

Short Research Note

## Habitat-dependent ecotype micro-distribution at the mid-shore in natural populations of *Littorina saxatilis*

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### Abstract

On the NW coast of Spain, there is a hybrid zone in natural populations of *L. saxatilis*, where the largest ecotype is adapted to the desiccation and heat stress of the upper shore, whereas the smaller one is adapted to the wave impacts of the lower shore. The two ecotypes meet and hybridise in a mid-shore area producing intermediate individuals or hybrids. It has been postulated that this hybrid zone is maintained by a selection-gradient model that assumes habitat-dependent selection for the different environments of upper and lower shore areas. In this study, we focus on the hybrid (mid-shore) area, where a transition occurs between upper-shore and lower-shore environments, in order to ascertain whether the habitat-dependent selection is maintained at the micro-habitat scale. We present data on snail density at several levels of the mid-shore (varying accordingly in the relative frequency of mussels and barnacles) obtained for three seasons in three consecutive years and three localities. In the mid-shore, the abundance of the RB ecotype increases with increasing tidal height, whereas the abundance of the SU ecotype increases with decreasing tidal height, suggesting that habitat-dependent selection is maintaining the ecotype micro-distribution.

Hybrid zones are produced when two or more populations distinct for some heritable trait, overlap and produce hybrids that are at least partially fertile (Arnold, 1997; but see wider definitions in Endler, 1977; Barton & Hewitt, 1985). These zones represent important scenarios from an evolutionary point of view, as they can be considered natural laboratories to check the adaptive value of new genetic combinations (Hewitt, 1988; Harrison, 1990; Arnold & Hodges, 1995; Nürnberger et al., 1995). Hybrid zones can be maintained by several mechanisms, from the tension zone model, where the hybrid zone is being maintained by the balance between dispersal and selection against hybrids (Barton & Hewitt, 1985), to the selection-gradient model, where parental and hybrid fitness depend basically on their

particular adaptability to different habitats (Moore & Price, 1993). On the rocky shores of the north-western coast of Spain, there is a striking polymorphism of the rough periwinkle *Littorina saxatilis* (Johannesson et al., 1993). Two distinct ecotypes are adapted to different shore levels and habitats: the large-sized ridged and banded ecotype (RB) is found among barnacles on the upper shore, while the small-sized smooth and unbanded ecotype (SU) is found on the lower shore among mussels. These two ecotypes and a variable number of intermediate forms (hybrids, HY) can be found on the mid-shore in true sympatry. The ecotypes have evolved a partial reproductive isolation apparently in sympatry, as a result of natural selection adapting each ecotype to its respective habitat (Rolán-Alvarez et al., 1997,

2004; Cruz et al., 2004a, b). Thus, the SU form is known to be more resistant to the wave impact, but to be less resistant to desiccation or heat stress than the RB ecotype (Rolán-Alvarez et al., 1997; Erlandsson et al., 1998).

It has been postulated that this polymorphism is maintained by a selection-gradient model, where parental and hybrid fitnesses depend on their particular adaptability to different habitats (Moore & Price, 1993). If this is correct, the ecotype distribution should also be present at a micro-habitat scale on the mid-shore, where a transition occurs between upper-shore and lower-shore environments. One previous study (Otero-Schmitt et al., 1997) showed that the micro-distribution of the ecotypes on the mid-shore area was dependent on the micro-habitat (mussel or barnacle patches). However, another study failed to show such a dependency (Kostylev et al., 1997), possibly because it did not consider the vertical shore gradient. In order to clarify whether the ecotype density is associated with the micro-habitat transition occurring on the mid-shore, we monitored the population density of the different ecotypes on the mid-shore at three localities during three seasons in three consecutive years.

