1 **A simulation study of the ecological speciation conditions in** 2 **the Galician marine snail *Littorina saxatilis***

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11

12 Abstract

13 In the last years the interest on evolutionary divergence at small spatial scales has
 14 increased and so did the study of speciation caused by ecologically-based divergent
 15 natural selection. The evolutionary interplay between gene flow and local adaptation
 16 can lead to low-dispersal locally adapted specialists. When this occurs the evolutionary
 17 interplay between gene flow and local adaptation could eventually lead to speciation.

18 The *L. saxatilis* system consists of two ecotypes displaying a microhabitat-associated
 19 intraspecific dimorphism along the wave-exposed rocky shores of Galicia. In spite of
 20 being a well-known system, the dynamics of the ecotype formation remains unclear and
 21 cannot be studied from empirical evidence alone. In this study, individual-based
 22 simulations were used to incorporate relevant ecological, spatial and genetic
 23 information, to check different evolutionary scenarios that could evolve non-random
 24 mating preferences and finally may facilitate speciation.

25 As main results, we observed the evolution of intermediate values of choice which
 26 matches estimates from empirical data of *L. saxatilis* in Galician shores and coincides
 27 with previous theoretical outcomes. Also, the use of the mating correlation as a proxy
 28 for assortative mating led to spuriously inferring greater reproductive isolation in the
 29 middle habitat than in the others, which does not happen when directly considering the
 30 choice values from the simulations. We also corroborate the well-known fact that the
 31 occurrence of speciation is influenced by the strength of selection. Taken together, this
 32 means, also according to other *L. saxatilis* systems, that speciation is not an immediate
 33 consequence of local divergent selection and mating preferences, but a fine tuning

among several factors including the ecological conditions in the shore levels, the selection strength, the mate choice stringency and cost to choosiness. The *L. saxatilis* system could correspond to a case of incomplete reproductive isolation, where choice intensity is intermediate and local adaptation within the habitat is strong. These results support previous interpretations of the *L. saxatilis* model system and indicate that further empirical studies would be interesting to test whether the mate choice mechanism functions as a similarity-like mechanism as has been shown in other littorinids.

42

43 **Introduction**

Speciation caused by ecologically based divergent natural selection can occur at small spatial scales. Microgeographic adaptation occurs when neighbour groups of individuals have adaptively diverged (Richardson et al., 2014) and this adaptation at small spatial scale may happen despite a high potential for mixing within the dispersal area. This idea has produced controversy between theoretical studies that show that natural selection can overcome the effects of gene flow (Dieckmann and Doebeli, 1999; Gavrilets, 2004; Barluenga et al., 2006; Hey, 2006; Bolnick and Fitzpatrick, 2007; Rolán-Alvarez, 2007; Debarre and Gandon, 2011; Weissing et al., 2011; Savolainen et al., 2013; Butlin et al., 2014; Richardson et al., 2014; Getz et al., 2016; Rettelbach et al., 2016; Servedio and Boughman, 2017; Kopp et al., 2018) and those that admit that this is feasible but that the conditions necessary for it to occur make it difficult to observe in the real world.

55 (Jiggins, 2006; Babik et al., 2009; Meyers et al., 2012; Martin, 2013; Savolainen et al.,
56 2013; Martin et al., 2015; Fernández-Meirama et al., 2017a; Foote, 2018).

57 There are various plausible scenarios for the evolutionary interplay between gene flow
58 and local adaptation. They may give rise to a monomorphic population, or to generalists
59 adapted to different habitats, or to polymorphic subpopulations of locally adapted
60 specialists, which, in case of low gene flow, could lead to speciation (Coyne and Orr,
61 2004).

62 However, for local adaptation to occur, a gene flow-reducing mechanism, such as non-
63 random mating due to mate choice, may be necessary in addition to selection.

64 Furthermore, to detect mate choice, it is important to take into account the true spatial
65 scale at which mate choice occurs to avoid a version of the so-called Simpson's paradox
66 in which the mating pattern existing in some data sets will disappear or be reversed
67 when the groups are combined. Thus measuring mate choice at the correct scale is key
68 to understanding this evolutionary process (Rolán-Alvarez et al., 2015b; Estévez et al.,
69 2018).

70 Local adaptation and speciation through non-random mating are expected to occur when
71 the traits involved in divergent selection and mate choice are the same, the so-called
72 magic trait (Gavrilets, 2004; Thibert-Plante and Gavrilets, 2013; Servedio and
73 Boughman, 2017; Richards et al., 2019).

74 Because of the long time scale necessary for local adaptation and mate choice evolution
75 under gene flow, simulation has become an essential part of speciation research. Recent
76 efforts on the study of the evolution of reproductive isolation focused on the complex
77 interactions that emerge in the presence of local adaptation, mate choice and sexual

selection under different genetic structure, migration model, etc. (Sadedin et al., 2009; Thibert-Plante and Hendry, 2009, 2011; Cotto and Servedio, 2017; Sachdeva and Barton, 2017; Perini et al., 2020).

The present work is based on *L. saxatilis* as model organism and used individual-based simulation to identify some relevant parameters, as local selection intensity, hybrid zone environment and demography, that can influence the evolution of local adaptation and non-random mating preferences. The *L. saxatilis* model case relies on the existence of two main ecotypes that have evolved by natural selection to adapt to distinct microhabitats in different geographical regions in parallel (see next section). The two ecotypes have evolved different average sizes, morphologies, behaviour and physiologies, and may potentially show partial reproductive isolation from each other due to different microhabitat and mating preferences (Rolán-Alvarez, 2007; Johannesson et al., 2010; Rolán-Alvarez et al., 2015a). This system is of particular interest in the Galician populations where these ecotypes live at different shore levels, while at the mid shore they meet and partially hybridize producing a hybrid zone that has been suggested as particularly suitable for ecological speciation in presence of gene flow (Rolán-Alvarez, 2007; Butlin et al., 2014; Boulding et al., 2017). Despite the number of empirical studies that have estimated biological and ecological parameters for this model system (reviewed in Pérez-Figueroa et al., 2005; Johannesson et al., 2010; Rolán-Alvarez et al., 2015a), there are several questions that remain unanswered (see below). Here, we perform spatially explicit computer simulations for studying the *L. saxatilis* Galician system incorporating the evolution of mate choice and reproductive isolation and using the parameter information available from empirical studies. Noteworthy, there were previous efforts of modelling another *L. saxatilis* system, the

102 Swedish one (Sadedin et al., 2009; Perini et al., 2020) which has also two ecotypes
 103 although under a different spatial and microhabitat distribution (see next section). Thus,
 104 although building on the Swedish 2009 modelling attempt, our model and simulations
 105 have different demographic and evolutionary settings, and utilize a different mating
 106 preference function for a better matching of the Galician demographic and evolutionary
 107 conditions (Rolán-Alvarez, 2007; Carvajal-Rodríguez and Rolán-Alvarez, 2014; Rolán-
 108 Alvarez et al., 2015a). The effect of introducing a cost of choosiness that was not
 109 considered in previous simulation studies was also included in our simulations.
 110 However, to validate our program we also replicate the 2009 Swedish model (see
 111 Program validation section).

