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Influence of spatial structure on genetic isolation in *Plebejus argus* populations (Lepidoptera: Lycaenidae)

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Populations of *Plebejus argus* were sampled in southwest Finland, both on the mainland and on islands, and in and around the Doñana National Park in southwest Spain. A total of 453 individuals coming from 14 locations were investigated using allozyme electrophoresis on a total of 10 polymorphic allozyme loci. Contrary to earlier studies, all conducted in Britain, our samples showed little differentiation between sampled locations. In Spain, the populations of the Donaña area showed no differentiation despite being up to 36 km apart; only the population to the south of the Guadalquivir river showed a significant difference to the others. In Finland the population on one island showed marked genetic differentiation from all the others, which showed little or no difference from each other. No isolation-by-distance effect could be detected in either system. We hypothesise that emigration–immigration events are more frequent in the Spanish and Finnish populations than in the British ones. We did, however, find two isolated populations, one in Spain and one in Finland; both were small and geographically isolated and shared evident drift.

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In industrialized countries natural habitats both decrease in abundance and increase in their patchiness. The isolation of habitat patches from each other is a major cause of long term decline of many species. For non-migratory butterflies, the recolonization of previously occupied habitats depends on the proximity to occupied patches (THOMAS and JONES 1993). Populations of widely distributed species with limited dispersal abilities seem to be particularly at risk from an increased fragmentation, as these have suffered more from an increased fragmentation than more vagile species (MAES and VAN DYCK 2001). The severe decline of many butterfly species in northwest Europe is partly due to an increased fragmentation of their habitats (VAN SWAAY and WARREN 1999).

In order to assess the impact of habitat fragmentation of populations, one should compare situations where the distribution of a species is naturally patchy with an area where the species has a more continuous habitat. The silver-studded blue *Plebejus argus* is a Lycaenid butterfly widely distributed in Europe. It may be locally abundant within its distribution range and is usually patchily distributed in Britain, where it has been intensively studied (THOMAS 1985, 1996; BROOKES et al. 1997; LEWIS et al. 1997; THOMAS et al. 1998). British populations show very limited dispersal abilities, resulting in mostly closed population structure. In other parts of Europe, the situation is quite different: in SW Spain, the species habitat is fairly continuous throughout the Doñana National park, where *Halimium halimifolium*, its local food plant, is abundant. The density of *P. argus* is also dependent upon the density of *Lasius niger*, its obligatory host ant species (JORDANO et al. 1992; SEYMOUR and JORDANO pers. comm.). In southern Finland, the species is present in numerous islets in the Baltic Sea, which may naturally have very small habitat patches. We hypothesize that the dispersal ability of this species may vary within its European range. On one hand, individuals from the most continuous habitats (i.e. in the Doñana N.P.) may disperse more than in naturally fragmented habitats, as they would be under less selection pressure against dispersal. On the other hand, in Finland, individuals on islands may be selected against dispersal, resulting in isolated populations. These islands must have been colonised by individuals crossing the sea, hence there is a possibility for an evolution of high dispersal abilities in a few individuals, resulting in a dual strategy of sedentarity and dispersal. This may depend on genotype and ecological and behavioural conditions (IMS and HJERMANN 2001). The aim of this study is to investigate dispersal from the point of view of its consequences on the genetic population structure of populations. Basically there can be three main possible spatial structures of population differentiation. (1) If populations show a marked isolation-by-distance effect, dispersal occurs locally in a stepping stone manner, most dispersing individuals moving only between neighbouring habitat patches.

(2) If populations are not genetically different from each other, then there is a high rate of dispersal, even between population far away from each other, resulting in a regional panmixia. (3) If populations are genetically different from each other but do not show isolation by distance effect, this may be due to occasional long distance colonisation where genetic differences occur due to founder effect. The aim of the present study is to identify the population structure of *Plebejus argus* in two contrasted landscape structures: the rather continuous habitat of Doñana National Park with the naturally patchily distributed populations of forest clearings and islands in south Finland.

MATERIAL AND METHODS

Study systems

Finland. — In southern Finland, *P. argus* is found both on the mainland and on numerous islands of the Finnish coastline, sometimes in very small habitat patches (Hyyryläinen, pers. comm.). The occupied habitat patches are localised either in forest clearings (mainland, and some of the islands) or rocky outcrops, as is the case on numerous islands. The host plants of *P. argus* in Finland are Ericaceae shrubs, *Vaccinium myrtillus*, *Arctostaphylos uva-ursi*, *Calluna vulgaris* and the associated ant is *Lasius platythorax* (Hyyryläinen, pers. comm).

