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Geographical variation in the yellow-legged gull: introgression or convergence from the herring gull?

J.-M. Pons[1], P.-A. Crochet[2], M. Thery[3] and A. Bermejo[4]

Abstract

Yellow-legged gulls *Larus michahellis* from the Atlantic Iberian coast exhibit some phenotypic similarities with the herring gull *L. argentatus* from Western Europe. To assess this phenomenon and its possible origin, we compared Mediterranean yellow-legged gulls, Atlantic Iberian yellow-legged gulls and herring gulls for several phenotypic traits (morphology, plumage), and used genetic data to determine the evolutionary history of the Atlantic Iberian yellow-legged gulls. Data from mitochondrial cytochrome *b* gene and microsatellite loci clearly indicate that Atlantic Iberian gulls are closely related to Mediterranean yellow-legged gulls, and do not show stronger signs of introgression with herring gulls relative to other populations of yellow-legged gulls. Atlantic Iberian yellow-legged gulls are more similar to herring gulls in body size and shape than to other yellow-legged gulls populations, but not in mantle colour and wing-tip pattern. Body size and other phenotypic and life history similarities with the herring gull (*L. argentatus argenteus*) such as voice, winter plumage and breeding phenology, previously described in several studies, might thus be interpreted as convergent characters. Within the yellow-legged gull, the high F_{st}-values obtained from four nuclear microsatellite loci indicate substantial population structure and reduced levels of gene flow between gull populations in Mediterranean France and Atlantic Iberia. Differences among these populations in breeding phenology and migration patterns, likely resulting from different local selection pressures, might contribute to this low level of gene flow.

Key words: *Larus argentatus* – *Larus michahellis* – microsatellites – mitochondrial DNA – phenotypic differentiation – southwest Palearctic

Introduction

Many species of birds exhibit strong variation in morphological characters between populations inhabiting different areas of their distribution (Zink and Remsen 1986). The significance of this variation has been discussed extensively, especially in the framework of the allopatric model of speciation, where this variation is seen as a first step toward speciation (Mayr 1963). This variation has often been interpreted as adaptive, i.e. resulting from the action of natural selection in different environments. However, random variation such as genetic drift combined with genetic isolation (isolation by distance or other factors reducing gene flow) could also result in geographical variation of phenotypic characters, and evidence for the adaptive nature of geographical variation is scant, especially in birds (Merilä and Crnokrak 2001). The occurrence of convergence, the expression of similar phenotypic traits in populations with independent evolutionary history but under similar environmental constraints, provides strong support for the action of natural selection on these traits. In this paper, we describe a case of possible morphological convergence between two species of Western European large white-headed gulls.

The systematics of the large white-headed gulls has been the subject of several recent papers, which have modified our understanding of the evolution on this group (de Knijff et al. 2001; Liebers et al. 2001; Crochet et al. 2002). Thus the yellow-legged gulls inhabiting the southwest of the Palaearctic region have been shown to comprise two species (Klein and Buchheim 1997), both distinct from the forms living in Northern Europe. The Caspian Gull *Larus cachinnans* (Pallas, 1811) inhabits the eastern part of the range, from the Black Sea basin eastward. The populations living in the Mediterranean basin, along the Atlantic coasts of Morocco and Iberia and in the Western Atlantic islands belong to the yellow-legged gull *L. michahellis* (see Sangster et al. 1998). The yellow-legged gull is currently split into two subspecies, *atlantis* (Dwight, 1922) restricted to the Macaronesian islands (del Hoyo et al. 1996; Snow and Perrins 1997) and *michahellis* (Naumann, 1840) a widespread continental form occurring along the Atlantic coasts of Iberia and Morocco as well as in the Mediterranean basin (Fig. 1).

Within *L. m. michahellis*, it has been suggested that populations from the northwestern Atlantic Iberian coasts differ from the Mediterranean populations in several phenotypic, ecological and ethological traits (see below). For several of these traits, the Atlantic Iberian populations are said to be more similar to the herring gull (*L. argentatus* Pontoppidan, 1763), a northern allopatric species (Teyssèdre 1983, 1984). This situation can be explained either by past introgression between yellow-legged gull and herring gull when the latter had probably a more southerly distribution during glacial maxima (Alcover et al. 1992; Covas and Blondel 1998) or by convergence. Comparisons of the amount and pattern of differentiation of phenotypic traits and genetic markers can separate between these alternative hypotheses. Introgression is a valuable hypothesis to test in this case since hybridization events and interspecific gene flow have been documented between different species of the '*argentatus-cachinnans-fuscus*' complex (Pierotti 1987; Liebers and Helbig 1999; Panov and Monzikov 1999; Liebers et al. 2001; Crochet et al. 2002).

Previous works have suggested that Atlantic Iberian populations of yellow-legged gull are closer to herring gull than to Mediterranean yellow-legged gull in voice (Teyssèdre 1984), breeding phenology (Minguez 1988; Munilla 1997a), pattern of post-breeding dispersion (Beaubrun 1988; Munilla 1997b) and winter plumage (Teyssèdre 1983). Our field observations confirm that Atlantic Iberian yellow-legged gull populations have a higher-pitched voice than Mediterranean yellow-legged gull populations (thus more similar to herring gull), breed

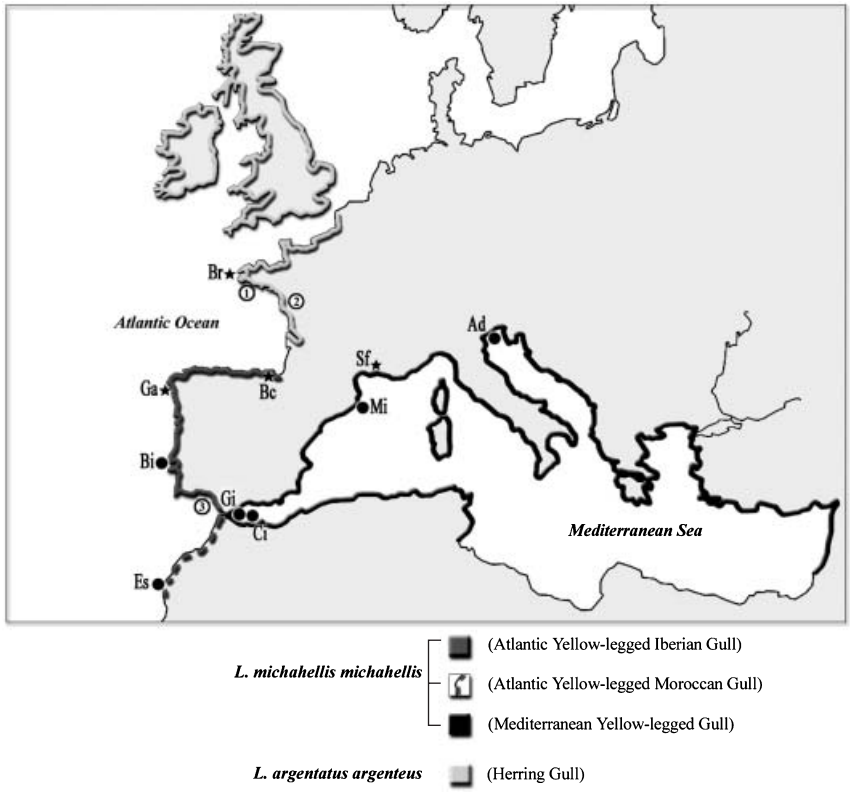

Fig. 1. Breeding distribution of *Larus argentatus* and *L. michahellis* in the southwest Palaearctic and studied localities. Black stars indicate locations of the colonies included in phenotypic and genetic analyses. Black dots indicate the localities for which only genetic data were available. (1) The herring gull breeds 200 km north from the breeding range of the Atlantic yellow-legged gull. (2) Along the Atlantic coast of France, south of Brittany, a small number of yellow-legged gulls coming from Mediterranean southern France breed in sympatry with the herring gull and do not hybridize although this has been observed elsewhere (Yésou 1991). (3) The Gibraltar straights appear to constitute a geographical limit between Atlantic and Mediterranean gulls. However, it is possible that the two forms meet in the poorly known populations distributed from the Portuguese border to the Atlantic side of the Gibraltar straight (A. Bermejo, pers. obs.). Adriatic (Ad), Basque Country (Bc), Berlenga islands (Bi), Britanny (Br), Cala Iris (Ci), Essaouira (Es), Galicia (Ga), Gibraltar (Gi), Medes island (Mi), South France (Sf)