112 Therefore, the main objective of this paper is try to answer the following questions
 113 which have been explicitly studied for the Galician system, although they can be
 114 extended to similar ecological speciation scenarios. Thus, given the conditions outlined
 115 in the Galician *L. saxatilis* model system (see details in Model and Methods but briefly,
 116 low dispersal ability, disruptive ecological adaptation, a magic trait, and an area of
 117 sympatry) our questions are:

- 118 1. Can we expect to find locally adapted specialists in the presence of gene flow?
- 119 2. Can mate choice (choosiness) and so full reproductive isolation, be evolved as a side
120 effect of ecological adaptation?
- 121 3. Can the intensity of mate choice be increased at the area where the ecotypes meet
122 because of reinforcement or other related mechanisms?

4. What is the impact of considering a cost in mate choice in relation to patterns of natural and sexual selection observed in nature?

Following, we briefly review the *L. saxatilis* species as a model organisms, then we explain the simulation model adapted to the Galician *L. saxatilis* scenario to answer the questions mentioned above.

Material and Methods

Model Organism

L. saxatilis is a marine snail gastropod living on North Atlantic rocky shores. The species crawls directly on rocks feeding on microalgae, diatoms and detritus. The species is gonochoric, shows internal fertilization and its ovoviviparous, showing direct development, i.e. with the female having a brood pouch with dozens to hundreds of embryos that are born as miniature snails. Due to these characteristics *L. saxatilis* shows low dispersal ability which also facilitates its capability to adapt to local conditions, and so local ecotypes have been frequently described on different localities and regions (Reid, 1996; Rolán-Alvarez, 2007; Johannesson et al., 2010; Rolán-Alvarez et al., 2015a). In particular, there is a striking sympatric polymorphism on Galician rocky shores (NorthWest Spain), where two ecotypes adapted to two different shore levels and microhabitats, coexist partially in sympatry at the mid shore (i.e. can frequently meet and mate partially assortatively, Rolán-Alvarez, 2007). A polymorphism determined by similar ecological forces (wave exposition and crab predation) is known in Britain and Sweden although the ecotypes are rarely (Britain) or never (Sweden) found in sympatry

there (Rolán-Alvarez et al., 2015a). In Galicia, an upper shore ecotype (named “Crab” in (Butlin et al., 2014)) lives on the barnacle belt, showing a larger and more robust shell, coloured with alternate ribs and black lines, that protects against crab predation (Rolán-Alvarez, 2007; Butlin et al., 2014; Boulding et al., 2017). This ecotype also shows a good resistance to desiccation, osmotic and sun stress. In addition, the lower shore ecotype (named “Wave” in (Butlin et al., 2014)) appears associated to the mussel belt, and has a smaller, softer and smoother shell, with bigger aperture to accommodate a massive muscular foot. This strong foot is necessary to maintain attached the snail to the substratum due to the strong waves that commonly impact at first instance on the lower shore. There are no crabs that can predate on this ecotype on the lower shore, and so, corresponding upper and lower shore microhabitats, represent a differential selective regime for the ecotype subpopulations. At the mid shore, where the above microhabitats overlap forming a patched distribution, both ecotypes meet and hybridize producing a number of intermediate morphological forms, although they show partial reproductive isolation with each ecotype mating preferentially with specimens of the same ecotype. These intermediate forms may usually resemble genetically to one or another ecotype though they occasionally can be truly intermediate genotypes (Galindo et al., 2013; Kess et al., 2018). The partial premating reproductive isolation seems to be linked to the size differences existing between these two ecotypes and could be a side effect of the size assortative mating typically observed in the species, being considered as a typical magic trait (Boulding et al., 2017; Fernández-Meirama et al., 2017a).

166 **Simulation Model**

167 There are few simulation studies on the interaction of natural selection with gene flow
 168 and the evolution of local adaptation in *Littorina* (Boulding, 1990; Pérez-Figueroa et al.,
 169 2005; Sadedin et al., 2009; Westram et al., 2018; Perini et al., 2020) and only the one
 170 from Sadedin and co-workers incorporates the evolution of mate choice and
 171 reproductive isolation.

172 The following model is a modification of the Sadedin et al. (2009) one, attending to the
 173 specific parameter values and spatial structure of the Galician *L. saxatilis* compared to
 174 the Swedish case. In the Swedish case the local morphological and behavioural
 175 adaptation occurs within islands in the Swedish archipelago in a horizontal wave
 176 exposure gradient through cliff and boulder habitats interspersed along the shore
 177 (Sadedin et al., 2009). On the contrary, the Galician ecotypes vary along a vertical,
 178 within locality micro geographic wave exposure gradient with different spatially
 179 varying selection favouring large sizes in upper-shore (wave- sheltered) and small sizes
 180 in lower-shore (wave-exposed). Unlike the Swedish case, the Galician ecotypes jointly
 181 with the intermediate forms, can be observed at approximately similar frequencies at the
 182 mid shore (Rolán-Alvarez, 2007; Galindo et al., 2013; Kess and Boulding, 2019).
 183 Migration rates of these snails are relatively low (less than 2 meters from the released
 184 point after one month), and the migration vectors of transplanted snails show a
 185 significant direction towards their native tidal height (Erlandsson et al., 1998).

186 The Galician simulation model begun with a pregnant female well adapted to the
 187 sheltered habitat arriving at the most upper deme in the shore, which is a realistic
 188 scenario of how *L. saxatilis* colonize new habitats (Janson, 1987). This founder female

produced offspring having the optimum phenotype for the wave-sheltered shore and a random mating value for the mating trait (see below). The model was spatially explicit in one dimension with three shore levels (upper, intermediate and lower).

Individuals were diploids with separate sexes, each individual was constituted by two different additive quantitative traits and a set of 8 microsatellite loci. The first quantitative trait is an ecological magic trait x i.e., it defines the individual fitness in a specific shore level and also is the target trait for the mate choice when mating is not random. An example of this kind of trait in *L. saxatilis* could be size (Boulding et al., 2017). The second trait c is a mate choice trait and is related with the sign of the preference for mate choice (positive or negative assortative) and also with the male choosiness. The choosiness is defined as the absolute value of the linear function $C = 2c - 1$ while the sign of the preference depends on the sign of C . If $C = 0$ ($c = 0.5$) there is random mating; negative values of C ($c < 0.5$) imply negative assortative mating, while positive values of C ($c > 0.5$) generate positive assortative mating (the absolute value, choosiness, varies between 0 and 1).

Each quantitative trait (x and c) was constituted by $L \in \{4, 8\}$ additive unlinked bi-allelic loci (see Table 1). Alleles may have value of 0 or 1. The trait value is scaled between 0 and 1 by summing over the alleles and dividing by L . Because we did not consider environmental variation the genotypic and phenotypic values were the same.

The generations were discrete non-overlapping and the simulation events occurred in the following order: birth, viability, mating, migration, reproduction and mutation. The parameter values can be consulted in Table 1.

