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A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors

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ABSTRACT: Electrophoretically detectable genetic variability of the Mediterranean mussel *Mytilus galloprovincialis* Lmk. was examined at 15 allozyme loci in 21 populations ranging from Santander (northern Spain) to Livorno (northwestern Italy). A major genetic break between Almeria and Alicante (southeastern Spain), as evidenced by 11 of 13 polymorphic loci examined, delimits 2 groups of populations with a high internal homogeneity. Roughly 75% of the total genetic differentiation was attributable to the divergence between these 2 groups of populations that displayed a genetic distance between them (D = 0.03) in the range of conspecific populations. This genetic break in *M. galloprovincialis* contrasts with earlier reports of genetic homogeneity among conspecific populations of the genus *Mytilus* over vast geographical distances, and represents an uncommon result in marine organisms with larval dispersal. The zone of genetic divergence in *M. galloprovincialis* corresponds to a discontinuity in the distribution of this mussel, and to the position of the well-defined Almeria-Oran oceanographic front, with a distributional boundary between Atlantic and Mediterranean communities. In this region, other marine species exhibit similar patterns of intraspecific divergence, suggesting the action of common biogeographic processes. It is proposed that contemporary influences on gene flow related to an ecological barrier, perhaps in combination with selective pressures associated to water mass differences, maintain the abrupt change in southeastern Spain.

KEY WORDS: *Mytilus* · Allozyme · Genetic discontinuity · Larval dispersal · Ecological barrier

INTRODUCTION

Studies on protein variation have revealed that a great variety of terrestrial species are divided into a mosaic of genetically distinct populations, separated by narrow zones of hybridization (Barton & Hewitt 1985, 1989). Such zones have been interpreted as the result of selection maintaining steep intraspecific clines in contiguous populations or, more frequently, as the result of secondary (post-glacial) contact and hybridization between previously isolated populations (Endler 1977, Barton & Hewitt 1985, Hewitt 1989). In marine invertebrates, reports of zones of contact among genetically distinct populations are rare. Most of these reports involve hybridization between closely related species (Schopf & Murphy 1973, Pesch 1974, Solignac 1976, Skibinski et al. 1978, 1983, Bert & Harrison 1988, Väinölä & Hvilson 1991, Sarver & Foltz 1993), and examples of transition zones at the intraspecific level are uncommon (Marcus 1977, Bulnheim & Scholl 1981, Väinölä & Varvio 1989, Avise 1992). Because most marine organisms have planktonic dispersal, the potential for high gene flow is thought to generally swamp the effects of forces acting to maintain genetic differentiation, except where historical geo- or ecophysical barriers have separated gene pools for periods of time (Johnson 1974, Tracey et al. 1975, Marcus 1977, Love & Larson 1978, Winans 1980, Davis et al. 1981, Beaumont 1982, Burton & Feldman 1982, Mork et al. 1985, Mitton et al. 1989, Väinölä & Varvio 1989, Macaranas et al. 1992, Planes 1993, Ayvazian et al. 1994). However, a larval pelagic strategy does not ensure geographically extensive homogeneity, since macro- and microgeographic clines have been com-

monly reported. Nevertheless, most of these reports involve 1 or a few loci (e.g. Schopf & Gooch 1971, Williams et al. 1973, Christiansen & Frydenberg 1974, Johnson 1974, Koehn et al. 1976, Marcus 1977, Levinton & Suchanek 1978, Fujio 1979, Sassaman & Yoshiyama 1979, Bulnheim & Scholl 1981, Buroker 1983, Koehn & Hilbish 1987, Saavedra et al. 1993), and examples of clinal variation for a large number of genes are rare (Väinölä & Varvio 1989, Ropson et al. 1990). While it has long been appreciated that natural selection may be responsible for this differentiation, the fact that dispersal capacities may not reflect levels of gene flow due to the action of extrinsic factors has received comparatively less consideration (Burton 1983, Hedgecock 1986, Bertness & Gaines 1993). At present, an important issue in evolutionary biology concerns whether general and predictable relationships exist between these extrinsic forces and the phylogeographic structures of species with larval dispersal.

The genus *Mytilus* is an excellent model for such studies, since much is known about its genetics, physiology and ecology (reviewed in Gosling 1992a), thus allowing a multidisciplinary approach in investigating the specific mechanisms operating to effect differentiation and adaptation events in species with long larval dispersal capabilities. Interestingly, the range of *M. galloprovincialis* in Europe extends throughout very different biogeographic provinces, from the Black Sea and Mediterranean to the Atlantic coast of France and the British Isles, as far as north as the Shetland and Orkney Islands (Koehn 1991, Gardner 1992, Gosling 1992c, Seed 1992). However, the analysis of macrogeographic variation in natural populations of *M. galloprovincialis* has received comparatively much less attention than in other *Mytilus* taxa (reviewed in Quesada 1992).

Here we present the results of an extensive survey on allozyme variation (15 loci, 21 samples, 2300 individuals) of natural populations of *Mytilus galloprovincialis* from southern Europe, the only region of the continent where this type of mussel is not intermixed with other *Mytilus* taxa (Koehn 1991, Gosling 1992b, Quesada 1992). The sample area covered a distance of approximately 4000 km, and samples were more densely distributed than in earlier studies of geographic variation on *M. galloprovincialis*. A major and unexpected multilocus genetic break distinguishing 2 groups of populations and associated with a well-defined biogeographic border are documented, and possible explanations for this result are examined. The results of this study suggest a new empirical appraisal of the influences of patterns of water currents, habitat and life history on the magnitude and pattern of genetic differentiation in marine species with high dispersal capabilities.

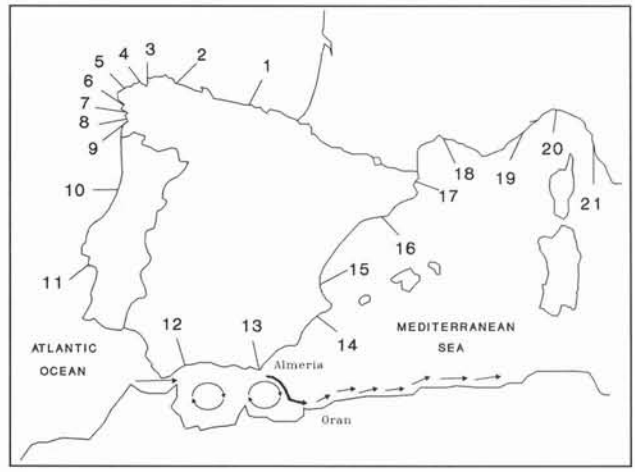

Fig. 1. Location of sampling sites along the southwest coasts of Europe and typical marine surface currents south of Spain (after Tintore et al. 1988). *Mytilus galloprovincialis* samples were collected from: (1) Santander, (2) Ribadeo, (3) Malata, (4) Sada, (5) Laxe, (6) Portosin, (7) Carril, (8) Rande, (9) Silleiro, (10) Aveiro, (11) Sesimbra, (12) Marbella, (13) Almeria, (14) Alicante, (15) Cullera, (16) Garraf, (17) LLansa, (18) Palavas, (19) Montecarlo, (20) Genova, (21) Livorno

MATERIALS AND METHODS

Sampling. Natural populations of adult mussels were collected from 21 sites during 1988 to 1990 along the southwest coasts of Europe, from Santander (northern Spain) to Livorno (northwestern Italy) (Fig. 1, Table 1). Specimens were sampled in the intertidal zone, except in Almeria and Alicante, where they were collected by divers from depths of 4 and 8 m respectively. Mussels were transported on ice to the laboratory, where they were dissected and stored at –70°C for up to 8 mo until analyzed by electrophoresis. No qualitative differences in allelic typing were found between fresh and frozen tissues.

Table 1. Sampling dates of *Mytilus galloprovincialis* populations. Population numbers are as in Fig. 1

Population	Sampling date	Population	Sampling date
1. Santander	Jul 1989	12. Marbella	Oct 1988
2. Ribadeo	Jun 1988	13. Almeria	Feb 1990
3. Malata	May 1988	14. Alicante	Feb 1990
4. Sada	Jan 1988	15. Cullera	Feb 1990
5. Laxe	Jan 1988	16. Garraf	Aug 1988
6. Portosin	Jul 1988	17. LLansa	Sep 1988
7. Carril	Jan 1988	18. Palavas	Sep 1988
8. Rande	Jan 1988	19. Montecarlo	Sep 1988
9. Silleiro	Mar 1988	20. Genova	Jul 1989
10. Aveiro	Sep 1988	21. Livorno	Jul 1989
11. Sesimbra	Sep 1988		

Protein electrophoresis. Soluble proteins were extracted by homogenizing individual tissues with an equal volume of 0.01 M dithiothreitol solution, and centrifuging this mixture at 7000 × *g* for 5 min. The supernatant was absorbed onto filter-paper wicks, which were placed into a slit cut in horizontal gels cooled to 4°C. We used the following buffer-isozyme combinations with digestive gland (G) or posterior adductor muscle (A) to resolve gene products of 15 presumptive loci, which include most of those reported to date in the genus *Mytilus*. A tris-citrate buffer, pH 7.0 (Ahmad et al. 1977) resolved isozymes of aspartate aminotransferase (*AAT-1, AAT-2* [D], E.C. 2.6.1.1), aminopeptidase (*AP* [D], E.C. 3.4.-.), isocitrate dehydrogensase (*IDH* [A], E.C. 1.1.1.42), leucine aminopeptidase-2 (*LAP-2* [D], E.C. 3.4.11.-) and 6-phosphogluconate dehydrogenase (*6PGDH* [A], E.C. 1.1.1.43). A sodium acetate buffer, pH 5.6 (Ahmad et al. 1977) resolved the products of esterase-D (*EST-D* [D], E.C. 3.1.1.1) and leucine aminopeptidase-1 (*LAP-1* [D], E.C. 3.4.11.-). A discontinuous tris-citrate, litium-borate, pH 8.5 (Grant & Cherry 1985) resolved diaphorase (*DIA* [D], E.C. 1.8.1.4), octopine dehydrogenase (*ODH* [D], E.C. 1.5.1.11) and superoxide dismutase (*SOD* [D], E.C. 1.15.1.1). A tris-maleic EDTA buffer, pH 7.4 (Shaw & Prasad 1970) was used for mannose phosphate isomerase (*MPI* [A], 5.3.1.8) and phosphoglucomutase (*PGM* [A], E.C. 5.4.2.2). A tris-borate-EDTA buffer, pH 8.0 (Ahmad et al. 1977) and pH 8.7 (Dando et al. 1981), resolved phosphoglucose isomerase (*PGI* [A], E.C. 5.3.1.9) and strombine dehydrogenase (*STDH* [A], E.C. 1.5.1.-) respectively. A total of 10 samples were characterized for all 15 loci, 6 samples were scored for 14 loci (*DIA* not assayed), and the remaining 5 samples were examined for a variable set of loci (12 to 14) (see Appendix).

