Distinct genetic subdivision in sympatric and sibling species of the genus *Littorina* (Gastropoda: Littorinidae)

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The genetic structure of two sibling and sympatric species of the genus *Littorina* was compared using allozymic loci. The two species are biologically and ecologically well-known and mostly show similar life history characteristics. Three populations of *L. mariae* Sacchi & Rastelli and *L. obtusata* (L.) were studied in the Muros-Noya Ria (Galicia, NW Spain). In addition, four microgeographical subsamples taken from one of the populations were analysed for each species. Age, sex and genotypes for nine polymorphic loci were studied in 1250 snails of both species.

L. mariae showed larger genetic population subdivision and lower heterozygosity levels for the loci studied than did *L. obtusata*. Heterozygote deficiencies were found in only a few cases in natural populations of both species, usually affecting the *Lap-1* locus. No significant genetic differences among age or sex classes were found. These results may be explained by the lower effective population size in *L. mariae* than in *L. obtusata*. Known differences between these species in generation interval and population density during the winter can cause the different effective population sizes suggested. These life history characteristics appear to provide the most likely explanations for the differences in genetic differentiation and heterozygosity between the two species. A previously unknown *L. mariae* morph from exposed shores is tentatively suggested to be conspecific.

Keywords: allozymes, flat periwinkles, genetic structure, *Littorina mariae*, *Littorina obtusata*, population subdivision.

Introduction

The use of allozymes as genetic markers has made possible detailed studies of population genetic structure in many animal and plant species (Ward, 1990; Lewontin, 1991). They have been used to show that species often display different hierarchical levels of population subdivision (Johnson & Black, 1991; Wolf, 1991), constraining or predisposing adaptive evolution (Wright, 1978; Slatkin, 1987). Moreover, undetected population structure can invalidate experimental field hypotheses. Thus, the study of a species' genetic structure is a necessary preliminary step in understanding the evolutionary potential of any taxon.

The degree of genetic subdivision (genetic structure) in any species can be described as an equilibrium between the evolutionary factors that promote genetic

cohesiveness and the factors that promote genetic differentiation; with some factors, for example natural selection, the influence can be in either direction (Nei, 1987; Slatkin, 1987). Some authors have also emphasized the factors extrinsic to the organism (biotic and abiotic) that can influence the degree of genetic relatedness among populations (Johnson & Black, 1991). It is desirable that any experiment designed to find the relative importance of the different factors includes some means of controlling them, at least partially. Comparative studies, among similar species with different genetic structures, have been used to infer the biological causes producing these differences (Berger, 1983; Ward, 1990). In these cases the uncontrolled factors are assumed to affect the compared species in the same way. A useful group for this kind of study is the North-Atlantic littorinids (Mollusca: Gastropoda), as they often have different genetic structures and they have been well-studied biologically and ecologically. In most cases the degree

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of population differentiation is related to the degree of gene flow among populations, inferred from dispersal capabilities or breeding systems (Berger, 1983; Janson, 1987a; Ward, 1990). This relationship may, however, be rather inaccurate in some species (Berger, 1977). The level of population subdivision is also sensitive to historical phenomena, such as bottlenecks, founder events (Berger, 1977; Janson, 1987b; Knight *et al.*, 1987) and selection (Berger, 1983; Johannesson & Johannesson, 1989). However, other relationships are not easily found in comparative studies because of the difficulty in using closely related species with comparable life history characteristics (Wolf, 1991). Here, the population genetic structures were compared, geographically and microgeographically, in two well-known sibling species living sympatrically in the same habitat.

Littorinid species which have direct development usually present more taxonomical problems than species having planktonic stages (Ward, 1990). On exposed Galician shores an unusual form of *Littorina mariae* morph has been found living on *Mastocarpus stellatus* (Rolán & Templado, 1987). The transition between the exposed and the typical form occurred abruptly in the area studied, suggesting a possible case of overlapping of valid species. In this study, these two forms were studied genetically for 10 allozymic loci to clarify their taxonomic status.

This paper reports different genetic structures in sympatric populations of the two sibling species *L. mariae* and *L. obtusata*. The facts known of the biology and ecology of these species allow suggestions to be made of the main causes of genetic differences between them. Moreover, results of the analysis of one sample of the *L. mariae* exposed morph from Galicia supports the suggestion that it is conspecific, perhaps an ecotype of this species.