212 **Spatial distribution**

213 The spatial distribution of individuals in the simulation tried to resemble the Galician
 214 rocky shore where *L. saxatilis* inhabits (Rolán-Alvarez, 2007). The population was
 215 simulated as a one-dimensional array with a total of 26 demes. The first 20 demes
 216 corresponded to the upper shore which is the sheltered habitat in the Galician coast, the
 217 next two demes were the intermediate habitat and the last four corresponded to the
 218 lower shore which is the exposed habitat. Each deme had a maximum capacity
 219 depending on the carrying capacity of its habitat. A deme in the lower-shore exposed
 220 habitat had a carrying capacity of $K = 15,000$ while the intermediate and sheltered had
 221 $K = 3750$ each. This distribution of demes per habitat tries to match the true habitat
 222 distribution and densities observed in Galicia (Rolán-Alvarez, 2007). However,
 223 symmetric models (with equal number of demes and population density) were also run
 224 in order to interpret the consequence of this asymmetry (see below). Only pregnant
 225 females were considered as migrants. A migrant female can move through one or two
 226 demes, the offspring will be born at the arrival deme. Each individual develops its
 227 whole life within a deme.

228 **Viability**

229 The survival condition of each individual depended on the distance between its
 230 ecological trait x and the optimum in the habitat where it was born. The optimum was 0,
 231 0.5 and 1 for the exposed, intermediate, and the sheltered shore respectively. We also
 232 considered the case of no selection in the intermediate habitat so that under this scenario
 233 a fraction of individuals may reproduce in a spatial region (two demes) that is neutral

with respect to ecological speciation (Cotto and Servedio, 2017). The fitness w_i of an individual i with phenotype x_i was given by (Sadedin et al., 2009)

$$w_i = \exp \left(\frac{-(x_i - \theta)^2}{2\sigma_s^2} \right) \quad (1)$$

where x_i is the value of the ecological trait, θ is the optimum in the habitat where the individual was born and σ_s is the inverse of selection strength.

The offspring survival was density-dependent following a Beverton-Holt model (Beverton and Holt, 1957). Thus, the individual viability depended on the total number of local juveniles N , the mean number ($b = 50$) of offspring of each individual and the carrying capacity (Sadedin et al., 2009)

$$v_i = \frac{1}{1 + \left(\frac{b}{2} - 1 \right) \frac{N}{w_i K}} \quad (2)$$

where w_i is the individual fitness (equation 1) and K is the carrying capacity of the deme.

Mating

In *L. saxatilis* mate choice is performed by males (Rolán-Alvarez et al., 2015a). In our simplest model (without cost), each male always mate with one of 10 females randomly chosen from its own deme. The probability of mating between the male and any of the 10 females is proportional to the FND ψ function (Ψ_{FND} , see equation 3).

$$\Psi_{FND} = \begin{cases} \exp\left(\frac{-C^2 D^2}{\sigma_a^2 D_{max}^2}\right), & \text{if } C > 0, \\ 1, & \text{if } C = 0, \\ \exp\left(\frac{-C^2 (D - D_{max})^2}{\sigma_a^2 D_{max}^2}\right), & \text{if } C < 0 \end{cases} \quad (3)$$

Ψ_{FND} depends on the value of the male mating trait c and the distance $D = |x_m - x_f|$ between the ecological traits of the male and the female candidate. The parameter C is a linear function of c , $C = 2c - 1$, and D_{max} is the maximum D (1 in this case) and σ_a is the tolerance or inverse strength of the male mating preference (Carvajal-Rodríguez and Rolán-Alvarez, 2014).

From (3), when $c > 0.5$ ($C > 0$) the mating is positive assortative, when $c < 0.5$ ($C < 0$) the mating is negative assortative, and it is random when the phenotype $c = 0.5$ ($C = 0$). The formulae in (3) is a modification of the original model of (Sadedin et al., 2009) to correct for the known problem that, when phenotypic values are equal to 0.5, positive assortative mating occurs regardless of the choice value (Carvajal-Rodríguez and Rolán-Alvarez, 2014).

As already explained, the choosiness is the absolute value of C while the sign of the preference depends on the sign of C . Thus, provided that some choosiness value evolved ($|C| > 0$), the resulting mate choice mechanism corresponds to a matching rule model combined with a magic trait (Kopp et al., 2018).

Under the mating without cost, each male always mates and produces offspring, independently of how choosy he is, so there will be mating even if all Ψ_{FND} values are very low. This is achieved by defining the mating probability as the FND function for a

272 given male and female pair relative to the total FND function for all females that the
273 given male encounters.

274 The following is an example of how mating is implemented without cost. Consider a
275 situation where a male i encounters female 1 and they have $\Psi_{FNDi1} = 10^{-3}$, and for any of
276 the remaining females in the deme the pair of male i with any female $j \neq 1$ has $\Psi_{FNDij} =$
277 10^{-4} . The sum of Ψ_{FND} values for this male with the ten females he encounters is $S = 10^{-3}$
278 $+ 9 \times 10^{-4} = 0.0019$. In the model without cost, the probability of mating for male i is
279 $\Pr(i \times 1) + 9\Pr(i \times j) = \Psi_{FNDi1}/S + 9\Psi_{FNDij}/S = S/S = 1$ where we divided by S due to the
280 condition that the male must mate. Thus, we see that the probability of male i mating
281 with female 1 is approximately 0.53 (i.e. $10^{-3}/S$) which is one order of magnitude greater
282 than with any other female, so it is proportional to the Ψ_{FND} values.

283 **Mate choice cost**

284 Given the value Ψ_{FND} of the preference function we considered also the case with cost to
285 choosiness (Bolnick, 2004). In the model with cost, the mating probability is equal to
286 the FND function for a given male and female pair (the coefficient of proportionality is
287 set to 1) and therefore an overly choosy male might not find a mate. Following our
288 previous example, male i randomly encounter female 1 plus 9 females from its deme
289 and will try to mate with all of them but now the mating probability coincides with the
290 Ψ_{FND} value and so, the probability of mating for male i is $\Pr(i \times 1) + 9\Pr(i \times j_{\neq 1}) = \Psi_{FNDi1} +$
291 $9\Psi_{FNDij} = S = 0.0019$. Therefore, there is more than a 99.8% chance that this male will
292 not mate. If no mating occurs the male is discarded and next male is considered. If no
293 mating takes place at all, the deme will be empty.

294 **Migration**

295 The life cycle occurs within the deme and ends after the reproduction. The pregnant
 296 females can disperse 0 (no migration), 1 or 2 demes in either direction with probability
 297 75%, 15% and 10% respectively. After migration, the female produce the offspring
 298 within the new deme and dies. These numbers were chosen in order to simulate a
 299 scenario of high gene flow under sympatry, as this was probably the starting point of the
 300 system before any local adaptation was evolved (Rolán-Alvarez, 2007).

301 **Reproduction**

302 After mating and migration, the number of offspring was obtained from a Poisson with
 303 mean b ($b = 50$ see Sadedin et al., 2009). Recall that males are the choosy sex so each
 304 male randomly encounter some females in the deme and successful mating will occur
 305 depending on the FND function i.e. on the phenotypes of male and female and on the
 306 choosiness of the male. If a specific mating is successful then the offspring number per
 307 mated male-female pair is sampled from Poisson distribution with mean 50. The genetic
 308 composition of the offspring depended on the independent segregation of the trait loci
 309 and microsatellites from the parental gametes.

