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Case studies and mathematical models of ecological speciation. 3: Ecotype formation in a Swedish snail

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Abstract

Formation of partially reproductively isolated ecotypes in the rough periwinkle, *Littorina saxatilis*, may be a case of incipient nonallopatric ecological speciation. To better understand the dynamics of ecotype formation, its timescale, driving forces and evolutionary consequences, we developed a spatially explicit, individual-based model incorporating relevant ecological, spatial and mate selection data for Swedish *L. saxatilis*. We explore the impact of bounded hybrid superiority, ecological scenarios and mate selection systems on ecotype formation, gene flow and the evolution of prezygotic isolation. Our model shows that ecotypes are expected to form rapidly in parapatry under conditions applicable to Swedish *L. saxatilis* and may proceed to speciation. However, evolution of nonrandom mating had complex behaviour. Ecotype evolution was inhibited by pre-existing mating preferences, but facilitated by the evolution of novel preferences. While in many scenarios positive assortative mating reduced gene flow between ecotypes, in others negative assortative mating arose, preferences were lost after ecotype formation, preferences were confined to one ecotype or the ancestral ecotype became extinct through sexual selection. Bounded hybrid superiority (as observed in nature) enhanced ecotype formation but increased gene flow. Our results highlight that ecotype formation and speciation are distinct processes: factors that contribute to ecotype formation can be detrimental to speciation and vice versa. The complex interactions observed between local adaptation and nonrandom mating imply that generalization from data is unreliable without quantitative theory for speciation.

Keywords: *Littorina*, parapatric, prezygotic, reproductive isolation, simulation

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Introduction

Recent syntheses provide a robust analytical framework for speciation theory (Kirkpatrick & Ravigné 2002; Servedio & Noor 2003; Gavrilets 2004) that is broadly in agreement with empirical data (Coyne & Orr 2004; Seehausen 2006; Bolnick & Fitzpatrick 2007; Hendry *et al.* 2007; Nosil *et al.* 2007; Gavrilets & Losos 2009). Of special recent interest are those cases where reproductive isolation evolves in the context of ecological selection and nonrandom mating with continuous gene flow, a process termed ecological speciation (Mayr 1947; Schluter 2000; Rundle & Nosil 2005). For many years, this process was considered to have limited plausibility (Mayr 1947), but exciting recent developments suggest that it may occur rapidly in both animals and plants (Barluenga *et al.* 2006; Savolainen *et al.* 2006), and that it can even drive adaptive radiation (Schluter 2000; Gavrilets & Vose 2005; Hendry *et al.* 2007; Langerhans *et al.* 2007; Jiggins 2008). Identifying conditions for ecological speciation, its patterns, timescales and driving forces has consequently become the focal point of current research in speciation (Bolnick 2004, 2006; Gavrilets & Vose 2007; Gavrilets *et al.* 2007; Smith & Benkman 2007; Steinfartz *et al.* 2007; Egan *et al.* 2008).

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Unfortunately, the complexity and diversity of processes influencing speciation imply that predictions from analytical models can rarely be applied directly to particular natural systems. Multiple evolutionary forces act simultaneously, similar outcomes can be achieved by different means, and small historical contingencies can irrevocably impact large-scale dynamics. The problem is compounded by the inaccessibility of natural populations to experimental manipulation on the scales required to test speciation theory. At this stage, numerical models form a crucial link between analytical models – which expose the fundamental underlying principles of speciation – and empirical data – which form our only evidence about how these principles are embodied in natural systems. Using simulations, we can represent detailed species-specific data and examine the sensitivity of the system to unknown parameters, allowing us to tune our models to the combinations of conditions that are most relevant in nature.

Recently, detailed simulations incorporating relevant ecological, behavioural, spatial and genetic data have been used to examine putative cases of ecological speciation of cichlids in a crater lake (Gavrilets *et al.* 2007; see also Barluenga *et al.* 2006), palms on an oceanic island (Gavrilets & Vose 2007; see also Savolainen *et al.* 2006) and hybrid speciation in butterflies (Duenez-Guzman *et al.* 2009). Other recent simulations have explored speciation via the co-evolution of habitat fidelity and assortative mating in walking-sticks (Nosil & Yukilevich 2008) and sensory drive in Lake Victoria cichlids (Kawata *et al.* 2007). By simulating real systems, these projects explored how general predictions of analytical models play out in realistic scenarios where numerous processes interact. In keeping with past models, these studies confirm that ecological speciation is plausible when loci have large effects, selection is neither too strong nor too weak, and nonrandom mating is influenced by ecological traits. At the same time, the models uncovered a number of unexpected limitations of data and verbal arguments, and revealed new dynamics generated by the interplay of spatial, ecological and genetic factors which were explicitly simulated.

Arguably one of the best-known case studies of ecological speciation is the rough periwinkle *Littorina saxatilis* (Johannesson *et al.* 1995; Rolan-Alvarez *et al.* 1999; Panova *et al.* 2006; Quesada *et al.* 2007; Johannesson *et al.* 2008). Here, we use a similar approach to Gavrilets *et al.* (2007), Gavrilets & Vose (2007) and Duenez-Guzman *et al.* (2009) to study the processes driving evolution of ecotypes and prezygotic isolation in *L. saxatilis* on Swedish shores. The Swedish *L. saxatilis* ecotypes exhibit several intriguing features, including strong positive assortative mating driven by male mating preferences (Hollander *et al.* 2005; Johannesson *et al.* 2008), spatial genetic structure at multiple scales (Mäkinen *et al.* 2008) and asymmetrical population sizes (Janson 1983). In systems with such strong spatial structure, locally adapted populations (ecotypes) may form without evolving premating reproductive isolation; when and if these ecotypes will become permanently isolated remains unknown (see review by Hendry *et al.* 2007). Thus, the relationship between ecotype formation and ecological speciation remains unclear. A further complication is bounded hybrid superiority (Moore 1977; Janson 1983). Our goals are to check the plausibility of several different scenarios that cannot be distinguished from empirical evidence alone, and to identify important parameters and features. This will guide the search for new empirical data within the *L. saxatilis* system and, potentially, provide direction for future analytical models to assist our understanding of ecological speciation in general. In the sections below, we first describe the relevant biology in more detail and then discuss the theoretical issues this raises. For a detailed review of *L. saxatilis* biology, see Johannesson (2003).

Biology of Swedish *L. saxatilis*

In the archipelago off the Swedish North Sea coast, the direct-developing intertidal snail *L. saxatilis* forms ecotypes that are adapted to cliff and boulder habitats interspersed along the shore (Janson 1982). On exposed cliffs where wave action is a major cause of mortality, *L. saxatilis* forms dense populations ($\approx$1100 snails/m^2) of small, thin-shelled snails with large apertures and feet providing strong adhesion (exposed ecotype). On boulder shores, where predatory crabs abound, *L. saxatilis* populations are relatively sparse ($\approx$290 snails/m^2), and snails are large and thick-shelled with small apertures (sheltered ecotype) (Johannesson & Johannesson 1995). Such cliff and boulder forms are mirrored in many other gastropods: in some cases they form separate species, while in others, differentiation is governed by phenotypic plasticity or heritable variation (Vermeij 1974; Johannesson 2003).