Materials and methods

Species studied

Littorina mariae and *L. obtusata* are two sibling species of North-Atlantic littorinids living in the Fucaceae belt of intertidal zones (Fretter & Graham, 1980). Some reproductive characteristics (Fretter & Graham, 1980; Reid, 1990) as well as some allozymic loci (Morris, 1979; Zaslavskaya *et al.*, 1992) can be used to distinguish the two species. They are dioecious and have direct development with crawling juveniles hatching from egg masses attached to seaweeds (Fretter & Graham, 1980). On Galician shores both species are found sympatrically (mainly on *Fucus vesiculosus*), although *L. mariae* prefers mid to lower intertidal zones whereas *L. obtusata* prefers mid to upper ones

(Rolán & Templado, 1987; Rolán-Alvarez, 1992). They show similar life history characteristics, although some differences are known: *L. mariae* is an annual species that feeds mainly on micro-epiphytes growing on seaweeds (Fretter & Graham, 1980; Williams, 1990, 1992) whereas *L. obtusata* survives between 2 and 10 years feeding directly on seaweeds (Fretter & Graham, 1980; Sergievsky, 1985). In addition, *L. mariae* is usually less affected by parasites and more affected by intertidal predators and other competing grazers than *L. obtusata*, which resists more stressful physical conditions during low tidal periods (Fretter & Graham, 1980; Williams, 1990, 1992).

Sampling

To find the geographical distribution of both species more than 32 samplings were carried out in the Ria of Muros-Noya (Fig. 1). Following this, three populations representative of the distribution of the two species in the Ria were selected: ABE (Abelleira, October 1989), AGU (Aguieira, October 1989) and PLB (Punta-Laxe-Brava, May 1990) (Fig. 1). Around 100 adult snails from the smallest possible area of *Fucus* were sampled in each population. This meant sampling areas of 1–2 m² for most cases, but about 50–200 m² in two samples of *L. mariae* (AGU and PLB, respectively). In the PLB population both species were sampled without overlapping; *L. mariae* was found on *Mastocarpus stellatus* whereas *L. obtusata* was found on *F. vesiculosus*.

Four microgeographical samples of both species were taken from the Abelleira population (July 1990). These snails were sampled over *Fucus* areas of about 150 m². Copulating pairs and, around them on the same or contiguous seaweeds, noncopulating snails were sampled in each area. This design was used in an attempt to study sexual selection in nature (Rolán-Alvarez *et al.*, unpublished data). Briefly, the noncopulating snails represented more than 77 per cent of the snails from the total population in *L. mariae* and more than 95 per cent in *L. obtusata*. The noncopulating subsamples of this later experiment were used to analyse the microgeographical genetic structure of these species. The species were classified according to shell characters in the field and this was later verified in the laboratory.

Variables and electrophoretic methods

Sex and age classes, and genotypes for 10 allozymic loci, were recorded for 1250 individuals of the two species. Three sex classes were used: males (M), females (F) and immature snails (I) (Fretter & Graham,

Fig. 1 Distribution of *Littorina mariae* and *L. obtusata* in the Ria of Muros-Noya. Arrows represent sampling areas.

1980; Reid, 1990). The two age classes used were based on the qualitative differences that can be observed during the shell growth: old (O) and young (Y) (Goodwin & Fish, 1977; Fretter & Graham, 1980).

Specimens were stored at -40°C or -70°C prior to dissection and electrophoresis. The posterior soft parts of each snail were homogenized in proportional volumes of 0.1 M Tris/HCl (pH 7.0). Electrophoresis was carried out using horizontal starch Sigma gels (12.5–14 per cent). The buffer systems used were continuous Tris-EDTA-borate (TEB) and Tris-EDTA-maleate (TEM) modified from Quesada (1992). Seven stain systems and ten loci were examined following Morris (1979) and Quesada (1992): phosphoglucose isomerase (E.C.5.3.1.9; *Pgi*), phosphoglucose mutase (E.C.5.4.2.2; *Pgm*), mannose phosphate isomerase (E.C.5.3.1.8; *Mpi*), esterases (E.C.3.1.1.1; *Es-2* and *Es-c*), arginine phosphokinase (E.C.2.7.3.3; *Ark* and *Ark-c*), aminopeptidase (E.C.3.4.13.-; Glycyl-L-leucine; *Ap*), leucine aminopeptidase (E.C.3.4.11.-; *Lap-1* and *Lap-2*). Loci and alleles were labelled according to their electrophoretic mobilities (Ward & Warwick, 1980).

