
Subcolonies	0.382 ± 0.045	12	0.375 ± 0.058	4

[a] Number of pairwise comparisons from legible fingerprints.

water. This result suggests that the genetic mating system matches the social mating system, as has been found in another locality for this species (Swatschek et al. 1994) as well as in several other species of procellariiforms (Northern Fulmar [*Fulmarus glacialis*], Hunter et al. 1992; Leach's Storm-Petrel [*Oceanodroma leucorhoa*], Mauck et al. 1995; but see Austin et al. 1993, Austin and Parkin 1996 for Short-tailed Shearwater [*Puffinus tenuirostris*]). Although EPCs were observed in Northern Fulmars (Hunter et al. 1992), no evidence of EPFs was found, possibly as a result of close mate guarding and multiple copulations to assure paternity (Hunter et al. 1992). In contrast, for nocturnal burrowing species such as Cory's Shearwater and Leach's Storm-Petrel, nothing is known about the occurrence of EPCs. Therefore, we do not know whether genetic monogamy reflects an absence of EPCs, the effectiveness of male tactics for paternity assurance, or both.

Genetic relationships within populations.—We detected high genetic similarity among individuals within populations, which indicated low levels of genetic variability. Similar results were found with a minisatellite marker in *C. d.*

TABLE 2. Mean genetic similarity values (S_{ij}) and F_{st} statistics between populations of Cory's Shearwaters by sex at different geographic scales (centered in bold). Numbers of pairwise comparisons are given in Table 1.

	Males			Females		
Population	n^a	S_{ij}	F_{st}	n^a	S_{ij}	F_{st}
		Subspecies				
Atlantic vs. mediterranean	10	0.82	0.231	—	—	—
		Macrogeographic				
All populations	10	0.89	0.183	—	—	—
Atlantic	2	0.78	0.280	—	—	—
Mediterranean	8	0.90	0.147	6	0.90	0.135
		Microgeographic				
Corsica	5	0.87	0.174	6	0.89	0.152
		Subcolonies[b]				
Lavezzi Island	4	0.99	0.016	—	—	—

[a] Number of sites.
[b] Sexes combined.

borealis (Da Silva and Granadeiro 1999), although their values were slightly lower. Low genetic variability is typical of inbred populations in birds (e.g. Burke and Bruford 1987, Triggs et al. 1992, Haig et al. 1993). Inbreeding is common in small populations owing to founder or bottleneck events and/or limited dispersal rates and consequent reductions in gene flow. In Cory's Shearwater, the extreme level of natal philopatry (Ristow et al. 1990, Thibault 1993, Borg and Cachia-Zammit 1997, Rabouam et al. 1998) must to some extent promote inbreeding. In addition, incestuous breeding has been reported in this species (Rabouam et al. 1998), and mating between close relatives is supported by the very high level of genetic relatedness between mates (Swatschek et al. 1994, this study).

Such high genetic relatedness has rarely been found in other birds (e.g. Bensch et al. 1994, Rätti et al. 1995) with the exception of one other procellariiform (Mauck et al. 1995) and a case of incestuous pairing in the House Sparrow (*Passer domesticus*; Wetton et al. 1987). Close genetic relatedness between mates was previously reported for Cory's Shearwater in Crete (Swatschek et al. 1994), although it was not as pronounced as in the Corsican birds. Differences in levels of genetic variability might arise from differences in effective population size; i.e. 1,000 pairs in Crete compared with fewer than 400 pairs on Lavezzi Island, Corsica. It remains unclear whether mating between close relatives results only from high levels of philopatry in this species, or whether it is also an assortative mating strategy (i.e. nonrandom mating).

Genetic relationships between populations.—DNA fingerprinting with a microsatellite marker revealed spatial structuring in the amount and distribution of genetic variation among populations of Cory's Shearwater. Previous studies based on allozymes and mtDNA revealed only slight divergence between *C. d. diomedea* and *C. d. borealis* (Randi et al. 1989, Wink et al. 1993, Heidrich et al. 1996). In addition, F_{st} values from microsatellites (0.183 for all populations; this study) and to a lesser extant minisatellites (up to 0.168; Da Silva and Granadeiro 1999) largely exceeded values obtained from allozyme divergence among populations (mean F_{st} = 0.083; Randi et al. 1989). Differences in the efficiency of genetic markers to detect variation depend on their degree of variability (i.e. largely on mutation rates). Minisatellites and microsatellites evolve more rapidly than allozymes and mtDNA and thus exhibit more variation. In Cory's Shearwater, the mutation rate of the microsatellite marker was within the range normally found in birds (e.g. Burke and Bruford 1987, Westneat 1990, Millard et al. 1994, Mauck et al. 1995).