4 weeks later than Mediterranean populations (thus at the same time as herring gull from Brittany, see Isenmann 1976 for Mediterranean yellow-legged gulls and Pons 1992 for herring gulls) and have a non-breeding plumage with extensive brownish streaks on the head and neck, similar to the non-breeding plumage of the herring gull, while Mediterranean yellow-legged gull show much more restricted greyish streaking on the head only.

In this paper, we examine the pattern of variation between herring gull and several populations of yellow-legged gull (Mediterranean and Atlantic Iberian populations) in additional characters: wing-tip pattern, biometry and mantle colour. We then evaluate the amount of genetic differentiation among the same populations using variation in allele frequencies of neutral molecular markers (mitochondrial DNA and four nuclear loci). If past introgression is responsible for the phenotypic resemblance of Atlantic Iberian yellow-legged gull and herring gulls, neutral markers should indicate that the Atlantic Iberian populations of yellow-legged gull have genetic contribution of herring gull and Mediterranean yellow-legged gull.

Materials and methods

Study localities

For the morphological data, our samples of Atlantic Iberian yellow-legged gulls originate from San Sebastian (Basque Country, North Spain) and Vionta (Galicia, Northwest Spain) (see Fig. 1, Table 1). They were compared with yellow-legged gulls from the Mediterranean area (Camargue, Southern France) and to herring gulls from Trébéron island and Ile aux dames (Brittany, Western France, see Fig. 1; Table 1).

Mitochondrial genetic data were collected from several populations of yellow-legged gulls from the Western Mediterranean (Camargue), the Atlantic-Mediterranean border (Gibraltar), Portugal (Berlenga islands) and Spain (San Sebastian, Vionta, Table 1 and Fig. 1). Herring gull samples were from Brittany also (Belle-Ile).

Microsatellites genetic data were collected from several colonies grouped in nine populations of yellow-legged gulls from the Northern Adriatic area, the Western Mediterranean and the Atlantic coasts of Morocco, Portugal and Spain (Table 1, Fig. 1). The precise localities are as follow: Cassa di Colmata near Mira, Salina di Cervia and Salina di Comacchion, NE Italy (North Adriatic); Ile Plane, Riou islands, near Marseille, S France (Marseille); Etang de Galabert and Etang des Impériaux, Camargue, S France (Camargue); Salins d'Aigues-Mortes, Southern France (Aigues-Mortes); Islas Medes, Gerona, NE Spain (Medes); Ile de Cala Iris, NE Morocco (Cala Iris); Ile d'Essaouira, SW Morocco (Essaouira); Ilha Berlenga Grande, Berlenga Islands, Portugal (Berlenga); Isla de Ons, NW Spain, Galicia, Pontevedra (Ons). Herring gulls samples were from Béniguet Island in Brittany (Finistère, W France), close to the place where morphological data were collected.

Phenotypic data

The phenotypic characters investigated were wing-tip patterns, mantle colour, body size and proportions. Biometric measurements were taken from live or culled breeding adults caught on their colonies

Table 1. Sample sizes per locality for phenotypic and genetic data

	Biometry	Colorimetry	Wing-tip	mtDNA	Microsatellites
Larus argentatus					
France (Britanny)					
Trébéron Island	109				
Ile aux Dames		15	32		
Beniguet Island					16
Belle-Ile				34	
L. michahellis					
Southern France					
Camargue	52	18	65	22	111
Aigues-Mortes					33
Marseille (Plane Island)					30
Spain					
Medes Island					19
Gibraltar				8	
Morocco (northeast)					
Cala Iris					20
Italia					
North Adriatic					29
Atlantic yellow-legged gull					
Spain (Basque Country)					
San Sebastian	34	13	18	20	
Spain (Galicia)					
Vionta	20	20	12	16	
Ons					29
Portugal					
Berlenga islands				13	30
Morocco (west)					
Essaouira					20

during the laying period. For yellow-legged gulls, colorimetric and wing-tip patterns data were obtained from live breeders (Atlantic Iberia) and from breeders culled during the laying period in Mediterranean France. For herring gulls, wing-tip patterns and colorimetric data were obtained from wings of breeders culled during control measures taking place in the laying period (P. Migot, pers. comm.). Most measurements were taken by the same observer (see below).

Wing-tip patterns

The wing-tip of adults of many large gull species exhibits a variable amount of black or dark on the outermost primaries and white spots on or near the primary tips (see Grant 1982 for a precise description). In the present study, primaries are numbered ascendantly, as is usually done with Larids (e.g. Coulson et al. 1982). To limit the effects of age on the wing-tip pattern (see Coulson et al. 1982; Allaine and Lebreton 1990), birds with a fourth-summer type plumage (i.e. with some speckling on wing coverts and/or rectrices, Monaghan and Duncan 1979) were excluded from analyses. Several variables were selected to describe the variations in the amount of black coloration on the wing-tip. The amount of black on the 10th primary (MIP10) and ninth primary (MIP9) was quantified using a melanism index comparable with those of Barth (1968) and Goethe (1961) except that we reduced the number of classes: our melanism index ranged from 1 to 9 for P10 and from 1 to 6 for P9. The other variables were the number of primaries with a black sub-terminal band or spot (NBP), the width of the black sub-terminal band across the webs of the feathers on the eighth (WBP8) and seventh primaries (WBP7). WBP8 and WBP7 were transformed into rank variables before being introduced in multivariate analysis.

Sexes were pooled because sexual dimorphism in wing-tip pattern has never been found in gulls (Mierauskas et al. 1991; Snell 1991; Conway 1995). For practical reasons we scored only one wing (generally the left) as there is usually no directional asymmetry in wing-tip patterns in large gulls (Conway 1995).

Colorimetric data

The colour of the mantle was assessed using a spectroradiometer (Ocean Optics, Inc. (Dunedin, FL, USA) PS 1000 diode array portable spectroradiometer). Reflectance curves were analysed with the segment method developed by Endler (1990) over the range 400–700 nm using the program Colour written by J.M. Rossi (laboratoire d'écologie, UMR 7625 CNRS, Université Pierre et Marie Curie, Paris). Three colorimetric variables were extracted: brightness (Q) between 400 nm and 700 nm, hue angle (H, dominant wavelength, corresponding to the colour name in every day speech), and chroma (C, purity or saturation of the colour) (see Endler 1990 for a detailed presentation of methods). To limit problems linked to possible seasonal variations in plumage colour (see Johnson et al. 1998), colorimetric measurements were made only with breeders trapped during the laying period.