Enzyme systems were resolved on 10 to 12% starch gels, except *PGI*, which was resolved on 6% polyacrylamide gels. The staining recipes for *ODH* and *STDH* were essentially those described by Dando et al. (1981) and Grant & Cherry (1985) respectively. For *DIA* the method given by Harris & Hopkinson (1976) was used. For all other systems the staining schemes were derived from Shaw & Prasad (1970). Subunit structures of enzymes inferred from the patterns of banding on gels were in agreement with those previously reported in the genus *Mytilus*, based on breeding data (Hvilson & Theisen 1984) and comparisons with related taxa (Fujio et al. 1983). Loci and alleles were labelled 1, 2, 3, etc., beginning with the less-anodally migrating bands. Two mussels were rerun in each gel to ensure the accuracy of relative electromorph mobilities between different samples and gels.

Data analysis. Heterogeneity among populations was tested using the Pearson chi-squared contingency test for allelic frequencies. For this analysis, classes with expected frequencies less than 5 were grouped (Haberman 1988).

Patterns of geographical variation were examined graphically at each locus by plotting allelic frequencies against geographical distances. To facilitate the visualization and comparison of the variation between alleles at very different frequencies, allelic frequencies were converted to standardized deviates (deviation from the mean allelic frequency in the whole data set expressed in standard deviation units). Clinal variation was tested by Pearson correlation coefficient of arcsine-transformed allele frequencies with geographical distances (Sokal & Rohlf 1981).

The relative amount of genetic variation among populations for individual loci and the whole data set of loci was assessed using hierarchical gene diversity coefficients (Nei 1973, 1987, Chakraborty 1980). The genetic differentiation was also examined by the unbiased estimates of genetic distances (Nei 1972), and the resultant matrix of pairwise standard genetic-distance values was used to generate UPGMA phenograms (Sneath & Sokal 1973). The overall gene flow was calculated from the estimated level of subdivision via the relation (Slatkin & Barton 1989): $G_{ST} = 1/(1+4Nm)$, where Nm is the average number of migrant individuals per generation. This method has been proposed as the most reliable indirect estimator of gene flow (Slatkin & Barton 1989).

Genotypic frequencies at each locus were assessed for goodness-of-fit to Hardy-Weinberg proportions by means of the unbiased *f*-statistic developed by Robertson & Hill (1984). The significance of *f* was evaluated using the test given by the ratio of the estimate to its standard error, which is more powerful than the traditional chi-squared test (Robertson & Hill 1984). We also tested for non-random assortment of genotypes at 2 loci by the Pearson chi-squared contingency test. For this analysis, only the most frequent alleles were considered in each calculation.

Most of the genetic parameters were calculated using the BIOSYS-1 computer program modified for an IBM-PC (Swofford & Selander 1981). Robertson & Hill's (1984) *f*-statistics were computed using the GENET-2 program (Quesada et al. 1992). The remaining statistical tests were carried out using the SPSS/PC package program (Nei et al. 1970). Tests were adjusted using the sequential Bonferroni method when many tests were performed simultaneously (Rice 1989).

RESULTS

Allele frequencies and sample sizes for each locus and site are presented in the Appendix. Out of a total

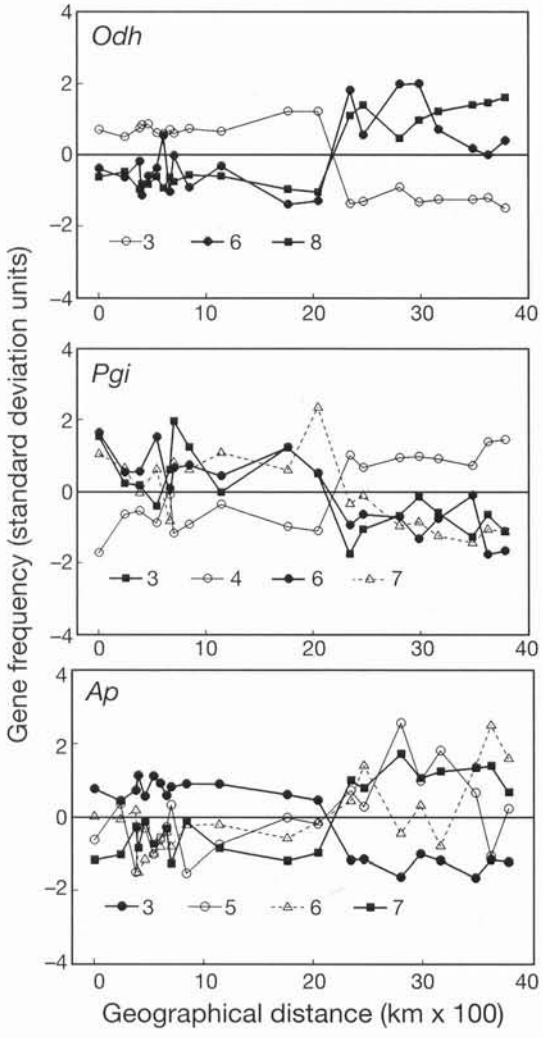

Fig. 2. *Mytilus galloprovincialis*. Geographic variation in allele frequencies at loci *Odh*, *Pgi* and *Ap*. Only the most frequent allele at each locus and alleles with significant geographical heterogeneity are plotted

of 15 loci, 2 (*Sod* and *Aat-2*) were monomorphic at the 95% level in all sites examined. The remaining 13 loci showed consistent polymorphism in the sample locations along the southwestern coasts of Europe. Mean heterozygosity over 15 loci ranged from 0.360 ± 0.069 (Santander) to 0.334 ± 0.068 (Livorno). The average number of alleles per locus was between 4.93 ± 0.58 (Almeria) and 4.13 ± 0.46 (Alicante).

Inspection of the allelic variation presented in this study reveals significant shifts in allelic frequencies between the samples collected between Santander and Almeria (area 1; sites 1 to 13) and those taken between Alicante and Livorno (area 2; sites 14 to 21), with differences between these 2 regions generally greater than those within either area (Table 2, Figs. 2 to 4). It must be noted, however, that the number of loci and alleles with significant geographical heterogeneity might be inflated. This effect is a consequence of the increase in type I error resulting from multiple significance test of the same null hypothesis (Miller 1981), as well as the non-independence of the tests performed for the alleles segregating within each locus. These problems may be avoided by using the sequential Bonferroni correction. This method controls the type I error by means of a tablewide significance level, and it does not require the independence of the single-significance tests (Holm 1979, Rice 1989). After carrying out this adjustment, both significant differences were found in the total region (12 loci) and in area 1 (3 loci), but no locus significant heterogeneity was observed in area 2 (Table 2). After the Bonferroni adjustment, correlation analysis of allele frequencies with geographical distance did not reveal significant clinal variation at any locus within populations from area 1 or area 2 (results not shown).

Regarding the pattern and magnitude of the geographical variation, the loci analyzed may be classified

Table 2. *Mytilus galloprovincialis*. Mean allele frequencies and significance levels for heterogeneity χ^2 [$p(\chi^2)$] in samples from area 1 (sites 1 to 13), area 2 (sites 14 to 21) and the total (sites 1 to 21). Significant tests after sequential Bonferroni correction are underlined (tablewide significant level = 0.05). Loci with a total significant heterogenity also showed significant differences in mean allele frequencies between areas 1 and 2 (arcsine *t*-testing). Alleles with an average frequency across all populations lower than 0.01 were not tested for heterogeneity. ns: non significant

Locus / Allele	Area 1 Mean ± SE	Area 1 $p(\chi^2)$	Area 1 $p(\chi^2)$	Area 2 Mean ± SE	Area 2 $p(\chi^2)$	Area 2 $p(\chi^2)$	Total $p(\chi^2)$	Total $p(\chi^2)$
Aat-1			ns			ns		0.0087
Aat-1^2	0.932 ± 0.006	ns		0.905 ± 0.007	ns		0.0306	
Aat-1^4	0.043 ± 0.005	ns		0.041 ± 0.004	ns		ns	
Aat-1^6	0.011 ± 0.002	ns		0.046 ± 0.005	ns		0.0000	
Ap			ns			ns		0.0000
Ap^3	0.410 ± 0.007	ns		0.163 ± 0.010	ns		0.0000	
Ap^5	0.351 ± 0.008	ns		0.419 ± 0.019	ns		0.0090	
Ap^6	0.174 ± 0.006	ns		0.238 ± 0.017	0.0111		0.0000	
Ap^7	0.048 ± 0.006	0.0028		0.157 ± 0.007	ns		0.0000	

Table 2 (continued)