310 **Mutation**

311 The new born underwent mutation after reproduction. The per individual haploid locus
 312 mutation rate was 10^{-3} for the stepwise mutation model of microsatellites (Slatkin,
 313 1995) and 10^{-5} for the additive locus (Sadedin et al., 2009).

The life cycle was allowed to run during 20,000 generations. We performed 20 replicates for each parameter combination and 48 different cases of parameter combinations for the Galician model. The number of runs for some cases was extended to 80,000 generations to evaluate long-term results, as this number of generations corresponds to an upper threshold for Galician *L.saxatilis* based on a generation time of 6 months and estimates of divergence time between ecotypes (Quesada et al., 2007).

Table 1. Model parameters

	Parameter	Symbol	Value
Demography	Generation number	T	20,000
	Carrying capacity exposed deme	K	15,000
	Intermediate and sheltered demes		3750
	Mean offspring number	b	50
	Between deme migration probability	m	
		0 demes	0.75
		1 demes	0.15
Genome Structure		2 demes	0.1
	Number of microsatellites		$L = 8$
	Ecological magic trait (L loci)	x	$L = \{4, 8\}$
	Male mating trait (L loci)	c	$L = \{4, 8\}$
Selection	Selection strength	σ_s	$\{0.15, 0.45, 1\}$
	Selection in middle habitat	θ	$\{\text{no}, \text{yes}\}$
Mating	Evaluated females per male	N_f	10
	Mating preference tolerance	σ_a	$\{0.05, 0.1\}$
	Mate choice cost		$\{\text{no}, \text{yes}\}$
Mutation	Neutral mutation rate per locus	μ_0	10^{-3}
	Trait mutation rate per locus	μ	10^{-5}

324 **Initial conditions**

325 As already indicated in the simulation settings, the experiment begun with a founder
 326 event in which the most upper deme of the sheltered habitat was colonized by the
 327 offspring of a single female adapted to this habitat ($x = 1$). The offspring number was 50
 328 (initial population size). All the individuals were homozygous for the quantitative traits
 329 and had the optimal ecological phenotype for this habitat ($x = 1$). The male mating trait
 330 was initially fixed at $c = 0.5$ (random mating). The microsatellite genotype was
 331 heterozygote for every locus and individual.

332

333 **Program validation**

334 To validate our implementation we replicated two other ecological speciation simulation
 335 models, namely, the model in (Sadedin et al., 2009) concerning the Swedish *L. saxatilis*
 336 ecotypes and the model in (Gavrilets et al., 2007) concerning the speciation process of
 337 cichlids in a crater lake (Barluenga et al., 2006). This replication effort comes in the
 338 spirit of exploring the robustness and reproducibility of individual-based models in
 339 order to augment credibility and consistency of computational modelling and simulation
 340 and thereby improving the evolutionary ecology theoretical field in general and
 341 speciation in particular (Massol and Debarre, 2015; Thiele and Grimm, 2015; David et
 342 al., 2017).

343 Regarding the replicated models, we have implemented only the mating system by
 344 similarity (matching rules sensu Kopp et al., 2018) with asymmetric resource
 345 availability. Also, regarding the cichlids model we have implemented only the two

patches space structure. The replication of such models allowed us to validate our implementation while at the same time it permitted to interpret the sensitivity of the evolutionary outcome to some of the assumptions in the model, as the type of preference function and the presence or absence of mate choice cost.

Analysis

We analyse the data obtained in the simulations using different variables. For example, the occurrence of ecological adaptation to the exposed habitat (lower shore) was recorded when the ecological trait changed its mean value by more than 75% ($x < 0.25$) as it is an arbitrary but reasonable indicator that most individuals have adapted to the new habitat and similar thresholds have been used in the past (Sadedin et al., 2009). Similarly, reproductive isolation evolution was recorded when the mean choosiness value was higher than 0.1 or, that is the same, the mean value of c was appreciably different from 0.5. ($c < 0.45$ or $c > 0.55$). In addition, we measured the genetic differentiation between habitats by means of the F_{ST} (Wright, 1951) and Q_{ST} indices (Leinonen et al., 2013). The fixation index F_{ST} was computed for the microsatellites as $F_{ST} = 1 - ((H_{low} + H_{mid} + H_{up})/3) / H_{total}$ where H_{shore} was the heterozygosity at the lower, middle or upper shore levels, and H_{total} was the total heterozygosity without considering the shore levels. Similarly, the quantitative genetic analogue of F_{ST} was computed for the ecological trait as $Q_{ST} = V_{between} / (V_{between} + 2 * V_{within})$ where V is the variance between or within habitats. Hence, if high mating discrimination happened in the middle habitat, $c \geq 0.9$, jointly with the occurrence of ecological adaptation, $Q_{ST} \geq 0.9$, then this was considered as reproductive isolation between the two ecotypes and can be interpreted as

368 a positive case of speciation caused by ecologically-based divergent natural selection
369 i.e. ecological speciation.

370 Simulation studies should be statistically analysed in order to be properly understood
371 and gain scientific credibility (Lotterhos et al., 2018). To evaluate the different
372 simulated scenarios in the Galician model, we investigated various simulation outcomes
373 (dependent variables) that could be influenced by *a priori* different combination of
374 simulation parameters (independent variables) under a classical multifactorial ANOVA
375 (see studied variables in Table 2). First, our independent variables were combined using
376 an orthogonal design (24 parameter combinations x 20 replicates per combination).
377 Notice that we do not use ANOVA as classical way for hypothesis testing, but rather as
378 an exploratory tool to compare the relative contribution of different factors (and
379 interactions) into the dependent ones. The relative contribution of each factor to the
380 overall variation was measured by the eta-squared coefficient (Sokal and Rohlf, 1981).
381 The eta-squared is finally expressed as % with respect to the sum of all etas in the
382 ANOVA (in order to allow comparison among different ANOVAs). In this way we can
383 identify the most interesting relationships (those with higher eta-squared i.e. higher
384 proportion of variance of the dependent variable explained) which can be detailed *a*
385 *posteriori* in the following sections. The per habitat average of the quantitative trait
386 phenotypic values, x and c , were used as dependent variables under this ANOVA
387 approach, and also the within habitat population size (N , considering all demes per
388 habitat), as well as the F_{ST} and Q_{ST} estimates described above. Additionally, we used the
389 Pearson correlation (r) of x values among partners in mates within habitat, as a way of
390 estimate the assortative mating for the ecological (magic) trait.

Table 2. Independent (factors) and dependent (response) variables for the ANOVA

Independent variables	Selection strength (σ_s)
	Mating strength (σ_a)
	Number of loci (L)
	Selection in the mid-shore (θ)
Dependent variables	Ecological trait (x)
	mating trait (c)
	Mating correlation for x (r)
	Population size (N)
	Differentiation measures (F_{ST} and Q_{ST})

Results

Program validation

When developing our Galician model we adapted it to replicate the 2×1 model (two habitats, 1 deme per habitat) in (Gavrilets et al., 2007) concerning the speciation process of cichlids in a crater lake (Barluenga et al., 2006). We obtained the same results regarding speciation events, choice trait and F_{ST} values, as reported from the original article. We also reproduced the similarity scenario for the Swedish *L. saxatilis* model (Sadedin et al., 2009) using their same mating preference function and obtained

qualitatively the same results regarding adaptation, neutral genetic differentiation, ecotype formation and non-random mating evolution.

