

DIFFERENTIATION IN RADULAR AND EMBRYONIC CHARACTERS, AND FURTHER COMMENTS ON GENE FLOW, BETWEEN TWO SYMPATRIC MORPHS OF *LITTORINA SAXATILIS* (OLIVI)

E. Rolán-Alvarez¹, E. Rolán² & K. Johannesson^{3*}

¹Departamento de Biología Fundamental (Genética),
Universidad de Vigo, 36200 Vigo, Spain

²Canovas del Castillo 22 6-f, 36202 Vigo, Spain

³Tjärnö Marine Biological Laboratory, S-452 96 Strömstad, Sweden

*Corresponding author

ABSTRACT

On exposed rocky shores of Galicia, two morphs of the direct-developing snail *Littorina saxatilis* are found associated with different microhabitats. A ridged and banded (RB) morph is found in upper, less wave-exposed parts of the shore, while a smooth and unbanded (SU) morph is present in low, heavily wave-stressed parts. They overlap in a mid-shore zone where hybrids are found, usually at low frequencies (<20%).

We compared shells of embryos from upper-shore RB females with those from low-shore SU females. We also compared embryonic shells from mid-shore RB and SU females, at two sites. The size of the nuclear whorl, the aperture width, the shell diameter and the number of whorls differed between embryos of the two morphs both in allopatry and in sympatry. Populations of the same morph from different microhabitats differed only in 1-2 of the characters. The number of cusps on the outer marginal tooth of the radula of adult females differed between both allopatric and sympatric populations of the two morphs.

We used allozyme data from Johannesson et al. (1993) to estimate gene flow (Nm) between morphs on a microgeographical scale (< 50 m). There was less gene flow between morphs than within morphs at similar distances. Gene flow between morphs was, however, large enough to prevent stochastic differentiation over tens of metres ($Nm > 4$). The results strongly support the suggestion that the two morphs are conspecific, although steep gradients of selection over micro-environmental transitions promote a number of polymorphic traits.

Keywords: hybridisation, polymorphic populations, microscale differentiation, allozymes.

INTRODUCTION

Littorina saxatilis (Olivi) has a low dispersal capability because of its non-pelagic development. As a result of low dispersal and of an extremely variable habi-

tat, the rocky intertidal, this species is highly polymorphic. A number of morphological traits differ between geographically close populations of this species, for example, adult size, shell shape, shell colour, number of penial glands (Janson 1982, Behrens Yamada 1987, Byers 1990, Johannesson & Johannesson 1990, Sergievsky 1992), as well as physiological and behavioural traits (Janson 1983, Sundell 1985). Typically, phenotypic changes in *L. saxatilis* accompany gradients of wave-exposure (Janson 1982 and 1983, Raffaelli 1982, Faller-Fritsch & Emson 1985, Cannon & Hughes 1992). One extreme case is found in Galicia, NW Spain, where two distinct morphs occupy different tidal levels on the same shores (Johannesson et al. 1993). The ridged and banded (RB) morph is found in the less wave-exposed upper parts of the shore, mainly on rocks and barnacles. The smooth and unbanded (SU) morph inhabits the heavily wave-exposed low-shore zone among blue mussels. However, both pure morphs and morphologically intermediate snails are mixed in the mid-shore zone of overlap, which may be only a few metres wide.

Johannesson et al. (1993) found that size and shell traits differed between RB and SU morphs, despite there being no consistent differences in allozyme distributions between them. They concluded that the two pure morphs were conspecific and suggested strong selection gradients (*sensu* Endler 1977), as the main reason for the polymorphism. Later studies supported this conclusion and added the information of an incomplete reproductive barrier between the two morphs (Johannesson et al. 1995a, Rolán-Alvarez et al. 1995).

In the present study, we report on differences in embryonic shell and radular traits between these RB and SU morphs in Galicia. Differences in embryonic shell sizes (or in shell sizes of hatching juveniles) between sheltered shore and exposed shore populations of *L. saxatilis* have previously been found (Raffaelli 1976, Roberts & Hughes 1980, Hughes 1980, Janson 1982). It seems as though large embryonic sizes are associated with wave-exposed areas. This is, however, non-intuitive, since juvenile and adult snails of sheltered areas grow faster and attain a much larger size than those of exposed areas (Janson 1982, Boulding 1990, Boulding & Van Alstyne 1993).

Differences between littorinid species in radular morphology have frequently been described (e.g. Bandel 1974, Rosewater 1979, Reid 1986, Boulding et al. 1993). A few studies have also described intraspecific differences among adults as well as ontogenetic differences (Reid *in press*, and references in there). In this study, we describe differences in cusp numbers between the two morphs of Galician *L. saxatilis*.

Allozyme variation indicates that the RB and the SU morphs are genetically very similar. Despite very different morphologies, there is no correlation between morphological distance and genetic distance among populations on a geographical scale (tens of kilometres) (Johannesson et al. 1993). This supports a conspecific status of the two morphs. In the present study, we have estimated average migration rates (Nm) on a microgeographical scale between

and within morphs. We used the allozyme data of Johannesson et al. (1993). The main conclusions from our earlier studies are strongly supported, and we now have complementary details of the gene flow.

