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Greater flamingo colonies around the Mediterranean form a single interbreeding population and share a common history

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The greater flamingo *Phoenicopterus roseus* is a long-lived colonial waterbird species, characterized by a large range encompassing three continents, a very limited number of breeding sites, and high dispersal abilities. We investigated both the phylogeographic history and the contemporary extent of genetic differentiation between eight different Mediterranean breeding colonies of greater flamingos sampled between 1995 and 2009, using both mitochondrial DNA and microsatellite markers. We found no significant differences in allelic richness or private allelic richness in relation to colony size. Overall, no genetic population differentiation was detected using either mitochondrial or microsatellite markers. F-statistics and Bayesian clustering methods did not support any significant genetic structure. Analysis of both mitochondrial DNA and microsatellites indicated that populations have undergone a bottleneck followed by rapid growth and expansion. The average time since expansion was estimated to be 696 421 yr (90% CI: 526 316–1 131 579 yr). We discuss our results in relation to both the possible historical events accounting for the present genetic structure and relevance to conservation and management of the species.

Predicting the genetic structure of colonial bird populations is not straightforward, as it depends both on contemporary ecological and/or behavioural features (such as the degree of natal philoptary and breeding dispersal, population size, and mating system) and on phylogeographic history (Tiedemann et al. 2004, Matthiopoulos et al. 2005, Milot et al. 2008, Overeem et al. 2008). For instance, stronger or weaker genetic structure than expected from ecological data (Milot et al. 2008) or from population history (Boessenkool et al. 2009) has been reported in seabirds. Moreover, contrasted patterns of genetic structure have been observed for closely-related species (Friesen et al. 2007). In contrast to seabirds, genetic structure has rarely been studied in colonial waterbirds that also show high dispersal abilities (but see Del Lama et al. 2002, Rocha et al. 2004, Reudink et al. 2011), but generally breed in less stable habitats. Exploring genetic diversity and differentiation in colonial waterbird species for which intercolony dispersal rates are known should thus provide insights in the respective roles of contemporary gene flow and population history in shaping their genetic structure.

The family Phoenicopteridae is particularly interesting as the six flamingo species that compose it have been described as being nomadic, breeding opportunistically in response to local favorable conditions (McCulloch et al. 2003, Childress et al. 2004, Amat et al. 2005, Johnson and Cézilly 2007). At the same time, although their geographic distributions cover large areas, all flamingo species tend to be very selective in their choice of breeding habitat, and generally form large colonies, often numbering thousands of individuals (Johnson and Cézilly 2007). Strong conspecific attraction coupled with aggregation at traditionally occupied breeding sites typically results in the vacancy of favorable alternative sites and a limited number of large breeding colonies. Little is known about the genetic structure of flamingo populations in relation to colonial breeding. A preliminary analysis based on mitochondrial DNA from a limited sample of 27 specimens has revealed little differentiation between the two main African populations of lesser flamingos *Phoenicopterus minor* from east and southern Africa (Zaccara et al. 2008). No data on population genetics are available for the other flamingo species.

Here we investigate both the phylogeographic history and the contemporary extent of genetic differentiation between breeding colonies of greater flamingos *P. roseus* in the Mediterranean. The greater flamingo is the most widespread species of the Phoenicopteridae family as it occurs on three continents (Europe, Africa and Asia), mainly in

brackish, saline and alkaline waters under tropical or temperate climatic conditions (Johnson and Cézilly 2007). However, only about 35, widely scattered, breeding sites are known for the species (Johnson and Cézilly 2007). In addition, under natural conditions, breeding sites are occupied on an irregular basis, depending on favourable climatic conditions and local water levels (Cézilly et al. 1995, Johnson and Cézilly 2007, Béchet and Johnson 2008). In the Mediterranean region, known breeding colonies of greater flamingos are located in Algeria, France, Italy, Morocco, Spain, Tunisia and Turkey. Long-term ringing and monitoring programs have been carried out since 1977 in France (Camargue) and since 1986 in Spain (Fuente de Piedra). More recently, similar programs have been launched at other breeding colonies around the Mediterranean.

Resightings of ringed flamingos have provided the basis for detailed analyses of demographic parameters in Camargue and Spain (Cézilly et al. 1996, Pradel et al. 1997, Tavecchia et al. 2001), mating system (Cézilly and Johnson 1995, Cézilly et al. 1997), and breeding behaviour (Cézilly 1993, Cézilly et al. 1994, Rendón et al. 2001, Schmaltz et al. 2011). Although flamingos become sexually mature at 3–4 yr of age, recruitment in the breeding segment of the population is progressive, with the latest birds starting breeding for the first time when 9 yr old (Pradel et al. 1997). Flamingos show low fecundity, laying a single egg per breeding attempt, high post-fledging survival (Johnson and Cézilly 2007), and adult annual survival rates above 0.93 and 0.97 for males and reproductively experienced females, respectively (Tavecchia et al. 2001). This is reflected in longevity records of 70 yr in captivity and at least 40 yr in the wild (Johnson and Cézilly 2007). In strong contrast to most long-lived Ciconiiforms (Cézilly et al. 2000), greater flamingos show no fidelity to mates between consecutive breeding years in the wild (Cézilly and Johnson 1995).

The true rate of breeding dispersal and, hence, gene flow between Mediterranean colonies of greater flamingos is difficult to estimate precisely from resightings of ringed birds because of low observer coverage in most of the Mediterranean (from over 400 000 resightings for over 30 000 ringed birds, 95% are from French and Spanish colonies, Johnson and Cézilly 2007) and intermittent breeding at several colonies. Despite the high levels of philopatry recorded for the two biggest and most stable colonies in the Mediterranean (Camargue, France and Fuente de Piedra, Spain; Nager et al. 1996), dispersal occurs regularly between closely-located breeding sites (Balkız et al. 2010), especially following low breeding success at the colony level (Nager et al. 1996), as observed in other colonial waterbird species (Dugger et al. 2010). A few long distance dispersal events have also been documented, between the western colonies (France, Spain or Italy) and the Gediz delta (east of Turkey) or the Banc d'Arguin (Mauritania; Balkız et al. 2007, Diawara et al. 2007). This pattern, associated with an irregular use of breeding sites through time, has led to the suggestion that *P. roseus* colonies in the Mediterranean function as a metapopulation (Balkız et al. 2007). However, the structure and dynamics of *P. roseus* populations do not conform to a metapopulation model sensu stricto because local extinctions do not result from a demographic extinction process, but from a temporal or definitive desertion of some breeding sites following poor environmental conditions (Matthiopoulos et al. 2005). In consequence, some breeders are more likely to disperse and reproduce in another colony instead of skipping breeding. As a result, local extinction-recolonisation of unstable breeding sites are expected to promote gene flow and prevent genetic differentiation through frequent 'reshuffling' of alleles in nearby colonies (Wade and McCauley 1988, Harrison and Hastings 1995, Pearce et al. 2005, Reudink et al. 2011). We therefore relied on both mitochondrial DNA and microsatellite markers to assess and compare genetic diversity levels between populations, infer past and present connections between colonies, and trace back population historical demography.