Statistical analysis

Variation in allelic and genotypic frequencies among samples for each locus and species were analysed using χ^2 homogeneity tests. A pseudoprobability χ^2 homogeneity test was employed when low sample size within cells (less than five) was expected (Zaykin & Pudovkin, 1993). Nei's unbiased heterozygosity index (H_e) was used to describe allozymic polymorphism in the genetic markers studied (Nei, 1987). Two F indices were used to indicate departure from Hardy-Weinberg expectations: the Wright's F and the Robertson & Hill (1984) F' indices. The F statistic was calculated using the BIOSYS programme (Swofford & Selander, 1981), and the pseudoprobability test for $D(-F)$ (Zaykin & Pudovkin, 1993) was used to obtain its significance. A BASIC program was used to calculate F' and its particular statistical test (Quesada *et al.*, 1992). A modification of the Bonferroni multitest correction was employed when many tests were carried out simultaneously (Rice, 1989).

The fixation F indices were used to study the genetic structure in each species, using the BIOSYS program (Swofford & Selander, 1981). This analysis decomposes the Hardy-Weinberg deviation for every locus into three components, F_{IS} , F_{ST} and F_{IT} (Wright, 1978; Nei, 1987). These estimators need fewer assumptions than alternative estimators (Nei, 1987). A pseudoprobability homogeneity χ^2 -test (Zaykin & Pudovkin, 1993) was used to test F_{ST} estimates. Comparisons among populations were made with Nei's unbiased genetic identity. The bootstrap confidence interval for each combination of pairs was also calculated (D. V. Zaykin & A. N. Tatarenkov, personal communication). An UPGMA dendrogram is presented for the genetic distances of the samples involved.

Results

Geographical analysis

The distribution of *L. mariae* and *L. obtusata* in the Ria of Muros-Noya is shown in Fig. 1. Both species have similar geographical distributions and appear sympatrically in most populations; however, in the more exposed shores *L. mariae* predominates whereas in the more sheltered ones *L. obtusata* appears alone. In addition, no significant differences were found in the distribution of the sexes between samples of each species. Furthermore, the male/female ratio did not differ significantly from one in any sample. However, significant differences with respect to the frequency of age classes among samples were found (Table 1).

Allozyme frequencies of the polymorphic loci were scored for each species from the Ria of Muros-Noya

(tables available from authors). Arginine kinase cathodal (*Ark-c*) was monomorphic in both species whereas seven loci were polymorphic in *L. mariae* and nine in *L. obtusata*. One diagnostic locus (*Ap*) and two loci with substantial differences (*Mpi* and *Ark*) were found between these two sibling species. *L. mariae* presented a mean Nei unbiased heterozygosity (H_e) of 0.108 (± 0.036) and 1.7 (± 0.22) alleles per locus for the 10 loci whereas *L. obtusata* showed a mean H_e of 0.216 (± 0.049) and 1.97 (± 0.03) alleles per locus. The heterozygosity per locus and population was significantly different between the two species in a Wilcoxon–Mann–Whitney test ($U=196$, $n_1=27$, $n_2=27$, $P<0.01$). However, this result may not be representative of the genome of each species because of the low number of loci employed in the study. Allelic frequency differences between males and females or old and young snails were analysed in each species for most loci and samples (except *L. mariae* PLB). No gene frequency differences between sex or age classes among samples were found after Bonferroni multitest corrections.

Estimators of the Hardy–Weinberg deviations are shown in Table 2. The F and F' statistics show similar results, with only a few significant F or F' estimates in these samples without Bonferroni corrections: five and six cases in 38 tests, respectively. The alternative statistical tests (for F or F') gave similar results, even if the pseudoprobability test was slightly more conservative (see Table 2). The significant cases may not be randomly distributed as most were heterozygote

deficiencies and appeared at the *Lap-1* locus. However, using the Bonferroni corrections only two F or F' estimates were significant: at the *Lap-1* locus in the samples of both species from Abelleira (see Table 2).