Biometric data

Five biometric variables, measured according to Migot (1986), were included in the analyses. Wing-length (WL) was measured to the nearest mm using a ruler, head and bill length (HL), culmen length (CL) and bill height at the nostrils (BHN) to the nearest 0.1 mm using a calliper and weight (W) to the nearest 10 g with a hand-held 1500 g Pesola (AG, Boar, Switzerland). In gulls, body weight can strongly vary during the breeding season (Monaghan and Metcalfe 1986). It is thus worthwhile to note that in the present study all breeders were trapped only during the laying period. This allows us to think that our weight data are homogeneous and comparable. All measurements were performed by JMP except for eight breeders from Vionta (Galicia) that were measured by A. Bermejo.

Because important sexual dimorphism in body size is a well-known feature in gulls (e.g. Harris and Hope Jones 1969; Ingolfsson 1969; Coulson et al. 1983), individuals were first sexed. Herring gulls from Brittany were sexed according to discriminant equations established by Migot (1986). For yellow legged gulls from Camargue, sex was determined by dissection. Using a single variable (head length), Bosch (1996) successfully sexed 99.4% of the sampled individuals in a colony of yellow-legged gulls from northeast Spain. When combining head length and bill height in a discriminant function, he correctly classified 100% of the sampled individuals. However, due to differences in shape and size between Mediterranean and Atlantic Iberia yellow-legged gulls, it was not possible to apply this discriminant equation to Atlantic Iberia birds. As no individuals of sex known by dissection were available for Atlantic Iberia gulls, culmen length was plotted against bill height at nostrils to graphically separate sexes. Given that we obtained two very distinct clusters of points, we were confident that this method was reliable for sexing individuals.

Data analyses

Normality of quantitative variables was first checked using the one sample Kolmogov-Smirnov test. Rank wing-tip variables were treated using Kruskall–Wallis analysis of variance and principal component analysis (PCA). Biometric and colorimetric data were compared using univariate (one way and two ways parametric ANOVA). In addition, standardized PCA using correlation matrix were performed to visualize how individuals from different geographic origin were distributed in a multivariate space. Complete or backward discriminant-function analyses (DFA) were also performed to classify individuals, gulls being grouped *a priori* according to their geographic origin. To test the robustness of the classification resulting from the DFAs, we used a Jackknife cross validation technique in which the classification function is computed from all individuals except the case being classified (Wilkinson 1999). All analyses were performed with the Systat 9 package (Wilkinson 1999).

Genetic data

Cytochrome b haplotypes

Samples used for DNA extraction included muscle tissue or feathers preserved in ethanol and blood stored in buffer (APS) or ethanol. Mitochondrial DNA extraction, amplification and sequencing were done either in Montpellier or in Paris. The protocols used in Montpellier have been previously published (Crochet et al. 2000; Crochet and Desmarais 2000). The protocols used in Paris are given below.

DNA was extracted from tissue samples using the CTAB procedure (Winnepenninckx et al. 1993). Part of the cytochrome *b* gene was amplified using amplification primers L14967 (5′-CATCCAAC ATCTCTGCTTGATGAAA-3′) and H15938 (5′-ATGAAGGGA TGTTCTACTGGTTG-3′). L refers to light strands and H refers to heavy strands, and the numbers refer to the position of the 3′ nucleotide of the primer on the White Leghorn chicken (*Gallus gallus*) mtDNA sequence (Desjardins and Morais 1990). The amplifications were performed in a final volume of 25 μL. Cycling conditions were 92°C for 40 s, 54°C for 40 s and 72°C for 60 s for 30 cycles. After purification (QiaQuick PCR Purification Kit, Qiagena (Holden, Germany)), direct sequencing of a segment of approximately 300 base pairs was performed on an automated sequencer following the supplier's procedures (Beckmann Coulter, Inc., Fullerton, CA, USA). Primers L14967 and H15503 (5′-GATCCTGTTTCGTGGAG-GAAGGGT-3′) were used as sequencing primers. Sequences have been deposited in the Genbank database (accession numbers: AF268493, AF268496, AF268495, AY391897).

Microsatellites

Four microsatellites loci developed for the North American herring gull (HG 27, HG 14, HG 18, HG 25) were successfully used on European herring gulls and yellow-legged gulls. Primer sequences, protocols used to amplify these microsatellites, and the number and sizes of alleles are described in Crochet et al., 2003.

Data analyses

The proportion of genetic variation distributed among populations was estimated using F_{st} for both cytochrome *b* and microsatellites data. Methods based on F_{st} are based only on differences in allele frequencies. When applied to the mitochondrial DNA sequence data, they only take into account the differences in haplotypes frequencies between the populations, not the phylogenetic relationships among the haplotypes. Other methods are available to take this information into account, such as Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992). In our case, AMOVA results were qualitatively similar to those based on F_{st} (results not shown) and will not be presented here. F_{st} values were estimated by the parameter θ (Weir and Cockerham 1984) using the Genetix 4.01 software (Belkhir et al. 2000, available at http://www.univ-montp2.fr/~genetix/genetix.htm). The significance of the θ values was assessed by comparing the observed values to the distribution of values obtained from 500 random permutations of the individuals' genotypes between the different populations.

Pairwise sequence divergence among cytochrome *b* haplotypes was measured by the Kimura two-parameter distance (Kimura 1981). An UPGMA tree based on the Reynolds' distances [$-\ln(1-F_{st})$, see Reynolds et al. 1983] was constructed using microsatellites data to visualise genetic relationships among yellow-legged gulls and between yellow-legged gulls and the herring gull.

Results

Phenotypic data

Wing-tip patterns

A PCA performed on 127 gulls showed a clear separation between herring gulls from Brittany and yellow-legged gulls from Atlantic Iberia and Southern France (see Fig. 2). Within yellow-legged gulls, populations of different geographic origins largely overlapped and did not form any distinct cluster. The first two axes explained 69% of the total variance (Table 2). The width of the black sub-terminal band on P7 and P8 (WBP7, WBP8) contributed most to the first axis, herring gulls from Brittany having narrower black bands on primaries than yellow-legged gulls (ANOVA on PC1: $F_{2,124} = 75.6$, $p < 0.0001$. Atlantic yellow-legged gulls, Mediterranean yellow-legged gulls versus herring gulls; *post hoc* tests, $p < 0.001$. No significant differences among yellow-legged gulls). Melanism scores MIP9 and MIP10 had the highest loadings on the second axis along which there was no clear separation between herring gulls and yellow-legged gulls (ANOVA on PC2: $F_{2,124} = 0.9$, $p = 0.41$).

In univariate analyses, yellow-legged gulls had a higher melanism index MIP10 than herring gulls (Table 3, Kruskall–

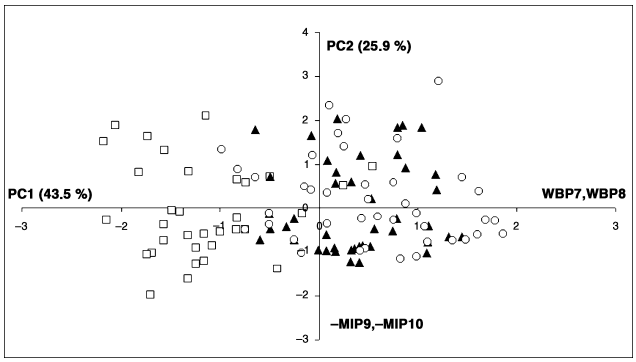

Fig. 2. Scatter plot of the first two principal components resulting from a principal component analysis performed on five wing-tip variables of 127 *Larus argentatus* from Brittany, Atlantic and Mediterranean yellow-legged gulls. White square, Brittany; black triangle, Atlantic Iberia; white circle, Mediterranean southern France

Table 2. Results of a standardized principal component analysis performed on 127 yellow-legged gulls and herring gulls using five wing tip variables. Geographic origin of birds; Brittany, Atlantic Iberian coast, Mediterranean southern France

	PC1	PC2
Eigen values	2.18	1.30
Total variance explained	43.15	25.97
Components loadings		
WBP7	0.92	0.06
WBP8	0.89	0.16
MIP9	−0.01	−0.85
MIP10	0.35	−0.74
NBP	0.64	0.09

Width sub-terminal black band (WBP7), P8 Width sub-terminal black band (WBP8), P9 melanism index (MIP9), P10 melanism index (MIP10), number of primaries with a sub-terminal black band (NBP).

