

Habitat choice in the flat periwinkle (*Littorina fabalis*) Iberian ecotypes: an experimental approach

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BACKGROUND

Although it is widely recognized that habitat choice can facilitate local adaptation, its contribution to speciation has been poorly studied.

Littorina species (marine intertidal gastropods) offer unique models to study the mechanisms underlying ecotype differentiation and ecological speciation.

In the flat periwinkle, *L. fabalis*, three ecotypes have been described in the Iberian Peninsula, each associated with different host algae/seagrass: *Fucus* sp. (FA), *Mastocarpus* sp. (MA), and *Zostera marina* (ZA) (Figs. 1, 2 and 3).

Whether this association results from consistent habitat preference/avoidance, represents the outcome of environmental selective pressures, like predation, and/or results simply from stochastic processes, has never been investigated.

In order to understand the contribution of habitat choice in *L. fabalis* ecotype formation, we conducted two series of laboratory experiments, where we measured *L. fabalis* immediate habitat choice within a 30 minutes interval and long-term choice within a six days interval.

Since the results were similar, here we present only those from the long-term choice experiment and discuss their evolutionary significance.



Figure 2. The three ecotypes of *L. fabalis* on their respective host algae. From left to right: ME, FE, ZE.



Figure 3. The three host plants of *L. fabalis* ecotypes in Iberia. From left to right: *M. stellata* (MA), *F. vesiculosus* (FA), *Z. marina* (ZA).

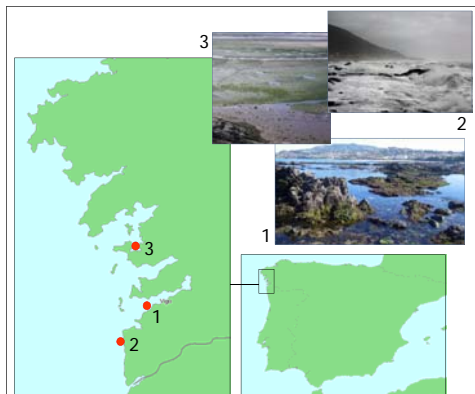


Figure 1. Sampling locations representing the different habitats where each of the three ecotypes of *L. fabalis* can be found. 1. Semi-sheltered habitat dominated by *Fucus vesiculosus* (FA), where the FE ecotype exists; 2. Exposed habitat dominated by *Mastocarpus stellata* (MA) inhabited by the ME ecotype; and 3. Sheltered habitat dominated by *Zostera marina* (ZA) the host algae of the ZE ecotype.

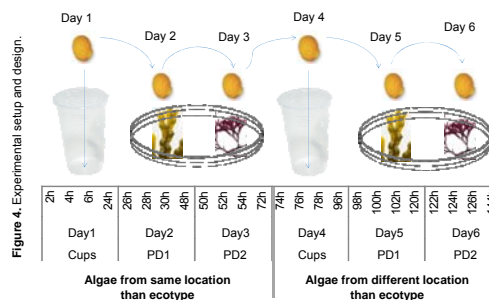


Figure 4. Experimental setup and design.

MATERIALS AND METHODS

SAMPLING

Individuals from the three ecotypes of *L. fabalis* were collected in 2011 and 2012 in six different locations from Galiza, Northwestern Iberia (Fig. 2).

EXPERIMENTS

Experiments were conducted using, approximately, 300 individuals (150 adults + 150 juveniles) of each ecotype.

Each snail was allowed to choose between two hosts for six periods of 24 hours each (6 Days) (Fig. 4). Three different snail/host combinations were used:

- FA vs. ZA with FE & ZE
- FA vs. MA with FE & ME
- MA vs. ZA with ME & ZE

Each experimental treatment differed in the following aspects:

- Snail starting point in the setup: in between host A and host B (No host); host A; host B.
- Host location: same/different beach from that of the snail.

DATA ANALYSIS

The choice of the individuals was recorded at four sampling points within each day (Fig. 4), but only the 24h choice was used in the analysis here presented.

Logistic regression GEE models were applied to the data, using the “exchangeable” correlation structure. $\alpha = 0.001$.