Selection for local adaptation is strong: *L. saxatilis* ecotypes survive approximately three times better in their optimal habitat, as do intermediates in intermediate habitat (Janson 1983). Several traits, such as growth rate, aperture size, shell thickness, spire height, maturation time, aggregation behaviour and reproductive rate, appear to be under differential selection between ecotypes (Janson 1982, 1983; Johannesson & Johannesson 1996; Hollander *et al.* 2006a). The genetic basis of these traits is unknown and they are unlikely to be independent. Phenotypic plasticity explains some differentiation between ecotypes, but most of the relevant variation is genetic (Johannesson & Johannesson 1996; Hollander *et al.* 2006b). Likewise, the cause of population density asymmetry between ecotypes is unknown. Sheltered-habitat snails are approximately four times larger and one-fourth as numerous compared with exposed-habitat snails, implying that differential resource use may explain the asymmetry.

Swedish *L. saxatilis* ecotypes mate assortatively. Males prefer mates with similar shell size and shape and mucous trail, leading to an ecotype-based assortative mating isolation index of 0.6 (that is, a 60% over-representation of homomorphic pairs in laboratory trials; Hollander *et al.* 2005; Johannesson *et al.* 2008).

Habitat patches occupied by the ecotypes form a fine mosaic along the shore (Janson 1983) alternating up to 10 times in 2 km. Patches are linked by narrow (4–10 m wide) zones where ecologically intermediate individuals have superior survivorship (Janson 1983). Because *L. saxatilis* lacks a planktonic larval stage, its dispersal distance is normally extremely short (1–4 m in a lifespan; Janson 1983). Rarely, individuals are dispersed long distances by wave action and rafting, allowing colonization of distant islands (Johannesson & Johannesson 1995). This dispersal pattern results in spatial genetic structure both within and between islands, demonstrated by allozyme and RAPD (random amplified polymorphic DNA) data (Johannesson & Tatarenkov 1997; Johannesson *et al.* 2004).

The exposed ecotype is believed to be ancestral because wave action is greater in exposed habitat, and cliffs appeared before boulder shores during postglacial uplift of the islands. Sheltered ecotypes could have arisen in several ways. First, they may have diverged in parapatry within islands. Alternatively, they may have arisen in isolation from the exposed ecotype on one or more different islands, later hybridizing until all trace of their independent origins has been lost (Grahame *et al.* 2006). There are empirical and theoretical reasons to believe that the ecotypes arose in parapatry within islands. Empirically, microsatellite alleles are more divergent between islands than between different ecotypes on the same island (Panova *et al.* 2006). Under an allopatric origin it is unexpected that differences in neutral markers between ecotypes within islands would be unique to each island (unless there was introgression of alleles under positive selection, for which there is no evidence). Theoretically, the allopatric hypothesis is likely only if gene flow prevents parapatric ecotype formation within islands. Given dispersal distances of 1−4 m and habitat patches ~200 m wide, a population founded in the sheltered habitat would expand into adjacent exposed habitat on the same island within at most 25 generations. Consequently, any new population would soon face the possibility of parapatric divergence, regardless of where its founder arrived. Rate of spread between islands is expected to be low based on the rafting mechanism and observed spatial genetic structure (Johannesson & Johannesson 1995; Johannesson & Tatarenkov 1997; Johannesson *et al.* 2004). Thus, unless parapatric divergence is strongly inhibited by gene flow, it is almost certainly the source of the ecotypes.

Ecotype formation in parapatry

Theories of spatial genetic variation have an extensive history, originating with Haldane's (1930) single-locus island model and later considering the spread of adaptive alleles in continuous space and cline formation across habitat boundaries (Fisher 1937, 1950; Haldane 1948; Bazykin 1972). Slatkin (1973) described the characteristic length of a single-locus cline, showing that selection strength and dispersal distance are crucial for cline formation. Later studies considered the behaviour of multilocus clines, concentrating on the resultant linkage disequilibria, cline shape, and barriers to neutral and adaptive gene flow (Slatkin 1975; Barton 1983, 1999; Barton & Gale 1993). Hard selection restricts conditions for ecotype evolution: if selection is too strong, successful colonization of alternative habitat becomes unlikely and selection merely stabilizes the pre-existing ecotype, while if selection is too weak, generalists evolve (Gavrilets & Vose 2007; Gavrilets *et al.* 2007). Together, these models imply that locally adapted ecotypes can evolve in parapatry, provided dispersal distance is short and ecological selection is neither too strong nor too weak (see also Endler 1977; Harrison 1990; Gavrilets 2004).

Bounded hybrid superiority has been suggested as a factor in the maintenance of hybrid zones in various taxa, such as northern flickers (*Colaptes auratus*; Moore & Buchanan 1985), big sagebrush (*Artemisia*; Wang *et al.* 1997), gulls (*Larus*; Good *et al.* 2000) and *Prunella* (Fritsche & Kaltz 2000). A zone of hybrid superiority may act as a stepping stone, allowing populations to evolve that are intermediately adapted and positioned, which eventually spread to the alternative habitat patch. Thus, colonization of the alternative habitat may be possible with relatively strong selection when a zone of hybrid superiority is present, and ecotype formation may be facilitated.

However, the zone of hybrid superiority might also inhibit speciation because selection may favour local gene flow between ecotypes at the zone of interaction. A two-allele stepping-stone model of bounded hybrid superiority at a single locus was analysed by Goodisman & Crozier (2001). Overdominance within the hybrid zone resulted in a flattening of the middle of the genetic cline. Linkage disequilibria at neutral loci were negligible, suggesting that bounded hybrid superiority provides little barrier to gene flow. This result contrasts with findings for tension zones (hybrid zones maintained by hybrid disadvantage), which constrain the spread of neutral variation while allowing adaptive alleles to introgress (Barton & Bengtsson 1986; Gavrilets & Cruzan 1998).

Nonrandom mating and speciation

Hendry *et al.* (2007) reviewed findings on the evolution of reproductive isolation in the context of local adaptation and immigration. In general, models indicate that partial reproductive barriers in the form of local adaptation, reduced dispersal and habitat preference may evolve rapidly when different habitats impose divergent selection. Such partial isolation is evident in many systems, including plants, invertebrates, birds and fish. However, it remains unclear whether ecotypes consistently progress to speciation (Hendry *et al.* 2007). The reproductive barriers that evolve most rapidly are associated with spatial or temporal separation due to habitat differences. Should habitat distribution or selection change, such barriers may dissolve. One mechanism that could lead to speciation of ecotypes is the evolution of assortative mating.

The role of assortative mating in speciation has long been controversial (Dobzhansky 1940; Spencer *et al.* 1986; Noor 1999; Servedio 2000). Models show that under some conditions, prezygotic isolation can evolve in sympatric populations subject to disruptive ecological selection (selection against intermediate phenotypes) (Lande & Kirkpatrick 1988; Via 2001; Gavrilets 2004). Speciation without geographic isolation may be especially likely when mate choice is based on ecological traits (often termed a 'magic trait' scenario; Gavrilets 2004). In a recent numerical model of ecological speciation in cichlids, Gavrilets *et al.* (2007) found that assortative mating sometimes evolved within the ancestral niche, resulting in the formation of sexual morphs. One of these sexual morphs then adapted to an alternative niche and assortative mating later strengthened, leading to speciation. This sequence of events occurred when selection was strong and the number of loci governing assortative mating was small. More generally, previous models suggest that increased assortative mating of locally adapted parapatric populations evolves when immigration is high, ecological selection is strong, and loci have large effects on adaptation and mate choice (see review by Gavrilets 2004).