Table 3. Geographic variation in wing-tip variables of yellow-legged gulls and herring gulls

	Yellow-legged gull				Herring gull	
	Atlantic Northwest Spain (Basque Country, Galicia)		Mediterranean France (Camargue)		Atlantic France (Brittany)	
	Median	Mean (SD) (*n*)	Median	Mean (SD) (*n*)	Median	Mean (SD) (*n*)
WBP7	40	40.22 (6.63) (31)	46	45.7 (6.37) (68)	26.25	26.40 (3.05) (32)
WBP8	71.6	72.59 (8.66) (31)	73.8	73.41 (8.12) (68)	51	51.19 (6.63) (32)
MIP9	5	3.96 (2.09) (44)	5	4.13 (1.74) (68)	5	4.63 (1.52) (32)
MIP10	7	6.55 (2.1) (43)	7	5.75 (1.99) (67)	6	5.5 (2.02) (32)
NBP	6 (6–7)[1]	6.23 (0.43) (43)	6 (6–7)	6.34 (0.51) (67)	6 (5–6)	5.9 (0.3) (31)

[1]Minimum–maximum.

Wallis test = 13.36, p = 0.001) and a higher number of primaries with a black spot (Kruskall–Wallis test = 17.87, p = 0.0001). In a similar way, yellow-legged gulls had a much larger black band on P7 and P8 than herring gulls [WBP7: $F_{(2,128)} = 120.20$, p = 0.0001. Sheffé's *post hoc* tests yellow-legged gulls versus herring gulls, p = 0.0001; WBP8: $F_{(2,128)} = 92.25$, p = 0.0001. Scheffè's *post hoc* tests yellow-legged gulls versus herring gulls, p = 0.0001]. On the other hand, there were significant differences among yellow-legged gulls only for MIP10 (Mann–Whitney test, $U = 1936.5$, p = 0.002), Atlantic birds having a higher P10 melanism index than their Mediterranean counterparts, and WBP7 (Scheffé's *post hoc* test Atlantic yellow-legged gulls versus Mediterranean gulls, p = 0.0001).

Mantle colour

A two-way analysis of variance was performed on the colour variables to test the effect of sex, geographical locality and interaction between these two principal effects (Table 4). A strong effect of locality was detected for the three colorimetric variables but neither sex effect nor interaction between locality and sex were significant. Herring gulls from Brittany had a much lighter plumage than yellow-legged gulls whereas nearly significant differences in brightness and chroma were found between Atlantic Iberian and Mediterranean gulls. Mediterranean birds tended to have a lighter and a less saturated plumage than Atlantic yellow-legged gulls. A similar trend was observed for the hue. Differences between herring gulls from Brittany and yellow-legged gulls from any origin were always highly significant whereas no statistical or only slight differences were found among yellow-legged gulls from diverse geographical origin.

To further investigate geographical variation in mantle colour, colorimetric variables were introduced in a Discriminant Function Analysis (Table 5). Multivariate ANOVA was strongly significant [Wilks lambda = 0.24; $F_{(6,126)} = 21.60$, p < 0.0001], the most discriminante variable being the brightness. Using the jackknife cross validation, all herring gulls were correctly classified whereas a high percentage of yellow-legged gulls were misclassified confirming that there was an overlapping in colorimetric characteristics of yellow-legged gulls from different geographical origin.

Morphometry

Two-way analyses of variance showed that there were highly significant differences in biometry according to geographical locality and sex, as well as a significant interaction between these two factors for several variables (Table 6). Results of subsequent analyses were thus presented for each sex. Scatter plots of principal components analyses performed on 106 females and 109 males showed that a similar pattern of variation in the PC1 × PC2 plane for males and females (females, Fig. 3a; males, Fig. 3b). A large part of the total variance was explained by the first axis, which can be interpreted as a 'size' axis, all variables being highly and positively correlated with the first axis (Table 7). A large

Table 4. Results of a two-way analysis of variance performed on three colorimetric variables for 66 yellow-legged gulls and herring gulls

	Yellow-legged		Herring gull			
	Atlantic Northwest Spain (Basque Country, Galicia) (*n* = 33) Mean (SD) (1)	Mediterranean (France, Camargue) (*n* = 18) Mean (SD) (2)	Atlantic France (Brittany) (*n* = 15) Mean (SD) (3)	*F*-ratio	df	p-value
Brightness (Q)	4584.14 (691.2)	4973.8 (830.1)	6922.6 (466.8)			
Locality				50.86	2,60	0.0001
Sex				1.36	1,60	0.25
Locality × sex				0.46	2,60	0.64
Chroma (C)	0.034 (0.008)	0.028 (0.006)	0.044 (0.014)			
Locality				9.62	2,60	0.0001
Sex				1.47	1,60	0.23
Locality × sex				0.35	2,60	0.71
Hue (H)	23.99 (5.66)	20.60 (7.05)	35.61 (8.11)			
Locality				19.28	2,60	0.0001
Sex				0.18	1,60	0.67
Locality × sex				0.20	2,60	0.82

Post hoc comparisons Scheffé's tests:
Brightness: (1) versus (2), p = 0.15; (1) versus (3), p = 0.0001; (2) versus (3), p = 0.0001.
Chroma: (1) versus (2), p = 0.09; (1) versus (3), p = 0.003; (2) versus (3), p = 0.0001.
Hue: (1) versus (2), p = 0.22; (1) versus (3), p = 0.0001; (2) versus (3), p = 0.0001.

	Atlantic (Iberia)	Atlantic (France)	Mediterranean (Southern France)	Percentage of correct
Atlantic (Iberia)	25 (23)	1 (1)	7 (9)	76 (70)
Atlantic (France)	0 (0)	16 (16)	0 (0)	100 (100)
Mediterranean (Southern France)	7 (8)	1 (2)	11 (9)	58 (53)

Table 5. Jacknifed classification matrix obtained with a stepwise discriminant analysis performed on three colorimetric variables for 68 yellow-legged gulls and herring gulls

Values in brackets are obtained with the Jackknife method.
The most discriminante variable is the Brightness (Q).