The F fixation indices of both species, in the samples studied, are shown in Table 3. The F_{IS} index represents an estimate of the mean F or F' values per locus for the samples analysed. Thus, it gave similar results to the mean values of F or F' shown in Table 2. However, a significant positive association between the F_{IS} values per locus of each species was observed, using a Kendall tau test ($\tau=0.809$, $n=7$, $P<0.05$).

Both *L. mariae* and *L. obtusata* showed substantial genetic subdivision in the Ria of Muros-Noya, with distinct loci in each species contributing to the population differentiation (Table 3). Interestingly, *L. mariae* (mean $F_{ST}=0.085$) showed more pronounced differentiation than *L. obtusata* (mean $F_{ST}=0.037$). All the F_{ST} values were significant in *L. mariae* after Bonferroni corrections whereas only five of the nine F_{ST} values were significant in *L. obtusata*. Furthermore, the *L. mariae* F_{ST} per locus estimates were significantly greater than for *L. obtusata*, using the Wilcoxon–Mann–Whitney test ($U=12.5$, $n_1=7$, $n_2=9$, $P<0.05$). However, one sample of *L. mariae* (PLB) comprised an atypical morph adapted to a completely distinct habitat. This population alone would be able to explain the different genetic subdivision between both species. A new test was devised to examine this possibility: the fixation F_{ST} index was compared in both species excluding the PLB samples.

Table 1 Percentage of sex and age classes in *Littorina mariae* and *L. obtusata* from the Ria of Muros-Noya

	<i>L. mariae</i>				<i>L. obtusata</i>			
	ABE	AGU	PLB	χ^2	ABE	AGU	PLB	χ^2
Sex								
M	54.8	47.5	—		56.8	44.2	38.0	
F	40.4	49.5	—		43.2	52.5	62.0	
I	4.8	3.0	—		—	3.3	—	
(<i>N</i>)	104	99		1.45	95	120	50	2.97
Age								
O	85.0	87.1	0		96.9	74.1	98.0	
Y	14.9	12.9	100		2.9	25.8	2.0	
(<i>N</i>)	107	101	103	238.21***	97	116	50	31.28***

(M) males, (F) females and (I) immature snails; (O) old and (Y) young snails; (ABE) Abelleira, (AGU) Agueira and (PLB) Punta-Laxe-Brava. The frequencies of sex and age classes between populations were compared using pseudoprobability χ^2 homogeneity tests (Zaykin & Pudovkin, 1993). (*N*) is sample size.

*** $P \leq 0.001$.

Table 2 Estimators of deviations from Hardy-Weinberg proportions per locus in three populations of *Littoria mariae* and *L. obtusata*

Locus		<i>L. mariae</i>			Mean	<i>L. obtusata</i>			Mean
		ABE	AGU	PLB		ABE	AGU	PLB	
<i>Pgi</i>	<i>F</i>	-0.046	-0.020	-0.120	-0.062	-0.016	-0.088	-0.064	-0.056
	<i>F'</i>	-0.020	-0.015	-0.115	-0.050	-0.011	-0.084	-0.054	-0.050
	(<i>N</i>)	116	100	103		96	116	50	
<i>Pgm</i>	<i>F</i>	—	0.119	—	0.119	0.026	0.013	0.062	0.034
	<i>F'</i>	—	0.070	—	0.070	0.032	0.017	0.072	0.040
	(<i>N</i>)		102			87	114	50	
<i>Mpi</i>	<i>F</i>	—	—	—	—	0.181*	0.018	-0.030	0.056
	<i>F'</i>	—	—	—	—	0.142**	0.018	-0.060	0.033
	(<i>N</i>)					88	112	36	
<i>Es-2</i>	<i>F</i>	0.068	—	—	0.068	0.156	-0.035	-0.075	0.015
	<i>F'</i>	0.043	—	—	0.043	0.162	-0.030	-0.066	0.022
	(<i>N</i>)	116				96	112	50	
<i>Es-c</i>	<i>F</i>	-0.016	0.135	—	0.059	0.080	-0.250**	0.114	-0.019
	<i>F'</i>	-0.016	0.140	—	0.062	0.086	-0.247**	0.126	-0.012
	(<i>N</i>)	111	101			94	115	50	
<i>Ark</i>	<i>F</i>	0.041	0.095	-0.101	0.012	0.080	-0.080	-0.068	-0.023
	<i>F'</i>	0.026	0.100	-0.095	0.012	0.086	-0.076	-0.058	0.016
	(<i>N</i>)	116	100	94		96	116	49	
<i>Lap-1</i>	<i>F</i>	0.175***	0.172*	-0.051	0.099	0.795***	0.226	0.027	0.349
	<i>F'</i>	0.385***	0.160**	-0.045	0.167	0.804***	0.134*	0.038	0.325
	(<i>N</i>)	111	90	83		96	116	50	
<i>Lap-2</i>	<i>F</i>	-0.027	-0.128	—	-0.077	-0.064	—	-0.042	-0.053
	<i>F'</i>	-0.022	-0.117	—	-0.047	-0.056	—	-0.032	-0.044
	(<i>N</i>)	116	39			65		50	