A much-contested view is that prezygotic isolation evolves in response to selection against hybridization, thus completing speciation (Dobzhansky 1940; see reviews by Spencer *et al.* 1986; Noor 1999; Servedio & Noor 2003). This process is generally termed reinforcement, regardless of whether hybrid disadvantage is due to local adaptation or intrinsic incompatibilities (see, for example, Servedio & Kirkpatrick 1997; Cain *et al.* 1999; Kirkpatrick & Servedio 1999; Servedio 2000; Kirkpatrick 2001). Selection against hybridization could occur in *L. saxatilis* ecotypes in sheltered and exposed habitats, but may be opposed by selection favouring intermediates in intermediate zones. Reinforcement is distinct from local adaptation of mating cues, which may play a larger role in prezygotic isolation (Servedio 2004). However, many models do not distinguish the two effects (Servedio & Kirkpatrick 1997; Cain *et al.* 1999; Servedio 2000; Kirkpatrick 2001). In *L. saxatilis*, as in these models, locally adapted traits form mating cues, so their results are relevant.

Models of mate choice evolution have used widely varying spatial assumptions, ranging from sympatry (e.g. Liou & Price 1994) to island (Servedio & Kirkpatrick 1997; Kirkpatrick & Servedio 1999; Kirkpatrick 2000, 2001; Servedio 2000, 2004, 2007), clinal (Lande 1981) and lattice-based mosaic models (Cain *et al.* 1999). Some of these models required weak selection (Kirkpatrick & Servedio 1999; Kirkpatrick 2000, 2001), a restrictive assumption (Servedio 2004). Servedio & Kirkpatrick (1997) suggested that prezygotic isolation evolves less easily with one-way than with two-way gene flow; however, this effect depends on whether mate choice is similarity-based (females prefer males who share their own phenotype) or matching-based (displays and preferences are separately determined) (Servedio 2000). These models used soft rather than hard selection, implying that selection did not impact population densities. Ability to colonize alternative habitat may restrict parapatric ecological speciation (Gavrilets & Vose 2007; Gavrilets *et al.* 2007), so hard selection is important for realism with *L. saxatilis*. Speciation models have commonly used absolute preferences, where individuals prefer a specific level of a trait. Preferences may also be open-ended, with extreme traits generating more extreme responses, due to sensory bias or sexual selection (Panhuis *et al.* 2001). In *L. saxatilis*, mate choice is primarily by males; Servedio (2007) used island and two-island models to show that reinforcement could occur through evolution of male mate choice.

The short dispersal distance of *L. saxatilis* implies that local gene flow may be influential for mate choice as well as ecology. Several models have examined how mate choice and reproductive isolation co-evolve on ecological gradients, using cline theory as a basis (Haldane 1948; Fisher 1950; Bazykin 1972; Slatkin 1973, 1975; Lande 1981). A spatially explicit lattice-based model by Cain *et al.* (1999) showed that mosaic hybrid zones facilitated the evolution of assortative mating based on ecological traits. Yukilevich & True (2006) used the two-island model to examine the co-evolution of habitat fidelity and assortative mating and showed that while migration modification was possible with strong selection, assortative mating evolved faster and more generally.

Bridging theory and data to model *L. saxatilis*

The spatial structuring seen in Swedish *L. saxatilis* is not an island, gradient or ordinary mosaic, but discrete habitat patches separated by narrow zones of bounded hybrid superiority. Analytical models show that bounded hybrid superiority does not provide a substantial barrier against neutral gene flow (Goodisman & Crozier 2001), potentially weakening assortative mating. However, microsatellite data indicate strong barriers to introgression between ecotypes in *L. saxatilis*, potentially providing a basis for mate choice divergence, especially if mate choice is directly influenced by ecological traits that are under strong differential selection on opposite sides of the hybrid zone.

The zone of hybrid superiority complicates the question of hybrid disadvantage and reinforcement in *L. saxatilis*. Because survival of the ecotypes in inappropriate habitat is small and dispersal between habitats is limited (Janson 1983), opportunities for mismating are rare. Ecological intermediates are disadvantaged in ecotype habitats, potentially driving reinforcement within sheltered and exposed patches. But in the intermediate zone, intermediate individuals are favoured, possibly selecting for disassortative mating. Evolution of mate choice in this scenario has not previously been modelled.

The genetics of mate choice in *L. saxatilis* are unknown; although we know that assortative mating occurs, it is unclear whether it is similarity-based, matching-based, absolute or open-ended. For this reason, we investigated each of these mate choice systems using models based on those proposed by Lande (1981) and further developed by Gavrilets (2004) and Gavrilets *et al.* (2007).

Our model had several aims. First, we strove to better understand the *L. saxatilis* system. Specifically, this included investigating (i) whether gene flow prevents ecotype formation in parapatry; (ii) whether selection against hybridization could have driven the evolution of prezygotic isolation between *L. saxatilis* ecotypes; and (iii) what conditions would favour or prevent evolution of the ecotypes and speciation.

Second, we aimed to understand more generally the interactions of spatial dynamics, mate choice evolution, sexual and natural selection in an ecologically and behaviourally realistic model. In particular, we aimed to explore the diversity of outcomes possible: are ecotype formation and speciation a foregone conclusion in *L. saxatilis*-like systems, or can slight changes in conditions lead to an entirely different fate? We also sought to examine the relationship between ecotype formation and speciation, investigating whether ecotypes lead inevitably to speciation or are themselves a persistent state.

Because we do not have complete empirical data about *L. saxatilis*, we tested the simulation for sensitivity to a range of parameter values and scenarios. All parameter values were chosen because they are plausible for *L. saxatilis*. In several cases, we explored multiple parameter values, because there is insufficient empirical data to say which is correct. Clearly, only one of the scenarios examined can be correct for *L. saxatilis*, but current empirical data does not allow us to choose between them or make any statement about their relative probability. When conditions are found where model results consistently deviate from empirical data, this suggests that these conditions are not applicable in nature. In this way, model results can be used to guide empirical research.

Model

The model was a spatial individual-based simulation similar to a previous model of cichlid speciation (Gavrilets *et al.* 2007). Individuals were assumed to reach reproductive maturity at 3 months and breed continuously until they died at 9 months, giving a mean generation time of 6 months (Janson 1982) represented by one model iteration. For simplicity, generations were discrete and nonoverlapping. Events occurred in the following order: birth, viability selection, mating, dispersal, reproduction and death. The simulation was written in C++. Pseudorandom numbers were generated using the mt19937 Mersenne Twister (Matsumoto & Nishimura 1998) and an algorithm by Vose (1991).

Individuals

Individuals were diploid with separate sexes and had three genetic traits. The ecological phenotype was represented as a single additive trait, x, which could also influence mating behaviour (termed a 'magic trait' model; Gavrilets 2004). Two other traits, termed f and c, could also influence mate choice. Trait f specified the ecological phenotype of females preferred by males, while trait c determined the strength and direction of mating preference (mating discrimination).

Each trait was determined by L additive independent diallelic loci with equal effect size. All traits were scaled between 0 and 1. Mutation rate was $\mu_t = 10^{-5}$ for trait loci. In addition, eight neutral microsatellite loci were simulated with stepwise mutation rate $\mu_m = 10^{-3}$ to assess the barrier to gene flow between ecotypes.

Spatial organization

One population was simulated as a one-dimensional array of demes: each deme represented 3 m of shore and $n = 128$ demes were simulated with looped boundaries (like the shore of an island). We simulated only one dimension because relevant traits for the ecotypes are vertically homogeneous (Johannesson & Johannesson 1995). Dispersal was usually local. Individuals dispersed 0, 1 or 2 demes in either direction with 60%, 30% and 10% probability respectively (values based on a mean movement rate of 1 m per month over 9 months in a random walk). In addition, there was a likelihood p_l of a long-distance dispersal event that moved the individual to a random location. Each deme had a habitat type: sheltered, exposed or intermediate. One patch of exposed and one patch of sheltered demes were simulated, and separated at each end by intermediate zones of width s_i. Each patch consisted of $s_h = 64$ adjacent demes of the same habitat.