Table 6. Result of two-ways analyses of variance performed on biometric variables for 215 Atlantic and Mediterranean yellow-legged gulls and herring gulls from Brittany

	F-ratio	df	p-value
Weight			
Locality	183.29	(2,209)	0.0001
Sex	386.33	(1,209)	0.0001
Locality × sex	7.37	(1,209)	0.001
Wing length			
Locality	346.71	(2,209)	0.0001
Sex	326.6	(1,209)	0.0001
Locality × sex	4.17	(1,209)	0.017
Head length			
Locality	207.1	(2,209)	0.0001
Sex	670.46	(1,209)	0.0001
Locality × sex	0.3	(1,209)	0.74
Culmen length			
Locality	38.34	(2,209)	0.0001
Sex	407.26	(1,209)	0.0001
Locality × sex	0.62	(1,209)	0.54
Bill height (at nostrils)			
Locality	358.55	(2,209)	0.0001
Sex	686.88	(1,209)	0.0001
Locality × sex	7.19	(1,209)	0.001

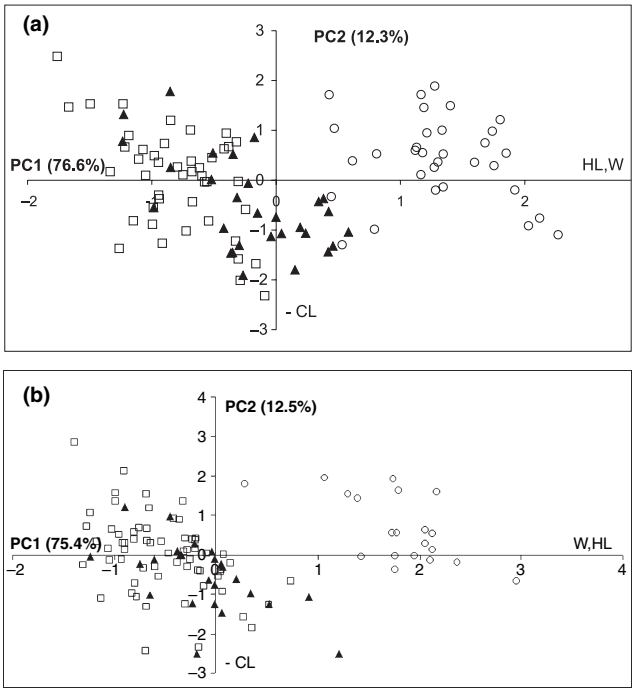

Fig. 3. (a) Scatter plot of the first two principal components resulting from a principal component analysis performed on biometric variables of 106 breeding females *Larus argentatus* from Brittany, Atlantic and Mediterranean yellow-legged gulls. White square, Brittany; black triangle, Atlantic Iberia; white circle, Mediterranean southern France. (b) Scatter plot of the first two principal components resulting from a principal component analysis performed on biometric variables of 109 breeding males *L. argentatus* from Brittany, Atlantic and Mediterranean yellow-legged gulls. White square, Brittany; black triangle, Atlantic Iberia; white circle, Mediterranean southern France

overlap occurred between herring gulls from Brittany and yellow-legged gulls from Atlantic Iberia whereas these two groups are clearly separated from the much larger Mediterranean birds. This separation is even more marked for males than for females (see Fig. 3a, b). An analysis of variance performed on the PC1 indicated that there were significant differences among the groups [females, $F_{(2,103)} = 198.81$, $p = 0.00001$; males, $F_{(2,106)} = 165.87$, $p = 0.00001$]. Each group was significantly different from each of the other group (Scheffé's *post hoc* tests, $p = 0.0001$ for all pair comparisons except Atlantic Iberia versus Atlantic France, $p = 0.004$).

The second axis, which can be interpreted as a 'shape' axis, also separated Mediterranean birds from Atlantic Iberian yellow-legged gulls [females, $F_{(2,103)} = 7.36$, $p = 0.001$; males, $F_{(2,106)} = 9.25$, $p = 0.0001$. Scheffé's *post hoc* tests: females, $p = 0.001$; males, $p = 0.0001$] whereas differences were only slightly significant between Atlantic yellow-legged gulls and herring gulls from Brittany (females, $p = 0.06$; males, $p = 0.04$). Hence the three groups are differentiable from one other on the 'size axis' whereas only two groups (Mediterranean/Atlantic Iberia, Brittany) are distinguishable on the second axis.

To further investigate geographical variation in morphometry, we performed a DFA (stepwise backward option) considering three a priori geographical groups (Atlantic Iberia, Atlantic France, Mediterranean France). Multivariate ANOVA was strongly significant for females (Wilk's lamda = 0.08, $F = 48.93$, df = 10, $p = 0.00001$) and for males (Wilk's lamda = 0.09, $F = 59.95$, df = 8, $p = 0.00001$). Using Jackknife cross validation, no birds from Atlantic Iberia were misclassified as Mediterranean yellow-legged gulls whereas only two females from Mediterranean France were misclassified as coming from Atlantic Iberia (Table 8). On the other hand, there was a higher percentage misclassified between the herring gull and the Atlantic yellow-legged populations. Results concerning males are similar but more clear-cut, the separation being complete among yellow-legged gulls (100% of correctly classified in every case) and less marked among Atlantic yellow-legged gulls and herring gulls (Table 8). These results indicate that Atlantic yellow-legged gulls breeding in Northern Atlantic Iberia are clearly distinct from birds breeding in Mediterranean France. This distinction is due to differences in size (as measured by wing length and weight) and, more importantly to shape, birds from Atlantic Iberia having shorter head and a thinner bill than their Mediterranean counterparts.

For each biometric variable, highly significant differences were found between Atlantic Iberian and Mediterranean gulls

Table 7. Results of standardized principal components analyses performed on adults Atlantic and Mediterranean yellow-legged gulls and herring gulls using five biometric variables. Geographic origin of birds; Brittany, Atlantic Iberian coast, Mediterranean southern France

	Females ($n = 106$)		Males ($n = 109$)	
	PC1	PC2	PC1	PC2
Eigen values	3.83	0.62	3.77	0.63
Total variance explained	76.65	12.34	75.44	12.53
Component loadings				
W	0.87	0.28	0.90	0.15
WL	0.91	0.19	0.88	0.20
HL	0.94	−0.18	0.93	−0.02
CL	0.76	−0.64	0.77	−0.61
BHN	0.90	0.26	0.86	0.40

Weight (W), wing length (WL), head length (HL), culmen length (CL), bill height at nostrils (BHN).

(Tables 9 and 10). Biometric differences among males were higher than differences found among females. On average, male and female Iberian gulls weighed 21 and 17% less and had a 6 and 5% shorter wing lengths, respectively, than Mediterranean gulls. Therefore, an Iberian male was approximately the same size as a Mediterranean female. On the other hand, Atlantic Iberian yellow-legged gulls were only slightly larger than herring gulls from Brittany, differences varying from 1.8 to 3.9% depending on variables, without being heavier (see Tables 9 and 10).

Genetic data

Mitochondrial Cytochrome b

A 308 base pairs fragment of the cytochrome *b* gene was sequenced from 113 specimens. The number of substitutions between the five identified haplotypes varied from one to six. Consequently the estimates of pairwise sequence divergence given by the Kimura two-parameter distance were low, varying from 0.3 to 2% (Table 11). Each haplotype has been named according to the taxon where it is most frequent (MIC, *michahellis*; ARG, *argentatus*; MAR, *marinus*, see Crochet 1998; Crochet et al. 2002).

Haplotype frequencies in each taxon are given in Fig. 4. Each population is characterized by one haplotype found at a very high frequency as exemplified by the haplotype MIC1 for yellow-legged gulls from Southern France and Atlantic Iberia. Individuals from these two areas shared the same haplotypes (MIC1 and MIC2) at very similar frequencies. On the other hand, yellow-legged gulls from Atlantic Iberia did not share any haplotypes with *L. argentatus*.