The significance of Wright's *F* estimator was tested using a pseudoproduct test (Zaykin & Pudovkin, 1993) and of Robertson and Hill's *F'* estimator the specific test designed for this purpose (Robertson & Hill, 1984). The underlined asterisks represent significant cases following Bonferroni corrections. (*N*) is sample size.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

L. mariae showed larger population genetic differentiation (mean $F_{ST} = 0.052$) than *L. obtusata* (mean $F_{ST} = 0.019$). However, the F_{ST} differences between species were not significant, although they were close to being significant ($U = 13$, $n_1 = 7$, $n_2 = 9$, $P = 0.071$). Furthermore, six of the seven F_{ST} per locus estimates in *L. mariae* were significant after Bonferroni corrections whereas only three of nine F_{ST} estimates were significant in *L. obtusata*.

Nei's unbiased genetic identities and distances were used to compare all combinations of pairs. The UPGMA tree of the genetic distances is shown in Fig. 2. The mean Nei genetic distance between the two species was 0.37, with all the populations of each species clustered together. Moreover, the genetic similarities of each pair-wise comparison were not statistically different within species, using bootstrap similarity confidence

intervals. This means that the *L. mariae* 'exposed' morph is genetically similar to the 'typical' morph of the species, and so the exposed morph may be included in *L. mariae*.

Microgeographical analysis

Tables of allelic frequencies of noncopulating *L. mariae* and *L. obtusata*, from the microgeographical study of Abelleira (July 1990), are available from the authors. No significant heterogeneity in allele frequencies was found between areas from the microgeographical study, and between them and previous samples of Abelleira (October 1989) from the geographical study. The fixation *F* indices for the four areas of the microgeographical study are shown in Table 4. The F_{IS} per locus estimates differed greatly

Table 3 Fixation F indices for both *Littorina mariae* and *L. obtusata* in the Ria of Muros-Noya

Locus	<i>L. mariae</i>			<i>L. obtusata</i>		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
<i>Pgi</i>	-0.070	-0.020	0.046***	-0.075	-0.033	0.039***
<i>Pgm</i>	0.119	0.151	0.037***	0.036	0.069	0.034***
<i>Mpi</i>	—	—	—	0.069	0.074	0.005
<i>Es-2</i>	0.068	0.107	0.042***	0.059	0.150	0.097***
<i>Es-c</i>	0.017	0.133	0.118***	-0.024	0.018	0.042***
<i>Ap</i>	—	—	—	-0.023	-0.021	0.002
<i>Ark</i>	-0.017	0.001	0.018**	-0.005	-0.002	0.004
<i>Lap-1</i>	0.154	0.250	0.114***	0.179	0.211	0.038***
<i>Lap-2</i>	-0.115	0.026	0.127***	-0.077	-0.051	0.024*
Mean	0.027	0.109	0.085	0.017	0.053	0.037

A pseudoprobability χ^2 homogeneity test (Zaykin & Pudovkin, 1993) was used to estimate the significance of F_{ST} . The underlined asterisks represent the significant cases following Bonferroni corrections. (N) is sample size.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Fig. 2 UPGMA cluster analysis for the three populations of *Littorina mariae* and *L. obtusata* species, using the unbiased Nei genetic distance coefficient.