We thank Manuel Domínguez of the Asociación Metalúrgica del Noroeste (AIMEN) for the SEM photographs, C. García, D. G. Reid and two anonymous referees for comments which improved the manuscript. This work was supported by the grant XUGA 20008B94 to E. R.-A. from Xunta de Galicia, and by grants from the Swedish Natural Research Council to K. J.

MATERIALS AND METHODS

Sampling and design

We sampled snails for analysing radular and embryonic shell characters in August 1994 from two localities (Silleiro and Portocelo, Galicia, NW Spain) a few kilometres apart. Additional samples of upper RB and low SU snails from Silleiro were obtained in April 1995. Both sites are exposed rocky shores with a maximum tidal amplitude of 3.5 m. We examined embryonic shells and radulae from five fertile females of each of the following groups: RB morph from upper shore, RB from mid shore, SU from mid shore, and SU from low shore. In upper and low-shore populations, we used females of the largest size in each morph. Females of approximately similar sizes were used in mid-shore sites. Thus in the sympatric populations we compared embryonic and radular traits between similarly sized females, while in comparing allopatric groups of different morphs we did not remove the existing size difference.

Adult females of *L. saxatilis* carry a varying number of embryos at different developmental stages, the total number of embryos being positively related to female size (Janson 1983). We analysed shell traits in the five most developed embryos of each female. In addition, we compared radular morphology between females of both morphs from the two mid-shore sites, and between upper RB females and low SU females from one of the sites (samples from Silleiro in April 95).

Embryo shell and adult radula

We obtained embryos by dissecting females immediately after sampling. The embryos were treated with a solution of sodium hypochlorite for 10 min to remove the periostracum and the soft parts. Thereafter, the embryos were cleaned in fresh water, air dried and attached to a plastic surface so that they could be examined in an apical view. We measured four different embryo shell dimensions with microscope equipped with an ocular scale (Fig. 1). Nucleus diameter (Figs. 1, 2a and 2b), aperture width (as seen in apical view), and shell diameter were measured with a precision of 0.007 mm. Number of whorls (following Verduin 1976) were measured with an error of 1/12 of a complete rev-

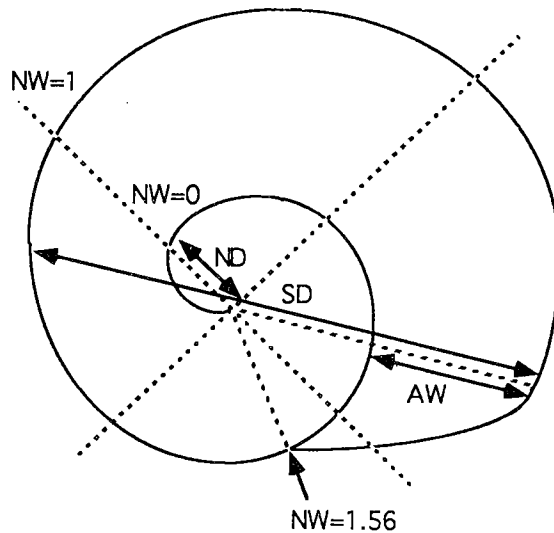


Fig. 1. Schematic drawing of an embryo shell seen in apical view showing measurements taken. NW, number of whorls, ND nuclear diameter, SD, shell diameter, and AW, aperture width.

olution. Some embryos were metal coated and photographed with scanning electron microscope (SEM).

We prepared the dissected radulae with NaOH (5%) to remove soft tissue, rinsed in fresh water and air dried before mounting for SEM. We counted the number of cusps of the outer marginal tooth from the SEM pictures at five different positions for each radula. The average of these was used as the individual count.

Estimation of gene flow

We used Wright's indirect estimate of gene flow based on genetic differentiation among local "island" populations, $Nm = 1/4(1/F_{ST} - 1)$. N is the average local population size, m is the average rate of immigration, and F_{ST} is a measure of genetic differentiation among local populations (Wright 1978). We used allozyme frequencies from Johannesson et al. (1993). This data set included pairs of parallel shore transects from each of three distant sites (tens of kilometres apart). Each transect included one upper shore (only the RB morph), one mid shore (RB, SU and hybrids) and one low shore sample (mainly the SU morph). In each sample an average of 48 individuals had been analysed for five polymorphic enzyme loci (see further descriptions in Johannesson et al. 1993). We calculated the average values of microscale genetic differentiation between morphs, and within morphs between habitats. From the F_{ST} values we obtained estimates of gene flow (Nm) between different subpopulations on a scale of tens of metres.

Table 1. Averages and standard deviations (in parenthesis) of embryo shell characters of ridged and banded (RB) and smooth and unbanded (SU) morphs of Galician *L. saxatilis*. Five embryos from each of five females per site, and two sites per sample were analysed. *N* is the total number of embryos studied. ANOVA_m is the *F* estimate of the morph factor in a three-way ANOVA (morph, population and female) and ANOVA_h is the *F* estimate of the habitat factor in a three-way ANOVA (habitat, population and female).