The θ_{st} value between *L. argentatus* (Brittany) and *L. michahellis* (all origins) was high and significant: θ_{st} (arg –

Table 8. Jacknifed classification matrix obtained with a backward stepwise discriminant analysis performed on biometric characters for 106 breeding females and 109 breeding males of yellow-legged gulls from Mediterranean Southern France, Atlantic Iberian and herring gulls from Atlantic France

	Atlantic (Iberia)	Atlantic (France)	Mediterranean (Southern France)	Percentage of correct
Females				
Atlantic (Iberia)	26 (24)	3 (5)	0 (0)	90 (83)
Atlantic (France)	4 (5)	41 (40)	0 (0)	91 (89)
Mediterranean (Southern France)	2 (2)	0 (0)	30 (30)	94 (94)
Males				
Atlantic (Iberia)	21 (20)	4 (5)	0 (0)	84 (80)
Atlantic (France)	6 (9)	58 (55)	0 (0)	91 (86)
Mediterranean (Southern France)	0 (0)	0 (0)	20 (20)	100 (100)

Values in brackets are obtained with the Jackknife method.
Five variables entered in the model [weight (W), wing length (WL), head length (HL), culmen length (CL), bill height at nostrils (BHN)].
Females: most discriminante variables are BHN > HL > W > WL > CL.
Males: most discriminante variables are WL > BHN > W > HL (CL has been removed in the final model).

Table 9. Geographic variation in biometric measurements of 106 yellow-legged gulls and herring gulls. Measurements made on breeding females trapped during the laying period

	Yellow-legged gulls				Herring gull		
	Atlantic Northwest Spain (Basque Country, Galicia[1]) ($n = 29$)	Mediterranean France (Camargue) ($n = 32$)			Atlantic France (Brittany) ($n = 42$)		
	Mean (SD)	Mean (SD)	p-Value[2]	D[3] (%)	Mean (SD)	p-Value[4]	D[5] (%)
Body weight	797.6 (50.6)	964.7 (72.5)	0.0001	−17	802.4 (50.2)	0.72	NS
Wing length	414.2 (7.6)	436.2 (9.7)	0.0001	−5	402.3 (7.0)	0.001	+2.9
Bill height (at nostrils)	16.1 (0.4)	18.0 (0.50)	0.0001	−10.6	15.7 (0.51)	0.001	+2.5
Culmen length	51.9 (1.9)	53.2 (1.6)	0.005	−2.4	50.8 (1.8%)	0.004	+2.1
Head length	115.6 (3.1)	121.1 (2.5)	0.0001	−4.5	111.3 (2.6)	0.0001	+3.9

[1]There were no significant differences in measurements between our Basque country and Galicia samples. Then data were pooled in an Atlantic Northwest Spain sample.
[2]p-Values of *t*-tests (Atlantic Northwest Spain versus Mediterranean France).
[3]Difference in percentage between means (Atlantic Northwest Spain versus Mediterranean France).
[4]p-Values of *t*-tests (Atlantic Northwest Spain versus Atlantic France).
[5]Difference in percentage between means (Atlantic Northwest Spain versus Atlantic France).

Table 10. Geographic variation in biometric measurements of 109 yellow-legged gulls and herring gulls. Measurements made on breeding males trapped during the laying period

	Yellow-legged gulls				Herring gull		
	Atlantic Northwest Spain (Basque Country, Galicia[1]) ($n = 25$)	Mediterranean France (Camargue) ($n = 20$)			Atlantic France (Brittany) ($n = 64$)		
	Mean (SD)	Mean (SD)	p-Value[2]	D[3] (%)	Mean (SD)	p-Value[4]	D[5] (%)
Males							
Body weight	949.4 (61.3)	1208.5 (78.9)	0.0001	−21.4	972.6 (71.2)	0.13	NS
Wing length	434.2 (10.5)	463.5 (7.5)	0.0001	−6.3	421.5 (8.2)	0.0001	+2.9
Bill height (at nostrils)	17.6 (0.5)	20.3 (0.5)	0.0001	−13.3	17.8 (0.6)	0.50	NS
Culmen length	57.2 (2.0)	59.3 (2.0)	0.001	−3.5	56.2 (2.1)	0.04	+1.8
Head length	126.4 (3.5)	132.7 (3.3)	0.0001	−4.7	122.1 (2.8)	0.0001	+ 3.4

[1]There were no significant differences in measurements between our Basque country and Galicia samples except a slight difference in the bill height ($\text{mean}_{\text{Galicia}} = 17.4$, SD = 0.4, $\text{mean}_{\text{Basque Country}} = 18.0$, SD = 0.4 ; *t*-test, p = 0.006). Then data were pooled in an Atlantic Northwest Spain sample.
[2]p-Values of *t*-tests (Atlantic Northwest Spain versus Mediterranean France).
[3]Difference in percentage between means (Atlantic Northwest Spain versus Mediterranean France).
[4]p-Values of *t*-tests (Atlantic Northwest Spain versus Atlantic France).
[5]Difference in percentage between means (Atlantic Northwest Spain versus Atlantic France).

Table 11. Pairwise Kimura two-parameters distances between cytochrome *b* haplotypes found in the herring gull and yellow-legged gulls (see text for the names of haplotypes)

	MIC1	MIC2	MAR
MIC2	0.003	–	
MAR	0.003	0.007	–
ARG	0.017	0.020	0.013

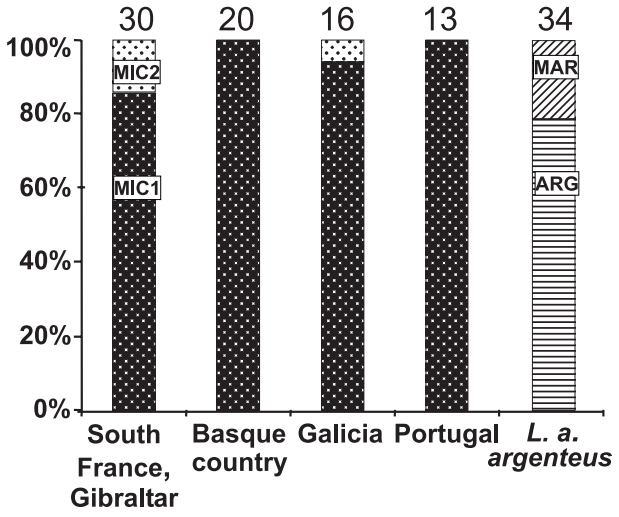

Fig. 4. Frequencies of the cytochrome *b* haplotypes found in 113 Atlantic and Mediterranean yellow-legged gulls and *Larus argentatus* from Brittany. Number of specimens above each histogram. See text for the names of haplotypes

mic) = 0.84, p < 0.001. Not surprisingly (see Fig. 4), a very low and non-significant θ_{st} value was found between populations of yellow-legged gulls from Southern France and Atlantic Iberia: θ_{st} (Southern France – Atlantic Iberia) = 0.002, p = 0.29.

Nuclear microsatellites

Alleles frequencies at the four microsatellites loci are given in Table 12. There were no significant deviation from Hardy–Weinberg equilibrium (no significant F_{is} values, permutation tests with 1000 replications as implemented in Genetix) for any locus after Bonferroni's correction for multiple testing.

Significant pairwise values of θ_{st} between populations of Mediterranean southern France and Atlantic Iberia (Galicia, Portugal) show that part of the variance in allele frequencies is due to geographic differentiation (Table 13). Galician and Portuguese populations were poorly differentiated while these Atlantic Iberian populations were significantly differentiated from the Mediterranean populations. The UPGMA tree based on the Reynolds' distances (Fig. 5) clearly indicates that the Iberian Atlantic populations fall within the yellow-legged gulls cluster and do not show any sign of introgression with the herring gull at these four nuclear loci.