Material and methods

Sampling

Eight breeding sites (Fig. 1, Table 1, 2) were sampled all over the Mediterranean basin between 1995 and 2009. Some breeding sites were sampled in different years, resulting in 20 sampling units (SU, Table 3). In both Fuente de Piedra

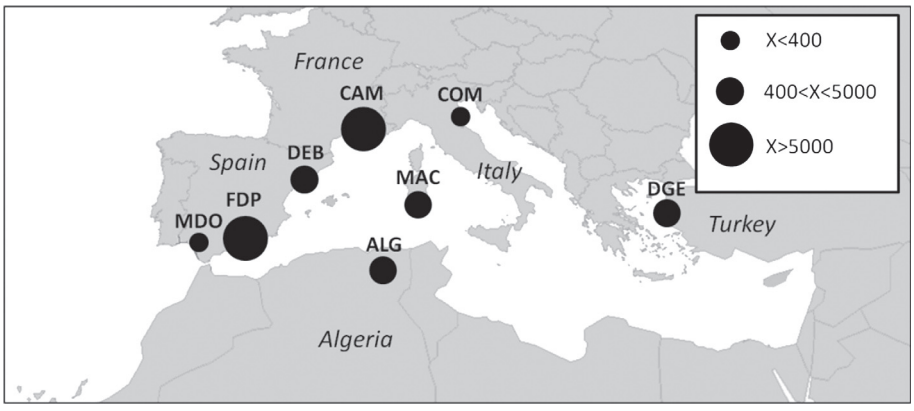

Figure 1. Locations and size of sampled breeding sites for greater flamingos in the Mediterranean basin. CAM: Camargue (France); FDP: Fuente de Piedra (Spain); DEB: Delta Ebro (Spain); MDO: Marismas del Odiel (Spain); MAC: Macchiareddu (Italy); COM: Comacchio (Italy); ALG: Garaet Ezzemoul (Algeria); DGE: Delta Gediz (Turkey); X: mean chick number on breeding years.

Table 1. Mitochondrial genetic diversity indices, sampling site (Fig. 1 for details) and date, sample size (n), number of haplotypes (k), allelic richness standardized for a sample size n = 21 (A_R), number of rare haplotypes, with frequency < 5% in the overall dataset (RH), haplotype diversity (h), number of segregating sites (S), nucleotidic diversity (π), and average number of differences between haplotype pairs (K) observed in 8 greater flamingo breeding sites .

Site	Date	n	k	A_R	RH	h	S	π	K
CAM	2003	21	6	4.02	4	0.61	5	0.0011	0.80
FDP	2007	25	6	3.31	4	0.53	5	0.0008	0.60
DEB	2007	25	5	2.45	3	0.30	4	0.0004	0.32
MDO	2008	23	2	1.96	1	0.17	1	0.0002	0.16
MAC	2007	25	3	1.00	0	0.23	2	0.0003	0.23
COM	2007	26	6	3.36	3	0.60	6	0.0012	0.87
ALG	2008	22	3	2.35	1	0.44	2	0.0006	0.47
DGE	2007	21	4	2.67	2	0.41	4	0.0007	0.54
Total		188	16	–	14	0.42	15	0.002	0.5

(Spain) and Garaet Ezzemoul (Algeria), birds breed in an intermittently flooded large depression, whereas all other Mediterranean breeding sites are located in commercial saltpans. The Camargue (France) breeding site is the most stable and productive (Johnson and Cézilly 2007), with almost continuous breeding over the last 40 yr (Béchet et al. 2012). In Spain, the Fuente de Piedra lagoon hosts the second most important breeding site but with no breeding in years of low rainfall resulting in 28 breeding attempts in the last 45 yr. All other breeding sites have been or known to be colonized by flamingos more recently (Gediz delta: 1982; Ebro delta: 1993; Macchiareddu (Cagliari): 1993; Comacchio: 1999; Garaet Ezzemoul: 2003; Marismas del Odiel: 2008), with irregular breeding since then for most of them. However, the novelty of some of these breeding sites does not mean that flamingos did not breed in the area in the recent past. In some cases, one new site might have been colonized following the abandonment of other nearby sites that were previously used, such as the Macchiareddu site (Italy), which has been used only since 1999 although breeding in this area is known since 1993. In addition, breeding attempts, sometimes by only a few tens or hundreds of pairs, can also occur at some other unsampled sites, most often with very low breeding success (Johnson and Cézilly 2007). For instance, at least 18 breeding attempts have been recorded in Algeria over the last eight years, while mass breeding occurred only at Garaet Ezzemoul (Boulekhssaïm et al. 2009, Samraoui et al. 2010).

A total of 762 chicks were sampled for either feathers or blood during ringing operations. Blood samples were obtained from punctuation of the brachial vein and stored in preservation buffer (Seutin et al. 1991), whereas feathers were stored in silicagel (Sigma). DNA was extracted using a standard phenol-chlorophorm method, and quality was UV assessed (Spectramax plus 384, Molecular devices) before diluting to ca 50 ng μl^{-1}.

Mitochondrial DNA sequencing and diversity

We amplified a 722 bp sequence of the cytochrome oxydase domain I for 188 individuals sampled at the 8 breeding sites (Table 1). This marker was selected since preliminary polymorphism tests indicated either similar level of polymorphism (Control Region, CR) or lower polymorphim (NADH dehydrogenase subunit 2 [ND2, 520 bp] or Cytochrome b [Cytb, 549 bp], Supplementary material Appendix 1). Primers were designed using PerlPrimer (Marshall 2004) based on the *Phoenicopterus ruber roseus* complete mitochondrial genome (NC_010089; Morgan-Richards et al. 2008): 'Pr-COIa-F' 5′-ACGCTTCAACACTCAGCCAT-3′, and 'Pr-COIa-R' 5′-TAATTCCAAAGCCTGGTAGG-3′. Amplification was carried out in 10 µl volume including 50 ng DNA template, 200 µM each dNTP, 200 nM each primer, 1× reaction buffer and 0.25 U HotMaster DNA polymerase (5-PRIME). A T3 thermocycler (Biometra) was used beginning with an initial denaturation at 94°C for 1.5 min, followed by

Table 2. Molecular definition and geographic distribution of the 16 haplotypes (h1–h16, GenBank Accession Number JF824492–JF824503) observed from 722 pb long mtDNA COI sequences in 8 *P. roseus* breeding sites.