Specimens for this study were obtained from three exposed rocky-shore localities of Galicia (NW Spain) 8 km apart: Silleiro, Centinela and Senín (further details of sampling sites can be found in Rolán-Alvarez et al., 1999) sampled during three consecutive years (1999–2001). Five different sampling areas (permanent experimental quadrats of  $32 \times 32$  cm) were studied across the vertical environmental gradient within the mid-shore (a subsection of the overall distribution, where both ecotypes were present at a frequency of at least 5%). Two experimental quadrats were defined at the extremes of this mid-shore area and three extra quadrats were defined equidistant between these two. Thus, levels 1–5 were established on the mid-shore covering a total distance of 4–8 m, where level 1 is the closest to the lower shore and level 5, the closest to the upper shore. All specimens of *L. saxatilis* present within each experimental area were picked. The quadrats were marked directly on the rock by a circular drill, and so we could resample the same areas at different times (every 3–4 months for 3 years). Individuals were sexed at the laboratory and classified

according to the presence/absence of bands and ridges (RB, HY or SU) and to the degree of development (adults or juveniles). The relative abundance of barnacles (*Chthamalus stellatus* and *C. montagui*) and mussels (*Mytilus galloprovincialis*) were estimated, in every experimental quadrat, dividing the area into 64 smaller quadrats of  $4 \times 4$  cm and counting the number of them, completely filled by barnacles or mussels.

We analysed how the snail density is affected by the factors *Locality* (random with three treatments: Silleiro, Centinela and Senín), *shore Level* (random with five treatments: Levels 1–5), *Season* (fixed with three treatments: spring, autumn and winter), and their interactions, by a three-way ANOVA (following Underwood, 1981). The replicates in this study were the data obtained in the three different years, because the polymorphism is rather stable across years and there were no significant differences in ecotype density across years in the same data set (not shown). Snail density can be influenced by migration, growth rate differences or viability. Because there are known differences among the *L. saxatilis* ecotypes (RB, HY and SU) and age classes (juvenile and adult) in migration and growth rate (Erlandsson et al., 1998; Johannesson et al., 1997), we analysed the data separately for each ecotype and each age class (six different analyses). The correlation between the hybrid density and the RB + SU density was estimated by the non-parametric Kendall–Tau correlation coefficient ( $\tau$ ) across samples (Sokal & Rohlf, 1995). The data were analysed using the SPSS/PC package (ver. 12.01).

The frequency of barnacles increases towards upper shore as shown by its correlation with shore level ( $\tau = 0.410$ ,  $df = 134$ ,  $p < 0.001$ ), whereas the frequency of mussels increases towards the lower shore ( $\tau = -0.708$ ,  $df = 134$ ,  $p < 0.001$ ). Thus, the mid-shore shows a continuous transition between upper and lower shore typical habitats. The analysis of snail density on the mid-shore is shown in Table 1 and Figure 1. The selection-gradient model predicts a relationship between ecotype and habitat distribution as well as between ecotype and season. Therefore, to test such a model we should focus on the significance of the factors *Level* and *Season* as well as their interactions with other factors.

Table 1. Analysis of the densities of adult and juvenile specimens of *Littorina saxatilis* by a three-way ANOVA with two fixed and one random factors: locality (random: Centinela, Senín and Silleiro), season (fixed: spring, autumn and winter) and level (fixed: with five vertical levels)