Nine habitat patches were sampled in southern Finland around the Hanko Peninsula (59°50′N, 23°15′E) in July 1999, of which the first five were located on the mainland and the last four on islands. 1: Santala; 2: Albläktsmossen; 3: Tvärminne A; 4: Tvärminne B; 5: Tvärminne C; 6: Ekö; 7: Kalvön; 8: Hermansö; 9: Algö (Fig. 1).

Spain. — In the Doñana National Park (37°00′N, 06°25′W; SW Spain, Huelva Province), *P. argus* is present on sandy ground, in heathland ('matorral') vegetation dominated by its local food plant *Halimium halimifolium* (JORDANO et al. 1992). Towards the east of the National Park, the species is also present at low density in the Natural Park, where it occurs in clearings within *Pinus maritima* forest. At Doñana, the host ant species of *P. argus* is *Lasius niger*. *P. argus* was sampled in 1999 from five as widely distributed habitat patches as possible, including one within a *Eucalyptus* forest in the north (Soto Grande), one within the pine forest in the natural park in the east (Laguna de Jiménez), and one at the other side of the Guadalquivir in the south (Sanlucar, Fig. 2).

The distance between sampled habitat patches varied from 1 to 16 km in SW Finland and from 6 to 47 km in SW Spain.

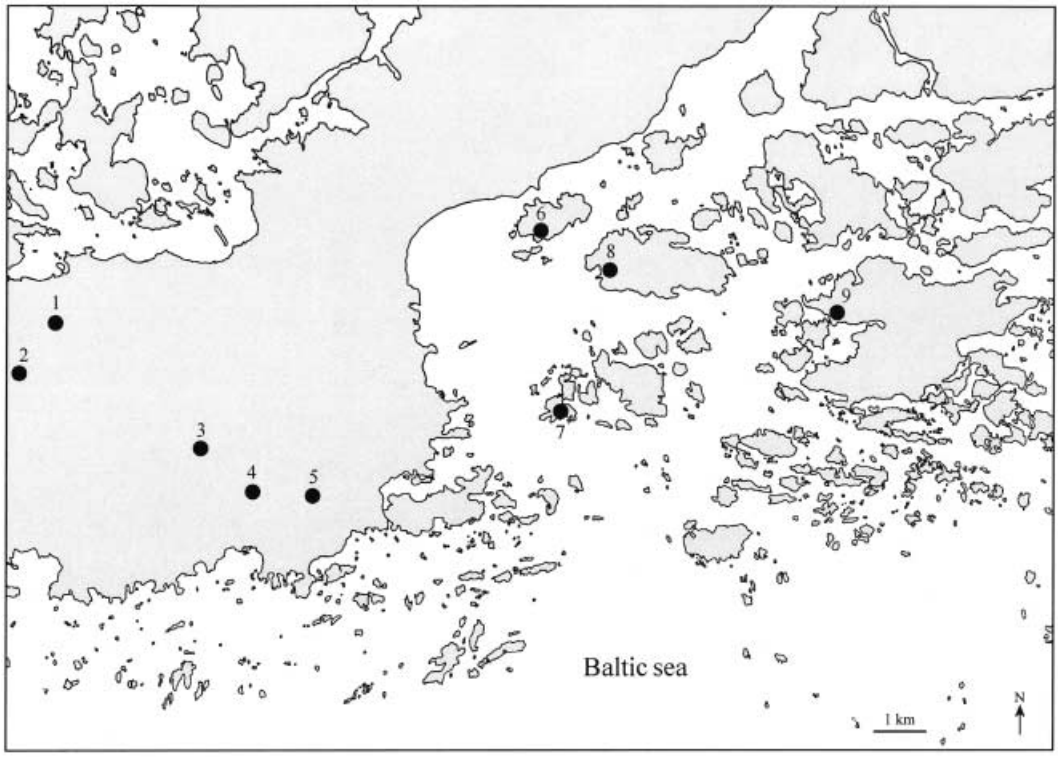

Fig. 1. Map of the studied area in Finland: 1: Santala; 2: Albläktsmossen; 3: Tvärminne A; 4: Tvärminne B; 5: Tvärminne C; 6: Ekö; 7: Kalvön; 8: Hermansö; 9: Älgö.