Locus / Allele	Area 1 Mean ± SE	Area 1 $p(\chi^2)$	$p(\chi^2)$	Area 2 Mean ± SE	Area 2 $p(\chi^2)$	$p(\chi^2)$	Total $p(\chi^2)$	$p(\chi^2)$
Dia			0.0249			0.0375		0.0000
Dia^1	0.010 ± 0.003	ns		0.024 ± 0.007	0.0005		0.0002	
Dia^2	0.091 ± 0.017	0.0006		0.121 ± 0.005	ns		0.0045	
Dia^4	0.658 ± 0.011	ns		0.554 ± 0.017	ns		0.0000	
Dia^6	0.216 ± 0.012	ns		0.279 ± 0.020	0.0022		0.0000	
Dia^7	0.017 ± 0.002	ns		0.013 ± 0.004	ns		ns	
Est-D			0.0430			ns		0.0000
Est-D^1	0.002 ± 0.001	0.0351		0.025 ± 0.006	ns		0.0000	
Est-D^2	0.039 ± 0.004	ns		0.017 ± 0.002	ns		0.0032	
Est-D^4	0.901 ± 0.009	0.0019		0.944 ± 0.006	ns		0.0000	
Est-D^6	0.046 ± 0.005	ns		0.014 ± 0.003	ns		0.0000	
Idh			ns			ns		ns
Idh^2	0.102 ± 0.005	ns		0.097 ± 0.007	ns		ns	
Idh^3	0.885 ± 0.005	ns		0.888 ± 0.008	ns		ns	
Idh^4	0.011 ± 0.003	ns		0.011 ± 0.003	ns		ns	
Lap-1			0.0410			ns		0.0000
Lap-1^2	0.009 ± 0.002	ns		0.019 ± 0.004	ns		0.0408	
Lap-1^3	0.031 ± 0.005	0.0434		0.039 ± 0.009	0.0131		0.0058	
Lap-1^4	0.023 ± 0.004	0.0277		0.079 ± 0.006	ns		0.0000	
Lap-1^5	0.410 ± 0.011	ns		0.398 ± 0.011	ns		ns	
Lap-1^6	0.491 ± 0.013	ns		0.437 ± 0.009	ns		0.0250	
Lap-1^7	0.033 ± 0.003	ns		0.026 ± 0.004	ns		ns	
Lap-2			ns			ns		0.0070
Lap-2^2	0.031 ± 0.004	ns		0.028 ± 0.005	ns		ns	
Lap-2^3	0.498 ± 0.008	ns		0.484 ± 0.012	ns		ns	
Lap-2^5	0.439 ± 0.009	ns		0.420 ± 0.009	ns		ns	
Lap-2^7	0.028 ± 0.003	ns		0.066 ± 0.007	ns		0.0000	
Mpi			0.0020			ns		0.0002
Mpi^2	0.944 ± 0.007	0.0020		0.965 ± 0.008	ns		0.0002	
Mpi^3	0.049 ± 0.006	0.0060		0.034 ± 0.008	ns		0.0011	
Odh			0.0042			0.0220		0.0000
Odh^3	0.558 ± 0.013	0.0045		0.131 ± 0.012	ns		0.0000	
Odh^6	0.128 ± 0.008	0.0293		0.216 ± 0.015	0.0165		0.0000	
Odh^8	0.295 ± 0.008	ns		0.642 ± 0.021	0.0160		0.0000	
6Pgdh			ns			ns		0.0236
6Pgdh^2	0.034 ± 0.004	ns		0.016 ± 0.002	ns		0.0467	
6Pgdh^4	0.926 ± 0.005	ns		0.956 ± 0.004	ns		0.0246	
6Pgdh^6	0.023 ± 0.004	0.0371		0.018 ± 0.003	ns		ns	
Pgi			0.0450			ns		0.0000
Pgi^2	0.021 ± 0.004	0.0223		0.006 ± 0.002	ns		0.0001	
Pgi^3	0.063 ± 0.005	ns		0.030 ± 0.003	ns		0.0045	
Pgi^4	0.559 ± 0.018	0.0013		0.788 ± 0.012	ns		0.0000	
Pgi^6	0.255 ± 0.010	ns		0.146 ± 0.012	ns		0.0000	
Pgi^7	0.065 ± 0.006	ns		0.025 ± 0.004	ns		0.0000	
Pgi^8	0.020 ± 0.005	0.0054		0.003 ± 0.001	ns		0.0000	
Pgm			ns			ns		0.0098
Pgm^2	0.014 ± 0.003	ns		0.037 ± 0.002	ns		0.0278	
Pgm^3	0.108 ± 0.005	ns		0.134 ± 0.005	ns		ns	
Pgm^4	0.601 ± 0.011	ns		0.523 ± 0.012	ns		0.0009	
Pgm^6	0.257 ± 0.010	ns		0.282 ± 0.008	ns		ns	
Stdh			0.0039			ns		0.0000
Stdh^2	0.116 ± 0.008	ns		0.184 ± 0.011	ns		0.0000	
Stdh^4	0.115 ± 0.012		0.0292	0.115 ± 0.014	0.0105		0.0035	
Stdh^5	0.026 ± 0.004		ns	0.007 ± 0.003	ns		0.0057	
Stdh^6	0.081 ± 0.005		ns	0.014 ± 0.006	ns		0.0000	
Stdh^7	0.623 ± 0.016		0.0442	0.660 ± 0.014	0.0442		0.0219	
Stdh^8	0.021 ± 0.005		0.0165	0.011 ± 0.003	0.0165		0.0060	

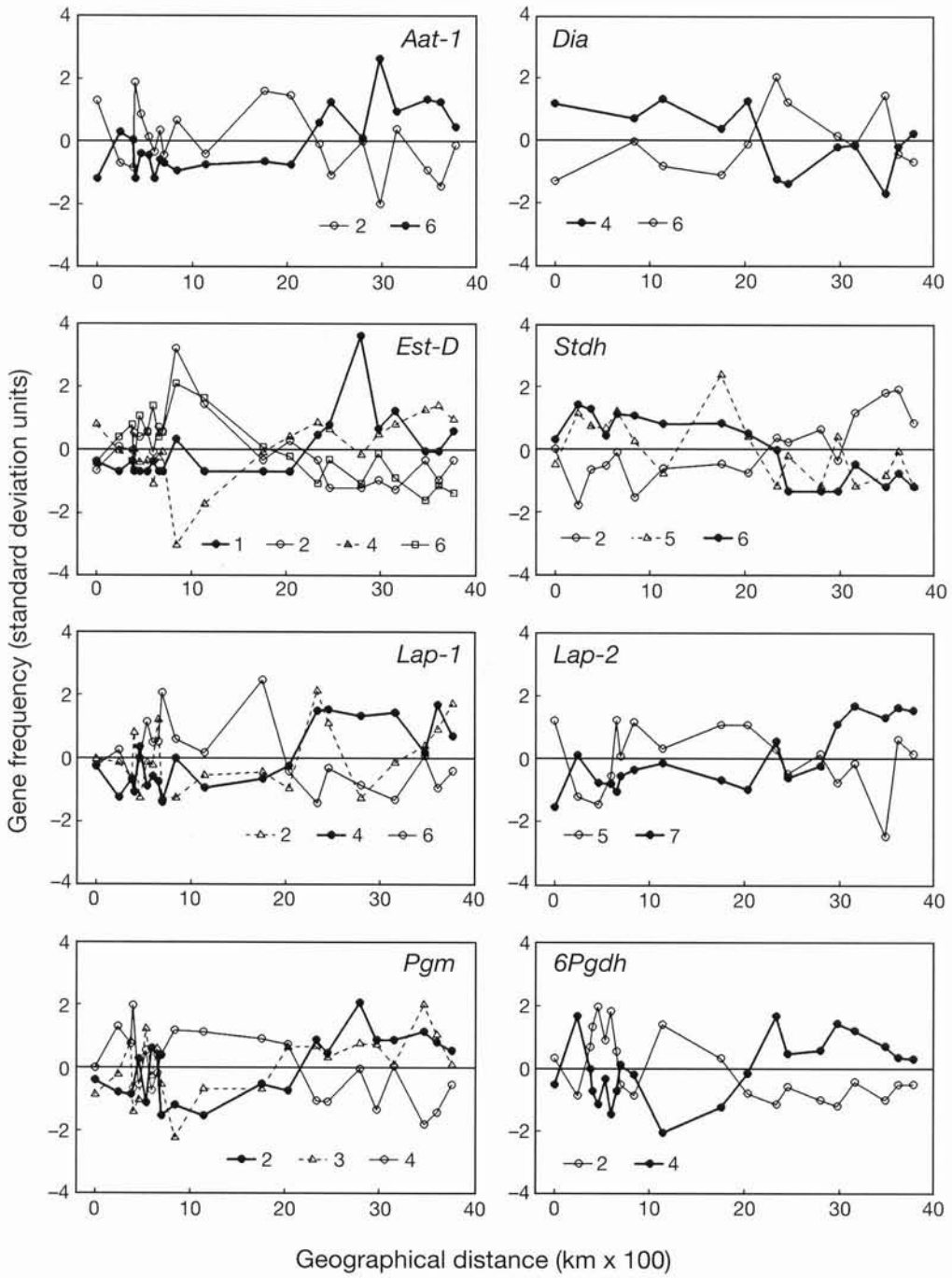

Fig. 3. *Mytilus galloprovincialis*. Geographical variation in allele frequencies at loci *Aat-1*, *Est-D*, *Lap-1*, *Pgm*, *Dia*, *Stdh*, *Lap-2* and *6Pgdh*. Only the most frequent allele at each locus and alleles with significant geographical heterogeneity are plotted

into 3 arbitrary groups. Two of these classes included the 11 loci which exhibited a significant sharp discontinuity in southeastern Spain, and the third one included the 4 loci which did not show this change.