Discussion

Intraspecific variability of the yellow-legged gull and systematic implications

Results of multivariate analyses indicate a substantial amount of geographical variation in phenotype of yellow-legged gulls inhabiting south-western Europe. Birds from the Mediterranean basin and birds from the North Atlantic coast of Spain differ in body size and proportions to the point that all Atlantic Iberian gulls can be identified as such, whereas other characters such as mantle colour and wing-tip pattern overlap between populations but show evidence of geographical variation. Although phenotypic plasticity has been shown to influence size in birds (Boag 1983, 1987; James 1983; Merilä and Wiggins 1995), the large amount of variation observed in our study and the numbers of characters concerned, including some plumage traits, suggest that genetic differences are probably involved.

On the Atlantic coast of France, a small number of yellow-legged gulls, most of them originating from southern France as shown by ringing recoveries (Marion et al. 1985), have been breeding in sympatry with the herring gull since the 1970s. These birds are phenotypically typical of 'Mediterranean' yellow-legged gulls and although they lay 2 weeks later than their Mediterranean counterparts in southern France, they still lay 2 weeks earlier than sympatric herring gulls and allopatric yellow-legged gulls breeding along the Atlantic Iberian shores (Minguez 1988; Yésou 1991; Munilla 1997a). This suggests that in gulls, laying date is partly genetically determined, as it

Table 12. Sample size and allele frequency for each population and each microsatellite locus

Locus	Allele	North Adriatic	Medes	Cala Iris	Berlenga	Ons	Aigues-Mortes	Camargue	Essaouira	Marseille	Herring gull
HG27		$n = 29$	$n = 19$	$n = 20$	$n = 30$	$n = 29$	$n = 33$	$n = 111$	$n = 20$	$n = 30$	$n = 16$
	114	0.0000	0.0263	0.0000	0.0000	0.0000	0.0152	0.0045	0.0000	0.0000	0.0000
	115	0.0172	0.0263	0.1000	0.0333	0.0345	0.1212	0.0225	0.2500	0.0667	0.0625
	116	0.4483	0.3158	0.4250	0.4333	0.5345	0.3636	0.4324	0.4500	0.4500	0.5625
	117	0.5345	0.5789	0.4750	0.5333	0.4310	0.5000	0.5405	0.3000	0.4667	0.3750
	118	0.0000	0.0526	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000
HG14		$n = 29$	$n = 19$	$n = 19$	$n = 30$	$n = 29$	$n = 32$	$n = 111$	$n = 20$	$n = 30$	$n = 16$
	126	0.0000	0.2105	0.0000	0.3000	0.1552	0.2344	0.2162	0.0000	0.2667	0.0000
	128	0.1207	0.0000	0.0000	0.0333	0.0517	0.0469	0.0225	0.3000	0.0167	0.1875
	130	0.0690	0.0789	0.0000	0.0000	0.0000	0.1719	0.1081	0.0250	0.1000	0.0000
	132	0.1034	0.1316	0.1316	0.0000	0.0862	0.1250	0.1441	0.0250	0.1167	0.1250
	134	0.6207	0.4474	0.5526	0.4000	0.2759	0.2969	0.3423	0.4000	0.4000	0.5000
	136	0.0862	0.1316	0.2895	0.1667	0.3793	0.1250	0.1667	0.1250	0.1000	0.1250
	138	0.0000	0.0000	0.0263	0.1000	0.0517	0.0000	0.0000	0.1250	0.0000	0.0625
HG18		$n = 29$	$n = 19$	$n = 18$	$n = 30$	$n = 29$	$n = 33$	$n = 111$	$n = 20$	$n = 29$	$n = 16$
	112	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0313
	114	0.0172	0.0263	0.0000	0.3500	0.2931	0.0455	0.0180	0.0750	0.0000	0.1875
	118	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	120	0.5862	0.5526	0.5556	0.3333	0.3793	0.5303	0.6126	0.4500	0.6207	0.2188
	122	0.0000	0.0000	0.0000	0.1333	0.0345	0.0000	0.0045	0.0000	0.0000	0.0313
	124	0.2414	0.3684	0.3889	0.1667	0.2931	0.3182	0.2523	0.4250	0.2414	0.5313
	126	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0090	0.0000	0.0000	0.0000
	128	0.1034	0.0263	0.0278	0.0167	0.0000	0.0758	0.0766	0.0500	0.0345	0.0000
	130	0.0345	0.0263	0.0278	0.0000	0.0000	0.0303	0.0270	0.0000	0.1034	0.0000
HG25		$n = 29$	$n = 18$	$n = 19$	$n = 28$	$n = 30$	$n = 33$	$n = 106$	$n = 16$	$n = 29$	$n = 14$
	119	0.0000	0.0000	0.0263	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	123	0.4828	0.5556	0.6316	0.4107	0.4833	0.4091	0.4717	0.7188	0.5000	0.0714
	125	0.1034	0.0833	0.0000	0.0714	0.0667	0.0000	0.0142	0.0000	0.0000	0.1786
	129	0.0000	0.0833	0.0526	0.0714	0.0333	0.0455	0.0189	0.0938	0.0172	0.6071
	131	0.4138	0.2778	0.2895	0.4464	0.4167	0.5455	0.4953	0.1875	0.4828	0.1429

Table 13. F_{st} values and Reynolds genetic distances (in brackets) between populations of yellow-legged gulls according to their geographic locality calculated with four microsatellites locus

	Mediterranean[1] (Southern France)	Atlantic Iberia (Galicia)
Galicia	**0.041** (0.042)	
Portugal	**0.044** (0.045)	0.012 (0.012)

[1]Yellow-legged gulls coming from Aigues-Mortes, Plane, Camargue were not genetically different and pooled together.
In bold, F_{st} values significant at the 0.05 level.

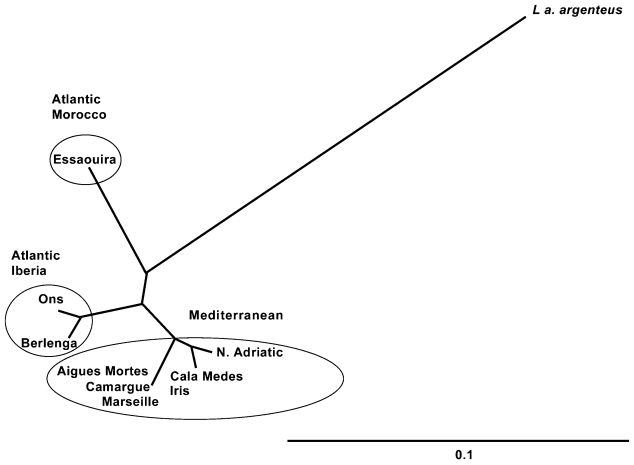

Fig. 5. An UPGMA tree based on four microsatellites nuclear loci showing Reynolds' genetic distances between yellow-legged gulls from Atlantic and Mediterranean localities and the herring gull. All yellow-legged gulls are gathered into two groups (Atlantic Iberia, Mediterranean) well differentiated from the herring gull

has been demonstrated in the case of the blue tit (Lambrechts and Dias 1993).

Teyssèdre (1984) recorded four types of vocalizations (the trumpeting call, the mew call, the call-note and the stoccato-call) from *L. fuscus* and herring gulls from Brittany and yellow-legged gulls from Mediterranean France, Atlantic France and Atlantic Iberia. Multivariate analyses of vocalizations found high statistical differences among populations, especially in the trumpeting and mew calls which are involved in mating behaviour. Herring gulls and Atlantic Iberia yellow-legged gulls, with 'high pitched' vocalizations including few harmonics, formed one group clearly separated from Mediterranean yellow-legged gulls and *L. fuscus* which were characterized by a lower pitched voice with many harmonics. These marked differences in vocalizations might constitute an isolating factor between Mediterranean and Atlantic yellow-legged gulls.