Morph	Nucleus diameter (mm)			Aperture width (mm)			Number of whorls			Shell diameter (mm)		
	Upper Low	Mid Mid	ANOVA _h	Upper Low	Mid Mid	ANOVA _h	Upper Low	Mid Mid	ANOVA _h	Upper Low	Mid Mid	ANOVA _h
RB	0.144 (0.011)	0.136 (0.010)	11.2**	0.127 (0.019)	0.151 (0.019)	27.2***	1.33 (0.106)	1.32 (0.073)	0.3 ns	0.567 (0.036)	0.552 (0.025)	1.3 ns
SU	0.125 (0.009)	0.126 (0.008)	0.2 ns	0.165 (0.021)	1.82 (0.021)	10.2**	1.43 (0.096)	1.42 (0.114)	0.3 ns	0.654 (0.044)	0.650 (0.048)	0.2 ns
<i>N</i>	50	50		50	50		50	50		80 ¹	100	
ANOVA _m	83.5***	20.2***		71.8***	44.5***		8.8**	10.3**		22.2***	64.2*** ²	

** $P < 0.01$, *** $P < 0.001$

1) The embryos of one low-shore female were lost before shell diameter was taken. To balance the test we randomly deleted one of the upper-shore females. 2) Cochran's test revealed heterogeneous variances but these disappeared after log-transformation. The *F*-value shown is for the transformed values.

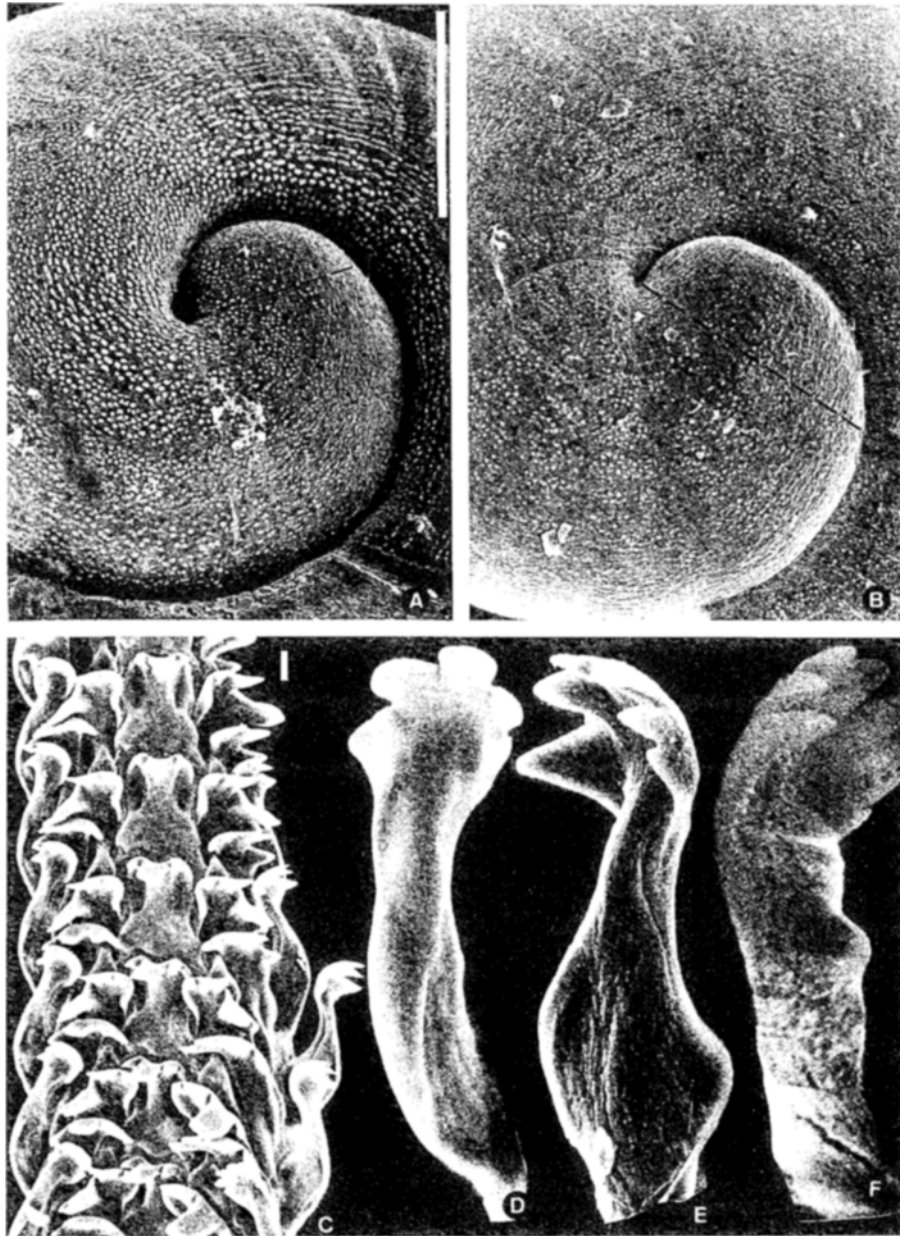


Fig. 2. Nucleus diameter (broken line) in an embryo shell from a low-shore smooth and unbanded (SU) female (A), and from an upper-shore ridged and banded (RB) female (B). White bar is 0.1 mm. Radula from an SU mid-shore *L. saxatilis* female (C). White bar is 0.1 mm. Outer marginal teeth from different radulae; upper-shore RB morph (D), mid-shore SU morph (E), and low-shore SU morph (F).