Class I loci showed the greatest abrupt variation. At each locus, 2 or more common alleles were strikingly affected by the genetic break. Loci included in this class are *Odh*, *Pgi* and *Ap* (Fig. 2). The discontinuity was particularly strong for *Odh*. At this locus, the frequency of the Odh^3 allele ranged from 0.500 to 0.658 throughout all the samples from Santander to Almeria, with an average value of 0.558 ± 0.013. East of Almeria, the frequency of this allele decreased abruptly from 0.658 to 0.107, and remained fairly invariant within area 2, with a mean frequency of 0.131 ± 0.012. The loci *Ap* and *Pgi* exhibited a concordant pattern of

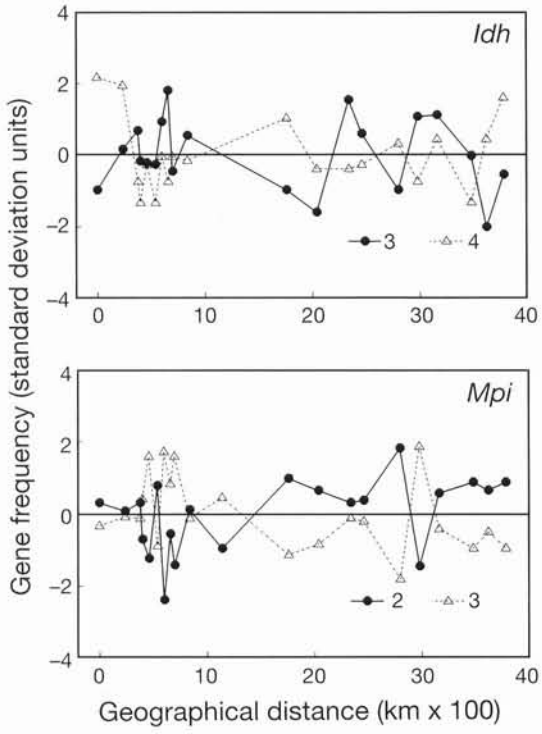

Fig. 4. *Mytilus galloprovincialis*. Geographical variation in allele frequencies at loci *Idh* and *Mpi*. Only most frequent alleles are plotted

abrupt transition in southeastern Spain, with an average change in frequency for the alleles most affected by the genetic break of 0.247 (Ap^3) and 0.229 (Pgi^4).

Class II loci presented an abrupt change in southeastern Spain of a lesser magnitude. Loci belonging to this class are *Aat-1*, *Est-D*, *Dia*, *Lap-1*, *Lap-2*, *Pgm*, *6Pgdh* and *Stdh* (Fig. 3). In these loci, the mean change in the frequency of the alleles more affected by the genetic discontinuity, ranged from 0.104 for Dia^4 to 0.023 for $Est\text{-}D^1$ and Pgm^2. Although the gene-frequency differences are modest in absolute terms, they are high in relative terms as measured by standard deviation units (SDU). When this transformation is used, a similar abrupt change across loci is apparent (Fig. 3), with an average variation of 1.68 ± 0.09 SDU for the most affected alleles at each locus, which is not significantly different from the average of 1.96 ± 0.09 SDU observed for class I loci (*U*-test= 1.04, $p > 0.05$)

Class III loci did not exhibit any significant genetic discontinuity in allelic frequencies throughout all the geographic areas studied. These loci were monomorphic (*Aat-2* and *Sod*) or presented a very frequent allele having the same electrophoretic mobility in both areas (*Idh* and *Mpi*) (Fig. 4).

The amount of genetic differentiation was small considering the large geographical area sampled. Only about 3% of the total gene diversity was due to differences among populations (Table 3). However, the magnitude of the G_{ST} estimates was very heterogeneous across loci, with class I loci exhibiting the highest values: *Odh* (0.124), *Pgi* (0.040) and *Ap* (0.033). By contrast, the remaining 10 polymorphic loci displayed very small and homogeneous G_{ST} estimates, most of them very close to 0.01. The divergence between area 1 and area 2 samples explains over 75% of the total heterogeneity, and is mainly attributable to class I loci. The degree of subdivision within areas explains the remaining 25% of the total differentiation, and the contribution of all loci is similar, including *Odh*, *Pgi* and *Ap*.

The abrupt change in allele frequencies in southeastern Spain is graphically summarised in the UPGMA dendrogram constructed from the 10 populations analyzed for the whole set of loci (Fig. 5A). All the samples collected from Santander to Almeria clustered in one phenetic group (area 1), and all the mussels taken from Alicante to Livorno clustered in the other (area 2). Within each cluster, mussel populations displayed a high genetic similarity, with a mean genetic distance for within-group comparisons of 0.001 ± 0.0002. This average increased to 0.032 ± 0.001 when pairs of populations from different areas were compared. The separation of area 1 and area 2 samples into 2 distinct groups is also mostly due to class I loci. When these loci are removed, the genetic distance between both areas decreases, but the same pattern of grouping persists at a lower clustering level, indicating the effect of other loci (Fig. 5B).

The estimates of the number of migrant individuals per generation (*Nm*) for area 1 and area 2 samples were 35.5 and 41.4 respectively. These values are very close to those previously reported in natural popula-

Table 3. *Mytilus galloprovincialis*. Gene diversity coefficients between populations (G_{ST}), between areas (G_{AT}) and between populations within areas (G_{SA})

Locus	G_{SA}	G_{AT}	G_{ST}
Aat-1	0.007	0.003	0.010
Ap	0.006	0.027	0.033
Dia	0.007	0.007	0.014
Est-D	0.008	0.006	0.014
Idh	0.004	0.000	0.004
Lap-1	0.006	0.002	0.008
Lap-2	0.003	0.001	0.004
Mpi	0.012	0.002	0.014
Odh	0.009	0.115	0.124
6Pgdh	0.005	0.003	0.008
Pgi	0.008	0.032	0.040
Pgm	0.005	0.003	0.008
Stdh	0.009	0.005	0.014
Mean	0.007	0.022	0.029

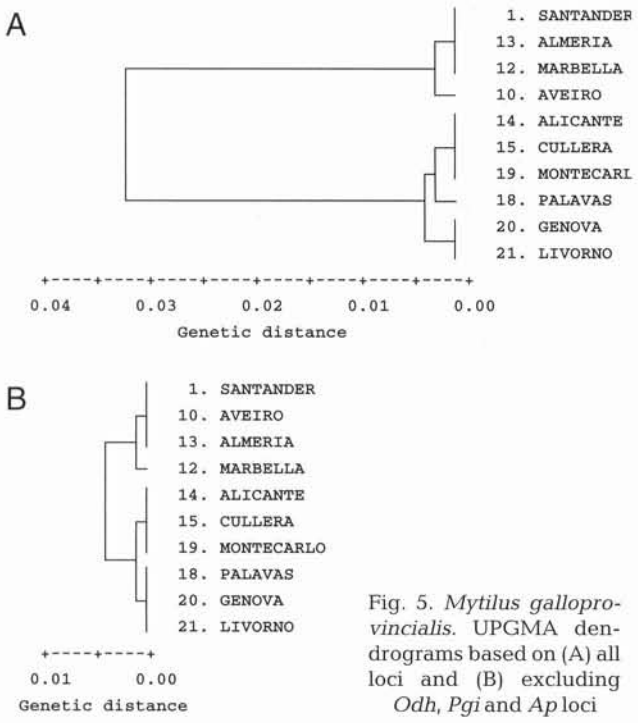

Fig. 5. *Mytilus galloprovincialis*. UPGMA dendrograms based on (A) all loci and (B) excluding *Odh*, *Pgi* and *Ap* loci

tions of *Mytilus edulis* (Nm = 42.0; Slatkin 1985a), and are indicative of a high gene flow, in accordance with the genetic homogeneity detected within each area. The estimate of Nm for the whole set of populations (Nm = 8.4) was approximately 4 to 5 times lower than the gene flow between populations within each area. When class I loci are excluded, the total Nm estimate (27.5) is also smaller than those obtained for area 1 (35.5) and area 2 (41.4). These results suggest some degree of isolation between the 2 groups of populations, although they are still indicative of a substantial gene flow. Nevertheless, the absolute magnitude of the total Nm estimate must be regarded with caution for several reasons. First of all, indirect methods of Nm estimation reflect the historical average of gene flow among populations that is necessary to generate the observed pattern of differentiation, and this usually results in an overestimate of the real gene flow (Slatkin 1985b). Secondly, the validity of the estimate of Nm depends on the applicability of the underlying assumptions, such as neutral loci or an island model of population structure. Such assumptions do not seem appropiate in the present context, since the discontinuities in allelic frequencies could be due to the absence of random mating between populations from areas 1 and 2, to selection or to a combination of both factors.

The presence of a restriction to gene flow is also suggested by the fact that a total of 15 rare alleles were not shared among area 1 and area 2 populations. Area 1 exhibited 11 of these exclusive alleles. Most of them were detected more than once in several populations, and their frequency was no higher than 0.025 nor lower than 0.005. The most representative distribution was that of the *Aat-1*[1] allele, which was present in 9 samples within area 1, with an average frequency of 0.012 ± 0.002. The 4 exclusive alleles found within area 2 were detected only once in an unique population.

Extensive heterozygote deficiencies or linkage disequilibrium within the transition zone at loci with strongest differentiation (class I loci), would indicate restriction of interbreeding among populations belonging to areas 1 and 2. Out of 12 tests showing significant deficiencies of heterozygotes after Bonferroni adjustment, 5 were observed in the samples closest to the genetic breakpoint: Almeria, Alicante and Cullera. However, only 2 correspond to loci with high genetic divergence: *Odh* and *Pgi*, in the Cullera sample (data detailed in Quesada 1992). Moreover, significant heterozygote deficiencies were also observed for *Pgi* outside of the transition zone in Rande and Genova samples, more than 1000 km away from the mussel breakpoint. These results clearly indicate that some mixing occurs in the transition zone, but that deficiencies of heterozygotes are no so extensive as would be expected in a non-interbreeding mixture of populations from both areas. In addition, tests of non-random assortment of genotypes showed little evidence for systematic associations between loci. Out of the 57 tests of genotypic dilocus associations performed between *Odh*, *Pgi* and *Ap*, only 2 were significant. These significant associations were detected for the pairwise combinations *Ap-Odh* in Livorno, and for *Ap-Pgi* in Montecarlo, very far from the transition region in southeastern Spain.