Source of variation	df	RB		HY		SU		Error component for <i>F</i> -test
		Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	
Level (LV)	4	<b>274</b> <sup>**</sup>	443 <sup>†</sup>	3 <sup>ns</sup>	14 <sup>ns</sup>	12,287 <sup>†</sup>	373 <sup>ns</sup>	(S × LC) + (LV × LC) – (S × LV × LC)
Season (S)	2	99 <sup>ns</sup>	<b>615</b> <sup>*</sup>	82 <sup>†</sup>	<b>191</b> <sup>*</sup>	2492 <sup>ns</sup>	832 <sup>†</sup>	S × LC
Locality (LC)	2	31 <sup>ns</sup>	22 <sup>ns</sup>	27 <sup>ns</sup>	24 <sup>ns</sup>	<b>41,135</b> <sup>**</sup>	860 <sup>†</sup>	(S × LC) + (LV × LC) – (S × LV × LC)
S × LV	8	20 <sup>ns</sup>	107 <sup>†</sup>	10 <sup>ns</sup>	6 <sup>ns</sup>	1040 <sup>ns</sup>	79 <sup>ns</sup>	S × LV × LC
S × LC	4	<b>45</b> <sup>*</sup>	48 <sup>ns</sup>	7 <sup>ns</sup>	15 <sup>ns</sup>	236 <sup>ns</sup>	<b>159</b> <sup>*</sup>	S × LV × LC
LC × LV	8	12 <sup>ns</sup>	61 <sup>ns</sup>	9 <sup>ns</sup>	15 <sup>ns</sup>	<b>3289</b> <sup>**</sup>	<b>109</b> <sup>*</sup>	S × LV × LC
S × LC × LV	16	13 <sup>ns</sup>	<b>50</b> <sup>*</sup>	9 <sup>ns</sup>	7 <sup>ns</sup>	607 <sup>ns</sup>	40 <sup>ns</sup>	Error
Error	90	12.8	20.5	9.9	12.3	1191	59	

The table shows the Mean Square (MS) of each factor and interaction. Superscript symbols refer to the probability of the *F*-test for the indicated MS ratio. The degrees of freedom (df) shown refer to each of the cells of the corresponding column. \*\*\**p* < 0.001, \*\**p* < 0.01, \**p* < 0.05, <sup>†</sup>*p* < 0.10, ns is non-significant for ANOVA. Numbers in bold face emphasise significant results.

The RB density increases towards the upper shore (although the result was weaker in juveniles), supporting that this ecotype lives directly associated with barnacles on the mid-shore. In fact, the overall RB density (both adults and juveniles) was positively correlated with barnacle density ( $\tau = 0.151$ ,  $df = 134$ ,  $p = 0.013$ ), but negatively correlated with mussel density ( $\tau = -0.142$ ,  $df = 134$ ,  $p = 0.022$ ). In adults, the interaction *Season by Locality* was also significant, because RB adults showed the highest density in autumn at Centinela and Senín, but in spring at Silleiro. In juveniles, the *three factor interaction* was significant, although in all cases, the highest densities were observed at the highest shore level (the interaction was caused by the lowest density which appeared in a few cases at intermediate shore levels).

The factor *Level* was found to be non-significant for SU density. In line with this, SU density was not correlated to barnacle density ( $\tau = 0.079$ ,  $df = 134$ ,  $p = 0.185$ ) or mussel density ( $\tau = 0.005$ ,  $df = 134$ ,  $p = 0.936$ ). However, there was a trend for SU density to decrease with increasing shore level (Fig. 1). Level 2 showed the highest densities in all cases while levels 5–6 showed the lowest densities. This suggests that SU density is somewhat related to the shore level, but not in a simple way, perhaps affected by the availability of refuges on the rocky shore surface (Kostylev et al., 1997). The factor *Locality*

(Silleiro showing larger density than the others) and the interaction *Locality by Level* were significant in adults, the latter due to differences in the decreasing densities across shore levels between localities. In SU juveniles, the *Locality by Level* interaction was also significant, showing the same trend as in adults. In addition, the interaction *Season by Locality* was also significant, due to the fact that the highest density was observed during autumn at Centinela, but during spring at the other localities. In summary, pure ecotypes are not distributed randomly across the vertical shore gradient on the mid-shore. The RB ecotype increases in frequency towards the upper shore level, where it is better adapted. On the contrary, the SU ecotype increases in frequency towards the lower shore level, though less clearly. The most probable interpretation of this pattern is that the ecotypes (at least the RB) have active preferences for choosing particular micro-habitats, supporting the selection-gradient model as a main contributor to the maintenance of the ecotype distribution on the mid-shore.