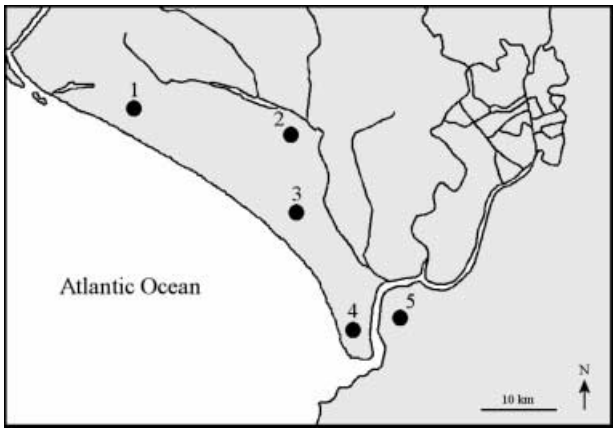

Fig. 2. Map of the studied area in the Doñana Natural and National Park (SW Spain): 1: Laguna de Jiménez; 2: Soto Grande; 3: Las Beles; 4: Marismillas; 5: Sanlucar.

Adults were captured in the field using a butterfly net (NABOKOV 2000), and were frozen alive the same day at −20°C or −80°C and later stored in a −80°C freezer. For the analysis, the abdomen and the thorax were squashed, homogenised on ice in 120 µl of pH 7.1 extraction buffer (saccharose 15 % w/v, 50 mM Tris HCl, Bromophenol blue as runner marker) and centrifuged at 14,000 r.p.m. during 8 min (NÈVE et al. 2000). Supernatants were frozen as 10 µl droplets as described in WYNNE and BROOKES (1992), with minor modifications. Genetic variability was examined using cellulose acetate electrophoresis methods using the buffer and staining recipes of RICHARDSON et al. (1986) and HEBERT and BEATON (1993).

Of the 15 enzyme loci tested, 10 proved to be scorable and polymorphic for the Finnish samples: glucose-6-phosphate isomerase (*PGI*, E.C. 5.3.1.9), phosphoglucomutase (*PGM*, E.C. 5.4.2.2), malic dehydrogenase $NADP^+$ enzyme (*ME*, E.C. 1.1.1.40), isocitrate dehydrogenase (*IDH*, E.C.), adenylate kinase (*AK*, E.C. 2.7.4.3), glutamate oxaloacetate transaminase (*GOT-s* & *GOT-f*, E.C. 2.6.1.1), phenylalanyl-proline peptidase (*PEP-Pp*, E.C. 3–4.11–13), leucyl-glycyl-glycine peptidase (*PEP-Lgg*, E.C. 3.4.11–13), malate dehydrogenase (*MDH*, E.C. 1.1.1.37) and 7 for the Spanish ones (*GOT-s*, *LGG* and *MDH* were monomorphic). Five further enzyme systems were also tested, but found to be either monomorphic or illegible: fumarate hydratase (*FUM*, E.C. 4.2.1.2), 6-phosphogluconate dehydrogenase (*6PGD*, E.C. 1.1.1.44), glucose-6-phosphate dehydrogenase (*G6PDH*, E.C. 1.1.1.49), mannose phosphate isomerase (*MPI*, E.C. 5.3.8.1), sorbitol dehydrogenase (*SDH*, E.C. 1.1.1.14)

Allelic and genotypic frequencies were calculated for each sample using GENEPOP 3.3 (RAYMOND and ROUSSET 1995b).

Genetic structure of populations was studied using several complementary approaches.

Habitat patches variability in allele frequencies was measured using estimates of F_{ST} (WRIGHT 1969), calculated as WEIR and COCKERHAM (1984). This coefficient θ was provided by the program FSTAT version 2.9.1 (GOUDET 1995). Standard deviations were obtained using the bootstapping procedure.

The pairwise differentiation of populations (Fisher test), the pairwise F_{ST} between samples using WEIR and COCKERHAMS θ (1984) method, the deviance from Hardy-Weinberg equilibrium, the test of isolation by distance and the estimation of the number of migrant per generation were computed with GENEPOP population genetics software (version 3.3, RAYMOND and ROUSSET 1995b).