Selection

The survival of individuals depended on the match between their ecological phenotype and the optimum phenotype in the patch where they were born (hard selection). Fitness w of an individual with ecological phenotype x was given by

$$\omega = \exp\left[-\frac{(x-\theta)^2}{2\sigma_s^2}\right],$$

where θ was the optimum phenotype. Within habitats, we used $\theta = 0$, 0.5 and 1 to specify optimum phenotypes within exposed, intermediate and sheltered habitats respectively. Parameter σ_s controlled the strength of selection by modulating the width of the distribution of surviving phenotypes. Smaller values thus indicate stronger selection. Empirical data show that locally maladapted individuals have survivorship ~30% of that of locally adapted ecotypes. For this reason, fitness w was scaled between 0.3 and 1. The relative survival values given by Janson (1983) can be approximated if $\sigma_s \leq 0.14$ (see Fig. S1).

Density-dependent population regulation (not selection) is relevant for the exposed ecotype, whose populations are limited by availability of cracks that protect from wave action. Although density dependence in the sheltered ecotype is less clear, limited food availability may be influential. Overall population density in the exposed habitat is approximately four times greater than in the sheltered habitat, but the reasons for this are uncertain: it may be due to characteristics of individuals or their habitat. We explored both scenarios. Because larger body size is a major component of adaptation to the sheltered habitat, for most experiments we assumed that individual resource use was proportional to ecological trait value x, with individuals with $x = 1$ using four times the resources used by individuals with $x = 0$. We term this scenario asymmetric resource use. We also considered an alternative scenario, in which carrying capacity was determined not by individual ecological traits, but by the habitat itself. In this case, termed asymmetric resource availability, carrying capacity K in sheltered demes was set to one-fourth of its value in exposed demes, but individuals used the same resources regardless of ecological trait values.

To determine offspring survival, we calculated a carrying capacity for the deme associated with each phenotype, $K = K_0 \times w$, where K_0 gives the maximum carrying capacity. Offspring viability, v, was then determined using the Beverton–Holt model (e.g. Kot 2001), which is influenced by the total volume of local juveniles N, the mean number of offspring of each individual b and the carrying capacity K:

$$\upsilon = \frac{1}{1 + (b/2 - 1)\frac{N}{K}}$$

Reproduction

For each surviving male, a Poisson-distributed number of offspring with mean $b = 50$ was generated. This parameter value was chosen to reflect laboratory observations (mean production of 0.3 to 0.9 offspring per female per day over a ~90-day reproductive life; K. Johannesson, unpublished data). Males, rather than females, determined reproductive rate because it was computationally convenient for offspring number to be determined in the same process as mate choice (which is performed by males in *L. saxatilis*). The variance in reproductive success among male and female *L. saxatilis* is unknown, and mean male and female reproductive success is equal, so this method entailed no loss of generality. All males mated so that there was no cost of choosiness.

The mother of each offspring was chosen by randomly sampling females in the same deme and accepting them with probability ψ. In addition to random mating, we looked at three nonrandom mating systems. Probability of mating was influenced by the three phenotypic traits, x (ecological trait of female), f (ecological trait preferred by male) and c (mating discrimination trait).

Mating preferences

The relative probability of mating between a male with traits f and c and a female with trait x was

$$\psi(x,f,c) = \begin{cases} \exp\left[-(2c-1)^2 \frac{(f-x)^2}{2\sigma_{\mathrm{a}}^2}\right], & \text{if } c<0.5, \\ 1, \quad \text{if } c = 0.5, \\ \exp\left[-(2c-1)^2 \frac{(f-(1-x))^2}{2\sigma_{\mathrm{a}}^2}\right], & \text{if } c<0.5, \end{cases}$$

where parameter σ_{a} scales the strength of male mating preferences. This equation, based on that of Lande (1981) and further developed by Gavrilets *et al.* (2007), implies that more extreme c phenotypes create stronger preferences. Males with $c = 0.5$ mated randomly. Trait f determines the value of the female ecological trait (x) preferred by a male. Trait c generates a preference for x similar to f when $c > 0.5$, and for x dissimilar to f when $c < 0.5$. A useful measure of the amount of mating discrimination is C, the deviation of the c trait from 0.5, which we define as $C = |2c - 1|$ (see also Gavrilets *et al.* 2007). See Supporting Information for further explanation and mating probability distributions generated using this function (Fig. S2).

The three nonrandom mating systems were specified as follows.

Matching. Traits x, f and c are all independent traits. This means that males can evolve to prefer females with large or small x, regardless of their own x. For example, a combination of either small f with $c < 0.5$ or large f with $c > 0.5$ trait values would generate positive assortative mating in the sheltered ecotype (which has large x), but negative assortative mating in the exposed ecotype (small x). Conversely, either large f with $c < 0.5$ or small f with $c > 0.5$ generates positive assortative mating in the exposed ecotype but negative assortative mating in the sheltered ecotype.

Similarity. Traits x and c are independent traits, but mating preference, f, is the x trait. This has the effect that males with $c > 0.5$ prefer mates who are ecologically similar to themselves (positive assortative mating), while males with $c < 0.5$ prefer mates who are ecologically dissimilar (negative assortative mating).

Open. Traits x and c are independent traits, while f has a fixed value of one. Males with $c > 0.5$ prefer mates who have the maximum possible x, whereas males with $c < 0.5$ prefer mates who have the minimum possible x. Functionally, this model closely resembles previous models of open-ended preference (e.g. Gavrilets 2004, p. 321). Under this model, positive assortative mating results when $c > 0.5$ in the sheltered ecotype and when $c < 0.5$ in the exposed ecotype; negative assortative mating results when $c < 0.5$ for sheltered and when $c > 0.5$ for exposed ecotypes.

Initial conditions

Our experiments examined evolution of the ecotypes on a single shore after a founder event where a patch of exposed habitat was colonized by the offspring of a single female adapted to exposed habitat (initial population size = 50). We chose these conditions because the most likely colonization event is the arrival of a gravid female of the exposed ecotype (Johannesson 1988). Initial populations were genetically uniform, with intermediate phenotypes for the c trait (initially random mating) and zero for the x trait (adapted to exposed habitat). The f trait was initially zero (that is, preference for the exposed ecotype) or 0.5 (intermediate preference). Mate choice in ancestral *L. saxatilis* is unknown, but some degree of size-assortative mating may have been present initially due to mechanical constraints and appears to be ancestral within the clade. Consequently, we compare both initially random mating ($i_c = 0.5$) and weak initial positive assortative mating ($i_c = 0.5 + 1/L$, where L is the number of loci influencing each trait).

Parameters

The simulation was run for 20 000 generations (based on a 6-month generation time and age of islands <10 000 years; Panova *et al.* 2006). Trait distributions were recorded every 100 generations; unless otherwise stated, results presented are for final-state data. Twenty replicates were completed in each experimental condition unless otherwise stated. A small number of replicates were run for 40 000 generations to assess long-term outcomes with standard conditions.

Parameters and their values are summarized in Table 1.