between species, except for *Es-c*, *Ap*, *Lap-1* and *Lap-2*. In both species these F_{IS} estimates were usually completely different from the F_{IS} estimates for the geographical study. There was no significant F_{ST} estimate, after Bonferroni corrections, in *L. mariae* or in *L. obtusata*, even though the F_{ST} estimates per locus in *L. obtusata* were significantly greater than the F_{ST} estimates per locus in *L. mariae* ($U = 13$, $n_1 = 9$, $n_2 = 9$, $P < 0.05$). However, the sample size for *L. obtusata* was lower than for *L. mariae* in the four samples and this can fully explain the genetic structure differences at the microgeographical level (see Chakraborty & Leimar, 1987).

Discussion

Natural populations of the sibling species *L. mariae* and *L. obtusata* showed different population subdivision and genetic variability in the Muros-Noya Ria. However, before discussing these data, the demo-

graphic structure and the existence of heterozygote deficiencies of the samples studied should be described.

The frequency of males and females was homogeneous whereas the frequency of old and young snails was heterogeneous among populations. The latter result was expected because the different samples were obtained in different seasons and years, and the frequency of age classes changes seasonally in these species (Goodwin & Fish, 1977; Fretter & Graham, 1980). However, these results do not affect the genetic analysis as there were no significant genetic differences between sex or age classes. In addition, the degree of genetic variability or population subdivision observed in *L. mariae* and *L. obtusata*, as well as the genetic distance estimate ($D = 0.37$) found between these species, was comparable to that found in other studies on these and similar species (see Ward, 1990; Zaslavskaya *et al.*, 1992).

Only a few significant cases of heterozygote deficiencies were observed in the species studied,

Table 4 Fixation F indices for *Littorina mariae* and *L. obtusata* species in the microgeographical study (Abelleira)

Locus	<i>L. mariae</i>			<i>L. obtusata</i>		
	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
<i>Pgi</i>	0.059	0.061	0.002	-0.011	-0.003	0.008
<i>Pgm</i>	-0.005	-0.001	0.003	0.043	0.050	0.008
<i>Mpi</i>	0.337	0.339	0.003	-0.019	-0.012	0.007
<i>Es-2</i>	-0.007	-0.004	0.003	0.091	0.112	0.023
<i>Es-c</i>	0.114	0.129	0.017**	0.057	0.061	0.004
<i>Ap</i>	-0.020	-0.013	0.007	-0.027	-0.010	0.016
<i>Ark</i>	0.185	0.187	0.003	-0.115	-0.104	0.009
<i>Lap-1</i>	0.137	0.143	0.007	0.277	0.281	0.005
<i>Lap-2</i>	-0.038	-0.038	0.001	-0.098	-0.085	0.013
Mean	0.098	0.104	0.007	0.014	0.025	0.011

A pseudoprobability χ^2 homogeneity test (Zaykin & Pudovkin, 1993) was used to estimate the significance of F_{ST} (Workman & Niswander, 1970). The underlined asterisks represent the significant cases following Bonferroni corrections. (N) is sample size.

** $P \leq 0.01$.

mostly at the *Lap-1* locus. This result agrees with similar studies on these and related littorinid species (see Ward, 1990). However, there are no causes that can be said with certainty to explain the particular behaviour of the *Lap-1* locus (for a review of the causes, see Gaffney *et al.*, 1990). Perhaps natural selection, in combination with habitat choice for the genotypes (or ecotypes) involved, can partially contribute to the heterozygote deficiencies in this locus (García-Dorado, 1986). More surprisingly, a significant association for the F_{IS} per locus was found between the species in the geographical study, but not in the microgeographical study. However, it is difficult to interpret this result. Perhaps the significant heterozygote deficiency in *Lap-1* in both species may explain the significant F_{IS} association from the geographical study. In fact, the F_{IS} per locus association between species is not significant if the *Lap-1* locus is excluded from the analysis.

In the Muros-Noya Ria *L. mariae* showed greater genetic subdivision and lower genetic variability for the loci studied than *L. obtusata* (Table 3). These are rather surprising results because both species are supposed to have similar life history characteristics (Fretter & Graham, 1980). The genetic variability result may be real (although it should be regarded with caution owing to the low number of loci studied) as most studies comparing heterozygosities between both sibling species found a similar trend (reviewed in Ward, 1990; Zaslavskaya *et al.*, 1992). The biological and ecological similarities of *L. mariae* and *L. obtusata* in

Galician populations allows us to suggest which life history characters affect each species separately.