RESULTS

Both the Finnish and Spanish populations were highly polymorphic; allele frequencies were calculated for each locus and population (Appendix 1 and 2).

Estimates of θ revealed low levels of variability among the populations sampled both in Finland (θ = 0.015) and in Spain (θ = 0.016, Table 1). If the analyses are done separately for island and mainland Finnish populations, the differentiation between samples (expressed in term of θ) is more important between the islands (θ = 0.025) than on the mainland, where it is not significant (θ = 0.011). For the Spanish analysis, if the Sanlucar sample is excluded so that only samples north of Guadalquivir are selected for

Table 1. *Estimations of tetha (θ) F statistics (WEIR and COCKERHAM 1984) calculated for islands, mainland and all samples in Finland, for all samples and for those situated in the north of Guadalquivir river in Spain. (*: 95 % confidence interval calculated by Bootstrapping over loci).*

	Finish samples			Spanish samples	
	All	Mainland	Island	All	north of Guadalquivir
θ	0.015 (0.003; 0.032)*	0.011 (−0.002; 0.031)*	0.025 (0.008; 0.042)*	0.016 (0.004; 0.031)*	0.001 (−0.007; 0.006)*

the calculation of θ, there is no genetic differentiation ($\theta = 0.001$).

In Finland, the exact tests for genotypic population differentiation for each sample pair reveal significant differences for a range of loci among populations (Table 2). The Kalvön island sample (FIN 7) is very different from all other Finnish population entities, either from the mainland or from other islands; this genetic isolation status is striking for the loci *PGI* and *PGM* ($P < 0.01$ or $P < 0.001$) towards all other samples and *GOT-f* ($P < 0.5$ or $P < 0.01$) towards four populations of which three are situated on the mainland (Table 2).

For Spanish samples, the Fisher tests of genotypic differentiation between sample pair (Table 3) shows no difference among samples of the Doñana park (populations north of Guadalquivir river). However, for the most southern sample Sanlucar, separated from the Doñana population by the Guadalquivir river, significant variations are detected for *AK*, *PGM*, *IDH* loci.

Mantel test did not indicate any isolation by distance effect between the nine Finnish samples, whatever the study scale (all samples $P = 0.64$, mainland only $P = 0.38$, and islands only $P = 0.52$). In Spain, there is no correlation between matrices of geographical and genetic distances ($P = 0.60$). Thus isolation-by-distance per se does not seem to be the cause of the differentiation observed either on Kalvön island, or in Sanlucar.

DISCUSSION

Levels of population differentiation are related to the degree of population isolation within the landscape. At the southern limit of its European range, *P. argus* is widely distributed throughout the xerophytic scrubland of the Doñana area, but the densities vary

Table 2. *Pairwise tests of genotypic differentiation (Fisher test; Raymond and Rousset 1995a) for the Finnish samples (below diagonal); differentiation level:. θ (Weir and Cockerham 1984) for each population pair at all loci, with their associated probabilities according to exact tests (above diagonal). *: P < 0.05; **: P < 0.01; ***: P < 0.001.*

	FIN 1	FIN 2	FIN 3	FIN 4	FIN 5	FIN 6	FIN 7	FIN 8	FIN 9
FIN 1	–	−0.0101	−0.0080	−0.0130	−0.0026	−0.0144	0.0306**	−0.0056	−0.0040
FIN 2	0	–	0.0078	−0.0032	0.0004	−0.0092	0.0299**	−0.0057	0.0214
FIN 3	*Got-s***	*Got-s** *Me**	–	−0.0043	0.0010	−0.0090	0.0475**	0.0027	−0.0016
FIN 4	0	0	0	–	0.0058	−0.0098	0.0462**	0.0065	−0.0007
FIN 5	0	*Me**	*Got-s***	0	–	−0.0101	0.0546**	−0.0085	0.0103
FIN 6	0	0	0	0	*Got-s**	–	0.0437**	−0.0092	−0.0033
FIN 7	*Pgi*** *Pgm**	*Pgi**** *Pgm** *Me** *Got-f**	*Pgi**** *Pgm*** *Got-f**	*Pgi**** *Pgm**	*Pgi*** *Pgm**** *Got-f***	*Pgi*** *Pgm*** *Got-f**	–	0.0408**	0.1005**
FIN 8	0	*Me** *Ak**	*Ak**	0	*Got-s**	0	*Pgi*** *Pgm****	–	0.0195
FIN 9	*Got-s***	0	0	0	*Got-s***	0	*Pgi**** *Pgm****	0	–