We aimed to duplicate the conditions under which ecotypes arose in *L. saxatilis*, while testing for sensitivity to the unknown parameters L (number of loci), σ_{s} (selection strength) and σ_{a} (accuracy of signal discrimination). We also examined the effects of long-distance dispersal probability p_{l}, initial mating traits i_c and i_f, and mate selection system, looking at systems with random, absolute similarity-based, absolute matching-based and open-ended preferences. Finally, we compared ecological scenarios where population density asymmetry between the ecotypes was caused by asymmetric resource use by snails of different ecotype (larger snails consume more resources) vs. asymmetric

Table 1 Parameters and their values in experiments

Parameter	Description	Values
L	Numbers of loci influencing traits	2, 4, 8*, 16
σ_s	Ecological selection (small σ_s = strong selection)	0.02, 0.08*, 0.14
σ_a	Variance in mate selection	0.05*, 0.1, 0.15
s_i	Intermediate zone width (in demes)	0, 4*, 8
p_l	Long-distance dispersal probability	10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}
i_c	*c*-trait phenotype of founders	0.5*, 0.5 + 1/L
i_f	*f*-trait phenotype of founders	0.0*, 0.5
Choice	Mate choice system	Random, matching, similarity, open
Model	Cause of population density asymmetry	Resource use*, resource availability
b	Mean offspring per male	50
K_0	Carrying capacity	1280
s_h	Habitat patch sizes	$64s_i$
n	Number of demes	128
μ_t	Mutation rate for trait loci	10^{-5}
μ_m	Mutation rate for microsatellite loci	10^{-3}

*Default values.

resource availability (resources were scarcer in the sheltered habitat).

Theoretical results

Qualitative results

Simulation outcomes can be broadly classified by the occurrence or failure of ecotype formation. That is, in some scenarios, populations in the sheltered habitat evolved ecological trait values adapted to that habitat; in others, populations in the sheltered habitat remained adapted to the exposed habitat. Intermediate outcomes (where populations were partially or temporarily adapted to the sheltered habitat) were rare. We considered populations adapted to the sheltered habitat if the mean value of the x trait in the sheltered habitat was >0.75. In most scenarios, ecotype formation occurred consistently or not at all, but in a few, the outcome was unpredictable. The co-evolution of traits across space and time in several key scenarios is shown in Fig. 1.

The most common outcome observed was that positive assortative mating co-evolved with ecotypes and was maintained until the simulation ended, but did not necessarily strengthen over time. Such a typical simulation run is shown in Fig. 1A. It can be seen that the x trait increased first within the intermediate zones, reaching its optimum value of around one-half within 400 generations. Populations then began to spread into the sheltered habitat. In the sheltered habitat, the c trait began to decline, indicating the evolution of preference for individuals with large x traits (because f was small). By 500 generations, the x trait had reached its maximum in the sheltered habitat, with a steep cline across the intermediate zones to zero in the exposed habitat. Simultaneously, the reduced c trait spread throughout the sheltered habitat, implying that positive assortative mating had evolved in the sheltered ecotype. However, random mating persisted in the exposed habitat. Moreover, rather than continuing to strengthen as the reinforcement hypothesis proposes, assortative mating later weakened, as indicated by the c trait regressing towards 0.5.

Notably, evolution of mating preferences was commonly asymmetric between ecotypes, with stronger preferences occurring in the sheltered ecotype than in the exposed ecotype. In Fig. 1A for example, the c trait deviates strongly from 0.5 in the sheltered, but not the exposed habitat. This effect was presumably due to the smaller relative population size in the sheltered ecotype resulting in strong selection against hybridization. In similarity-based and open-ended mate choice scenarios, symmetric evolution of assortative mating was seen when the number of loci involved was intermediate (L = 4–8; Fig. S10).

Although evolution of ecotypes with assortative mating was the most common outcome, very different results were possible in specific conditions. Several of these possibilities are shown in the remaining panels of Fig. 1 and described below.

Mating preferences for the other ecotype evolved in one scenario with population density asymmetry generated by resource use (Fig. 1B). This occurred when mating was similarity-based, selection was weak (σ_s = 0.14), the intermediate zones were wide (s_i = 8) and four loci regulated mate choice (L = 4). Ecotypes evolved rapidly in this scenario and persisted throughout the simulation. Visual inspection of the data (Fig. 1B) shows that mating preference was most extreme around the intermediate zones, suggesting that its evolution was driven by spatially localized sexual selection favouring hybridization.