	Polymorphic sites[a]																Geographic distribution of haplotypes								
	4	6	9	1	2	3	3	4	4	5	5	5	6	6	6										
Hapl	3	7	4	8	4	3	6	2	8	3	7	8	2	4	9		CAM 2003	FDP 2007	DEB 2007	MDO 2008	MAC 2007	COM 2007	ALG 2008	DGE 2007	Total
h1	G	A	T	T	T	C	G	T	T	T	C	A	A	C	C		13	17	21	21	22	16	16	16	142
h2	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.		3	1							4
h3	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.							2		1	3
h4	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.									1	1
h5	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.			1							1
h6	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.		2	4	1	2	2	4	5	3	23
h7	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.		1								1
h8	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.			1				2	1		4
h9	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.		1				1				2
h10	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.							1			1
h11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T			1							1
h12	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.		1								1
h13	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.				1						1
h14	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.				1						1
h15	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.				1						1
h16	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.							1			1

Hapl: haplotype name; [a]vertical numbers show the position of the polymorphic site relative to the complete *P. roseus* mtDNA genome sequence (NC_010089). Dots show homology with haplotype h1.

Table 3. Genetic diversity indices, averaged over 13 microsatellite loci, for 8 greater flamingo breeding sites, some being sampled on more than one occasion (Year), average number of alleles (A), average number of rare alleles (RA), allelic richness (A_R^{18}, A_R^{27}) and private allelic richness (PA_R^{18}, PA_R^{27}) standardized respectively for a sample size n = 18 and n = 27 by a rarefaction method, mean expected and observed heterozygosity (respectively He and Ho), and p-value of test for deficit in heterozygote relative to Hardy–Weinberg Equilibrium (HWE).

Site	Year	n	A	RA	A_R^{18}	A_R^{27}	PA_R^{18}	PA_R^{27}	He	Ho	HWE
CAM	1995	39	7.00	7.23	6.11	6.63	0.06	0.07	0.72	0.69	0.045[a]
	1996	35	6.62	5.38	5.80	6.29	0.06	0.07	0.71	0.67	0.012[a]
	1997	39	7.00	9.08	6.05	6.50	0.05	0.08	0.72	0.70	0.086
	1998	39	7.15	8.38	6.34	6.80	0.12	0.16	0.73	0.69	0.009[a]
	2008	33	6.23	4.46	5.78	6.16	0.00	0.00	0.66	0.67	0.809
	2009	30	6.77	4.38	6.14	6.59	0.03	0.01	0.70	0.74	0.988
FDP	1996	37	6.69	4.92	5.86	6.41	0.04	0.06	0.69	0.69	0.522
	1999	42	6.62	6.38	5.88	6.31	0.02	0.01	0.71	0.71	0.668
	2007	29	6.62	4.38	6.02	6.56	0.03	0.02	0.71	0.66	0.016[a]
	2009	42	6.85	5.62	5.95	6.44	0.10	0.12	0.70	0.73	0.983
DEB	2007	40	6.69	5.62	5.95	6.42	0.06	0.07	0.71	0.72	0.723
	2008	22	5.85	6.69	6.29	na	0.00	na	0.70	0.69	0.434
MDO	2008	41	7.00	5.31	6.12	6.58	0.05	0.00	0.70	0.70	0.968
MAC	2007	36	7.00	6.31	6.20	6.76	0.03	0.00	0.73	0.73	0.607
COM	2007	30	6.62	1.85	5.38	6.59	0.10	0.04	0.70	0.74	0.415
	2008	28	6.92	6.08	6.06	6.63	0.02	0.03	0.70	0.70	0.418
ALG	2008	40	7.00	3.00	5.67	6.71	0.00	0.00	0.70	0.71	0.234
	2009	45	7.08	5.85	6.13	6.56	0.04	0.05	0.70	0.72	0.394
DGE	2007	19	5.39	5.92	6.01	na	0.04	na	0.67	0.67	0.875
	2009	54	7.31	7.54	5.98	6.52	0.07	0.09	0.71	0.71	0.508

[a]: deficit in heterozygote not significant after Bonferroni correction.
na. not applied as sampling size is <27.

34 cycles consisting of 30 s at 94°C, 45 s at 46°C and 45 s at 65°C, and a final extension step at 65°C for 10 min. PCR products were sequenced by Macrogen (South Korea), using Big Dye Sequencing protocol (Applied Biosystems 3730xl). Sequences were edited and aligned manually using BioEdit 7.0.9 (Hall 1999). Genetic diversity was estimated by the haplotype number (k), the allelic richness (A_R; for a standardized population size of n = 21), the rare haplotype number (RH; with a global frequency $<5\%$), and the haplotype diversity (h). Molecular diversity was also estimated by the number of polymorphic sites (S), the nucleotide diversity (π) and the mean number of differences between haplotype pairs (K). A minimum spanning network was generated. All analyses were computed with either ARLEQUIN 3.1 (Excoffier et al. 2005) or DnaSP 5.10 (Librado and Rozas 2009).

Microsatellites typing and diversity

Seven hundred and sixty two individuals (Table 3) were amplified for 15 microsatellites loci specifically designed for *Phoenicopterus roseus*: PrA2, PrD3, PrD4, PrD5, PrD7, PrD9, PrA102, PrA110, PrA113, PrC101, PrC109, PrD102, PrD108, PrD121 and PrD126 (An et al. 2010). Genotyping was performed on a 96 capillary sequencer ABI3730XL (GENTYANE, INRA, France). Alleles were scored using GeneMapper 4.0 (Applied Biosystems).

Linkage disequilibrium and deviation from Hardy–Weinberg equilibrium were tested with FSTAT 2.9.3 for each SU and for the whole data set (Goudet 1995). Bonferroni corrections (Rice 1989) were applied to correct for multiple simultaneous comparisons. The program MICRO-CHECKER (van Oosterhout et al. 2004) was used to test for genotyping errors.

Levels of genetic variability were estimated for each SU such as the average number of alleles (A), the average number of rare alleles (RA; with frequency below 5% in the whole data set), the allelic richness (A_R), and the average frequency of private alleles (PA_R; alleles specific to a given sampling unit). The last two indices (A_R and PA_R) were standardized by a rarefaction method (Petit et al. 1998) both for a sample size of n = 18 and n = 27. The former sample size allowed computing A_R and PA_R for every sampling units whereas the latter excluded two SU (DEB2008 and DGE2007) with the smallest sample size. These indices were computed by HP-RARE 1.0 (Kalinowski 2004, 2005) or FSTAT 2.9.3. Differences in A_R and PA_R according to colony size were assessed using non parametric tests (Kruskal–Wallis analysis of variance), with colony size being classified as 'small', 'medium' or 'large' if the average number of chicks per year in which breeding did occur (X) were respectively $X<400$, $400<X<5000$, or $X>5000$ (Fig. 1; Johnson and Cézilly 2007). Differences for A_R and PA_R were also investigated between eastern and western Mediterranean colonies (DGE2007 and DGE2009 vs all the others) for a sample size of n = 27, using the hierarchy option in HP-RARE 1.0 and performing a Wilcoxon test across loci as recommended in Kalinowski (2004).

Population genetic structure analyses

For mitochondrial data, pairwise θ_{ST} and Fisher's exact test were computed with ARLEQUIN 3.1 for the 8 breeding sites. Numbers of migrants between breeding sites were derived from θ_{ST} values using the following equation: $M = (1-\theta_{ST})/(2\theta_{ST})$ (Excoffier et al. 2005).