Table 3. *Pairwise tests of genotypic differentiation (Fisher test; Raymond and Rousset 1995a) for the Spanish samples (below diagonal); differentiation level: θ (Weir and Cockerham 1984) for each population pair at all loci, with their associated probabilities according to exact tests (above diagonal). *: P < 0.05; **: P < 0.01; ***: P < 0.001.*

	SP 1	SP 2	SP 3	SP 4	SP 5
SP 1	–	−0.0058	−0.0017	0.0099	0.0304**
SP 2	0	–	−0.0015	0.0136	0.0279**
SP 3	0	0	–	0.0226**	0.0354**
SP 4	0	0	0	–	0.0483**
SP 5	*Pgm** *Ak**	*Ak*** *Idh**	*Ak**** *Idh**	*Me**	–

greatly within two orders of magnitude, in accordance mainly with the local abundance of the host ant *Lasius niger* (Jordano and Seymour, unpubl.). Individuals wander regularly between foraging, resting and breeding microhabitats (G. Nève, unpubl.), as the distribution of the food and resting resources for adults explain the regular movements of adult butterflies (Van Dyck and Matthysen 1999). Furthermore, their density may be very high (> 100 individuals/m^2) and their sheer local abundance may also trigger dispersal, as has been observed in other butterfly species (Shapiro 1970; Baguette et al. 1998). The populations of *P. argus* of the Doñana area north of the Guadalquivir river do not significantly differ from each other suggesting that this area may be viewed genetically as one unit, due to significant gene flow between populations. Only the Sanlucar population shows genetic distinctiveness, probably due to the Guadalquivir river being an efficient barrier to dispersal in *P. argus*.

In Finland, only populations from islands showed a marked genotypic differentiation ($\theta = 0.025$); populations from the mainland showed no overall differentiation (nonsignificant θ). This was unexpected, as the mainland populations live in forest clearings separated by clearly unsuitable, mainly forested, habitat, in contrast with the more continuous heathland habitat of Spain. The differenciation of island populations results probably from occasional extinction and recolonisation processes, leading to a subsequent genetic drift of populations according to their isolation and size (Gilpin 1991). In previous studies of *P. argus*, it was found that it exhibits a very limited gene flow, resulting from a limited dispersal ability (Thomas and Harrison 1992; Brookes et al. 1997; Lewis et al. 1997). In north Wales, no colonisation has been recorded between habitat patches separated by 600 m of unfavourable habitat and dispersal over 1 km is extremely rare (Thomas and Harrison 1992); *P. argus* is not present either in small (< 0.05 ha) or in isolated (> 1 km) patches (Thomas and Harrison 1992; Thomas et al. 1992) and a migration event over few km is considered very unlikely. Furthermore, in Wales, *P. argus* specimens from large habitat patches show a lower ratio of thorax to abdomen sizes, suggesting the local selection of a reduction of dispersal ability, compared to individuals from small habitat patches (Thomas et al. 1998). This differentiation of selection would be efficient only if gene flow is sufficiently low between populations (Altukhov 1991).

From genetical data only, the homogenous structure and the lack of isolation by distance found in the Spanish samples, and to a lesser extent in Finland, characterise species with open population structure and large dispersal power (Hastings and Harrison 1994; Vandewoestijne et al. 1999). *P. argus* populations in Wales are considered as 'classical' metapopulations (Thomas and Harrison 1992; Hanski and Thomas 1994), with a limited gene flow allowing differentiation of populations from different habitat patches, whereas in Doñana the habitat patches are nearly continuous and support local populations between which gene flow counteracts genetic drift. The low differentiation of Finnish populations would likewise be the result of regular migration or recolonisation events. We therefore hypothesise that suitable habitats patches are so densely distributed in the Finnish system that the high migration rate does not allow genetic differentiation, even over long distances, due to migration probably taking place in a 'stepping stone' fashion; this, however, is not a general case, as *Philaenus spumarius* (Homoptera), also studied in islands off Tvärminne Zoological Station, showed levels of heterozygosity correlated with both the degree of isolation and size of populations (Saura et al. 1973).