DISCUSSION

This allozyme survey in *Mytilus galloprovincialis* shows a major genetic break in southeastern Spain that delimits 2 groups of populations with a high internal homogeneity. Sharp discontinuities detected in allele frequencies at 11 of 13 polymorphic loci coincide spatially in the region between Almeria and Alicante, over 300 km apart, which is a relatively narrow zone with respect to the dispersal distance. Roughly 75% of the total genetic differentiation was attributable to the divergence between both areas. However, the genetic distance between these 2 regions was small (0.03), in the range expected for conspecific populations (0.0 to 0.05; Ferguson 1980), and much lower than genetic distances reported between the well-recognized *Mytilus* taxa, where distances based on 16 to 23 loci ranged from 0.16 to 0.28 (Skibinski et al. 1980, Grant & Cherry 1985, Väinölä & Hvilson 1991). Moreover, the

observed pattern of heterozygote deficiencies and the absence of significant associations among genotypes near the transition zone suggest that the 2 types of mussels successfully interbreed. Thus, our results are far from suggesting differentiation at the species level, in agreement with earlier taxonomic studies on European *M. galloprovincialis*, based on morphological and allozyme data (Koehn 1991, Gardner 1992, Gosling 1992b, Seed 1992).

The results of this study contrast with previous surveys on *Mytilus* of fairly genetic homogeneity over vast geographical distances, except the sharp cline in *M. edulis* for the *Lap* locus at the entrance to Long Island Sound (USA) and at Cape Cod (Massachusetts, USA) (Koehn 1991, Gosling 1992c). Our data demonstrate, for the first time, that extensive differentiation at many loci is possible in conspecific populations of *Mytilus* over relatively short distances. On the other hand, the sharp multilocus cline in *M. galloprovincialis* represents an uncommon result in marine organisms with larval dispersal, since only a few studies report allozyme clines at many loci (Väinölä & Varvio 1989, Ropson et al. 1990), and none involve well-defined narrow contact zones.

Previous surveys of allozymic variation in *Mytilus galloprovincialis* have already revealed some evidence of genetic divergence for 1 to 2 loci in disjunct Atlantic and Mediterranean populations (Varvio et al. 1988, Sanjuan et al. 1990). These earlier results thus support our finding of genetically distinct eastern and western breakpoint populations in European *M. galloprovincialis*. The present study extends the differentiation up to 11 loci, and determines the transition zone and type of change between these populations.

Since *Mytilus galloprovincialis* is characterized by a long pelagic larvae stage of around 3 to 4 wk (Lutz & Kennish 1992), the sharp genetic change observed in southeastern Spain could not persists without strong natural selection and/or the presence of environmental factors limiting larval dispersal and gene flow. Three observations strongly support the hypothesis that there is restriction of gene flow between the populations belonging to both areas: (1) The change occurs at many loci, as expected from an isolation process affecting the entire genome. (2) The genetic break is concordant with geographical position for all 11 loci, that exhibit a parallel abrupt change with no intermediate frequencies. (3) Many rare alleles (14% of the total) are not shared between the 2 areas. Alternatively, estimates of gene flow, although consistent with a reduction in migration, indicate that gene flow is still substantial.

Theoretically, if no external selective differences existed, the position of the transition zone would be attracted to an area where gene flow is reduced (Endler 1977, Barton & Hewitt 1985). Much evidence suggests that the transition zone could, in fact, represent an ecological barrier, and restrict gene flow between *Mytilus* populations located at both sides of the genetic breakpoint. Recent studies of satellite imagery indicate that the main path of inflowing Atlantic water into the Mediterranean through the Straits of Gibraltar is around 2 large anticyclonic gyres in the Alboran Sea (Tintore et al. 1988). The convergence of the Eastern Gyre with the resident Mediterranean water near Cape Gata (Almeria) determines a well-defined frontal zone (Fig. 1), where the main current is deflected southward toward Oran (Algeria), then creating the Algerian Current (Arnone & La Violette 1986, Tintore et al. 1988, Arnone et al. 1990). This oceanographic front (the Almeria-Oran Front) is associated with strong southeastward currents (average speeds of 40 cm s^{-1} and maximum speeds of 60 cm s^{-1}), and dramatic changes in salinity (2 psu) and temperature (1.4°C) within a 2 km distance, with effects that involve the upper 300 m water layer (Lohrenz et al. 1988, Tintore et al. 1988, Arnone et al. 1990). It is suggested that the direction of the strong surface water currents, coupled with the ecological gradients associated with this oceanographic boundary pose a barrier to the dispersal of planktonic mussel larvae, physiologically acclimated to their native environment, and intolerant to sudden environmental changes (Bayne 1965, 1976, Lutz & Kennish 1992). Thus, crossing this oceanographic front might be fatal to larvae, irrespective of their genotype, so that apparent dispersal capacity would not be realized.

A second factor supporting an ecological barrier is that the zone of genetic change corresponds to an area of low density for mussel populations (Quesada 1992). In fact, no evidence of mussel presence was found along the coastline between Almeria and Alicante after detailed sampling. Garcia-Raso et al. (1992) and fishermen confirm this result. The extensive extinctions of filtering marine organisms associated with the catastrophic-storm-related phenomena that periodically occur in this region (Garcia-Raso et al. 1992), in conjunction with extremely high summer temperatures and aridity that may reduce mussel survival (Capel 1981, Tsuchiya 1983, Seed & Suchanek 1992), suggest that the observed discontinuity in distribution corresponds to an area unsuitable for mussels. In conclusion, the coastline between Almeria and Alicante represents a density trough, probably contributing to isolation among mussel populations and providing another cause of coincident clines (Hewitt 1989, Quesada 1992).

No significant clinal variation was found within the western or eastern breakpoint regions, which display similar but smaller environmental gradients (such as salinity and temperature), to those observed in the

transition zone (Collier 1970, Rodriguez 1982, Tintore et al. 1988). This could indicate that most of the divergence between regions does not reflect an adaptive response to an underlying environmental gradient. Most probably, however, mussel populations on both sides of the genetic breakpoint are also genetically adapted to their present native environment, although much of this adaptedness could have been acquired after isolation. If such a situation occurs, coincident clines involving multiple loci themselves can act as barriers to gene flow, even for neutral loci, due to linkage disequilibrium between the selected and neutral loci (Barton 1979, 1982, Barton & Hewitt 1985). In summary, we suggest that the genetic breakpoint corresponds closely in geographical position to an ecological barrier that, perhaps in conjunction with coincident clines, restricts gene flow among mussel populations from western and eastern regions. This means that functionally independent ecological and genetic factors may act together in the maintenance of genetic isolation, and in determining the position of the contact zone.

Southeastern Spain is an area widely recognized as a biogeographic border between Mediterranean and Atlantic biotic-communities (Rodriguez et al. 1979, Conde & Seoane 1982, Peres 1989). Because the intraspecific break in the genus *Mytilus* is closely coincident with this biogeographic limit, it is suggested that both phenomena may be due to a common set of factors. A similar geographical correspondence between intraspecific divergence and this distributional limit has been observed for the barnacle *Chthamalus montagui* (Dando & Southward 1981) and the fish *Gobious paganellus* (Amores et al. 1990), as evidenced by allozyme and chromosome polymorphisms respectively. Hence, similar historical and contemporary phenomena may be responsible for these patterns of variation. The picture in southeastern Spain resembles that observed for other marine species in other well-defined biogeographic borders (Väinölä & Varvio 1989, Avise 1992). Thus, our results support the hypothesis suggested by Avise et al. (1987), that when phylogenetic discontinuities occur within widely distributed species, they tend to be concordant with the boundaries between traditional recognized zoogeographic provinces.

The large-scale climatic and eustatic changes which occurred in the Atlantic and Mediterranean areas over the Pleistocene could have initiated the genetic divergence among *Mytilus* populations (Quesada 1992). In this period the Mediterranean was affected by wide temperature glacial/interglacial fluctuations (Thunell 1979), and water exchange between the Atlantic and Mediterranean was much altered (Pielou 1979, Loubere 1982, Rodriguez 1982). Moreover, the narrow pathways between the various basins of the Mediterranean must frequently have been narrower and more restricted that at present due to the eustatic lowering of the Glacial sea levels (Thiede 1978, Pielou 1979, Rodriguez 1982), thus allowing *Mytilus* populations to have split. The transition zone in southeastern Spain might represent a post-glacial contact of allopatrically divergent populations, when oceanographic circulation became similar to present. However, the alternative hypothesis of an *in situ* origin for the mussel genetic divergence cannot be excluded with the present data set.

In conclusion, we propose that the genetic divergence in *Mytilus* populations is maintained by contemporary influences on gene flow due to an ecological barrier, perhaps in combination with selective pressures associated with water mass differences. The results of this study support that extrinsic forces, such as climatic and oceanographic events, may be the major causal elements determining genetic differentiation and geographical distribution among populations of marine species with large population sizes and pelagic larval dispersal.