Finally, we repeated the simulations with the Swedish model but using the preference function defined in (3) instead of the original one in (Sadedin et al., 2009). We obtained some new cases of evolution of negative assortative mating and fewer invasion and adaptation cases. These results are expected since it is known that the original function in (Sadedin et al., 2009) has an anomalous negative assortative mating behaviour (Debarre, 2012) implying less negative assortative mating cases than expected (Carvajal-Rodríguez and Rolán-Alvarez, 2014). However, the main conclusions in Sadedin remained, namely, ecotypes may persist indefinitely with moderate genetic differentiation without speciation. As Sadedin *et al* highlights, both ecological divergence and the evolution of reproductive isolation, are crucial to ecological speciation, but since they are driven by different forces that can even conflict, the prediction about ecological speciation requires highly detailed data jointly with a quantitative simulation model specially designed for the problem at hand as we did with what we have called the Galician model.

Galician model

The results are first explored by factorial ANOVA, in order to emphasize the main factors (and interactions) contributing the most (highest variance explained) to the dependent variables, and secondly the details of these effects are fully described in next sections.

Parameter effects: Orthogonal analysis of variance

The relative importance of the different simulated parameters, as depicted by the selection and mating strength, number of genes, etc, was summarized by the ANOVA from which the factors and interactions that explained the larger percentage of variance in the evolutionary outcome can be outlined (Table 3, also see supplementary material for the full table of the factorial ANOVA).

Table 3. ANOVA results showing % of variance (% partial eta squared; see text) explained by each factor for the ecological (x) and mating (c) traits, mating correlation (r), population size (N) and differentiation measures (F_{ST} and Q_{ST}). Only the two most important factor interactions are presented. The values within cells correspond to the % at lower, middle, upper shore, by this order, except for differentiation (F_{ST} and Q_{ST}) that are between shore levels. The asterisk indicates significance at the 0.001 level.

Factor	Response					
	x	c	r	N	F_{ST}	Q_{ST}
σ_s	40*, 35*, 26*	26*, 20, 58*	46*, 64*, 45*	30*, 29*, 35*	48*	38*
σ_a	3, 1, 9*	1, 0, 1	5*, 9*, 0	1, 0, 1	2*	1
L	0, 9*, 0	9*, 9, 0	8*, 1, 18*	7*, 1*, 21*	7*	18*
θ	4, 3*, 1	12*, 9, 3	2, 0, 0	12*, 34*, 0	0	22*
$L \times \sigma_s$	42*, 13*, 31*	16*, 16, 30*	33*, 15*, 30*	12*, 2*, 33*	31*	30*
$\theta \times \sigma_s$	2, 14*, 0	19*, 17, 0	2, 2, 0	20*, 28*, 2	0	31*

The selection strength σ_s was clearly the key element with highest effect in adaptation, choice evolution, population size and differentiation. The number of genes had typically

a significant although lower impact on most dependent variables. The mating strength (σ_a) and selection on the mid shore (θ) showed in general low impact on most variables. On the other hand, the interaction of selection with the number of genes, and with less importance the $\theta \times \sigma_s$ interaction, had also great importance for most variables. Some factors require special attention in certain variables. For example, the number of genes (L) shows a moderate impact in the mating correlation (r) and both F_{ST} and Q_{ST} variables. In the following we present these outcomes more in detail.

Ecotype formation: Habitat colonization and adaptation

The founder population had the ecological trait perfectly adapted ($x = 1$) to the sheltered (upper shore) habitat. As time passed some individuals migrated to the intermediate and lower habitats where the ecological conditions were different so that the optimal value for the exposed habitat (lower shore) was $x = 0$. In the previous section, we have emphasized that σ_s and $L \times \sigma_s$ were the most relevant factors determining the evolution of the ecological trait x (Table 3). We will now describe these patterns in further detail. In Figure 1 we may appreciate the summary of the adaptation process after 20,000 generations, through the interplay between migration and selection within habitat, averaged over the different simulated scenarios and demes.

First, adaptation to the upper shore (Figure 1, white bars, $x \geq 0.75$) happened in virtually all cases which is not surprising since the founder individual was perfectly adapted to this habitat.

Habitat colonization and adaptation to the lower shore happened under weak to intermediate selection ($\sigma_s \in \{1, 0.45\}$), irrespective of the value of the other

parameters. Under strong selection ($\sigma_s = 0.15$) adaptation occurred in cases with few loci ($L=4$) and non-neutral hybrid zone (optimum 0.5) and also under neutral hybrid zone and mating strength $\sigma_a=0.1$. For cases corresponding to scenarios under strong selection and more number of loci ($L=8$) adaptation only occurred when the hybrid zone was non-neutral. Thus, we may appreciate that more than 80% of the simulated cases successfully colonized the new habitat and adapted to it having the optimal ecological phenotype (Figure 1, black bars, $x \leq 0.15$, Fig. 1).

The middle shore (Figure 1, grey bar), had some cases where generalists ($0.4 < x < 0.6$) evolved. The evolution of generalists in the middle shore corresponded to scenarios with non-neutral hybrid zone and strong selection ($\sigma_s = 0.15$) with adaptation in the lower shore. Other cases under strong selection evolved ecological trait phenotypes close to the upper shore optimum (Figure 1, $x > 0.75$). In the latter cases, there was not colonization of the lower shore and they only differ from cases in which generalists evolved in that the ecological trait was neutral in the hybrid zone. Thus, under strong selection in the upper and lower shores, the environmental conditions of the hybrid zone as defined by the selection in the mid-shore (θ) are key to the evolution of generalists and the colonization of the lower shore.

However, more than 60% of the cases in the middle habitat had an ecological trait value closer to the optimum for the lower habitat (Figure 1, $x < 0.25$). This asymmetry, i.e. ecological trait values in the intermediate habitat being closer to the lower shore than to the upper shore optimum, can be explained by the higher carrying capacity of the lower habitat which implied a higher gene flow from lower to middle than from upper to middle through the vertical axis of the Galician coast.

Fig 1. Mean value per habitat of the ecological trait for individuals living in the sheltered (upper), intermediate and exposed (lower) habitats.

To confirm the cause of the asymmetric effect for adaptation in the middle habitat, we studied a specific symmetric case corresponding to $L = 4$ loci under intermediate selection ($\sigma_s = 0.45$) and neutral hybrid zone. We simulate 100 replicates of the Galician model and also 100 replicates of a symmetric model with the same number of demes in the upper and lower shore and equal per deme carrying capacity in the three habitats (supplementary Table S1). We found no differences in adaptation to the lower shore and confirmed the effect of differential migration to adaptation to the middle shore where the mean ecological trait value was 0.15 ± 0.04 for the Galician model while it was 0.48 ± 0.07 for the Symmetric model (supplementary Table S2).