Banding data have established that natal philopatry is high in Cory's Shearwater and that dispersal occurs mainly between subcolonies within populations (Thibault 1993, Rabouam et al. 1998). These observations were confirmed by our genetic analysis in that subcolonies were not genetically differentiated in either sex. However, dispersal alone cannot explain the overall pattern of genetic variation among populations of Cory's Shearwater. Because the species is highly philopatric, we expect decreasing gene flow and increasing differentiation with increasing distance between populations. We observed this pattern when calculating differentiation at the macrogeographic scale. However, this trend disappeared when populations from different subspecies were considered separately, suggesting that additional factors beyond dispersal contributed to shape the genetic structure of populations. We suggest that different historic or demographic events have an important role in the genetic structuring of different populations of Cory's Shearwater, as supported by the three following arguments.

First, the greater genetic divergence within *C. d. borealis* than within *C. d. diomedea* suggests that the Mediterranean populations achieved isolation only recently (see also Randi et al. 1989). Colonization of Mediterranean islands must have occurred after the last glacial period about 10,000 years ago, which is after Atlantic populations were already established (Voous 1976). Moreover, gene flow may be ongoing among Mediterranean populations, undetectable by observations of banded birds, but common enough to maintain low levels of genetic differentiation. Randi et al. (1989) estimated an exchange of 4 to 19 individuals per generation among breeding colonies, and Heidrich et al. (1996) detected two maternal lines within the Mediterranean subspecies, thus supporting the view that females disperse among populations. In addition, Mediterranean populations may

not have reached demographic equilibrium (Randi et al. 1989) owing to repeated perturbations by humans and introduced predators (Thibault 1995).

Second, Mediterranean and Atlantic populations appear to be more similar to one another than are populations within the Atlantic. This supports the hypothesis that the Mediterranean subspecies has recently radiated from a founder group of Atlantic individuals (see Voous 1976). Immigration from the Atlantic to the Mediterranean still occurs: three individuals of *C. d. borealis* recently were recovered in the Mediterranean, two of which bred successfully (Lo Valvo and Massa 1988, De Juana 1994, Thibault and Bretagnolle 1997).

Third, within the Mediterranean, populations are more differentiated at the microgeographic scale of Corsica than at the broadest macrogeographic scale of the basin. This apparently paradoxical result parallels that found by Friesen et al. (1996) in the Common Murre (*Uria aalge*). In that situation, substructuring within populations (microgeographic scale) exceeded structuring among populations (macrogeographic scale). Because effective population sizes of seabird colonies are lower than those of populations, and assuming that migration rates among colonies (within populations) exceed those between populations, Friesen et al. (1996) showed that if the factor of difference between migration rates is less than that between effective population sizes, substructuring within populations will exceed structuring among populations. Their results, however, relied on an island model of dispersal and genetic equilibrium among and within populations, conditions that are not certain for Cory's Shearwaters. Moreover, migration rates and population sizes may vary in time and space, and the resulting demographic instability has been shown to play a large role in partitioning genetic variation (e.g. Wade and McCauley 1988, Whitlock 1992).

Populations of Cory's Shearwater have a discrete distribution, i.e. archipelagos with several colonies (islands) and subcolonies within islands. Subcolonies are further subjected to variable levels of predation by humans and rats, which are two main factors in population regulation in this species (Thibault et al. 1997). As a result of such perturbations (which are heterogeneous in space and time) and differences in demographic factors (breeding success, effective population size and migration rate), bottleneck events, and random drift may have promoted rapid genetic differentiation among small colonies on islands within the Corsican archipelago (see also Baker and Moeed 1987, Whitlock 1992).

Da Silva and Granadeiro (1999) recently found that, unlike Mediterranean populations of Cory's Shearwater, Atlantic populations show reduced differentiation at minisatellite loci. Their analysis was based on eight Atlantic populations (vs. two Atlantic populations in our study). They suggested that a level of gene flow that is low enough to be undetected by banding studies but sufficient to prevent divergence could account for this lack of population genetic structuring. However, gene flow probably occurs in both subspecies, and because Atlantic populations were established well before Mediterranean ones, the former should exhibit greater genetic differentiation. Moreover, levels of population differentiation (F_{st}) obtained for *C. d. borealis* (0.02 to 0.094 and 0.007 to 0.168 for two different enzymes) by Da Silva and Granadeiro (1999) were lower than our values for *C. d. diomedea* (0.147 to 0.280). Da Silva and Granadeiro's (1999) values were, surprisingly, comparable to those found using mtDNA (Randi et al. 1989) despite the fact that minisatellites should evolve more rapidly than mtDNA. This latter finding contrasts with other studies that compared mtDNA and minisatellites in their efficiency to detect variability (Triggs et al. 1992, Degnan 1993, Fleischer et al. 1994, Freeman-Gallant 1996). Thus, we suggest that microsatellite markers were more efficient in detecting patterns of genetic structuring among populations of Cory's Shearwater. However, contrasting histories in the Atlantic and Mediterranean require that we sample and compare mtDNA of more Atlantic populations to develop a better picture of population structure and phylogeography of Cory's Shearwater in this region.

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