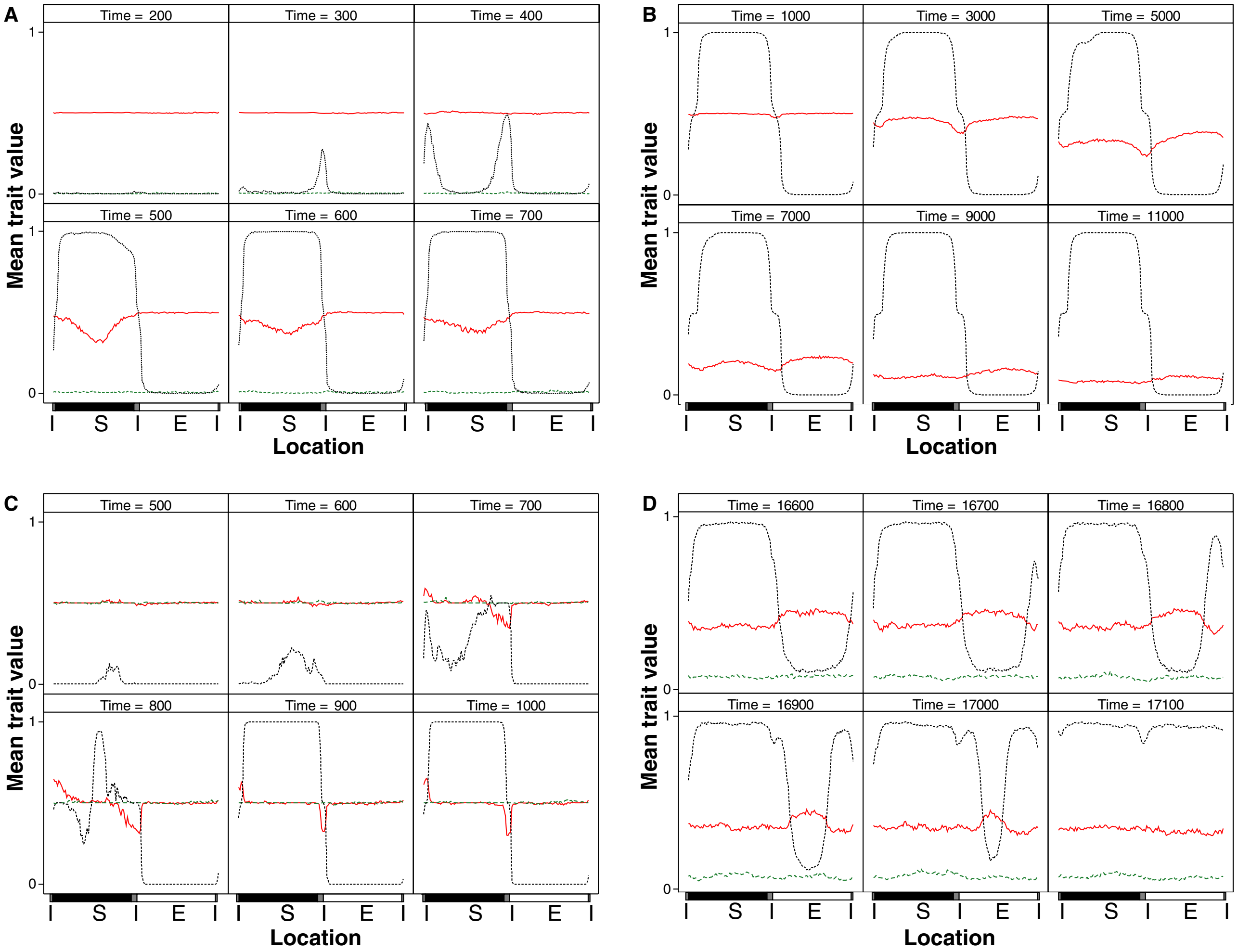

Fig. 1 Examples of spatial genetic evolution in different conditions. Panels indicate time intervals with generation number indicated above each panel. Location is represented on the *x*-axis. Within graphs, the locations on the left side are sheltered habitat (black, marked S) and those on the right are exposed (white, marked E), separated by intermediate zones at the centre and ends (grey, marked I). Mean values of the ecological trait *x* (..........), preference trait *f* (–––) and the mating discrimination trait *c* (———) at each location are shown on the *y*-axis. Deviation from 0.5 in the *c* trait indicates nonrandom mating. Nondefault conditions or parameter values are given in braces below. (A) Ecotype formation accompanied by evolution of assortative matching-based mating in the sheltered ecotype. Assortative mating in this case results from the combination of large *x*, small *f* and *c* below 0.5. (B) Ecotype formation followed by the evolution of negative assortative mating (generated by $c < 0.5$ with similarity-based mating) (conditions: $\sigma_s = 0.14$, $s_i = 8$, $L = 4$). (C) Ecotype formation accompanied by the evolution of assortative mating that is later lost outside the intermediate zones (conditions: matching-based mating, $i_f = 0.5$). (D) Ecotype formation followed by the sheltered ecotype driving the exposed ecotype to extinction (conditions: resource availability asymmetry, matching-based mating, $L = 16$, $\sigma_s = 0.14$).

In other cases, assortative mating evolved but was later confined to intermediate zones (Fig. 1C). This was a common outcome when the *f* trait was initially intermediate ($i_f = 0.5$). First, the sheltered ecotype evolved with assortative mating as seen in Fig. 1A. However, at each end of the sheltered habitat, assortative mating was generated by different trait combinations (large *c* and *f* at the left margin, and small *c* and *f* on the right margin). These different mate choice systems spread into the sheltered habitat, met and recombined. The recombination generated individuals who mated disassortatively or at random (by combining large *c* and small *f*, or vice versa, and by producing individuals with intermediate *c*). As a result, assortative mating was ultimately lost within the sheltered habitat, but maintained at habitat boundaries. This finding duplicates a commonly observed natural pattern where assortative mating is stronger at parapatric borders than in more central populations (Jiggins & Mallet 2000).

Where population density asymmetry was generated by resource availability rather than resource use, asymmetry in mating preferences was more extreme. If selection was weak and the sheltered ecotype mated assortatively while random mating persisted in the exposed ecotype, the sheltered ecotype often invaded the exposed habitat and drove the exposed ecotype to extinction through sexual selection (Fig. 1D). This occurred in some runs when habitats were asymmetric, mate selection was open-ended or matching-based and the *f* trait was initially zero, selection was weak and the number of loci involved was large. First, sheltered ecotype evolved with assortative mating ($c < 0.5$). However, the exposed ecotype continued to mate at random ($c = 0.5$). Then the sheltered ecotype gradually spread across the hybrid zone, maintaining its mating preferences and eliminating the exposed ecotype as it spread.

Parameter effects

More detailed results are presented in Figs S3–S10.

Ecotypes evolved under a wide range of conditions in the model (Fig. 2). Provided that ecological selection was comparatively weak, ecotypes evolved rapidly even if mating was random. With strong selection, ecotype formation could be facilitated by wide intermediate zones and loci of large effect. Nonrandom mating also strongly facilitated ecotype formation in most conditions. However, accurate discrimination among signals was required for the evolution of nonrandom mating ($\sigma_a = 0.05$); results when $\sigma_a \geq 0.1$ were indistinguishable from those found with random mating (see Fig. S3. This result agrees with findings from similar previous models (Gavrilets *et al.* 2007; Gavrilets & Vose 2007).

We measured the timing and frequency of ecotype formation. For runs where ecotypes formed, we measured the strength of mating discrimination *C*. We also measured the level of genetic divergence between the ecotypes (F_{ST}, calculated as per Michalakis & Excoffier (1996)) every 100 generations. Although mating preference evolution was most strongly influenced by the mate choice system and number of loci, divergence between ecotypes was most influenced by the width of the intermediate zones and selection strength.

Ecotypes usually formed rapidly, within <1000 generations. Figure 3 shows the overall frequency distribution of ecotype formation time, grouped by its most influential factors, which were selection strength and intermediate zone width (see Fig. S4, for complete data). Ecotypes formed relatively slowly when selection was strong ($F_{2,1506} = 37.00$, $P < 0.001$), the intermediate zones were narrow ($F_{2,1506} = 24.39$, $P < 0.001$) or the number of loci involved was large ($F_{3,1506} = 11.18$, $P < 0.001$). In most conditions, mate choice system did not clearly influence ecotype formation time ($F_{3,1506} = 0.97$, $P > 0.05$). The timescale for ecotype formation observed here is of similar order to that observed in previous speciation models such as in study by Gavrilets & Vose (2005), and is congruent with evidence for the rapid origin of partial reproductive barriers in a range of taxa (see review by Hendry *et al.* (2007)).

A key question in speciation theory is the relation between assortative mating and the cessation of gene flow. To address this question, we examined the relationship between the evolution of mating discrimination and genetic divergence in scenarios where assortative mating evolved (Fig. 4; see also Fig. S5). We found that mating discrimination and F_{ST} were correlated across experimental conditions (pooled data: $R^2 = 36.3\%$, $P < 0.001$) and F_{ST} was often higher in scenarios with any form of nonrandom mating than when random mating was enforced. Moreover, scenarios where

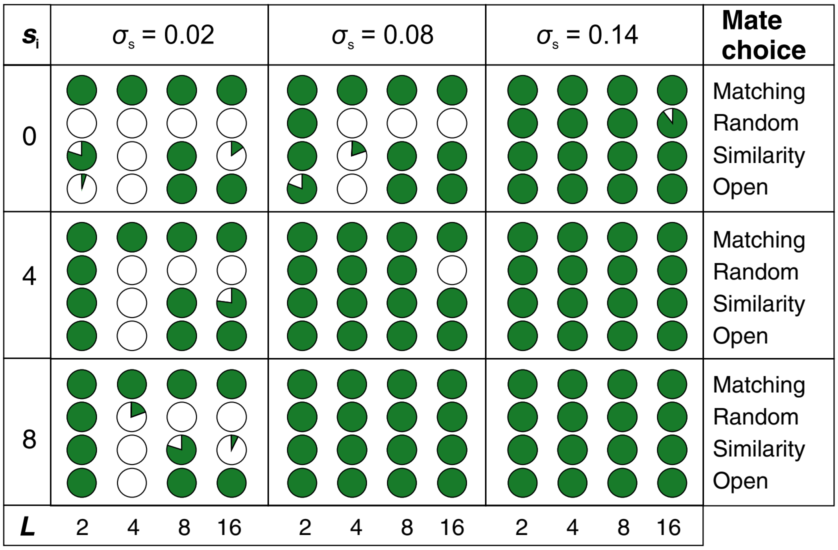

Fig. 2 Pie charts showing the proportion of simulations runs where ecotypes formed (shaded) in each set of conditions. The *x*-axis panels indicate selection strength σ_s (lower values indicate stronger selection) and number of loci *L*, while the *y*-axis panels show intermediate zone width s_i and mate choice system. Ecotype formation occurred under broad conditions and was facilitated by nonrandom mating, wide intermediate zones and weak selection.