510 The asymmetric effect is a significant difference with the Swedish model where
 511 intermediate zones are non neutral (optimum 0.5) and their distribution is not localized
 512 just in the middle point of the vertical coast axis but in the circular horizontal borders
 513 between sheltered and exposed so before each sheltered and at the end of each exposed
 514 in a circular (as in an island coast) distribution.

515 In addition to the ecological trait values, we also studied the time to adaptation to the
 516 exposed habitat. Basically there were only two opposite outcomes, most scenarios
 517 produced fast adaptation in less than 1000 generations but some did not reach
 518 adaptation in 20,000 generations (they appear as the right-most bar in Fig.2). As before,
 519 the strength of selection was the fundamental factor affecting the speed of adaptation.
 520 Most fast adaptation cases involve an intermediate-low selection strength while most
 521 cases of non-adaptation corresponded to strong selection.

523 **Fig 2.** Generations until adaptation to the lower shore ($X < 0.25$) for the different scenarios assayed.
 524

There was also an interaction effect between the strength of selection and the number of loci ($L \times \sigma_s$ in Table 3), implying that under strongest selection ($\sigma_s = 0.15$) there was twice the probability of adaptation under the fewer loci scenario ($L = 4$) than under the greater one ($L=8$).

Mating trait

We have already emphasized that it was σ_s and $\sigma_s \times L$ the most consistent factors determining the evolution of c (Table 3). We will now describe how these effects occur in detail. The phenotypic value for the male mating trait, c , was initially 0.5 that implies random mating (i.e. no choosiness $|C| = |2c - 1| = 0$). Values of c above 0.5 imply positive assortative while below 0.5 imply negative assortative mating. To discard noisy variation around 0.5 we considered that positive mate choice evolved when the mean c values were above 0.55 while negative choice evolved when mean values were below 0.45. Thus, from Fig. 3 we appreciate that positive assortative mate choice ($c > 0.55$) evolved in the three habitats in most cases although there were some cases (about 15% in the three habitats) with negative mate choice ($c < 0.45$, Fig. 3).

Fig 3. Mean value of the mate choice trait for individuals living in the upper (sheltered), middle and lower (exposed) habitats.

There were a clear pattern behind the negative assortative mating scenarios, the combination of few loci ($L = 4$) with strong selection ($\sigma_s = 0.15$) (Table S3). Negative assortative mating only evolved under strong selection scenarios and the most favourable scenarios include few loci, low tolerance ($\sigma_a = 0.05$) and the selective middle zone. It seems that under this scenario it was useful to evolve a preference for the different type in order to maintain the polymorphisms avoiding the fast fixation of sub-optimal genotype combinations. In fact, these negative assortative mating cases did not evolve fast, i.e. it took at least 1500-10,000 generations until the x trait was below 0.25 in the exposed shore and after 20,000 generations the mean fitness still was sub-optimal ($w < 0.9$) in the three habitats.

When selection was not so strong and/or the number of loci was high, the result was mostly positive assortative mating (70% of cases in the three habitats with average choosiness of 0.45, $c = 0.725$, see next section).

As with the ecological trait, we also studied the time required to evolve choice in the lower habitat. Interestingly, the time needed for this character to evolve is much longer than for the ecological trait.

Mating trait versus mating correlation

The increase of reproductive isolation in speciation scenarios is typically estimated by means of size (or ecologically related traits) assortative mating (Jiang et al., 2013; Janicke et al., 2019). However, the evolution of assortative mating can be only produced by means of the mate choice (choosiness in our model). Therefore, it is of special interest to compare a genetic trait that has evolved, such as male mating trait (c) values, with the observed mating pattern, as measured by the correlation (r) between male and female phenotypes x (ecological trait) in mated pairs, since this is a common measure of assortative mating (Fig. 4). This could help to identify potential situations in which demographic effects may give the wrong impression of mate choice evolution.

Fig 4. Mating trait value (c , abscissa axis) versus the mating pattern as measured by the correlation (r , ordinate axis) for the ecological trait in mated pairs.

There was good agreement between the sign of the choice and the mating pattern described by the correlation. Regarding choosiness, higher $|C|$ values implied higher absolute correlation value and vice versa. The agreement was quite good in the range $0.2 < c < 0.8$ i.e. a choosiness below 0.6 ($|C| < 0.6$). With choosiness values above 0.6 ($c \leq 0.2$ and $c \geq 0.8$ values) there was a saturation of the correlation value reaching its maximum in several cases. Also noteworthy is that there were some cases with high choosiness that presented very low correlation (points close to the abscissa at the right-hand end of Fig. 4). These latter cases corresponded to scenarios where the ecological trait was virtually fixed.

In general, about 1/3 of the cases, (33%, 34% and 29% for the lower, middle and upper shore respectively) reached intermediate (0.4-0.6) positive choosiness values as a result

of the trade-off between divergence and sexual selection thus favouring local adaptation under migration (Rettelbach et al., 2013; Cotto and Servedio, 2017; Sachdeva and Barton, 2017).

As with the ecological trait, we studied the possible effect of the asymmetry of the Galician model in the evolution of the mating trait and observed that in this case the asymmetry in carrying capacity seems to have little or no effect on the evolution of the mating trait (supplementary Table S2). The values obtained for the mating trait matches well the empirical estimation of mate choice values for *L. saxatilis* and other littorinids (Fernández-Meirama et al., 2017a, 2017b).

Neutral (F_{ST}) and quantitative (Q_{ST}) genetic differentiation

Regarding the relative contribution of the distinct factors to the population differentiation, the analysis of variance (Table 3) showed that the selection strength (σ_s) and its interaction with number of loci ($L \times \sigma_s$) had the highest effect on both neutral and quantitative differentiation. In addition, all interactions in the table involving strength of selection (σ_s) as well as the number of loci (L) showed a large impact on quantitative differentiation (Q_{ST}). This suggest the great importance of the strength of selection (as a factor and in interactions) in determining the variance explained in Q_{ST} . However, the ANOVA did not investigate the relationship between F_{ST} and Q_{ST} .

The Q_{ST} vs F_{ST} comparison (Fig. 5) presented the typical pattern of local adaptation in the presence of gene flow, with high Q_{ST} versus low F_{ST} values (Leinonen et al., 2013). The only exception occurs when adaptation is not reached within the 20,000 generation

608 interval (e.g. strong selection with $L=8$ loci and neutral hybrid zone, see the ecotype
609 formation section), in that cases the Q_{ST} values remained low.

610 **Fig 5.** Neutral versus quantitative genetic differentiation

611 Intermediate habitat effect

612 We have modelled two different ecological scenarios for the intermediate habitat (2
613 demes). In the selective-middle habitat scenario there is positive selection for the
614 ecological trait with the optimum in 0.5. In the neutral-middle habitat scenario the
615 ecological trait was neutral. The latter is a realistic scenario not usually considered in
616 ecological speciation modelling studies (but see Cotto and Servedio, 2017). The kind of
617 scenario (selective or not) had a minor impact (affecting mean trait values) regarding

the ecological trait (x) in the middle shore but a moderate to high impact for c (at lower shore), N (from low to mid shore) and Q_{ST} (Table 3).