For microsatellite data, F_{ST}, R_{ST}, and Fisher's exact tests were computed for pairs of SU to estimate spatial and temporal genetic structuring. F_{ST} and R_{ST} were calculated using ARLEQUIN 3.1 (Excoffier et al. 2005), whereas Fisher's exact tests were computed using FSTAT 2.9.3 (Goudet 1995). Since differentiation tests involved 190 pairwise combinations of SU, the probability of finding at least one significant value by chance with $\alpha = 0.05$ was extremely high ($p = 1-(1-0.05)^{190} = 0.999$). Levels of significance were therefore assessed using Bonferonni (Rice 1989), as well as Benjamini–Yekutiely (BY; Benjamini and Yekutieli 2001) corrections for multiple comparisons. Bonferroni correction is regarded as the most conservative method, whereas BY provides a better compromise between type 1 and 2 errors (Narum 2006). Isolation by distance was investigated using a Mantel test implemented in GENEPOP by comparing pairwise genetic distances ($F_{ST}/(1-F_{ST})$) to the natural logarithm of geographic distances between breeding sites. We assumed no geographical barrier of gene flow as flamingos are known to be able to fly across the Mediterranean (Green et al. 1989, Barbraud et al. 2003). We estimated gene flow ($4N_em$) among sampling sites (one cohort per sampling site: TDV08, FDP09, DEB07, MAC07, MDO08, COM08, ALG09, DGE09) and theta ($\Theta = 4N_e\mu$) within each breeding site using the coalescent approach implemented in MIGRATE (Beerli and Felsenstein 2001). For each run, we used 10 short chains of 400 000 sampled and 20 000 recorded trees, followed by 3 long chains of 4 000 000 sampled and 200 000 recorded trees. We chose adaptive heating with temperatures set to 1, 1.5, 3 and 10 000 in order to improve sampling of tree space. We assumed full migration model and symmetrical gene flow. We conducted these analyses four times. Initial estimates of Θ and gene flow were generated from F_{ST} values on the first run, and the maximum-likelihood estimates from the previous run were used as the initial estimates of these parameters for the subsequent three runs to confirm that final chains converged to the same estimates (as determined by overlapping 95% confidence intervals). Estimates are reported from the last run only.

Furthermore, we used the Bayesian clustering method implemented in the software STRUCTURE 2.1 (Pritchard et al. 2000) to identify the most probable number of genetic units K. Five runs for each value of K (1–20) were performed to assess consistency of likelihood estimations ($Pr(X/K = k)$). Other parameters were 1.0×10^5 MCMC iterations with an initial burn-in of 1.0×10^5 steps. We chose the admixture model with correlated allele frequencies (Falush et al. 2003) since we had no a priori reasons to exclude mixed ancestry of individuals and results strongly suggested current breeding groups to have derived from the same ancestral population. We then computed the posterior value of K ($Pr(K = k/X)$) following Eq. 3 in Pritchard et al. (2000) using R ver. 2.7.2 (R Development core Team).

Historical demography analysis

Historical demography analyses for both mtDNA and microsatellite data were performed for the whole data set considered as one population, since no differentiation between colonies was detected (see Results). Neutrality tests included Fu's Fs (Fu 1997), Tajima's D (Tajima 1989a, b), Fu and Li's F* and D* statistics (Fu and Li 1993).

Mismatch distribution and raggedness index were also performed (Rogers and Harpending 1992, Harpending et al. 1993). Time since population growth was inferred from the mismatch distribution (Rogers and Harpending 1992). The expansion time (τ) in mutational units was obtained with 90% confidence interval by parametric bootstrapping (1000 replicates). All analyses were computed with either ARLEQUIN 3.1 (Excoffier et al. 2005) or DnaSP 5.10 (Librado and Rozas 2009). Then, time in years since expansion (t) was calculated as $t = (\tau/2u) \times g$, with u being the haplotype mutation rate and g being the generation time of the species. A conventional molecular clock of 2% nucleotide substitution per site per million years was retained (Quinn 1992). Generation time was calculated as $g = \mu + [s/(1-s)]$, where μ is the average age at first breeding and s is the adult survival rate (Sæther et al. 2005). Considering a survival rate of 0.97 for adult reproductive females (Tavecchia et al. 2001), and 6 yr as the age at which 50% of individuals have been recruited in the breeding segment of the population (Pradel et al. 1997), generation time was estimated to be 38 yr.

To detect past demographic expansion based on microsatellite data, we used a method analogous to mismatch distributions, the imbalance index ($\ln\beta$) statistic, which relies on an imbalance between the variance of differences in allele size and heterozygosity in populations which have undergone demographic growth (Kimmel et al. 1998, King et al. 2000). We calculated the variance estimator θ_V and the homozygosity estimator θ_H from Eq. (1) and (3) of Kimmel et al. (1998). The difference between the natural logarithms of these two estimators, averaged over all microsatellite loci, is the imbalance index ($\ln\beta$). Kimmel et al. (1998) showed that a $\ln\beta > 1$ is a signature of population expansion preceded by a bottleneck whereas values of $\ln\beta < 1$ are characteristic of a population initially at mutation-drift equilibrium which gradually or suddenly increased in size. We also tested for the presence of an excess in heterozygosity in the population as expected after a bottleneck compared to a population at mutation-drift equilibrium with BOTTLENECK 1.2.02 (Cornuet and Luikart 1996). After few generations this genetic signature tends to disappear as the population reaches mutation-drift equilibrium again. We ran BOTTLENECK for the two kinds of mutation models appropriate for microsatellite data: the single mutation model (SMM; Luikart and Cornuet 1998) and the two-phased model (TPM; Di Rienzo et al. 1994). Following recommendations of Piry et al. (1999), we ran the TPM with 95% of single-step mutation and a variance of 12 among multiple steps, and used a signed-rank Wilcoxon test to assess significance of the heterozygosity excess.

Results

Mitochondrial DNA variation

Fifteen polymorphic sites were found for the mtDNA COI marker, defining 16 haplotypes (Table 2) presenting a star-like pattern illustrated by the minimum spanning network (MSN; Fig. 2). These haplotypes only differed from 1 to 4

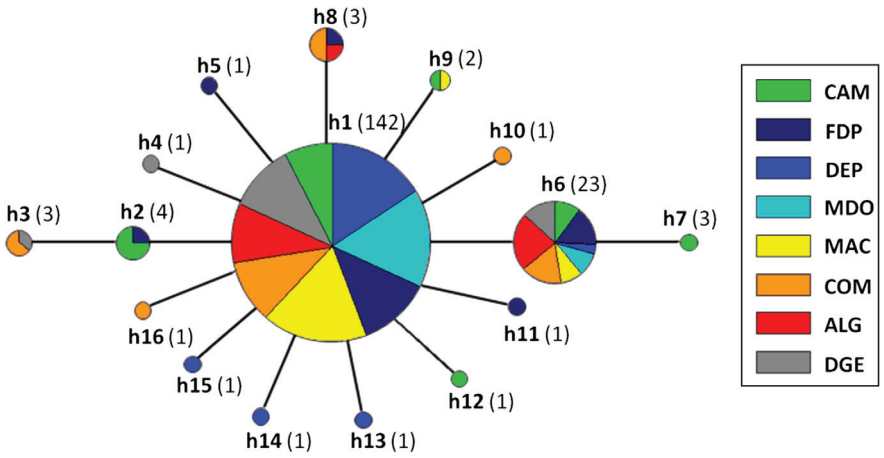

Figure 2. Minimum spanning network (MSN) of greater flamingo haplotypes based on 722 bp long mtDNA COI sequences. Each circle represent one haplotype (haplotype name beside the circle), with the area being proportional to the number of individuals sharing that haplotype (in parentheses). Lines between two haplotypes indicate one nucleotide difference. Slices within circle illustrate the haplotype distribution between the 8 sampled breeding sites (distinguished with different colors).