Appendix. Allele frequencies and sample sizes (N) of *Mytilus galloprovincialis* populations. Population numbers are as in Fig. 1

Locus	Population	Allele 1	2	3	4	5	6	7	8	9	N
Aat-1	1. Santander	0.010	0.954	0.000	0.036	0.000	0.000	–	–	–	98
	2. Ribadeo	0.000	0.904	0.000	0.066	0.000	0.030	–	–	–	99
	3. Malata	0.010	0.900	0.000	0.065	0.000	0.025	–	–	–	100
	4. Sada	0.000	0.969	0.000	0.031	0.000	0.000	–	–	–	16
	5. Laxe	0.016	0.943	0.000	0.021	0.005	0.016	–	–	–	96
	6. Portosin	0.025	0.925	0.000	0.035	0.000	0.015	–	–	–	100
	7. Carril	0.013	0.913	0.000	0.060	0.013	0.000	–	–	–	75
	8. Rande	0.016	0.930	0.000	0.033	0.008	0.012	–	–	–	122
	9. Silleiro	0.010	0.910	0.015	0.040	0.015	0.010	–	–	–	100
	10. Aveiro	0.005	0.938	0.000	0.046	0.005	0.005	–	–	–	97
	11. Sesimbra	0.000	0.911	0.000	0.080	0.000	0.009	–	–	–	56
	12. Marbella	0.000	0.962	0.011	0.016	0.000	0.011	–	–	–	92
	13. Almeria	0.006	0.958	0.003	0.024	0.000	0.009	–	–	–	167

Appendix (continued)

Locus	Population	Allele 1	2	3	4	5	6	7	8	9	N
Aat-1	14. Alicante	0.000	0.919	0.000	0.041	0.005	0.036	–	–	–	111
	15. Cullera	0.000	0.894	0.000	0.053	0.004	0.049	–	–	–	113
	16. Garraf	0.000	0.921	0.000	0.026	0.026	0.026	–	–	–	19
	17. LLansa	0.000	0.871	0.005	0.041	0.005	0.077	–	–	–	97
	18. Palavas	0.000	0.931	0.005	0.021	0.000	0.043	–	–	–	94
	19. Montecarlo	0.000	0.898	0.000	0.034	0.017	0.051	–	–	–	88
	20. Genova	0.000	0.885	0.000	0.060	0.005	0.049	–	–	–	91
	21. Livorno	0.000	0.918	0.000	0.049	0.000	0.033	–	–	–	91
Aat-2	1. Santander	0.000	1.000	0.000	–	–	–	–	–	–	91
	2. Ribadeo	0.005	0.995	0.000	–	–	–	–	–	–	98
	3. Malata	0.005	0.995	0.000	–	–	–	–	–	–	98
	4. Sada	0.000	1.000	0.000	–	–	–	–	–	–	20
	5. Laxe	0.006	0.994	0.000	–	–	–	–	–	–	80
	6. Portosin	0.000	1.000	0.000	–	–	–	–	–	–	92
	7. Carril	0.006	0.994	0.000	–	–	–	–	–	–	78
	8. Rande	0.004	0.996	0.000	–	–	–	–	–	–	121
	9. Silleiro	0.000	1.000	0.000	–	–	–	–	–	–	95
	10. Aveiro	0.012	0.988	0.000	–	–	–	–	–	–	82
	11. Sesimbra	0.011	0.989	0.000	–	–	–	–	–	–	46
	12. Marbella	0.006	0.994	0.000	–	–	–	–	–	–	86
	13. Almeria	0.000	1.000	0.000	–	–	–	–	–	–	142
	14. Alicante	0.000	1.000	0.000	–	–	–	–	–	–	118
	15. Cullera	0.000	1.000	0.000	–	–	–	–	–	–	93
	16. Garraf	0.025	0.975	0.000	–	–	–	–	–	–	20
	17. LLansa	0.011	0.989	0.000	–	–	–	–	–	–	95
	18. Palavas	0.000	1.000	0.000	–	–	–	–	–	–	91
	19. Montecarlo	0.009	0.983	0.009	–	–	–	–	–	–	58
	20. Genova	0.000	1.000	0.000	–	–	–	–	–	–	90
	21. Livorno	0.000	1.000	0.000	–	–	–	–	–	–	97
Ap	1. Santander	0.000	0.011	0.409	0.000	0.344	0.199	0.022	0.016	–	93
	2. Ribadeo	0.005	0.000	0.370	0.005	0.395	0.195	0.030	0.000	–	100
	3. Malata	0.011	0.005	0.404	0.000	0.298	0.207	0.074	0.000	–	94
	4. Sada	0.004	0.012	0.455	0.000	0.361	0.127	0.041	0.000	–	122
	5. Laxe	0.005	0.014	0.384	0.000	0.361	0.144	0.083	0.005	–	108
	6. Portosin	0.016	0.000	0.453	0.005	0.323	0.151	0.047	0.005	–	96
	7. Carril	0.000	0.005	0.429	0.000	0.344	0.160	0.057	0.005	–	106
	8. Rande	0.000	0.000	0.388	0.000	0.362	0.179	0.071	0.000	–	112
	9. Silleiro	0.011	0.000	0.417	0.000	0.394	0.161	0.017	0.000	–	90
	10. Aveiro	0.000	0.005	0.427	0.000	0.297	0.188	0.083	0.000	–	96
	11. Sesimbra	0.007	0.000	0.426	0.000	0.338	0.189	0.041	0.000	–	74
	12. Marbella	0.000	0.038	0.392	0.000	0.376	0.172	0.022	0.000	–	93
	13. Almeria	0.000	0.025	0.374	0.006	0.368	0.193	0.034	0.000	–	163
	14. Alicante	0.004	0.013	0.174	0.000	0.415	0.220	0.148	0.025	–	118
	15. Cullera	0.000	0.014	0.177	0.000	0.392	0.266	0.136	0.014	–	246
	16. Garraf	0.000	0.000	0.119	0.000	0.512	0.179	0.190	0.000	–	42
	17. LLansa	0.000	0.000	0.196	0.000	0.430	0.215	0.152	0.006	–	79
	18. Palavas	0.000	0.011	0.174	0.000	0.473	0.163	0.163	0.016	–	92
	19. Montecarlo	0.000	0.026	0.117	0.000	0.413	0.265	0.168	0.010	–	98
	20. Genova	0.000	0.005	0.177	0.000	0.323	0.318	0.172	0.005	–	96
	21. Livorno	0.000	0.015	0.170	0.000	0.390	0.275	0.130	0.020	–	100
Dia	1. Santander	0.006	0.122	0.006	0.672	0.000	0.183	0.011	0.000	–	90
	10. Aveiro	0.005	0.068	0.016	0.641	0.000	0.250	0.021	0.000	–	96
	11. Sesimbra	0.007	0.076	0.000	0.681	0.000	0.208	0.021	0.007	–	72
	12. Marbella	0.021	0.146	0.000	0.620	0.005	0.193	0.016	0.000	–	96
	13. Almeria	0.011	0.042	0.000	0.676	0.000	0.246	0.018	0.007	–	142
	14. Alicante	0.004	0.092	0.000	0.519	0.000	0.362	0.023	0.000	–	130
	15. Cullera	0.003	0.140	0.003	0.510	0.000	0.318	0.022	0.003	–	157
	17. LLansa	0.026	0.123	0.006	0.584	0.000	0.260	0.000	0.000	–	77
	18. Palavas	0.016	0.121	0.005	0.588	0.000	0.242	0.027	0.000	–	91
	19. Montecarlo	0.044	0.133	0.000	0.487	0.000	0.329	0.006	0.000	–	79
	20. Genova	0.056	0.117	0.011	0.583	0.000	0.228	0.006	0.000	–	90
	21. Livorno	0.020	0.120	0.020	0.610	0.005	0.215	0.010	0.000	–	100
Est-D	1. Santander	0.005	0.020	0.000	0.944	0.005	0.025	0.000	0.000	0.000	99
	2. Ribadeo	0.000	0.032	0.005	0.916	0.005	0.042	0.000	0.000	0.000	95
	3. Malata	0.005	0.030	0.005	0.905	0.005	0.050	0.000	0.000	0.000	100
	4. Sada	0.000	0.039	0.000	0.917	0.020	0.020	0.000	0.005	0.000	102
	5. Laxe	0.000	0.037	0.000	0.903	0.005	0.056	0.000	0.000	0.000	108
	6. Portosin	0.000	0.040	0.000	0.905	0.010	0.045	0.000	0.000	0.000	100

Continued on next page

Appendix (continued)