There are no direct estimates of the dispersal capabilities or migration rates in these species but laboratory observations regarding mobility of juveniles and adults, as well as female capability to store sperm (Rolán-Alvarez, 1992, 1993), suggest them to be similar. Other features, such as egg masses or juveniles rafting on seaweeds (Jokiel, 1990), if they exist, would be very similar because the species feed on the same algae in Galicia (Rolán & Templado, 1987; Rolán-Alvarez, 1992). Moreover, *L. mariae* and *L. obtusata* have similar geographical distributions in the Muros-Noya Ria (Fig. 1).

Natural selection has been claimed as an important factor producing population genetic differentiation but it is impossible to test all the potential ways of influencing this factor in natural populations (Kimura, 1983). Moreover, allozymic polymorphism appears to depend fundamentally on stochastic factors, even though a smaller part of the variability may be caused by natural selection (Kimura, 1983; Skibinski *et al.*, 1993). Some ecological selective hypotheses have been claimed to explain allozymic polymorphism, but not genetic differentiation, in natural populations (reviewed in Kimura, 1983). Nevertheless, these selective hypotheses do not successfully explain the results of this study, as *L. obtusata* and *L. mariae* have rather similar habitats and life histories on Galician shores. Moreover, it is very difficult to explain the differences between *L. mariae* and *L. obtusata* in heterozygosity

and genetic differentiation, simultaneously, by any selective hypothesis. In addition, when different morphs or ecotypes are included in the analysis, natural selection may have, at least indirectly, a greater effect on the observed genetic polymorphism. The *L. mariae* PLB population illustrates a particular adaptation to a different habitat. Thus, following the selective argument, we would expect an important contribution from this population to the genetic subdivision in this species. However, we found the same pattern excluding the PLB samples from both species. These facts do not support the suggestion that natural selection is a major contributing factor causing differences in genetic structure between *L. mariae* and *L. obtusata*.

Every factor that influences the effective population size, by means of genetic drift, may also be responsible for the differences in genetic structure and variability between species (Kimura, 1983; Slatkin, 1987). Mating system is one of the most important life history characteristics that can influence the effective population size, but *L. mariae* and *L. obtusata* appear to display similar mating systems (Fretter & Graham, 1980; Rolán-Alvarez, 1992). However, there are some differences in life histories that suggest different effective population sizes in each species. They appear to have similar population densities on Galician shores, although *L. mariae* has a lower population density during the winter (Rolán & Templado, 1987; Rolán-Alvarez, 1993). Population bottlenecks or founder events have been used to explain reductions in heterozygosity level or genetic differentiation in natural populations (Berger, 1977; Ward, 1990), but the effectiveness of the process depends on the importance and the frequency of the bottlenecks as well as the rate of population recovery (Kimura, 1983; Nei, 1987). It would be necessary to assume significant bottlenecks every winter in *L. mariae* to explain the allozymic variability and genetic structure differences with respect to *L. obtusata*. Thus, this phenomenon may contribute to the species differences but it does not appear to be the only factor.

Hill (1972) showed that in dioecious organisms the effective population size depends on the number of males and females and the generation interval of the species. So, species with longer generation intervals will have larger effective population sizes and will be less affected by genetic drift (Hill, 1972; Kimura, 1983). The sex ratio was similar in all populations of the two species, but it is known that there are substantial differences between *L. mariae* and *L. obtusata* in the generation interval (Fretter & Graham, 1980). A similar trend apparently occurs on Galician shores, with *L. mariae* mostly being annual and *L. obtusata* perennial (two to a few years) (Rolán-Alvarez, 1992). If

it is assumed that *L. mariae* and *L. obtusata* have exactly the same life history characteristics apart from the generation interval, then because of this fact alone, they would show different genetic structures and heterozygosity levels (Hill, 1972).

The exact generation interval and population density through seasons are not known in *L. mariae* and *L. obtusata* on Galician coasts. Thus, further studies in natural populations, on longevity as well as population densities, are necessary before quantitative estimates of the contribution of these factors to the genetic structure in each species can be obtained.

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