nucleotides and therefore could be considered as belonging to a single lineage. The haplotype (h1) displaying a central position in the MSN was abundant (found in 142 individuals) and widespread, occurring in every sampled colony. Genetic diversity indices are indicated in Table 1.

Microsatellite variation

Microsatellite loci appeared to be inherited independently of each other, as no evidence for linkage disequilibrium was found in any pair of loci. Two loci (PrC01 and PrD102) showed significant heterozygote deficit after Bonferroni correction due to the presence of null alleles and were excluded from all subsequent analyses. Of the remaining loci, 4 SU showed a deficit in heterozygotes, mostly due to the influence of a single locus (PrA102), although this was not significant after Bonferroni correction. The total number of alleles per locus varied from 5 (PrD121) to 12 (PrA102), and mean expected heterozygosity per locus varied from 0.41 (PrC109) to 0.86 (PrA102). Microsatellite diversity indices per SU are indicated in Table 3. Whichever sample size was chosen for standardization, there were no significant differences in allelic richness (A_R^{18}: $\chi^2 = 0.41$, DF = 2, $p = 0.82$; A_R^{27}: $\chi^2 = 2.59$, DF = 2, $p = 0.27$) or private allelic richness (PA_R^{18}: $\chi^2 = 0.73$, DF = 2, $p = 0.69$; PA_R^{27}: $\chi^2 = 1.61$, $p = 0.45$) in relation to colony size. Furthermore, no significant differences were found in allelic richness (A_R^{27}: $\chi^2 = 0.24$, DF = 1, $p = 0.63$) or private allelic richness (PA_R^{27}: $\chi^2 = 0.72$, DF = 1, $p = 0.40$) between eastern and western colonies.

Spatial and temporal genetic population structure

No genetic population differentiation was detected for mitochondrial or microsatellite dataset. For mtDNA, pairwise θ_{ST}, global θ_{ST} ($\theta_{ST} = 0.015$; standard error = 0.017) and Fisher's exact test values were all not significant (Supplementary material Appendix 1, Table A1). Estimates of the number of migrants between breeding sites inferred from pairwise θ_{ST} values ranged between 29 and infinity. For microsatellites, both pairwise F_{ST} (Table 4), and Fisher's exact test (Supplementary material Appendix 1, Table A2) were not significant after correction for multiple comparisons, even when using the least conservative method (Benjamini–Yekutieli). No significant correlation between genetic and geographic distances was found (Mantel test: Spearman rank correlation coefficient = −0.0006; $p = 0.53$). Bayesian clustering methods found no genetic structure and indicated both maximum likelihood and highest posterior probability for only one genetic cluster ($K = 1$; $P(K = 1) = 1$). Θ values estimated from microsatellite data were quite similar for all breeding sites, ranging from 0.91 to 1.06 (Table 5), and therefore could not be related to colony size or location. Estimated number of migrants fluctuated between 0.56 and 7.44. Most confidence intervals overlaped, but lowest and highest estimates were significantly different. The lowest values were found from the Marismas del Odiel (Spain) to the Camargue (France), and from the Saline di Comacchio (Italy) to the Marismas del Odiel, whereas the highest values were observed from Machiareddu (Sardinia) to the Gediz delta (Turkey) and from the Gediz delta to Fuente de Piedra (Spain). Interestingly, one of the highest gene flow estimates was found between distant colonies (DGE to FDP), whereas one of the lowest estimate was observed between two nearby colonies (MDO to CAM).

Historical demography

The mismatch distribution of pairwise nucleotide differences combining mtDNA COI haplotypes from all colonies produced a distinctive unimodal curve characteristic of populations having undergone a bottleneck followed by expansion (Fig. 3). In addition, this distribution did not deviate significantly from the expected distribution obtained under a sudden expansion model (SSD = 0.0012, $p = 0.55$; Harpending's raggedness index = 0.1384, $p = 0.55$). The average time since expansion was estimated to be 696 421 years (ranging from 526 316 to 1 131 579 yr), from τ values calculated over 1000 bootstrap replicates ($\tau = 0.527$, 90% CI: 0.40–0.86). All neutrality test statistics exhibited significant negative values (Fu's $Fs = -18.23$, $p < 0.00$; Tajima's $D = -2.07$, $p < 0.00$; Fu and Li's $D* = -4.48$, $p < 0.02$;

Table 4. Pairwise F_{ST} (below diagonal) and associated p values computed over 10 000 permutations (above diagonal) for 13 microsatellites between 20 sampling units (SU) of the greater flamingo.