Locus	Population	Allele 1	2	3	4	5	6	7	8	9	N
Est-D	7. Carril	0.005	0.029	0.000	0.880	0.005	0.063	0.005	0.010	0.005	104
	8. Rande	0.000	0.042	0.000	0.908	0.008	0.042	0.000	0.000	0.000	120
	9. Silleiro	0.000	0.040	0.000	0.914	0.000	0.045	0.000	0.000	0.000	99
	10. Aveiro	0.016	0.083	0.005	0.813	0.000	0.078	0.000	0.000	0.005	96
	11. Sesimbra	0.000	0.054	0.000	0.858	0.020	0.068	0.000	0.000	0.000	74
	12. Marbella	0.000	0.025	0.005	0.914	0.020	0.035	0.000	0.000	0.000	99
	13. Almeria	0.000	0.035	0.000	0.931	0.006	0.029	0.000	0.000	0.000	173
	14. Alicante	0.018	0.025	0.000	0.946	0.000	0.011	0.000	0.000	0.000	138
	15. Cullera	0.023	0.011	0.000	0.939	0.000	0.027	0.000	0.000	0.000	280
	16. Garraf	0.067	0.011	0.000	0.911	0.000	0.011	0.000	0.000	0.000	45
	17. LLansa	0.021	0.015	0.000	0.933	0.000	0.031	0.000	0.000	0.000	97
	18. Palavas	0.030	0.010	0.000	0.944	0.000	0.015	0.000	0.000	0.000	99
	19. Montecarlo	0.010	0.025	0.000	0.960	0.005	0.000	0.000	0.000	0.000	100
	20. Genova	0.010	0.015	0.000	0.965	0.000	0.010	0.000	0.000	0.000	100
	21. Livorno	0.020	0.025	0.000	0.950	0.000	0.005	0.000	0.000	0.000	100
Idh	1. Santander	0.000	0.105	0.865	0.030	0.000	0.000	–	–	–	100
	2. Ribadeo	0.000	0.083	0.889	0.028	0.000	0.000	–	–	–	90
	3. Malata	0.000	0.095	0.900	0.005	0.000	0.000	–	–	–	100
	4. Sada	0.000	0.118	0.882	0.000	0.000	0.000	–	–	–	76
	5. Laxe	0.000	0.110	0.881	0.009	0.000	0.000	–	–	–	109
	6. Portosin	0.005	0.115	0.880	0.000	0.000	0.000	–	–	–	100
	7. Carril	0.000	0.084	0.905	0.011	0.000	0.000	–	–	–	95
	8. Rande	0.005	0.067	0.924	0.005	0.000	0.000	–	–	–	105
	9. Silleiro	0.000	0.113	0.876	0.011	0.000	0.000	–	–	–	93
	10. Aveiro	0.000	0.093	0.897	0.010	0.000	0.000	–	–	–	97
	12. Marbella	0.005	0.110	0.865	0.020	0.000	0.000	–	–	–	100
	13. Almeria	0.000	0.133	0.852	0.008	0.008	0.000	–	–	–	64
	14. Alicante	0.000	0.074	0.918	0.008	0.000	0.000	–	–	–	61
	15. Cullera	0.000	0.093	0.898	0.009	0.000	0.000	–	–	–	54
	16. Garraf	0.000	0.122	0.865	0.014	0.000	0.000	–	–	–	37
	17. LLansa	0.000	0.087	0.908	0.005	0.000	0.000	–	–	–	98
	18. Palavas	0.000	0.071	0.909	0.015	0.000	0.005	–	–	–	99
	19. Montecarlo	0.005	0.110	0.885	0.000	0.000	0.000	–	–	–	100
	20. Genova	0.010	0.126	0.843	0.015	0.005	0.000	–	–	–	99
	21. Livorno	0.005	0.096	0.874	0.025	0.000	0.000	–	–	–	99
Lap-1	1. Santander	0.000	0.012	0.029	0.035	0.436	0.459	0.029	0.000	–	86
	2. Ribadeo	0.000	0.011	0.011	0.005	0.440	0.484	0.049	0.000	–	91
	3. Malata	0.000	0.006	0.040	0.023	0.449	0.438	0.045	0.000	–	88
	4. Sada	0.000	0.020	0.059	0.010	0.455	0.421	0.035	0.000	–	101
	5. Laxe	0.000	0.000	0.034	0.053	0.403	0.471	0.039	0.000	–	103
	6. Portosin	0.000	0.011	0.049	0.016	0.359	0.527	0.038	0.000	–	92
	7. Carril	0.000	0.010	0.029	0.025	0.436	0.495	0.005	0.000	–	102
	8. Rande	0.008	0.024	0.048	0.020	0.363	0.496	0.040	0.000	–	124
	9. Silleiro	0.000	0.000	0.033	0.000	0.375	0.571	0.022	0.000	–	92
	10. Aveiro	0.000	0.000	0.047	0.042	0.380	0.500	0.026	0.005	–	96
	11. Sesimbra	0.014	0.007	0.014	0.014	0.426	0.480	0.047	0.000	–	74
	12. Marbella	0.000	0.008	0.000	0.023	0.348	0.591	0.030	0.000	–	66
	13. Almeria	0.003	0.003	0.016	0.035	0.461	0.452	0.029	0.000	–	155
	14. Alicante	0.000	0.033	0.022	0.088	0.430	0.404	0.022	0.000	–	136
	15. Cullera	0.008	0.023	0.033	0.089	0.376	0.457	0.012	0.002	–	242
	16. Garraf	0.000	0.000	0.069	0.083	0.375	0.431	0.042	0.000	–	36
	18. Palavas	0.000	0.011	0.065	0.086	0.409	0.409	0.022	0.000	–	93
	19. Montecarlo	0.000	0.016	0.008	0.048	0.435	0.476	0.016	0.000	–	62
	20. Genova	0.000	0.021	0.016	0.094	0.406	0.427	0.036	0.000	–	96
	21. Livorno	0.000	0.029	0.058	0.064	0.355	0.453	0.029	0.012	–	86
Lap-2	1. Santander	0.000	0.056	0.472	0.000	0.465	0.000	0.007	0.000	0.000	71
	2. Ribadeo	0.000	0.021	0.537	0.000	0.395	0.000	0.047	0.000	0.000	95
	3. Malata	0.005	0.036	0.536	0.010	0.388	0.000	0.026	0.000	0.000	98
	6. Portosin	0.010	0.040	0.510	0.000	0.414	0.000	0.025	0.000	0.000	99
	8. Rande	0.000	0.029	0.485	0.000	0.466	0.000	0.019	0.000	0.000	103
	9. Silleiro	0.000	0.016	0.521	0.000	0.432	0.000	0.031	0.000	0.000	96
	10. Aveiro	0.000	0.026	0.469	0.000	0.464	0.000	0.036	0.005	0.000	98
	11. Sesimbra	0.000	0.034	0.486	0.000	0.439	0.000	0.041	0.000	0.000	74
	12. Marbella	0.011	0.006	0.494	0.000	0.461	0.000	0.028	0.000	0.000	90
	13. Almeria	0.000	0.045	0.473	0.000	0.461	0.000	0.021	0.000	0.000	168
	14. Alicante	0.000	0.011	0.489	0.000	0.438	0.000	0.058	0.000	0.004	138
	15. Cullera	0.000	0.040	0.512	0.002	0.416	0.000	0.030	0.000	0.000	214
	16. Garraf	0.000	0.053	0.474	0.000	0.434	0.000	0.039	0.000	0.000	38

Appendix (continued)

Locus	Population	Allele 1	2	3	4	5	6	7	8	9	N
Lap-2	17. LLansa	0.000	0.010	0.510	0.000	0.408	0.000	0.071	0.000	0.000	98
	18. Palavas	0.005	0.015	0.465	0.005	0.425	0.000	0.085	0.000	0.000	100
	19. Montecarlo	0.000	0.025	0.540	0.000	0.359	0.000	0.076	0.000	0.000	99
	20. Genova	0.000	0.026	0.442	0.000	0.447	0.000	0.084	0.000	0.000	95
	21. Livorno	0.000	0.044	0.440	0.000	0.434	0.000	0.082	0.000	0.000	91
Mpi	1. Santander	0.005	0.960	0.035	0.000	–	–	–	–	–	100
	2. Ribadeo	0.000	0.954	0.041	0.005	–	–	–	–	–	98
	3. Malata	0.000	0.960	0.040	0.000	–	–	–	–	–	87
	4. Sada	0.010	0.933	0.053	0.005	–	–	–	–	–	104
	5. Laxe	0.000	0.919	0.081	0.000	–	–	–	–	–	105
	6. Portosin	0.005	0.973	0.022	0.000	–	–	–	–	–	93
	7. Carril	0.022	0.888	0.084	0.006	–	–	–	–	–	89
	8. Rande	0.000	0.937	0.063	0.000	–	–	–	–	–	119
	9. Silleiro	0.000	0.914	0.081	0.005	–	–	–	–	–	99
	10. Aveiro	0.005	0.955	0.040	0.000	–	–	–	–	–	99
	11. Sesimbra	0.020	0.926	0.054	0.000	–	–	–	–	–	74
	12. Marbella	0.005	0.978	0.016	0.000	–	–	–	–	–	91
	13. Almeria	0.008	0.969	0.023	0.000	–	–	–	–	–	131
	14. Alicante	0.000	0.960	0.040	0.000	–	–	–	–	–	99
	15. Cullera	0.000	0.962	0.038	0.000	–	–	–	–	–	173
	16. Garraf	0.000	1.000	0.000	0.000	–	–	–	–	–	16
	17. LLansa	0.000	0.913	0.087	0.000	–	–	–	–	–	52
	18. Palavas	0.000	0.967	0.033	0.000	–	–	–	–	–	75
	19. Montecarlo	0.005	0.975	0.020	0.000	–	–	–	–	–	100
	20. Genova	0.000	0.969	0.031	0.000	–	–	–	–	–	97
	21. Livorno	0.005	0.975	0.020	0.000	–	–	–	–	–	99
Odh	1. Santander	0.000	0.000	0.543	0.000	0.000	0.140	0.000	0.317	0.000	93
	2. Ribadeo	0.005	0.000	0.500	0.000	0.005	0.126	0.000	0.343	0.020	99
	3. Malata	0.011	0.000	0.554	0.000	0.000	0.151	0.000	0.258	0.027	93
	4. Sada	0.020	0.000	0.574	0.000	0.004	0.098	0.008	0.283	0.012	122
	5. Laxe	0.000	0.000	0.578	0.005	0.009	0.128	0.000	0.280	0.000	109
	6. Portosin	0.015	0.000	0.525	0.000	0.000	0.141	0.000	0.318	0.000	99
	7. Carril	0.005	0.000	0.518	0.000	0.000	0.191	0.000	0.264	0.023	110
	8. Rande	0.012	0.000	0.544	0.000	0.004	0.104	0.000	0.316	0.020	125
	9. Silleiro	0.015	0.000	0.521	0.000	0.005	0.160	0.000	0.294	0.005	97
	10. Aveiro	0.000	0.000	0.551	0.000	0.005	0.111	0.000	0.328	0.005	99
	11. Sesimbra	0.000	0.000	0.534	0.000	0.000	0.144	0.000	0.322	0.000	73
	12. Marbella	0.000	0.000	0.655	0.000	0.000	0.085	0.000	0.260	0.000	100
	13. Almeria	0.003	0.000	0.658	0.000	0.000	0.091	0.000	0.246	0.003	171
	14. Alicante	0.000	0.000	0.107	0.000	0.000	0.263	0.000	0.622	0.008	131
	15. Cullera	0.004	0.000	0.121	0.000	0.000	0.193	0.000	0.675	0.008	257
	16. Garraf	0.000	0.000	0.205	0.000	0.011	0.273	0.000	0.511	0.000	44
	17. LLansa	0.000	0.000	0.118	0.000	0.000	0.274	0.000	0.602	0.005	93
	18. Palavas	0.005	0.000	0.136	0.000	0.000	0.202	0.000	0.646	0.010	99
	19. Montecarlo	0.000	0.000	0.133	0.000	0.005	0.173	0.000	0.679	0.010	98
	20. Genova	0.000	0.000	0.143	0.000	0.000	0.163	0.005	0.689	0.000	98
	21. Livorno	0.000	0.000	0.085	0.000	0.005	0.185	0.000	0.715	0.010	100
6Pgdh	1. Santander	0.000	0.032	0.011	0.926	0.005	0.026	0.000	0.000	–	95
	2. Ribadeo	0.005	0.015	0.000	0.974	0.000	0.005	0.000	0.000	–	97
	3. Malata	0.005	0.037	0.011	0.937	0.005	0.000	0.005	0.000	–	95
	4. Sada	0.009	0.046	0.009	0.921	0.000	0.014	0.000	0.000	–	108
	5. Laxe	0.005	0.055	0.000	0.912	0.005	0.022	0.000	0.000	–	91
	6. Portosin	0.005	0.040	0.005	0.930	0.010	0.010	0.000	0.000	–	100
	7. Carril	0.005	0.053	0.011	0.905	0.016	0.011	0.000	0.000	–	95
	8. Rande	0.000	0.035	0.012	0.921	0.000	0.028	0.004	0.000	–	127
	9. Silleiro	0.005	0.020	0.000	0.940	0.000	0.035	0.000	0.000	–	100
	10. Aveiro	0.000	0.015	0.010	0.933	0.010	0.031	0.000	0.000	–	97
	11. Sesimbra	0.000	0.047	0.027	0.892	0.000	0.034	0.000	0.000	–	74
	12. Marbella	0.011	0.032	0.005	0.910	0.000	0.043	0.000	0.000	–	94
	13. Almeria	0.003	0.016	0.000	0.934	0.003	0.044	0.000	0.000	–	160
	14. Alicante	0.000	0.011	0.000	0.974	0.004	0.011	0.000	0.000	–	135
	15. Cullera	0.000	0.019	0.011	0.948	0.000	0.019	0.000	0.004	–	134
	16. Garraf	0.000	0.013	0.000	0.950	0.000	0.025	0.013	0.000	–	40
	17. LLansa	0.000	0.010	0.000	0.969	0.010	0.010	0.000	0.000	–	98
	18. Palavas	0.005	0.021	0.000	0.964	0.000	0.010	0.000	0.000	–	97
	19. Montecarlo	0.000	0.013	0.000	0.953	0.000	0.033	0.000	0.000	–	75
	20. Genova	0.000	0.020	0.005	0.945	0.005	0.020	0.005	0.000	–	100
	21. Livorno	0.000	0.020	0.020	0.944	0.000	0.015	0.000	0.000	–	99