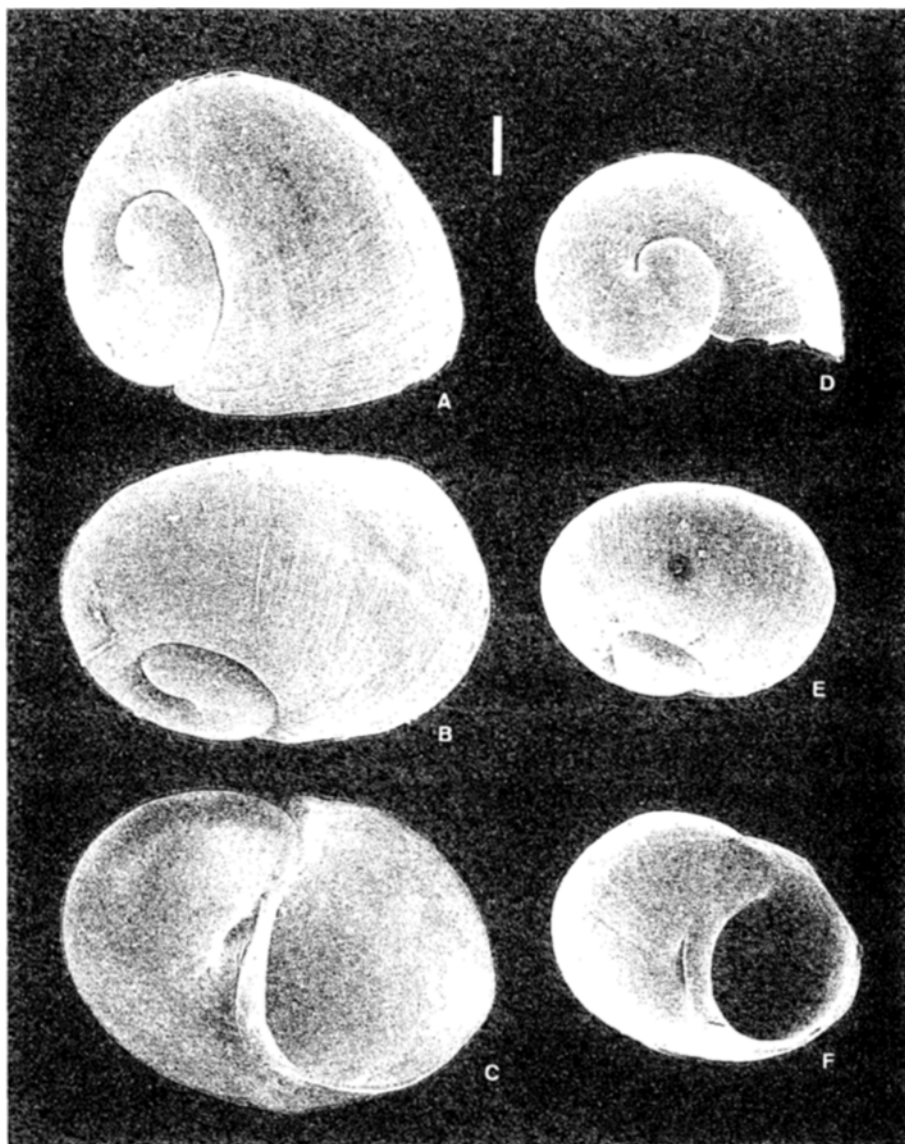


Fig. 3. Embryo shells from Galician *L. saxatilis*. A-C are from low-shore females of smooth and unbanded (SU) morph, and D-F are from upper-shore females of ridged and banded (RB) morph. White bar is 0.1 mm.

RESULTS

Embryonic shell and adult radula

The mean size of females varied between populations; RB females from upper shore being 11.5 mm in shell length (standard deviation:1.84), SU females

from low shore being 6.28 (0.77), RB females from mid shore being 6.75 (0.69), and SU females from mid shore being 5.98 (0.62). We compared group averages of the four embryo characters (nucleus diameter, width of aperture, number of whorls and shell diameter) with three-way analysis of variance (ANOVA). The factors were morph (fixed), population (random), and female (nested within interaction), or habitat (shore level, fixed), population (random) and female (nested within interaction). All traits except one (see Table 1) showed homogeneous variances among groups ($P > 0.05$, Cochran's test). The female factor was usually significant, while the population factor was significant in only once case (for shell diameter, $P < 0.05$), and the interaction factor was never significant ($P > 0.10$). The morph or habitat factors were tested against the interaction plus the female nested factor.

There were significant differences in all four embryo characters between both RB morph of upper shore and SU morph of low shore, and between RB and SU morph of mid shore (Table 1). Thus, the nucleus diameter was larger in the RB morph than in the SU morph, both in allopatry and in sympatry. The aperture width, the shell diameter, and the number of whorls, were all smaller in the RB morph than in the SU morph, both in allopatric and sympatric populations (Table 1, Fig. 3). We also found three out of eight within morph comparisons to be significant. Thus nucleus diameter and width of aperture differed between RB from upper and mid shore, while the latter trait also differed between SU from low and mid shore (Table 1).

The radulae were similar in both morphs, except for the number of cusps on the outer marginal tooth (Fig. 2 C-F). We compared counts of cusp number with a three-way ANOVA (factors morph, population, and snail nested within the interaction) when comparing mid-shore populations, and a two-way ANOVA (factors morph and snail) when comparing the upper- and low-shore

Table 2. Average number of radular cusps of outer marginal teeth (standard deviation in parenthesis) of ridged and banded (RB) and smooth and unbanded (SU) females of Galician *L. saxatilis* from two sites. Five teeth of each individual and five individuals of each sample were analysed. *N* is the number of teeth analysed. Mid shore samples were studied in two localities, but upper and lower shore samples in one.