The absence of isolation by distance structure at the regional scale in both studied areas does not prevent occasional genetic differentiation of populations. This is the case in populations not geographically distant but separated from the others by a major barrier to dispersal. In Spain, only the Sanlucar (SP 5) sample is significantly different from all other Spanish samples; this population is separated from the Doñana populations by the Guadalquivir River (ca 1 km wide). It seems that this population has evolved separately from the Doñana population for a long time, as its heterozygosity is higher than for the Doñana populations and it displays 4 private alleles. In contrast, the *P. argus* population from the small Finnish island Kalvön (FIN 7) shows a significative genetic divergence to all other populations, but it holds only one private allele and it shows one of the lowest observed heterozygosities. Low heterozygosity and high genetic divergence may be expected from its relative isolation and from the very small size of its local habitat patch (ca 100 m^2), and consequently of a probably small *P. argus* population. It is noteworthy that in both Spanish and Finnish systems, isolated populations were associated with a water barrier. We therefore suspect that migration over land, including unfavourable habitats such as forests, is significant in both Spanish and Finnish systems, but that movements over water bodies such as sea or river may be more difficult. The Kalvön island combines the two factors which explain its genetic isolation position: low immigration (and emigration) success due to its isolation and small habitat-patch size. The lower divergence of other islands is probably due to the fact that these support more and larger patches, and that these island patches are

probably connected to each other or to the mainland through ecological stepping stones (SUTCLIFFE and THOMAS 1996; TRAVIS and DYTHAM 1999).

It appears that differences in dispersal capacity and population structure recorded between regions of *P. argus* range (S Finland and SW Spain vs Britain), are attributable to differences in the distribution and sizes of habitat patches. The local dispersal behaviour and consequently the metapopulation dynamics of *P. argus* may differ considerably between regions, depending on the local spatio-temporal distribution and history of its habitat patches.

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APPENDIX 1

Allozyme alleles frequencies for Finnish samples of *Plebejus argus*. Ho, mean observed heterozygosity (95 % confidence interval).

Locus	Allele	MAINLAND					ISLANDS			
N		FIN 1 34	FIN 2 36	FIN 3 36	FIN 4 38	FIN 5 39	FIN 6 32	FIN 7 33	FIN 8 34	FIN 9 28
Pgi	A	0.000	0.028	0.028	0.013	0.013	0.000	0.000	0.000	0.000
	B	0.294	0.361	0.194	0.276	0.333	0.297	0.288	0.324	0.214
	C	0.029	0.000	0.014	0.013	0.013	0.000	0.000	0.029	0.000
	D	0.000	0.000	0.000	0.000	0.013	0.016	0.000	0.015	0.000
	E	0.000	0.000	0.028	0.000	0.013	0.000	0.000	0.000	0.000
	F	0.618	0.542	0.667	0.658	0.551	0.609	0.439	0.529	0.750
	G	0.059	0.069	0.069	0.039	0.064	0.078	0.167	0.074	0.036
	H	0.000	0.000	0.000	0.000	0.000	0.000	0.106	0.000	0.000
	I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000
Pgm	A	0.279	0.278	0.319	0.276	0.385	0.328	0.106	0.353	0.375
	B	0.721	0.722	0.681	0.724	0.603	0.656	0.894	0.647	0.607
	C	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
	D	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.018
Me	A	0.000	0.000	0.028	0.000	0.000	0.031	0.000	0.000	0.000
	B	0.206	0.167	0.250	0.171	0.256	0.188	0.303	0.235	0.155
	C	0.750	0.750	0.722	0.803	0.744	0.750	0.697	0.676	0.828
	D	0.044	0.083	0.000	0.026	0.000	0.031	0.000	0.088	0.017
Idh	A	0.015	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000
	B	0.970	1.000	0.986	0.974	1.000	1.000	1.000	1.000	1.000
	C	0.015	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000
Ak	A	0.544	0.611	0.472	0.513	0.500	0.609	0.515	0.662	0.621
	B	0.456	0.389	0.528	0.487	0.500	0.391	0.485	0.338	0.379
Got-s	A	0.015	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
	B	0.912	0.903	0.833	0.908	0.974	0.844	0.939	0.897	0.845
	C	0.015	0.042	0.167	0.066	0.013	0.094	0.061	0.103	0.155
	D	0.059	0.056	0.000	0.026	0.000	0.063	0.000	0.000	0.000
Got-f	A	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
	B	0.000	0.000	0.000	0.013	0.000	0.016	0.000	0.000	0.000
	C	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000
	D	0.882	0.778	0.792	0.816	0.718	0.750	0.924	0.794	0.879
	E	0.000	0.014	0.014	0.013	0.013	0.016	0.000	0.059	0.000
	F	0.000	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000
	G	0.118	0.208	0.181	0.158	0.218	0.203	0.076	0.147	0.121
	H	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000
Pp	A	0.000	0.028	0.014	0.000	0.013	0.000	0.000	0.044	0.000
	B	0.029	0.069	0.056	0.095	0.167	0.113	0.045	0.088	0.107
	C	0.118	0.139	0.097	0.095	0.077	0.000	0.121	0.088	0.143
	D	0.779	0.736	0.736	0.770	0.628	0.774	0.758	0.765	0.679
	E	0.074	0.028	0.097	0.041	0.115	0.113	0.076	0.015	0.071
Lgg	A	0.000	0.000	0.014	0.000	0.026	0.063	0.000	0.000	0.000
	B	0.044	0.028	0.069	0.118	0.103	0.188	0.061	0.162	0.034
	C	0.206	0.472	0.153	0.132	0.333	0.422	0.318	0.309	0.172
	D	0.735	0.458	0.764	0.750	0.513	0.328	0.606	0.515	0.741
	E	0.015	0.042	0.000	0.000	0.026	0.000	0.015	0.015	0.052
Mdh	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.052
	B	0.971	0.972	0.972	0.987	1.000	0.984	1.000	1.000	0.914
	C	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	D	0.029	0.028	0.028	0.000	0.000	0.016	0.000	0.000	0.034
Ho		0.268 (0.060)	0.272 (0.061)	0.286 (0.049)	0.277 (0.054)	0.346 (0.085)	0.267 (0.053)	0.267 (0.073)	0.329 (0.073)	0.308 (0.046)