Atlantic Iberian populations of the yellow-legged gull have thus constant morphological differences, some of which are diagnostic (body proportions, this study; voice, Teyssèdre 1984), and pattern of variation in nuclear microsatellites indicate that in their recent history and/or current pattern of gene flow they form a distinct cluster respectively to Mediterranean populations. The amount of genetic differentiation is

nevertheless rather low and they do not constitute a distinct evolutionary unit (lack of reciprocal monophyly for any type of marker, no original mitochondrial or nuclear DNA variants). Various systematic options could be adopted to account for the observed variation among yellow-legged gulls populations. Liebers et al. (2001) proposed to lump all populations from Macaronesian islands, Spain and Morocco into the subspecies *atlantis*. This treatment acknowledges the differences between Mediterranean and Atlantic Iberian populations but overlooks the variation among Atlantic populations: there are some indications of phenotypic divergence between birds from the Azores and birds from Atlantic Iberia and Southern Morocco (Beaubrun 1988), while preliminary results (J.M. Pons et al., pers. comm.) indicates further differences between yellow-legged gulls from Atlantic Iberia and Atlantic Morocco. Furthermore, Liebers et al. (2001) documented significant genetic differentiation between their 'Southern *atlantis*' (the populations traditionally included in the subspecies *atlantis*) and their 'Northern *atlantis*' (the Atlantic Iberian populations). The alternative treatment would be to recognize the Atlantic Iberian populations as a distinct subspecies, based on diagnostic phenotypic characters and original history. The available name for the yellow-legged gull of the Atlantic Iberian populations is *lusitanius* (Joiris 1978), based on birds from the harbour of Peniche, in front of the Berlenga Islands (Portugal). Although this description is extremely poor, this name is valid under the rules of the International Code of Zoological Nomenclature. We prefer to keep an open position on this subject pending a more complete treatment of genetic and phenotypic characters of the yellow-legged gull in the south-western Palaearctic, including birds from the Macaronesian islands and birds from other populations along the Atlantic coast between Morocco and northern Spain in order to establish affinities between all insular and continental forms.

The fact that the phenotypic differentiation between Atlantic Iberian and Mediterranean yellow-legged gulls corresponds to a significant differentiation at nuclear markers suggests that this reduction of gene flow participates in the maintaining of the phenotypic divergence. In addition to isolation by distance, the delay in breeding phenology between Atlantic and Mediterranean birds (Beaubrun 1988; Minguez 1988; Munilla 1997a) may constitute a substantial barrier to current gene flow.

Similarities between herring gull and atlantic yellow-legged gull

On the basis of several behavioural and phenotypic traits, it had been suggested that Atlantic yellow-legged gulls from northwest Spain were more similar to *L. a. argenteus* than to yellow-legged gulls from the Mediterranean basin (Teyssèdre 1983, 1984; Carrera et al. 1987; Minguez and Ganuza 1995). Our results provide additional evidence that Atlantic Iberian yellow-legged gulls are closer to herring gull than to Mediterranean yellow-legged gulls, although somewhat intermediate between them, in size and shape. However, in wing-tip patterns and mantle colour, no such trend was detected. Neutral molecular markers indicate that Atlantic Iberian populations of the yellow-legged gull are significantly differentiated from their Mediterranean counterparts, but do not show any sign of introgression from herring gull: no herring gull haplotypes were found in yellow-legged gulls from the Northern Atlantic Iberian coast, and there was no sign of intergradation at microsatellite loci.

These results demonstrate that Atlantic Iberian populations of the yellow-legged gull do not share similar characters with herring gulls as a result of introgression, as this had been suggested previously. The similarities between these unrelated populations can be explained by retention of ancestral characters or by convergent evolution. Retention of ancestral characters would be a possible explanation if herring gull and yellow-legged gulls were each other's closest relatives, as none of the other species share their common characteristics. Alternatively, the environmental similarities between the Atlantic coasts of northern Spain and western France compared with the Mediterranean coasts, which differ in several climatic and oceanographic features (Zotier et al. 1999), could provide similar selection pressures resulting in independent (convergent) evolution of similar traits in herring gulls and the Atlantic populations of the yellow-legged gulls. Presently available data on the evolution of the large white-headed gulls give conflicting results and these hypotheses cannot be evaluated (de Knijff et al. 2001; Liebers et al. 2001; Crochet et al. 2002).

Geographical variation in body size has often been interpreted in the framework of Bergman's rule as a consequence of environmental factors related to latitude (James 1991). This explanation does not hold in the present case as Atlantic Iberian and Mediterranean yellow-legged gulls live at similar latitudes, whereas herring gulls live further north. In the herring gull, it has been proposed that a stabilizing selection pressure operates on body size, individuals ranking in extreme size classes having higher mortality rates than their medium size counterparts (Monaghan and Metcalfe 1986) and food has been identified as a cause of natural selection on body size in the Darwin Finch (*Geospiza fortis*) (Grant and Grant 1993). The smaller body size of Atlantic Iberian yellow-legged gulls and herring gulls could thus be an adaptation to food resources, since the diet of the Atlantic Spain and France gulls are more similar to each other than to Mediterranean gulls (Munilla 1997a). However reciprocal transplant experimentations would be necessary to firmly test this food hypothesis.

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Résumé

Variation géographique des goélands à pattes jaunes ibériques: introgression ou convergence avec le Goéland argenté?

Les goélands à pattes jaunes (*Larus michahellis*) du littoral atlantique ibérique partagent plusieurs similarités phénotypiques avec le Goéland argenté (*Larus argentatus*) nichant plus au nord en Europe de l'Ouest. Afin d'évaluer prècisément ce phénomène et d'en comprendre l'origine, nous avons comparé les caractères phénotypiques (morphologie, plumage) des goélands à pattes jaunes de la côte atlantique ibérique avec ceux du Goéland leucophée méditerranéen (*Larus michahellis*) et du Goéland argenté. Par ailleurs, nous avons utilisé des marqueurs génétiques pour déterminer l'histoire évolutive des goélands à pattes jaunes du littoral atlantique ibérique. Les résultats obtenus à partir de l'ADN mitochondrial et de quatre microsatellites indiquent clairement que les goélands à pattes jaunes ibériques de l'atlantique sont fortement apparentés au Goéland leucophée et qu'il n'existe pas de signes récents d'introgression avec le Goéland argenté. Les goélands à pattes jaunes ibériques ressemblent davantage au Goéland argenté qu'au Goéland leucophée pour la taille et la forme corporelles mais pas pour ce qui est des patrons alaires ni de la couleur du manteau. Outre la taille corporelle, les similarités phénotypiques (voix, plumage hivernal) et des traits d'histoire de vie (phénologie de la reproduction, patrons de dispersion), déjà décrites dans la littérature, résultent donc probablement d'un phénomène de convergence. Au sein des goélands à pattes jaunes, les valeurs élevées de Fst obtenues avec les marqueurs microsatellites révèlent une forte structuration génétique des populations et indiquent un flux de gènes réduit entre les populations méditerranéennes et atlantiques. Les différences de phénologie de la reproduction et de patrons de migration entre ces populations résultant probablement de pressions de sélection locales différentes pourraient contribuer à ce faible flux de gènes.

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