Morph	Upper Low	Mid Mid	ANOVA _h
RB	6.04 (0.35)	6.60 (0.73)	1.9 ns
SU	7.08 (0.28)	7.22 (0.68)	3.9 ns
<i>N</i>	50	100	
ANOVA _m	67.6***	5.2*	

* $P < 0.05$, *** $P < 0.001$

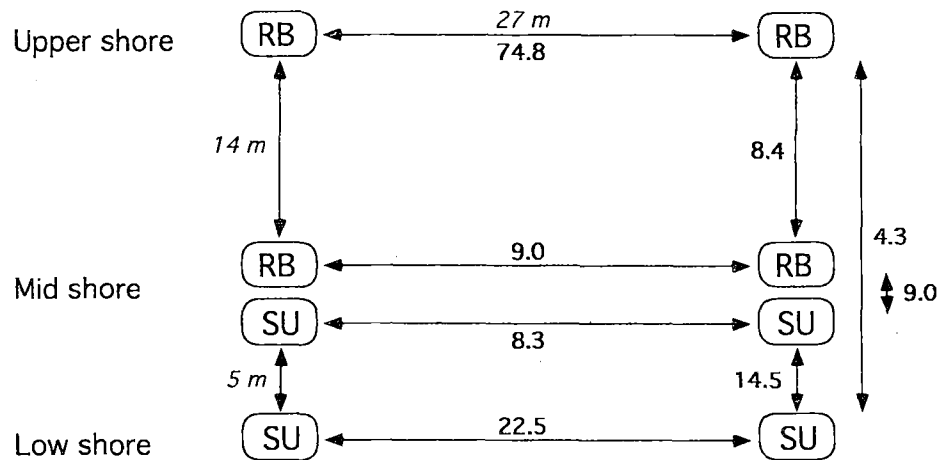


Fig. 4. Nm estimates of gene flow among populations of the two distinct Galician rocky shore morphs of *L. saxatilis*. RB, the ridged and banded morph and SU, the smooth and unbanded morph. The allozyme data on which these estimates are based were from three pairs of parallel transects from localities 25 - 77 km apart (see Johannesson et al. 1993). Each estimate is an average of 3-6 individual Nm estimates, and based on allozyme variation in five highly polymorphic loci. The average distances between populations are indicated.

populations. Furthermore, within morph differences were compared with a two factor ANOVA using habitat and snail (nested within shore level) as factors. The snail factor was usually significant, but the interaction was not. The morph factor was significant in both sympatric and allopatric populations (Table 2). That is, the number of cusps in the SU morph was higher than in the RB morph, both in sympatric and allopatric populations (Table 2). There were no significant differences in the number of cusps between mid-shore sites (Table 2).

Estimation of gene flow

The distribution of alleles has been presented elsewhere (Johannesson et al. 1993). In the present study we have, however, extended the analyses of the genetic data to include detailed estimates of gene flow (Nm) between morphs and within morphs between habitats. On a scale of tens of metres the gene flow within and between morphs varies a lot. The gene flow between upper shore RB and low shore SU individuals is, for example, considerably less than between populations of the same morph and habitat at similar distances (Fig. 4). Comparing the F_{ST} estimates reveals these differences to be significant (Table 3). Thus there is a partial barrier to gene flow between the two morphs in the mid-shore zone of overlap. On the other hand, the gene flow between sympatric populations of the two morphs tends to be larger than between allopatric populations (Fig. 4). The tendency is only significant at the $\alpha=0.1$ level

Table 3. Average F_{ST} , estimating genetic differentiation (standard deviations in parenthesis), between pairs of populations of Galician *L. saxatilis*. In "RB upper-SU low", F_{ST} values between upper shore ridged and banded (RB) and low shore smooth and unbanded (SU) populations of the same transect are averaged over six transects. In "RB mid-SU mid" F_{ST} values between sympatric mid shore populations of the two morphs are averaged over the six mid shore sites. In "Within RB/SU" F_{ST} between populations of the same morph (RB or SU) at distances of 13 - 45 m are averaged over three RB and three SU pairs. (Allozyme data from Johannesson et al. 1993). Group means are compared with t-tests.

Loci	RB upper-SU low	RB mid-SU mid	Within RB/SU
<i>Pgi</i>	0.052	0.018	0.007
<i>s</i>	(0.020)	(0.006)	(0.004)
<i>N</i>	6	6	6
<i>Mpi</i>	0.021	0.023	0.015
<i>s</i>	(0.013)	(0.012)	(0.008)
<i>N</i>	4	3	3
<i>Aat</i> ⁻¹	0.079	0.056	0.007
<i>s</i>	(0.031)	(0.020)	(0.005)
<i>N</i>	6	6	6
<i>Pgm</i> ⁻¹	0.067	0.017	0.020
<i>s</i>	(0.023)	(0.007)	(0.006)
<i>N</i>	6	6	5
<i>Pnp</i>	0.031	0.015	0.004
<i>s</i>	(0.016)	(0.007)	(0.003)
<i>N</i>	6	6	6
Mean	0.0500	0.0258	0.0106
<i>s</i>	(0.0242)	(0.0171)	(0.0067)
<i>N</i>	5	5	5
$\begin{array}{ccc} <-----> & & <-----> \\ t=1.82, df=8, P\approx 0.1 & & t=1.85, df=8, P\approx 0.1 \\ <-----> & & <-----> \\ & t=3.50, df=8, P<0.01 \end{array}$			

(Table 3), but may be true as the power of the test is low. If it is true, this is direct evidence for gene flow, albeit an impeded one, between the two contrasting morphs in the mid-shore zone.