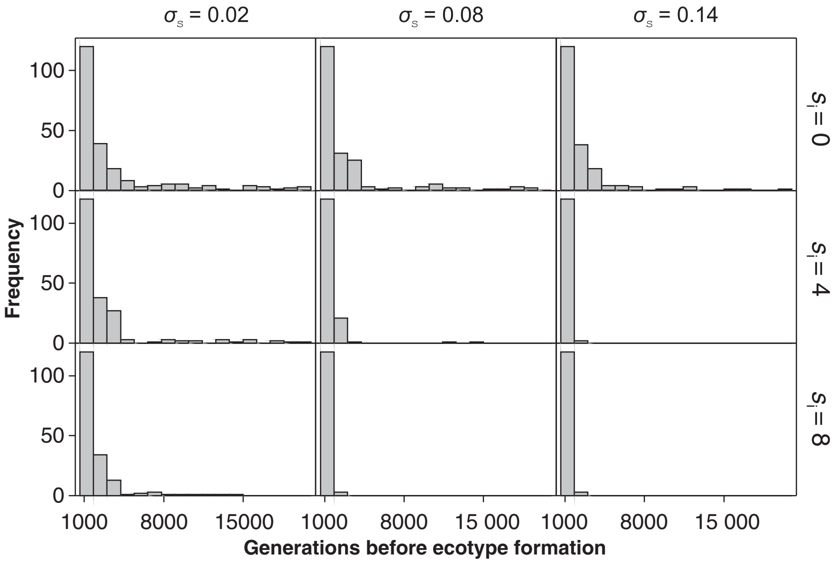

Fig. 3 Frequency distribution for ecotype formation time. Panels indicate selection strength σ_s (lower values indicate stronger selection) and intermediate zone width s_i (the two most influential parameters).

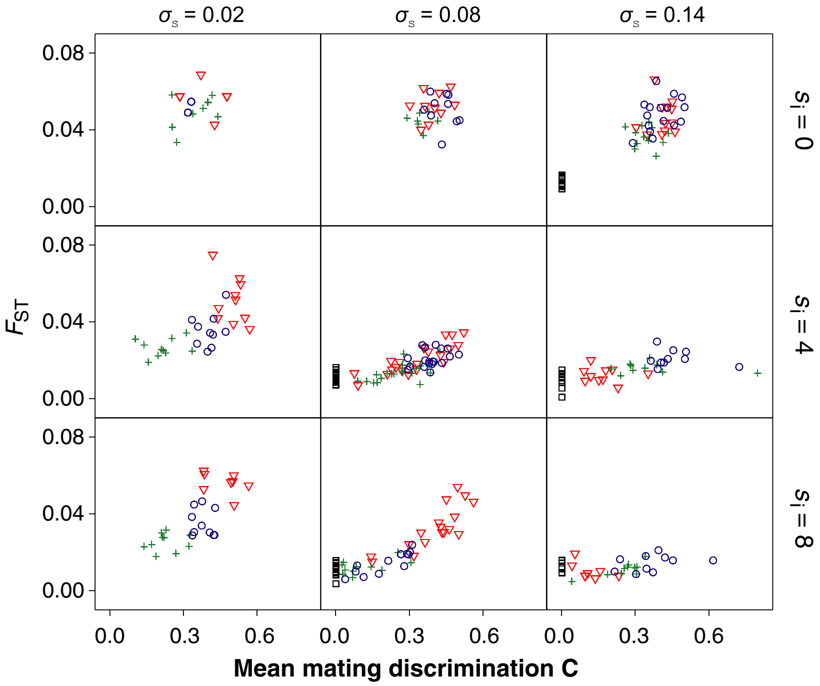

Fig. 4 The relationship between mating discrimination *C* and genetic divergence F_{ST} of ecotypes. Symbols show mate choice system (+ = matching; O = open; Δ = similarity; ☐ = random). *C* was by definition zero with random mating. Scenarios with higher *C* often had higher F_{ST}, indicating effective prezygotic isolation. However, within scenarios, correlations between *C* and F_{ST} were often negligible.

greater assortative mating evolved often also had higher F_{ST}. However, within scenarios, the relationship between F_{ST} and mating discrimination was commonly nonsignificant. Thus, we found only limited evidence for assortative mating directly reducing gene flow. This result is congruent with findings from other recent models of ecological speciation (Gavrilets & Vose 2007; Gavrilets *et al.* 2007).

Extending runs to 40 000 generations, we found that the rate of increase in both F_{ST} and mating discrimination declined over time and for most scenarios plateaued ~20 000–30 000 generations. Mean maximum F_{ST} values usually remained below 0.05. This plateau effect in F_{ST} is expected because absolute reproductive isolation is not possible within the model (males always mate). However, the failure to evolve strong mating preferences in most scenarios suggests that speciation may by unlikely in this system.

Figure 5 shows main effects plots for mating discrimination *C* and genetic divergence of the ecotypes (F_{ST}). Analysis of variance using a general linear model indicated significant main effects of all parameters but no

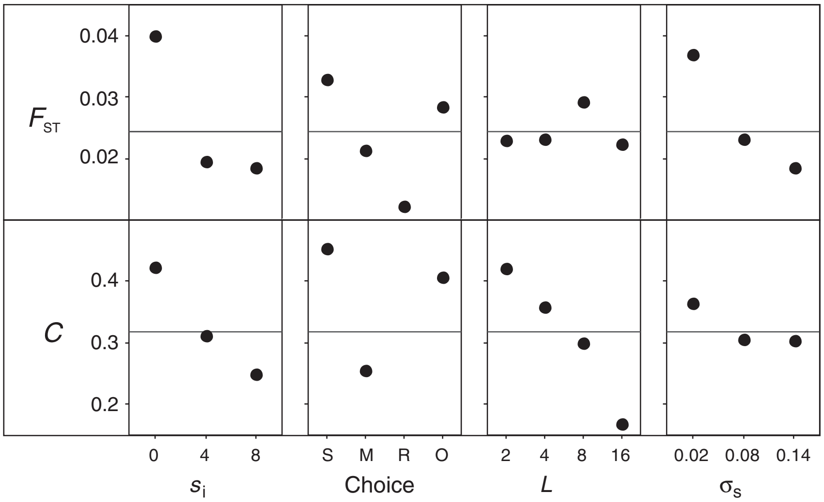

Fig. 5 Data means for genetic divergence F_{ST} and mating discrimination C. Plots show mean final-states of F_{ST} and C for each level of s_i, mate choice, L and σ_s. Within graphs, data are pooled across levels of other independent variables and a reference line indicates the grand mean (F_{ST}: 0.0244; C: 0.280). Analysis of variance found no significant interactions. See Supporting Information for ANOVA tables.

significant interactions (details in Supporting Information). Mate choice system and number of loci were most influential for mating discrimination ($F_{2,2654} = 167.53$, $F_{3,2654} = 221.14$, $P < 0.001$), while intermediate zone width s_i, selection strength σ_s and mate choice system were the strongest determinants of gene flow ($F_{2,2877} = 1294.41$, $F_{2,2877} = 718.1$, $F_{2,2877} = 530.93$ respectively; $P < 0.001$). Figures S6 and S7 show the evolution of F_{ST} and mating discrimination over time respectively.

Selection strength σ_s and number of loci L

Ecotype formation occurred most rapidly and frequently with relatively weak ecological selection ($\sigma_s > 0.08$): strong selection could sometimes prevent colonization of the intermediate habitat within the time frame of the simulation. However, once ecotypes had formed, stronger selection resulted in increased genetic divergence of the ecotypes due to decreased gene flow. Weaker ecological selection was nonetheless associated with increased assortative mating, suggesting that immigration between the ecotypes leading to selection against hybridization was a driving force in mate choice evolution.