APPENDIX 2

Allozyme alleles frequencies in Spanish populations sampled. The other three loci studied in Finland (*Mdh; Lgg; Got-s*) are monomorphic in the studied Spanish samples. Ho, mean observed heterozygosity (95 % confidence interval).

Locus N	Allele	SP 1 35	SP 2 33	SP 3 36	SP 4 4	SP 5 35
Pgi	A	0.000	0.000	0.000	0.000	0.014
	B	0.414	0.561	0.431	0.375	0.443
	C	0.014	0.015	0.042	0.000	0.014
	D	0.029	0.015	0.000	0.000	0.000
	E	0.529	0.409	0.528	0.625	0.529
	F	0.014	0.000	0.000	0.000	0.000
Pgm	A	0.014	0.000	0.000	0.000	0.000
	B	0.329	0.424	0.306	0.375	0.557
	C	0.000	0.091	0.069	0.125	0.057
	D	0.243	0.167	0.236	0.125	0.114
	E	0.071	0.061	0.069	0.000	0.057
	F	0.343	0.258	0.319	0.375	0.214
Ak	A	0.800	0.818	0.875	0.875	0.614
	B	0.200	0.182	0.125	0.125	0.386
Idh	A	0.457	0.364	0.347	0.375	0.543
	B	0.543	0.636	0.653	0.625	0.443
	C	0.000	0.000	0.000	0.000	0.014
Me	A	0.771	0.727	0.667	1.000	0.529
	B	0.043	0.061	0.000	0.000	0.086
	C	0.014	0.015	0.014	0.000	0.014
	D	0.171	0.197	0.319	0.000	0.371
Got-f	A	0.000	0.000	0.000	0.000	0.014
	B	0.000	0.045	0.014	0.000	0.000
	C	0.000	0.000	0.014	0.000	0.000
	D	0.057	0.030	0.000	0.000	0.000
	E	0.857	0.818	0.944	1.000	0.957
	F	0.086	0.061	0.028	0.000	0.029
	G	0.000	0.045	0.000	0.000	0.000
Pp	A	0.057	0.061	0.000	0.000	0.029
	B	0.043	0.091	0.139	0.250	0.143
	C	0.343	0.333	0.361	0.750	0.414
	D	0.557	0.515	0.500	0.000	0.386
	E	0.000	0.000	0.000	0.000	0.029
Ho		0.282 (0.073)	0.312 (0.086)	0.357 (0.108)	0.214 (0.101)	0.408 (0.099)