Both under the selective and neutral-middle scenarios, there were in the middle shore, a higher percentage of cases with mean ecological phenotype closer to the lower zone optimum (47 or 64% for selective or neutral-middle respectively) than to the upper zone optimum (0 or 11% for selective or neutral-middle respectively). This asymmetry was already evident in Fig. 1, and was related with the Galician microhabitat configuration jointly with the higher carrying capacity of the lower habitat that provoked a higher number of migrants arriving from this habitat (Table S2). This result matches the empirical observation regarding the size of hybrids in the mid-shore which even though they are genetically more heterogeneous than Crab (upper shore) or Wave (lower shore) ecotypes they tend to be closer to the lower shore phenotype (Galindo et al., 2013; Diz et al., 2021).

Regarding the impact of the middle scenario over the colonization of the lower shore, there were more colonization cases of the exposed habitat when there was selection in the middle shore (97% of those cases) than when the middle shore was neutral (79% of those cases). In fact the analysis of variance (Table 3) showed that the selection strength (σ_s) and its interaction with the middle scenario ($\theta \times \sigma_s$) had significant effect for the x trait in the middle zone.

Regarding the mating trait and mating correlation, the middle habitat scenario did not contribute to explain any significant variance for the mating trait or the correlation over the simulations (Table 3 and compare rows within Table S4 and Table S5). Also, the choosiness between the different habitats were quite similar (compare columns in Table

S4). However there were important differences for the correlation between habitats. The mean mating correlation was 0.24 in the exposed area, 0.55 in the middle and 0.34 in the sheltered area. However, these general mean values are affected by cases with negative choice and also by cases with poor adaptation. Obviously, when excluding negative choice scenarios the correlation increases but the pattern with higher correlation in the middle habitat still holds. Specifically for the intermediate selection cases, the correlation values in the middle habitat can be above 0.85 (Table S5). The effect was even higher when the symmetric model is considered (Table S6) so that the higher correlation in the middle habitat was not an effect of the asymmetry in the Galician model.

Finally, regarding genetic differentiation, F_{ST} was not affected by the presence or absence of selection in the intermediate habitat, contrary to quantitative differentiation (Q_{ST}) which was 20% higher in average when the middle habitat was selective ($Q_{ST} = 0.93$), compared to when the middle was neutral ($Q_{ST} = 0.74$). The type of middle habitat scenario explained 22% of the variance in Q_{ST} over the simulations (Table 3).

Ecological speciation

We defined as a proxy for complete reproductive isolation the concurrence of high local adaptation ($Q_{ST} \geq 0.9$, see Fig. 5) and high middle-shore choosiness (see Fig. 4, $c \geq 0.9$ i.e. positive assortative mating with choosiness $|C| \geq 0.8$). This reproductive isolation caused by ecologically-based divergent natural selection is called ecological speciation. Thus, when taking jointly the evolution of adaptation and assortative mating, we observed that there were ecological speciation in about 5% of the simulations. All the ecological speciation cases happened under the few selective loci ($L=4$) scenario.

However, they were not uniformly distributed for the different selection strengths with all speciation cases linked to intermediate or weak selection strength. We increased the simulation runs to 100 to check this result and observed an 11-15% percentage of speciation for intermediate and weak selection while there were no speciation for the strong selection cases ($\sigma_s = 0.15$, Table S7). These same results were also obtained in the long-term (80,000 generations).

Mate choice cost

As before, the relative importance of the different evolutionary scenarios was summarized by the ANOVA (supplementary Table S8). The strength of selection was again the most important factor influencing the dependent variables. The factor $L \times \sigma_s$, i.e. the interaction between number of loci and strength of selection, had decreased importance. In general many more factors were now non significant in several variables, which suggest that with cost less adaptation and evolution of variables is obtained.

In the previous scenarios there were no cost for being choosy. Adding a cost to the mate choice implies that the overly choosy males may remain unmated. As a result there were no evolutionary outcome in the form of negative choice neither negative assortative mating (negative mating correlation). Also, less positive choice was evolved in the three habitats (see Table 4).

Table 4. Mean choosiness $|C|$ linked to positive assortative mating (negative values excluded) for the different habitats with selective or neutral middle scenario and with or without mate choice cost. The values are averages for the different L and σ_a .

No cost	Cost
---------	------

	Lower	Midle	Upper	Lower	Middle	Upper
Selective middle	0.42	0.42	0.44	0.34	0.34	0.34
Neutral middle	0.49	0.47	0.47	0.39	0.38	0.39

Finally, there were fewer colonization events in the presence of cost. Under the selective-middle habitat scenario 83% of the simulations with cost underwent colonization, compared to 97% without cost. The same pattern occurred under the neutral-middle habitat scenario, 66% of colonization with cost versus 79% without cost. However, when colonization occurred the fitness in the exposed habitat was higher under cost scenarios (mean fitness 99.7%) indicating that adaptation was better in these cases. Finally, we do not find ecological speciation when mate choice cost was included but in just one run of the case with weak selection ($\sigma_s = 1$), low number of loci ($L=4$), higher tolerance ($\sigma_a = 0.1$) and selective-middle scenario. This case implies a 0.2% compared to 5% speciation cases when cost was absent.

Discussion

We have analysed the evolution of local adaptation and mate choice in a system displaying an extreme microhabitat environmental heterogeneity. This system resembles the *L. saxatilis* microhabitat-associated dimorphism along the wave-exposed rocky shores of Galicia. *L. saxatilis* dimorphism is a good example of microgeographic

adaptation and incipient-speciation which occurs despite high gene flow based on the species expected levels of dispersal (Rolán-Alvarez 2007; Richardson et al., 2014). In our individual-based simulations we investigated the dynamics of the ecotype formation through the inter-deme and habitat spatial and temporal scales. This deme-based fine spatial scale model showed how the strength of selection and the evolution of choosiness may interact in order to produce local adaptation and reproductive isolation (Cotto and Servedio, 2017).

Our results are consistent with the key role of natural selection in the ecological speciation processes (Barton, 2010). The strength of selection on its own or through interaction with other factors explains most of the variance in the dependent variables over the scenarios simulated for both with and without mate choice cost. That is, most of the differences in ecological and mating trait values are explained by the strength of selection.

We have seen how the population, as the individuals disperse through inter-habitat demes, evolve different phenotypic values for habitat specialization. Given the high environmental heterogeneity between the sheltered and exposed habitats we obtained specialists for these habitats. The outcome was slightly different in the middle habitat, where generalist or specialist evolved depending on how the environmental heterogeneity was defined. When the middle habitat was neutral this facilitated the co-existence of specialists from both shore levels although with a higher % from the lower shore due to the higher number of migrants coming from this shore. On the contrary, when the middle habitat was non-neutral with an optimum in-between both extreme shore levels, which implies a less-heterogeneous environment, more intermediate

phenotypes evolved. These results are consistent with previous studies showing the positive relationship between local adaptation and environmental heterogeneity (Berdahl et al., 2015; Svandal et al., 2015).

Concerning our question number 1 about the pattern of local adaptation under gene flow, which expects high quantitative genetic divergence for certain traits, without a general divergence at the genome level, we confirmed the expected pattern and observed high quantitative trait genetic divergence (Q_{ST}) jointly with low neutral genetic differentiation (F_{ST}).