	CAM 1995	CAM 1996	CAM 1997	CAM 1998	CAM 2008	CAM 2009	FDP 1996	FDP 1999	FDP 2007	FDP 2009	DEB 2007	DEB 2008	MDO 2008	MAC 2007	COM 2007	COM 2008	ALG 2008	ALG 2009	DGE 2007	DGE 2009
CAM 1995	*	0.451	0.153	0.586	0.234	0.991	0.856	0.216	0.748	0.297	0.568	0.802	0.153	0.487	0.081	0.342	0.306	0.748	0.991	0.757
CAM 1996	−0.002	*	0.955	0.937	0.487	0.991	0.757	0.811	0.883	0.694	0.396	0.469	0.333	0.523	0.441	0.991	0.541	0.351	0.991	0.640
CAM 1997	0.006	−0.007	*	0.604	**0.045**	0.982	0.288	0.225	0.523	0.090	0.090	0.360	0.369	0.189	0.550	0.559	0.135	**0.045**	0.982	0.081
CAM 1998	−0.002	−0.008	−0.001	*	0.360	0.991	0.766	0.874	0.982	0.451	0.090	0.451	0.414	0.090	0.333	0.964	0.829	0.243	0.991	0.973
CAM 2008	0.001	−0.001	**0.009**	0.000	*	0.505	0.279	0.613	0.856	0.982	0.216	0.748	0.631	**0.029**	0.072	0.252	0.081	**0.036**	0.946	0.559
CAM 2009	−0.044	−0.027	−0.017	−0.024	−0.003	*	0.991	0.991	0.982	0.982	0.991	0.991	0.820	0.991	0.604	0.973	0.541	0.991	0.505	0.991
FDP 1996	−0.006	−0.007	0.002	−0.004	0.000	−0.032	*	0.676	0.694	0.703	0.541	0.559	0.288	0.451	0.207	0.622	0.739	0.595	0.991	0.496
FDP 1999	0.002	−0.005	0.002	−0.005	−0.005	−0.021	−0.005	*	0.631	0.523	0.054	0.369	0.153	0.063	0.063	0.973	0.189	0.090	0.991	0.739
FDP 2007	−0.006	−0.008	0.000	−0.010	−0.008	−0.027	−0.005	−0.005	*	0.775	0.153	0.856	0.586	0.180	0.207	0.829	0.784	0.685	0.991	0.856
FDP 2009	0.002	−0.004	0.007	−0.001	−0.011	−0.018	−0.005	−0.003	−0.005	*	0.324	0.324	0.505	**0.036**	0.252	0.730	0.081	0.054	0.991	0.451
DEB 2007	−0.002	0.000	0.009	0.008	0.002	−0.034	−0.002	0.008	0.005	0.001	*	0.586	**0.036**	0.649	0.387	0.162	0.153	0.234	0.982	0.225
DEB 2008	−0.006	−0.001	0.001	0.001	−0.007	−0.039	−0.003	0.002	−0.007	0.002	−0.003	*	0.405	0.595	0.207	0.270	0.622	0.874	0.991	0.568
MDO 2008	0.006	0.001	0.001	0.000	−0.004	−0.008	0.003	0.003	−0.003	−0.002	**0.012**	0.000	*	**0.012**	0.261	0.234	**0.018**	**0.015**	0.991	0.225
MAC 2007	−0.002	−0.001	0.004	0.008	**0.015**	−0.031	0.000	0.013	0.005	**0.012**	−0.005	−0.005	**0.018**	*	0.297	0.153	0.378	0.550	0.991	0.243
COM 2007	0.005	−0.003	−0.003	0.002	0.007	−0.007	0.003	0.006	0.003	0.001	−0.001	0.002	0.001	0.001	*	0.360	0.052	**0.018**	0.496	0.081
COM 2008	0.001	−0.011	−0.002	−0.009	0.001	−0.016	−0.004	−0.009	−0.007	−0.005	0.005	0.006	0.004	0.006	0.001	*	0.279	0.090	0.955	0.568
ALG 2008	0.001	−0.004	0.006	−0.005	0.011	−0.004	−0.006	0.002	−0.006	0.006	0.003	−0.004	**0.012**	0.000	0.010	0.002	*	0.730	0.982	0.631
ALG 2009	−0.005	0.002	**0.011**	0.004	**0.010**	−0.030	−0.004	0.007	−0.003	0.009	0.003	−0.007	**0.016**	−0.003	**0.017**	0.008	−0.005	*	0.991	0.460
DGE 2007	−0.035	−0.029	−0.017	−0.033	−0.023	−0.005	−0.035	−0.029	−0.041	−0.030	−0.027	−0.042	−0.024	−0.021	−0.007	−0.018	−0.021	−0.033	*	0.991
DGE 2009	−0.004	−0.003	0.005	−0.007	−0.002	−0.025	−0.002	−0.004	−0.007	0.000	0.004	−0.003	0.003	0.004	0.005	−0.003	−0.003	−0.002	−0.031	*

Values in bold were non significant after Benjamini–Yekutieli correction for 190 multiple tests comparisons ($\alpha = 0.008$).

Table 5. Estimates of theta ($\Theta = 4N_e\mu$) and gene flow ($4N_em$) between 8 greater flamingo sampled breeding sites with 95% confidence intervals (in parentheses) estimated with program MIGRATE.

		$4Nm$							
Population i	Θ	CAM 2008 → i	FDP 2009 → i	DEB 2007 → i	MDO 2008 → i	MAC 2007 → i	COM 2008 → i	ALG 2009 → i	DGE 2009 → i
CAM 2008	0.94 (0.88–1.00)	- -	4.92 (4.21–5.76)	1.25 (0.96–1.60)	0.56 (0.40–0.75)	1.99 (1.60–2.48)	2.5 (1.96–2.96)	1.08 (0.83–1.41)	1.68 (1.33–2.12)
FDP 2009	0.95 (0.91–1.02)	1.68 (1.28–2.16)	- -	4.32 (3.61–5.14)	2.93 (2.36–3.60)	3.72 (3.05–4.52)	3.2 (2.56–3.92)	4.89 (4.08–5.76)	6.72 (5.73–7.88)
DEB 2007	0.91 (0.86–0.98)	3.08 (2.48–3.86)	2.8 (2.24–3.44)	- -	0.8 (0.56–1.16)	4.84 (4.04–5.9)	1.56 (1.20–2.04)	4.64 (3.88–5.56)	6.48 (5.52–7.60)
MDO 2008	0.99 (0.92–1.06)	5.04 (4.20–6.04)	5.96 (5.03–7.04)	2.36 (1.76–2.81)	- -	4.84 (4.01–5.80)	0.57 (0.36–0.84)	4.08 (3.36–4.92)	5.72 (4.80–6.20)
MAC 2007	1.04 (0.98–1.11)	3.2 (2.64–3.82)	1.72 (1.16–2.16)	3.56 (2.96–4.28)	5.04 (4.24–5.96)	- -	2.12 (1.68–2.64)	4.85 (4.08–5.72)	2.36 (1.89–2.88)
COM 2008	0.99 (0.92–1.06)	4.91 (4.12–5.96)	4.43 (3.68–5.32)	1.84 (1.41–2.33)	4.05 (3.32–4.92)	3.32 (2.68–4.08)	- -	4.28 (3.52–5.12)	2.12 (1.63–2.68)
ALG 2009	1 (0.94–1.06)	2.12 (1.68–2.64)	2.5 (2.08–3.61)	2.21 (1.76–2.77)	6.32 (5.36–7.48)	2.96 (2.41–3.64)	3.85 (3.59–4.64)	- -	5.04 (4.25–5.59)
DGE 2009	1.06 (1.00–1.12)	4.9 (4.04–5.72)	1.36 (0.77–1.76)	4.45 (3.72–5.29)	1.99 (1.58–2.45)	7.44 (7.21–8.63)	5.65 (4.77–6.68)	2.24 (1.79–2.75)	- -

Fu and Li's $F* = -4.28$, $p < 0.02$) indicative of divergence from mutation-drift equilibrium caused by population growth.

When considering microsatellite data for one single population at the scale of the Mediterranean, the positive value of Lnβ indicated again a bottleneck followed by rapid population growth ($Ln\theta_V = 4.04$; $Ln\theta_{Po} = -1.66$; $Ln\beta = 5.70$). However, analyses from BOTTLENECK provided evidence that this reduction in effective population size was not recent. Depending on the mutation model (TPM or SMM), 9 or 10 loci displayed a deficit in heterozygotes, whereas all the other loci exhibited an excess of heterozygotes. Accordingly, a one-tailed Wilcoxon test provided no evidence for heterozygosity excess compared to mutation-drift equilibrium (TPM: $p = 0.11$; SMM: $p = 0.10$).