In hybrids, the pattern was very simple, for only the factor *Season* in juveniles was significant (but suggestive in adults too; see Table 1), which happened because densities were highest in spring and lowest in winter. This could be related to the best overall reproductive rates due to favourable environmental conditions during spring (Reid, 1996), but it could also be due to natural selection

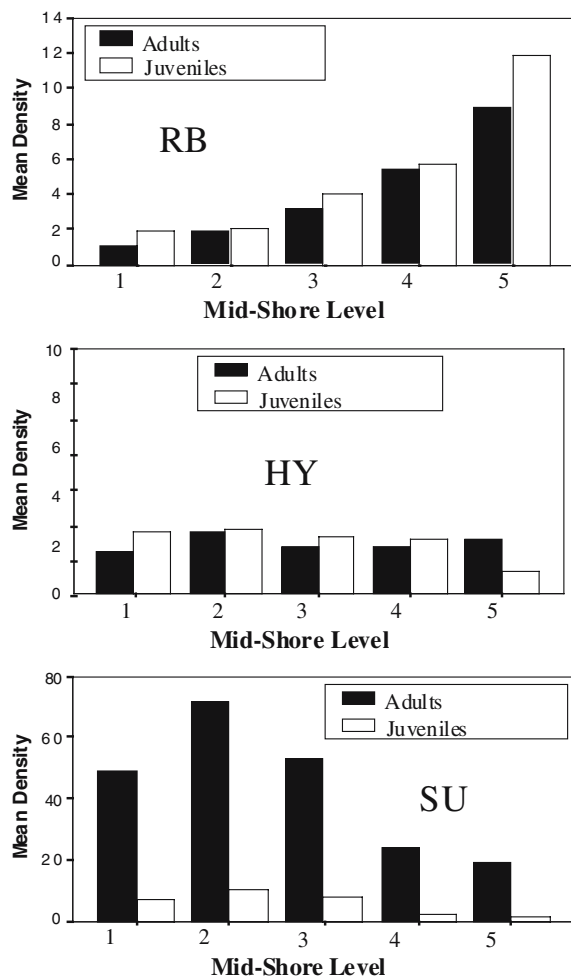


Figure 1. Snail density across the vertical shore gradient on the mid-shore for the three ecotypes (RB, HY and SU) averaged across localities, seasons and years. Level 1 is the closest to the lower shore and level 5 the closest to the upper shore.

affecting the hybrids differentially across seasons. The overall hybrid (adults + juveniles) density was not correlated with either barnacle ( $\tau = -0.006$ ,  $df = 134$ ,  $p = 0.992$ ) or mussel density ( $\tau = -0.013$ ,  $df = 134$ ,  $p = 0.839$ ), showing that hybrid density has no relationship with micro-habitat availability. Thus, either the hybrids have lost the genetic characteristics that allow them to choose their most suitable habitat or there is no such habitat for them. Any of these possibilities suggest that natural selection may affect hybrids in a way different from that with pure ecotypes. Cruz et al. (2001) showed that different traits in hybrids are differently affected by sexual

selection, some showing linear (directional selection), while others showing a quadratic (disruptive selection) fitness function.

Finally, there was a positive correlation between hybrid density and pure ecotype (RB + SU) density ( $\tau = 0.344$ ,  $df = 134$ ,  $p < 0.001$ ), suggesting that the hybrids are not trapped in areas of low density. Thus, hybrids are produced predominantly at higher frequencies of the pure ecotypes, in disagreement with a tension zone model prediction (Barton & Hewitt, 1985). In conclusion, our results suggest that the snail micro-distribution across the mid-shore environmental gradient is caused by a mechanism consistent with the selection-gradient model. Other hybrid zone models, like the mosaic hybrid zone model (it incorporates selection-gradient and tension zone mechanisms simultaneously; see Harrison & Rand, 1989; Arnold, 1997) are not supported, as there is no evidence favouring any postzygotic handicap in wild or laboratory born  $F_1$  hybrids with respect to the pure ecotypes (Rolán-Alvarez et al., 2004).

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