RESULTS

Did the snails preferably choose to settle on their host algae/seagrass?

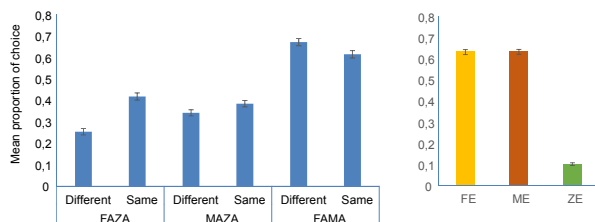


Figure 5: Mean proportion of choice of own host by Host-Pair/Host-Location (p<7.2e-14). FAZA: Different vs Same: p<2e-16. FAZA vs MAZA: p<0.25. FAZA vs FAMA: p<2e-16. MAZA vs FAMA: p<2e-16. With \pm standard error of the mean.

Figure 6: Mean proportion of choice of own host by Ecotype (p<2e-16). FE vs ME: p<0.96. FE vs ZE: p<2e-16. ME vs ZE: p<2e-16. With \pm standard error of the mean.

Which host algae/seagrass did the snails prefer?

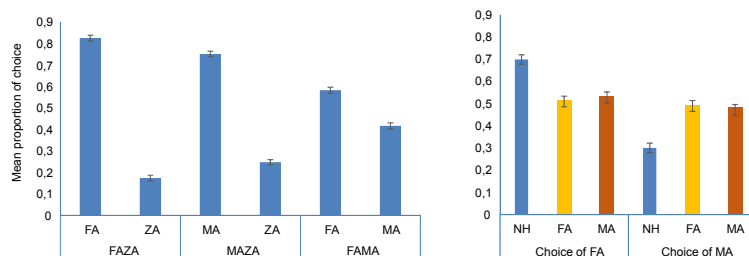


Figure 7: Mean proportion of choice for each host, within Host-Pair. FAZA: FA vs ZA: p<4.6e-14. MAZA: MA vs ZA: p<2e-16. FAMA: FA vs MA: p<2e-16. With \pm standard error of the mean. Within each Host-Pair, other variables were also significant: Ecotype, Host-Location and Snail starting point. Ecotype*Age is also significant in FAMA.

Figure 8: Mean proportion of choice FA and MA in FAMA, by Snail Starting-Point. NH = no-host, FA = starting on FA, MA = starting on MA. NH vs FA: p<1.1e-09. NH vs MA: p<4.1e-08. FA vs MA: p<0.768. With \pm standard error of the mean.

MAIN RESULTS and DISCUSSION

Did the snails preferably choose to settle on their host algae/seagrass?

- Snails from the FE and ME ecotype show a preference for their host algae.
- Snails from the ZE ecotype show avoidance for their host seagrass.
- The proportion of choice in FAMA is therefore higher than in FAZA and MAZA.
- Host-Location has a significant effect in FAZA, with higher proportion of choice when the host comes from the same beach as the snails.

Which host algae/seagrass did the snails prefer?

- Within each Host-Pair, all snails clearly avoid ZA.
- In FAMA there is a preference for FA, which is probably the ancestral host of all three ecotypes.
- Within FAMA there is also a Snail Starting-Point effect: when the snails started prospecting from the middle of the plastic cups (Fig. 4), they chose FA more often. When they started on FA or MA they tended to choose evenly among FA and MA.

Discussion

•65 to 70% of ME and FE chose their own host algae. Habitat choice thus seems to contribute to the genetic isolation of these ecotypes, favouring the process of ecological speciation. However, there is still opportunity for gene flow between ecotypes. The starting-point for habitat prospecting coupled with host geographic distribution and abundance need to be considered when interpreting patterns of gene flow between ecotypes.

•Only 10% of ZE chose their own host seagrass. These snails seem to prefer either FA or MA, but not their own host ZA. The reasons for ZA avoidance by ZE individuals are currently unknown.

•Other factors still need to be evaluated, in order to explain the observed association between ecotypes and their hosts' colour: predation; plasticity; imprinting; assortative mating; social learning.

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