Discussion

Genetic diversity

Only 16 mtDNA COI haplotypes were found, which is low given the high total sample size ($n = 188$ individuals) and sequence length (722 bp). Data for three others mtDNA targets (ND2, cytb and CR) assayed for polymorphism in two SU (FDP2007 and DGE2007) confirm this trend (Supplementary material Appendix 1, Table A1 and A2). Indeed, combining the two SU, the number of haplotypes for CR (10) is similar to COI (8) with similar sample size (32–38 individuals). ND2 and cytb were characterized by a low diversity with only four and three haplotypes respectively (see Waits et al. 2003 for an example of low diversity observed at several mitochondrial markers in the double-crested cormorant *Phalacrocorax auritus*). The prevailing hypothesis to explain low levels of mitochondrial haplotypic diversity associated with low nucleotidic diversity is a bottleneck responsible for allelic loss. For instance, rapid depletion in haplotype number has been shown in the endangered whooping crane *Grus americana* in which population size has been drastically reduced to 14 individuals, leading to the loss of about two-thirds of the haplotypes present in the pre-bottlenecked population (Glenn et al. 1999). Alternatively, a selective sweep may lead to the same molecular signature in mtDNA polymorphism. If it is impossible to distinguish between the two processes (demography vs selection) when only mtDNA data are used (Depaulis et al. 2003), the fact that a bottleneck was also detected for neutral markers (microsatellites, $Ln\beta = 5.70$) supports the hypothesis of a bottleneck as the main source of the observed pattern. However, a selective sweep can not discarded as possible additional source of reduction in polymorphism for mtDNA. The only data available for congeneric species indicate higher variation in the lesser flamingo *P. minor*, with higher levels of both haplotype and nucleotide diversity (respectively 0.83 and 0.0015–0.0017) despite relying on a smaller sample size (27 individuals only), and using a mitochondrial marker (ND2) of shorter length (Zaccara et al. 2008). Future investigation of the levels of mitochondrial diversity in the more closely-related Caribbean flamingo *P. ruber* would help to assess to what extent the low polymorphism observed in *P. roseus* is due to ancestral depletion or dates back to speciation. Microsatellite data provide evidence for a bottleneck ($Ln\beta = 5.70$), although not a recent one as we found no evidence for an excess of heterozygotes (Nei et al. 1975, Maruyama and Fuerst 1985). Because our microsatellite markers have not yet been used

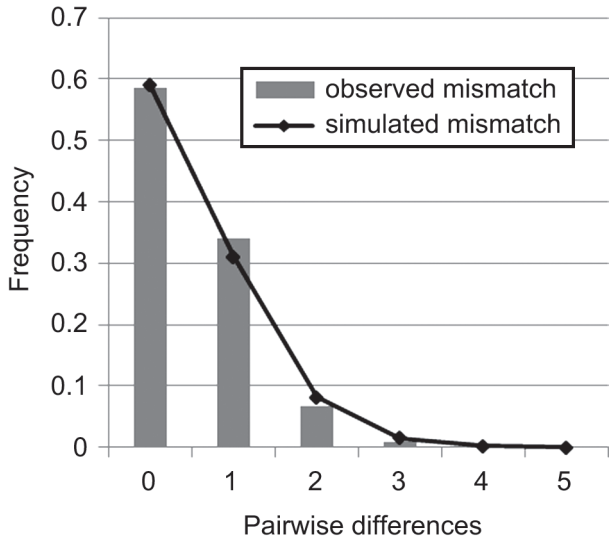

Figure 3. Pairwise nucleotides mismatch distribution for mtDNA COI *P. roseus* haplotypes. Solid line indicates expected distribution under the sudden expansion model of Rogers (1995).

on other flamingo species, interspecific comparisons of the levels of genetic diversity are not possible. However, expected heterozygosity levels are of the same magnitude (Goodstrey et al. 1998, Burg and Croxall 2001, Reudink et al. 2011) or higher (Rocha et al. 2004, Jones et al. 2005, van Bekkum et al. 2006, Overeem et al. 2008) than those observed in other long-lived bird species. No difference in allelic richness was found between breeding sites, and genetic diversity levels did not depend on colony size. This suggests that sufficient gene flow occurs between breeding sites to prevent local bottlenecks, even in the smallest colonies, thus maintaining high local effective population size (Broquet et al. 2010), as confirmed by the similar Θ values obtained for all breeding sites. The same pattern would be expected in an alternative scenario where all recent colonies would have been formed by a reasonably large number of founder individuals (the gene flow being effectively the colonization of the sites). However, the later scenario is unlikely as the colonies are known to have been colonized at different times and to vary markedly in their carrying capacity.

Genetic structure

We found genetic homogeneity of allelic frequencies across the whole Mediterranean for both mitochondrial and microsatellite markers. Moreover, microsatellite data indicated no departure from Hardy–Weinberg equilibrium. Temporal sampling of microsatellites markers at several sites showed temporal stability of allelic composition. Gene flow between breeding sites thus appears to be high and uninfluenced by geographical distances, invalidating the hypothesis of isolation by distance. The consistency between the patterns obtained by mitochondrial and microsatellite markers indicates similar levels of dispersal in both sexes, as previously indicated by demographic studies (Nager et al. 1996). Therefore we can confidently consider that all greater flamingos' colonies in the Mediterranean are part of a single genetically homogenous and panmictic population.

Until now, population structure and dynamics of the greater flamingo in the Mediterranean had been investigated only by capture–mark–recapture (CMR) methods, based on the resightings of ringed birds. Several studies identified structured patterns of movements, natal philopatry and breeding site fidelity, thus invalidating randomness of dispersal in this species primarily considered as nomadic (Nager et al. 1996, Rendón et al. 2001, Balkız et al. 2007, 2010). In fact, levels of natal philopatry, breeding site fidelity and hence dispersal are influenced by various factors, such as breeding site quality (annual stability of breeding conditions and breeding success; Nager et al. 1996, Rendón et al. 2001), intensity of intraspecific competition (Rendón et al. 2001), and individual breeding experience (Balkız et al. 2010). Some long-distance dispersal events have also been reported (Balkız et al. 2007, Diawara et al. 2007), but reciprocity of these exchanges is difficult to assess because of much lower ringing and resighting effort outside the north-western Mediterranean.