DISCUSSION

Smooth and unbanded (SU) females from low, more heavily wave-exposed parts of shores, carried embryos with higher number of whorls, with larger shell diameter, with smaller nucleus, and wider apertures than did ridged and banded (RB) females from upper, less wave-exposed parts of shores. The same differences were also present between the two morphs from sympatric mid-shore sites.

Embryonic characters, except size, have rarely been measured before. Smith (1981) as well as Grahame & Mill (1993), however, found variation in shell characters among embryos from different British sites and concluded that this reflected inherited differences in shell characters. Sizes of hatching embryos of *L. saxatilis* are generally greater in exposed as compared to protected shores (Raffaelli 1976, Roberts & Hughes 1980, Janson 1982), and may differ even between contrasting habitats (cliffs and boulders) of the same locality (Hart & Begon 1982). Our results show that embryonic characters may even differ between upper and lower parts of the same piece of shore.

The difference in shell diameter, reflecting differences in embryo size, between the two morphs may be genetic or non-genetic (e.g. maternal) in origin. A maternal influence would, however, probably have resulted in size differences in the opposite direction. This is because in Galicia female *L. saxatilis* of the low shore are much smaller (Johannesson et al. 1993) and grow at a much slower rate than females of the upper shore (Johannesson, Rolán-Alvarez & Erlandsson, unpubl.). The contrasting sizes between embryos and adults of the same morph may obviously be adaptive, and the physiological explanation to it may be simple enough. Reid (in press) reports "giant embryos" from northerly populations of *L. saxatilis* where embryos may reach sizes of more than 1 mm before they leave the mother's pouch. It thus seems a possibility that the larger size of the low shore embryos is due to them being retained in the embryo pocket for a while after hatching rather than due to an increased growth during the early development.

The embryo size differences between the morphs, as well as the differences in the other three embryonic characters, persisted in sympatric mid-shore populations (Table 1). This suggests that the differences are mainly genetic, and thus it seems probable that they are caused by differential selection. Why small embryos are favoured in upper shore ("wave-protected") areas and large embryos in low shore ("wave-exposed") areas is, however, unclear to us. The large width of the aperture of the low shore embryos may promote a firm attachment as does a wide aperture among adult snails of wave-exposed sites (Johannesson 1986, Crothers 1992, McMahon 1992). The narrow aperture of the upper shore embryos may, as in adults, reduce desiccation stress (Atkinson & Newbury 1984), and perhaps restrict the possibility for crabs to peel off the shell (Johannesson 1986).

According to Bandel (1974) *L. saxatilis* has five cusps of the outer marginal tooth. Raffaelli (1979) reports that adult *L. saxatilis* have 5-6 cusps of this tooth while juveniles have 7-8. The latter result suggests the presence of a size factor, because the small barnacle ecotype earlier referred to as *L. neglecta* had the same number of cusps (7-8) as the juvenile *L. saxatilis*. In our study the upper shore RB morph at Silheiro usually had six cusps while the low shore SU morph usually had seven. The difference between the different published records (including ours) suggests intraspecific variation also in this character.

Possibly, part of the difference between upper shore RB morph and low shore SU morph may be due to adult size differences of the two groups. However, the difference between the morphs in the mid shore sites (Table 2) could not have been due solely to size differences as in this site females of similar sizes were compared.

It has traditionally been assumed that the radula is a useful taxonomic and systematic character of gastropods (Bandel 1974, Rosewater 1979), however, Raffaelli (1979) and Reid & Golikov (1991) concluded that radular characters were not taxonomically useful in closely related littorinid species. Intraspecific differences whether genetic or not obviously threatens the taxonomic interpretation. For example, a difference in cusp numbers between two closely related Pacific species, the sheltered shore *L. sitkana* and the exposed shore *L. sp.* (the former having 7-8 cusps and the latter 9-10; Boulding et al. 1993), may equally well be due to local adaptations as to stochastic differences between separate evolutionary lineages.

This study adds embryonic and radular characters to the list of known shell characters that differ between the two Galician morphs of *L. saxatilis* (Johannesson et al. 1993), and if these differences are mainly genetic rather than non-genetic, as argued above, the differences must be maintained by differential selection as the gene flow between the morphs seems to be large enough to balance the effect of random genetic drift ($Nm \geq 4$).

A basic assumption underlying the estimates of F_{ST} and Nm is that the genetic variation in e.g. allozyme loci is neutral, or nearly so (although Slatkin & Barton 1989, have pointed out that the F_{ST} estimates are fairly robust despite moderate levels of selection). At least one of our studied loci (*Aat*, see Johannesson et al. 1993) is strongly selected elsewhere (Johannesson et al. 1995b). Any selected differences in the Galician sites would tend to increase F_{ST} and decrease Nm . Thus our current Nm estimates are, if anything, too low.