We found that the time-scale for ecological adaptation and the occurrence of ecological speciation were influenced by the strength of selection. If selection was strong the time for adaptation was longer. Moreover, weak or intermediate selection and few selective loci were favoured under ecological speciation. However it should be noted that we modelled independent loci, i.e., few unlinked loci may represent a relatively wide genomic region depending on the linkage relationships of the species. Obviously, the fewer the loci, the smaller the genomic region. Our results matches previous one for the Swedish model in (Sadedin et al., 2009) where ecotype formation was slower when the number of loci involved was larger and also recent results for the *L. saxatilis* Swedish system that shows the importance of adaptive recombination suppression within inversions to facilitate ecotype evolution and adaptation (Koch et al., 2021). Moreover, recent work (Kautt et al., 2020) has highlighted that simple genetic architecture, as the one we modelled, not necessarily lead to speciation even with magic traits which is also consistent with our findings. It seems however that polygenic selection might be more

efficient in driving sympatric speciation under some circumstances however this setting was out of the scope of the present study.

Most of the simulations evolved intermediate values of choosiness as a result of the trade-off between the effect of natural and sexual selection. Similar results have been obtained previously under some theoretical models and specific parameter range (see e.g. Rettelbach et al., 2013; Cotto and Servedio, 2017; Sachdeva and Barton, 2017 and references therein) and it is interesting that we have obtained the same result with a model for the specific case of *L. saxatilis* in the Galician coast using empirical estimates of the parameters (when available) and that the intermediate choosiness obtained in the simulations matches quite well empirical estimates of *L. saxatilis* and other littorinids (Fernández-Meirama et al., 2017a, 2017b; López-Cortegano et al., 2020).

Therefore, regarding our question number 2 on mating choice evolution as an effect of ecological speciation we may say that yes, under *L. saxatilis* Galician-like conditions, choosiness evolved during the process of ecological adaptation. Most of the evolved choosiness corresponded to positive assortative mating although negative assortative mating evolved under the combination of few loci and strong selection. As a general pattern, intermediate selection and few selective loci favoured the evolution of choosiness. However, this does not mean that the evolution of choosiness will irremediably cause ecological speciation. Actually, choosiness was typically maintained at intermediate values.

This was caused because there was an interplay between the evolution of assortative mating and local selective pressures. We modelled the evolution of choosiness as an ecologically neutral trait, although having as the target trait a magic trait, with realistic

migration estimated for *L. saxatilis* in the Galician coast (Rolán-Alvarez et al., 2015a). These conditions imply that the evolution of choosiness is constrained by the maintenance of the trait polymorphism required for evolving local adaptation which is in turn affected by the current choosiness. If choosiness is strong, then rare, non optimal, phenotypes would mate between themselves. Therefore, during the process of local adaptation the alleles coding for strong choosiness are eliminated by viability selection. Under this setting, the highest amount of divergence occurs at intermediate choosiness values for a wide range of parameter values, which has been also observed in other studies (Rettelbach et al., 2013; Cotto and Servedio, 2017; Sachdeva and Barton, 2017). As far as we know, this is a new outcome regarding the evolution of mate choice for the *L. saxatilis* systems and is consistent with our intermediate estimates of assortative mating (Johannesson et al., 1995; Rolán-Alvarez et al., 1999, 2004; Cruz et al., 2004) and mate choice (Fernández-Meirama et al., 2017a, 2017b) for the Galician system. Positive assortative mating evolved in the three simulated habitats.

We also studied the correspondence between the preference based on the *c* trait and the assortative mating correlation measure. We already know that both measures matched well if the sampling is done at the correct scale where choice occurs (Estévez et al., 2018). However, we found important differences when comparing the choosiness values (recall that choosiness is a linear function of the *c* trait) with the value of the assortative mating correlation measure, especially when interpreted in the context of reinforcement. Reinforcement is defined in a broad sense as the evolution of traits that minimize hybrid formation in response to selection (Servedio et al., 2013; Pfennig, 2016). The effect of selection can be indirect i.e. reinforcement occurs due to selection against hybrids that indirectly results in prezygotic isolation or on the contrary, it involves the effect of

natural or sexual selection acting directly on the prezygotic phenotypes involved in mate attraction. In the latter case, reinforcement is better called reproductive interference (Butlin and Ritchie, 2013; Hochkirch, 2013; Shaw and Mendelson, 2013).

Actually, we found higher mating correlation in the middle habitat than in the lower and upper ones. Therefore, if we use mating correlation as a proxy for assortative mating (Jiang et al., 2013; Janicke et al., 2019) we would say that we are detecting reproductive interference.

However, choosiness in the middle habitat, where hybrids can be formed, was very similar to choosiness in the other habitats, and so actually we cannot conclude that reproductive interference was evolved in our scenarios.

This putative reinforcement-like pattern cannot be due to a character displacement for the ecological trait (x) since there were no more extreme phenotypes in the middle area than in the other two. However, there was higher variation for the ecological trait in the middle habitat than in the others (coefficient of variation was at least one order of magnitude higher). Therefore, we should infer a higher sensitivity of the correlation measure to outliers (Pernet et al., 2013) which on the other hand would also explain the observed saturation effect of r over c . Actually, estimates from *L. saxatilis* empirical data indicate that mate choice has not increased in populations where the two ecotypes meet compared with those with only one ecotype (Fernández-Meirama et al., 2017a).

All of the above corroborates that evolving high mate choice levels is difficult in presence of high gene flow. Therefore, regarding our question number 3 in the introduction, we may say that mate choice does not increase in the middle habitat under the simulated conditions of the Galician *L. saxatilis* system.

Finally, we considered the question number 4 about the impact of adding a cost to the mate choice and we observed in concordance with previous studies (Kopp and Hermisson, 2008), less reproductive isolation cases and just residual ecological speciation when the cost was added. As expected, when choosiness evolved its value was lower, but still relevant to have an ecological impact. In addition, there were an increasing importance on how the middle habitat was modelled and its impact on adaptation. When the middle habitat was neutral regarding the ecological trait, there were twice more adaptation failures than when the middle habitat was selective because when selection favours an intermediate phenotype this probably facilitated the transition to the optimal phenotypes for the lower shore (Schneider and Burger, 2006).

In summary, it seems that the *L. saxatilis* model system may correspond well to an incomplete ecological speciation case with intermediate choosiness and strong within habitat adaptation. The results obtained in our model system suggest that, the evolution of ecological speciation is not an immediate consequence of local divergent selection and mating preferences, but a fine tuning among the environmental conditions in the microhabitat and the hybrid middle zone, the genetic basis of the traits, the selection intensity and the mate choice stringency and cost. Similar results which have been obtained for other ecological speciation model cases (Safran et al., 2013; Cotto and Servedio, 2017) suggest that incomplete ecological speciation may be not rare.

These results may guide the search for new empirical data providing direction for future models to assist our understanding of the *L. saxatilis* system and ecological speciation in general. For example it would be interesting to check the particular condition that could facilitate higher values of choosiness and so putatively complete the ecological

844 speciation process in this model system or even test whether the mate choice
845 mechanism functions as a similarity-like mechanism as has been shown in other
846 littorinids (Lau et al., 2021).

847

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