Hence, in that respect our genetic results contrast with CMR data as they did not detect isolation by distance, nor significant differences in gene flow between close or distant colonies (Balkız et al. 2007, 2010, Diawara et al. 2007). A similar pattern has recently been reported for another colonial waterbird species, the white pelican *Pelecanus erythrorhynchos*, for which CMR data suggested the coexistence of two isolated metapopulation systems, while genetic data indicated panmixia (Reudink et al. 2011). Discrepancies between patterns observed from CMR and genetic data are not rare, noticeably in seabirds (reviewed by Friesen et al. 2007). Comparisons between results obtained with the two methods often show that even if high natal and breeding philopatry levels are observed, population genetic structure can be homogeneous at various spatial scales (Roeder et al. 2001, Inchausti and Weimerskirch 2002, Burg and Croxall 2004, van Bekkum et al. 2006, Milot et al. 2008). The discrepancy between CMR and genetic data reflects the different time scales for which they are informative. If CMR provides an estimate of ongoing dispersal rates, genetic data estimate gene flow between populations on both an ecological an evolutionary time scale. Thus, our genetic data suggests that gene flow between Mediterranean colonies is high enough to prevent differentiation on an evolutionary time scale. However, we cannot infer the level of colony interdependence (i.e. true dispersal rates, level of colonies connectivity, chance of recolonisation of deserted colonies by nearby ones) without additional demographic analyses (Waples 1998).

Although data on the genetic structure of waterbird populations remain scarce, they are indicative of a similar situation to the one observed in the greater flamingo (wood stork *Mycteria americana*: Rocha et al. 2004; lesser flamingo: Zaccara et al. 2008; roseate spoonbill *Ajaja ajaja*: Santos et al. 2008; white pelican: Reudink et al. 2011). However, this pattern cannot be generalized to all colonial waterbirds, as other studies have reported high levels of genetic differentiation at continental scales, eventually leading to the distinction between different subspecies (great cormorant *Phalacrocorax carbo*: Goodstrey et al. 1998; sandhill crane *Grus canadensis*: Rhymer et al. 2001; lesser white-fronted goose *Anser erythropus*: Ruokonen et al. 2004). The peculiar mating system of greater flamingos, social monogamy with

no pair-bond over consecutive breeding seasons (Cézilly and Johnson 1995), may result in a extensive mixing of individuals at breeding colonies through time and thus may have also contributed to the observed genetic structure.

Historical demography and phylogeography

From mtDNA data, Fu's Fs, Fu and Li's F*, and Fu and Li's D* (Fu 1997) were all significantly negative, being compatible with either population expansion or background selection. However, synonymous polymorphisms were observed for COI (coding for the same amino acids) as well as other mtDNA targets sequenced as part of this study (ND2 and Cytb) which is not in favor of selection. In addition, all following features were in accordance with recent demographic expansion of the population: 1) a significantly negative Tajima's D test (which specifically tests for a population expansion; Tajima 1989a), 2) a star-shaped haplotype network (Avise 2000) and 3) an unimodal mismatch distribution with both SSD and Harpending's raggedness index (Rogers and Harpending 1992, Harpending et al. 1993) being non-significant. Finally, data for microsatellite (which are neutral) were also concordant with a rapid expansion with an imbalance index ($Ln\beta = 5.70$) higher than one (Kimmel et al. 1998, King et al. 2000). Therefore, both mtDNA and microsatellite data support an historical reduction in the effective size of the Mediterranean population, followed by a population expansion. As this demographic expansion was estimated to have started ca 693 421 yr ago (90% CI: 526 316–1 131 579), the several glacial (stadial) cycles that occurred since then may have had no marked genetic impact. Finally, mtDNA data showed that only one phylogeographic lineage is actually present in the Mediterranean. Therefore, contemporary Mediterranean flamingos might have either persisted in the whole Mediterranean area or in part of it, or may have recolonized at some point in their history the Mediterranean area from one single glacial refuge.

All through the Pleistocene, the paleo-environment of the Mediterranean basin went through large fluctuations in terms of average summer temperature, precipitation regime, and sea level (Martrat et al. 2004, Kuhlemann et al. 2008). Therefore, the suitability of the area for the breeding of flamingos may have varied drastically. Assuming that the ecological requirements of the species have not changed through time, temperatures, at least in the northern part of the Mediterranean basin, might have regularly been too low (Martrat et al. 2004, Jost et al. 2005, Kuhlemann et al. 2008), and the area too dry (Elenga et al. 2000) to provide favorable conditions for breeding. Exceptionally cold winters are known to induce mass mortality, as observed in the south of France in the winter of 1984–1985, when temperatures reached −18°C (Johnson and Cézilly 2007). All these features make unlikely a continuous presence of greater flamingos in the Mediterranean basin during the entire Pleistocene. An alternative hypothesis then consists in the past existence of a refuge area, different from the Mediterranean basin, which offered favorable local conditions for flamingos during the Pleistocene. This area may have corresponded to the African rift lakes. All three lakes in the area currently used as breeding sites, i.e. lake Magadi, lake Natron and lake Elmenteita, were already present during the Pleistocene (Owen et al. 2009). For example, evidence suggests that water levels during the last glacial maximum did not differ from present day, even though the region was then characterized by a drier climate (Barker and Gasse 2003). In addition, decrease in water levels and alkalinisation may have occurred in other African rift lakes at some point in their history (Cohen et al. 2007, Ryner et al. 2007, Stager and Johnson 2008), making them favorable for breeding by greater flamingos. A formal test of the two alternative hypotheses is however beyond the scope of this study, and would require obtaining samples over the whole range of the species.

Implications for conservation

Although the world population of the greater flamingo is estimated at about 500 000 individuals (Johnson and Cézilly 2007), the species remains vulnerable, particularly in the face of wetland loss throughout its distribution range and the low number of breeding sites (Béchet et al. 2009, 2012). Defining to what extent spatially and temporally discontinuous reproductive units should be considered as a single population is therefore of prime importance for conservation, particularly in the case of such a species with discontinuous breeding distribution and high potential for dispersal (Genovart et al. 2007, Gómez-Díaz et al. 2009). Here, the homogeneity of genetic structure attested by both microsatellite and mitochondrial markers indicates that all Mediterranean colonies of the greater flamingo should now be considered as part of a single evolutionary significant unit (ESU; Moritz 1994). In terms of genetic diversity, if the low polymorphism observed for mitochondrial markers might not be particularly informative (Reed 2010), heterozygosity at microsatellite markers suggest that the genetic potential of the Mediterranean population to adapt to ecological changes is high, although this would have to be confirmed by further investigations using non neutral loci, such as, for instance, genes from the major histocompatibility complex (Bos et al. 2008, André et al. 2011). Finally, since the size and history of colonies did not affect the levels of genetic diversity, no particular breeding site could be given conservation priority based on genetic information only. Conservationists should rather rely on demographic data, especially colony productivity and exchanges with other colonies ensuring demographic and genetic stability of the population to establish conservation priorities. On this basis, the Camargue and Fuente de Piedra, the two oldest and biggest colonies, for which exchanges with other west Mediterranean breeding sites are well known, are prime candidates and already targets of active conservation actions (Rendón-Martos and Johnson 1996, Johnson 1997, Béchet and Johnson 2008). However, additional data is needed to assess the level of demographic interdependence between breeding sites (Béchet et al. 2006), the importance of understudied colonies in Algeria (Boucheker et al. 2011) and Turkey (Balkız et al. 2007) for the rest of the Mediterranean populations, and possible connections with other areas (African and Middle-East populations).

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Supplementary material (Appendix J5549 at <www.oikosoffice.lu.se/appendix>). Appendix 1.