Possibly, the polymorphic characters are genetically more or less fixed in upper and low-shore zones. The mid shore, on the other hand, is a microgeographic hybrid zone with a mixture of pure morphs (RB and SU) and hybrids. In this zone there is presumably no differential selection. Gene flow between the two morphs, which tends to reduce genetic differences, is instead opposed by partial assortative mating and migration of pure morphs into the mid-shore zone. Furthermore, the mid-shore zone may not be truly homogeneous but rather a mosaic of different patches of microhabitats each favouring one of the morphs. However, the scale of patchiness in the mid shore is fine grained rather than coarse grained (sensu Levins 1968), as the action radii of most snails over a few weeks are larger or much larger than the scale of habitat patchiness (Erlandsson, Johannesson & Rolán-Alvarez unpubl.). Thus differential selection in a patchy mid-shore environment may not be so important to the polymorphism unless snails of different morphs actively choose different microhabitats.

The differences between the SU and RB morphs in embryonic and adult characters, and the partial reproductive barrier between the two, may produce obvious doubts about their conspecific status. However, as we have shown earlier, and as is supported by the estimates of Nm of this study, there is gene flow between the two forms. This gene flow produces what seems to be viable hybrids in the mid-shore areas, and tends to decrease the character differences between pure morphs in the mid shore (Johannesson et al. 1993, this study). Furthermore, the local gene flow between the different morphs is larger than the gene flow within morphs on a geographic scale, which shows that the morphs are truly conspecific (Johannesson et al. 1993). Following the biological species concept, these two morphs are clearly conspecific with their present level of gene flow. Moreover, the same conclusion would be achieved applying two alternative species concepts, the recognition species concept and the cohesion species concept (Lambert & Paterson 1983, Templeton 1989). The pure morphs maintain only partially distinct recognition mating systems (Johannesson et al. 1995a, Rolán-Alvarez et al. 1995). Under the cohesion species concept they would likewise be considered conspecific, as the phenotypic, and, in particular, the genetic cohesion of RB and SU morphs, are not independent of each other.

REFERENCES

- Atkinson, W. D. & S. F. Newbury, 1984. The adaptations of the rough winkle, *Littorina rudis*, to desiccation and to dislodgement by wind and waves. - *J. Anim. Ecol.* **53**: 93-105.
- Bandel, K., 1974. Studies on Littorinidae from the Atlantic. - *Veliger* **17**: 92-114.
- Behrens Yamada, S., 1987. Geographic variation in the growth rates of *Littorina littorea* and *L. saxatilis*. - *Mar. Biol.* **96**: 529-534.
- Boulding, E. G., 1990. Are the opposing selection pressures on exposed and protected shores sufficient to maintain genetic differentiation between gastropod populations with high intermigration rates? - *Hydrobiologia* **193**: 41-52.
- Boulding, E. G. & K. L. Van Alstyne, 1993. Mechanisms of differential survival and growth of two species of *Littorina* on wave-exposed and on protected shores. - *J. Exp. Mar. Biol. Ecol.* **169**: 139-166.
- Boulding, E. G., J. Buckland-Nicks & K. L. Van Alstyne, 1993. Morphological and allozyme variation in *Littorina sitkana* and related *Littorina* species from the Northeastern Pacific. - *Veliger* **36**: 43-68.
- Byers, B. A., 1990. Shell colour polymorphism associated with substrate colour in the intertidal snail *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae). - *Biol. J. Linn. Soc.* **40**: 3-10.
- Cannon, J. P. & R. N. Hughes, 1992. Resistance to environmental stressors in *Littorina saxatilis* (Olivi) and *L. neglecta* Bean. - In J. Grahame, P. J. Mill & D. G. Reid (eds): *Proc. 3rd Int. Symp. Littorinid Biol.*, pp 61-68. Malacological Society of London.
- Crothers, J. H., 1992. Shell size and shape variation in *Littorina littorea* (L.) from West Somerset. - In J. Grahame, P. J. Mill & D. G. Reid (eds): *Proc. 3rd Int. Symp. Littorinid Biol.*, pp 91-98. Malacological Society of London.
- Ender, J. A., 1977. Geographic variation, speciation, and clines. - Princeton University Press, Princeton, N. J. 336 pp.
- Faller-Fritsch, R. J. & R. H. Emson, 1985. Causes and patterns of mortality in *Littorina rudis*