Continued on next page

Appendix (continued)

Locus	Population	Allele 1	2	3	4	5	6	7	8	9	N
Pgi	1. Santander	0.021	0.021	0.080	0.447	0.032	0.309	0.074	0.011	0.005	94
	2. Ribadeo	0.000	0.053	0.053	0.580	0.005	0.239	0.064	0.005	0.000	94
	3. Malata	0.006	0.029	0.052	0.592	0.000	0.241	0.046	0.034	0.000	87
	6. Portosin	0.000	0.006	0.040	0.551	0.011	0.301	0.063	0.028	0.000	88
	8. Rande	0.009	0.000	0.061	0.670	0.004	0.200	0.026	0.026	0.004	115
	9. Silleiro	0.000	0.016	0.089	0.516	0.011	0.247	0.068	0.053	0.000	95
	10. Aveiro	0.000	0.021	0.074	0.547	0.021	0.253	0.063	0.021	0.000	95
	11. Sesimbra	0.000	0.014	0.048	0.616	0.014	0.233	0.075	0.000	0.000	73
	12. Marbella	0.006	0.017	0.074	0.540	0.006	0.284	0.063	0.011	0.000	88
	13. Almeria	0.007	0.033	0.059	0.526	0.016	0.239	0.108	0.007	0.007	153
	14. Alicante	0.000	0.013	0.013	0.787	0.000	0.148	0.039	0.000	0.000	115
	15. Cullera	0.002	0.005	0.027	0.745	0.002	0.167	0.045	0.005	0.002	276
	16. Garraf	0.000	0.000	0.035	0.779	0.000	0.163	0.023	0.000	0.000	43
	17. LLansa	0.000	0.010	0.046	0.784	0.005	0.124	0.026	0.005	0.000	97
	18. Palavas	0.000	0.011	0.037	0.777	0.000	0.160	0.016	0.000	0.000	94
	19. Montecarlo	0.000	0.000	0.023	0.753	0.000	0.201	0.011	0.011	0.000	87
	20. Genova	0.000	0.005	0.036	0.835	0.005	0.098	0.021	0.000	0.000	97
	21. Livorno	0.000	0.000	0.026	0.844	0.006	0.104	0.019	0.000	0.000	77
Pgm	1. Santander	0.000	0.017	0.099	0.570	0.000	0.297	0.017	0.000	0.000	86
	2. Ribadeo	0.005	0.011	0.113	0.640	0.000	0.215	0.000	0.016	0.000	93
	3. Malata	0.000	0.010	0.136	0.611	0.000	0.207	0.030	0.005	0.000	99
	4. Sada	0.000	0.013	0.087	0.675	0.000	0.200	0.013	0.013	0.000	40
	5. Laxe	0.000	0.027	0.095	0.541	0.007	0.324	0.000	0.007	0.000	74
	6. Portosin	0.000	0.006	0.145	0.599	0.000	0.227	0.012	0.012	0.000	86
	7. Carril	0.000	0.032	0.112	0.532	0.005	0.293	0.011	0.016	0.000	94
	8. Rande	0.000	0.029	0.131	0.561	0.000	0.270	0.008	0.000	0.000	122
	9. Silleiro	0.000	0.000	0.106	0.591	0.005	0.283	0.015	0.000	0.000	99
	10. Aveiro	0.000	0.005	0.069	0.633	0.000	0.271	0.016	0.000	0.005	94
	11. Sesimbra	0.014	0.000	0.103	0.630	0.000	0.253	0.000	0.000	0.000	73
	12. Marbella	0.000	0.015	0.103	0.619	0.000	0.253	0.010	0.000	0.000	97
	13. Almeria	0.003	0.012	0.104	0.610	0.006	0.251	0.012	0.003	0.000	173
	14. Alicante	0.020	0.036	0.133	0.515	0.000	0.276	0.010	0.010	0.000	98
	15. Cullera	0.002	0.030	0.125	0.513	0.000	0.302	0.019	0.009	0.000	268
	16. Garraf	0.000	0.054	0.135	0.568	0.000	0.243	0.000	0.000	0.000	37
	17. LLansa	0.000	0.036	0.134	0.500	0.000	0.304	0.021	0.005	0.000	97
	18. Palavas	0.010	0.036	0.120	0.573	0.000	0.255	0.000	0.005	0.000	96
	19. Montecarlo	0.020	0.040	0.162	0.475	0.000	0.293	0.010	0.000	0.000	99
	20. Genova	0.005	0.035	0.141	0.495	0.000	0.313	0.000	0.010	0.000	99
	21. Livorno	0.010	0.031	0.120	0.542	0.000	0.271	0.016	0.010	0.000	96
Sod	1. Santander	1.000	–	–	–	–	–	–	–	–	60
	2. Ribadeo	1.000	–	–	–	–	–	–	–	–	98
	3. Malata	1.000	–	–	–	–	–	–	–	–	80
	4. Sada	1.000	–	–	–	–	–	–	–	–	100
	5. Laxe	1.000	–	–	–	–	–	–	–	–	110
	6. Portosin	1.000	–	–	–	–	–	–	–	–	99
	7. Carril	1.000	–	–	–	–	–	–	–	–	80
	8. Rande	1.000	–	–	–	–	–	–	–	–	122
	9. Silleiro	1.000	–	–	–	–	–	–	–	–	96
	10. Aveiro	1.000	–	–	–	–	–	–	–	–	40
	11. Sesimbra	1.000	–	–	–	–	–	–	–	–	38
	12. Marbella	1.000	–	–	–	–	–	–	–	–	40
	13. Almeria	1.000	–	–	–	–	–	–	–	–	118
	14. Alicante	1.000	–	–	–	–	–	–	–	–	139
	15. Cullera	1.000	–	–	–	–	–	–	–	–	240
	16. Garraf	1.000	–	–	–	–	–	–	–	–	46
	17. LLansa	1.000	–	–	–	–	–	–	–	–	60
	18. Palavas	1.000	–	–	–	–	–	–	–	–	40
	19. Montecarlo	1.000	–	–	–	–	–	–	–	–	40
	20. Genova	1.000	–	–	–	–	–	–	–	–	40
	21. Livorno	1.000	–	–	–	–	–	–	–	–	40
Stdh	1. Santander	0.000	0.148	0.000	0.112	0.010	0.061	0.653	0.005	0.010	98
	2. Ribadeo	0.011	0.068	0.023	0.051	0.034	0.102	0.676	0.017	0.017	88
	3. Malata	0.000	0.118	0.000	0.139	0.028	0.097	0.576	0.028	0.014	72
	6. Portosin	0.000	0.124	0.011	0.124	0.027	0.065	0.624	0.022	0.005	93
	8. Rande	0.013	0.143	0.004	0.087	0.035	0.091	0.565	0.057	0.004	115
	9. Silleiro	0.011	0.079	0.000	0.084	0.021	0.089	0.689	0.016	0.011	95
	10. Aveiro	0.006	0.120	0.000	0.114	0.006	0.072	0.669	0.012	0.000	83
	12. Marbella	0.011	0.126	0.000	0.144	0.052	0.080	0.557	0.029	0.000	87

Appendix (continued)

Locus	Population	Allele									N
		1	2	3	4	5	6	7	8	9	
Stdh	13. Almería	0.000	0.114	0.000	0.182	0.023	0.068	0.602	0.000	0.011	44
	14. Alicante	0.000	0.163	0.000	0.144	0.000	0.048	0.644	0.000	0.000	52
	15. Cullera	0.000	0.157	0.000	0.100	0.014	0.000	0.700	0.029	0.000	35
	16. Garraf	0.000	0.175	0.000	0.175	0.000	0.000	0.637	0.000	0.013	40
	17. LLansa	0.000	0.131	0.000	0.142	0.023	0.000	0.670	0.011	0.023	88
	18. Palavas	0.005	0.199	0.000	0.051	0.000	0.031	0.694	0.020	0.000	98
	19. Montecarlo	0.005	0.227	0.010	0.101	0.005	0.005	0.636	0.010	0.000	99
	20. Genova	0.005	0.232	0.000	0.137	0.016	0.021	0.584	0.005	0.000	95
	21. Livorno	0.005	0.184	0.000	0.071	0.000	0.005	0.714	0.010	0.010	98

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