- (Maton) in relation to intraspecific variation: a review. - In P. G. Moore & R. Seed (eds): The ecology of rocky coasts, pp. 157-177. St. Edmundsbury Press, Edmunds UK.
- Grahame, J. & P. J. Mill, 1993. Shell shape variation in rough periwinkles: Genotypic and phenotypic effects. - In J. C. Aldrich (ed.): Quantified Phenotypic Responses in Morphology and Physiology, pp. 25-30. JAPAGA, Ashford UK.
- Hart, A. & M. Begon, 1982. The status of general reproductive-strategy theories, illustrated in winkles. - *Oecologia (Berl.)* **52**: 37-42.
- Hughes, R. N., 1980. Population dynamics, growth and reproductive rates of *Littorina nigrolineata* Gray from a moderately sheltered locality in North Wales. - *J. Exp. Mar. Biol. Ecol.* **44**: 211-228.
- Janson, K., 1982. Genetic and environmental effects on the growth rate of *Littorina saxatilis*. - *Mar. Biol.* **69**: 73-78.
- Janson, K., 1983. Selection and migration in two distinct phenotypes of *Littorina saxatilis* in Sweden. - *Oecologia (Berlin)* **59**: 58-61.
- Johannesson, B., 1986. Shell morphology of *Littorina saxatilis* Olivi: the relative importance of physical factors and predation. - *J. Exp. Mar. Biol. Ecol.* **102**: 183-195.
- Johannesson, B. & K. Johannesson, 1990. *Littorina neglecta* Bean, a morphological form within the variable species *Littorina saxatilis* (Olivi)? - *Hydrobiologia* **193**: 71-87.
- Johannesson, K., B. Johannesson & U. Lundgren, 1995b. Strong natural selection causes micro-scale allozyme variation in a marine snail. - *Proc. Natl. Acad. Sci. USA* **92**: 2602-2606.
- Johannesson, K., B. Johannesson & E. Rolán-Alvarez, 1993. Morphological differentiation and genetic cohesiveness over a micro-environmental gradient in the marine snail *Littorina saxatilis*. - *Evolution* **47**: 1770-1787.
- Johannesson, K., E. Rolán-Alvarez & A. Ekendahl, 1995a. Incipient reproductive isolation between two sympatric morphs of the intertidal snail *Littorina saxatilis*. - *Evolution* **49**: 1180-1190.
- Lambert, D. M. & H. E. H. Paterson, 1983. On 'Bridging the gap between race and species': the isolation concept and an alternative. - *Proc. Linn. Soc. N. S. W.* **107**: 501-514.
- Levins, R., 1968. Evolution in changing environments. - Princeton University Press, Princeton, N. J. 120 pp.
- McMahon, R. F., 1992. Microgeographic variation in the shell morphometrics of *Nodilittorina unifasciata* from Southwestern Australia in relation to wave exposure of shore. - In J. Grahame, P. J. Mill & D. G. Reid (eds): Proc. 3rd Int. Symp. Littorinid Biol., pp 107-118. Malacological Society of London.
- Raffaelli, D. G., 1976. The determination of zonation patterns of *Littorina neritoides* and the *Littorina saxatilis* species-complex. - Ph.D. thesis, University of Wales.
- Raffaelli, D. G., 1979. The taxonomy of the *Littorina saxatilis* species-complex, with particular reference to the systematic status of *Littorina patula* Jeffrys. - *Zool. J. Linn. Soc.* **65**: 219-232.
- Raffaelli, D. G., 1982. Recent ecological research on some European species of *Littorina*. - *J. Moll. Stud.* **48**: 342-354.
- Reid, D. G., 1986. The littorinid molluscs of mangrove forests in the Indo-Pacific region. - British Museum (Natural History), London. 228 pp.
- Reid, D. G., 1996. Systematics and evolution of *Littorina*. - Ray Society, London (in press).
- Reid, D. G. & A. N. Golikov, 1991. *Littorina naticoides*, new species, with notes on the other smooth-shelled *Littorina* species from the northwestern Pacific. - *Nautilus* **105**: 7-15.
- Roberts, D. J. & R. N. Hughes, 1980. Growth and reproductive rates of *Littorina rudis* from three contrasted shores in North Wales, UK. - *Mar. Biol.* **58**: 47-54.
- Rolán-Alvarez, E., K. Johannesson & A. Ekendahl, 1995. Frequency- and density-dependent sexual selection in natural populations of Galician *Littorina saxatilis* (Olivi). - *Hydrobiologia* **309**: 167-172.
- Rosewater, J., 1979. A close look at *Littorina radulae*. - The Bulletin of the Malacological Union. Inc.: 5-8.

- Sergievsy, S. O., 1992. A review of ecophysiological studies of the colour polymorphism of *Littorina obtusata* (L.) and *L. saxatilis* (Olivi) in the White sea. - In J. Grahame, P. J. Mill & D. G. Reid (eds): Proc. 3rd Int. Symp. Littorinid Biol., pp 235-246. Malacological Society of London.
- Slatkin, M. & N. H. Barton, 1989. A comparison of three indirect methods for estimating average levels of gene flow. - *Evolution* 43: 1349-1368.
- Smith, J. E., 1981. The natural history and taxonomy of shell variation in the periwinkles *Littorina saxatilis* and *Littorina rudis*. - *J. mar. biol. Ass. U. K.* 61: 215-241.
- Sundell, K., 1985. Adaptability of two phenotypes of *Littorina saxatilis* (Olivi) to different salinities. - *J. Exp. Mar. Biol. Ecol.* 92: 115-123.
- Templeton, A. R., 1989. The meaning of species and speciation: a genetic perspective. - In D. Otte & J. A. Endler (eds): *Speciation and its consequences*, pp. 3-27. Sinauer, MA.
- Verduin, A., 1976. On the systematics of recent *Rissoa* of the subgenus *Turboelle* Gray, 1847, from the Mediterranean and European Atlantic coasts. - *Basteria* 40: 21-73.
- Wright, S., 1978. *Evolution and the genetics of populations*, vol 4: Variability within and among natural populations. - University of Chicago press, Chicago. 580 pp.