In many conditions studied, larger numbers of loci reduced the likelihood and speed of ecotype formation and the extent of assortative mating. F_{ST} was maximal at intermediate numbers of loci. That ecotype formation should occur more easily with relatively weak hard selection and few loci agrees with previous analytical and numerical results (e.g. see review by Gavrilets 2004; Gavrilets *et al.* 2007). When individual loci have small effects, the number of mutations required to survive in alternative habitat is large, so differently adapted populations are unlikely to become established. Similarly, when selection is strong, mutations are removed more rapidly, so the likelihood of crossing a fitness valley is smaller. However, we found that with strong selection and open-ended or similarity-based mate choice, ecotype formation was least probable in four-locus systems (see Fig. 2). This effect results from the specific interaction of sexual and ecological selection in this scenario. When mate choice is influenced by many loci, even if those loci have small effects, chance variations permit the formation of local clusters of individuals with slightly divergent mating preferences; if located around the intermediate zone, these relatively isolated clusters could facilitate local ecological adaptation and, subsequently, ecotype formation.

Width of the intermediate zones s_i

Ecotype formation occurred more rapidly and consistently when a zone of hybrid superiority was present; the width of the zone did not strongly influence ecotype formation. Genetic divergence was greater when the zone was absent; again, zone width did not influence this result. Assortative mating weakened with wider zones, except when mating was similarity-based.

Mate choice

Nonrandom mating simultaneously facilitated ecotype formation and enhanced reproductive isolation. Ecotypes evolved most consistently when mate choice was based on matching traits, and less often in scenarios with similarity-based or open-ended preferences. However, both assortative mating and genetic divergence were usually strong when mate choice was similarity-based or open-ended, and weak when preferences were based on matching traits.

Initial positive assortative mating ($i_c = 0.5 + 1/L$, indicating a weak mating preference) greatly delayed or prevented ecotype formation, even when mate choice was similarity-based (Fig. S8). Indeed, ecotype formation with initial positive assortative mating was both rarer and slower than with random mating. This effect was caused by sexual selection reducing variation in the ecological trait within the exposed habitat. However, once ecotypes formed, genetic divergence and later evolution of mate choice were largely unaffected by initial mate choice state.

In contrast, the initial value of the f trait, i_f, influenced the long-term evolution of assortative mating. When i_f matched the initial ecological trait value ($i_x = i_f = 0$), assortative mating usually persisted and sometimes strengthened with time. However, when i_f was intermediate ($i_f = 0.5$), assortative mating often facilitated ecotype evolution but failed to persist, and random mating ultimately prevailed outside the intermediate zones (Fig. 1B).

Long-distance dispersal p_l

Ecotype formation occurred freely when p_l fell below a threshold frequency (~10% of individuals) and consistently failed when dispersal exceeded this level (see Fig. S9). Empirical data on rafting frequency in *L. saxatilis* suggest it falls well below this threshold (Panova *et al.* 2006). Assortative mating strengthened with increased long-distance dispersal, but genetic divergence between the ecotypes was not significantly affected.

Discussion

We used a spatially explicit, individual-based simulation model to disentangle the interacting processes that influence evolution in *Littorina saxatilis*, generating quantitative predictions for timescales, genetic divergence and mate choice evolution during ecotype formation and speciation. Examining a wide range of empirically plausible scenarios, and analysing sensitivity to unknown parameters, allows us to connect our findings to key predictions from analytical theory, without sacrificing the ecological detail needed to understand the specific evolution of *L. saxatilis*.

Overall, our results show that ecotype formation occurs easily in parapatry under conditions applicable to Swedish *L. saxatilis*, but does not necessarily progress to speciation. This finding is consistent with genetic evidence that ecotypes have arisen in parapatry (Panova *et al.* 2006). Assortative mating within ecotypes evolved commonly in the model, as seen in nature. Gene flow between ecotypes in the model was also comparable with estimates from empirical data. Thus, in general, our results agree well with empirical observations. However, there were a number of counter-intuitive outcomes within particular parameter ranges, and even minor changes in some conditions could inhibit ecotype formation or initiate speciation. These findings highlight the delicate balance of forces at work in the maintenance of ecotypes and the hazards of prediction without quantitative theory. They also suggest that the biology of *L. saxatilis* falls within specific parameter ranges that consistently generate model outcomes similar to those observed in nature, whereas other parameter ranges give different predictions. Our model results matched empirical data when mating was similarity-based, resource use limited population density, selection was relatively weak (within the plausible range for *L. saxatilis*, which still implies strong selection), and the number of loci governing mate choice and ecological variation was intermediate. Thus, the model may help to guide future empirical work.

Next, we discuss the implications of our findings about the interacting processes of ecotype formation, mate choice evolution and speciation, both in *L. saxatilis* and generally.

Ecotype formation

It might be suggested that gene flow could restrict ecotype formation in *L. saxatilis*. Our results imply that this is highly unlikely. In most conditions studied, ecotypes arose rapidly and persisted indefinitely; within the plausible ranges of parameter values, ecotype formation was rarely inhibited by gene flow. Ecotype formation was facilitated by a wider zone of bounded hybrid superiority, as suggested by previous work (Goodisman & Crozier 2001), and by weaker selection. Both of these factors actually increased gene flow. The main inhibitor of ecotype formation was not gene flow, but an inability to colonize the intermediate zone or alternative habitat due to strong ecological selection. This effect was exacerbated if loci had small effects, in agreement with existing theory. There is no empirical evidence that ability to colonize the alternate habitat constrains ecotype formation in *L. saxatilis*, so the results imply that selection falls at the weaker end of the plausible range for *L. saxatilis* (still relatively strong selection).

Assortative mating, when it evolves subsequent to population divergence, has long been considered to provide a mechanism for reinforcing selection, thus facilitating speciation and adaptive divergence generally. Our results show that when assortative mating is present in the ancestral population, it can instead inhibit divergence. In the current model, the existence of assortative mating in the ancestral ecotype was a strong inhibitor of ecotype formation when ecological selection was strong. This effect occurred because pre-existing positive assortative mating reduced variation in the parental ecotype, making ecotypes slower and less likely to evolve than if mating was random. That is, sexual selection suppressed the spread of novel adaptive alleles. In the case of reinforcement, a scenario can be envisaged where reinforcing selection for the evolution of novel, divergent mating preferences faces conflicting sexual selection to maintain pre-existing, shared preferences.

On the other hand, when mating was initially random, positive assortative mating evolving in the simulation was a powerful facilitator of ecotype formation. In this situation, the form of mate selection chosen was influential, with ecotypes arising most easily when mates were chosen by matching traits. If (as has been previously suggested) we assume that assortative mating is indeed an ancestral trait in *L. saxatilis* ecotypes, the simulation data suggest that matching-based or similarity-based mate choice are likely in *L. saxatilis*, because these mate choice systems consistently allow ecotype formation within plausible timescales even when assortative mating is initially present. The observation that evolution of assortative mating was usually asymmetric under matching-based mating adds weight to the idea that similarity-based mating is the likely mechanism in *L. saxatilis*. Body size influences mating decisions in *L. saxatilis* (Hollander *et al.* 2005) and provides a simple basis for similarity-based mating. Physical incompatibility may contribute to similarity-based mating if, for example, the exposed ecotype male is too small to mate successfully with the sheltered female.

We found that frequent long-distance dispersal (exceeding 10% of individuals) could prevent ecotype formation by eliminating spatial genetic structure. This observation may explain why *Littorina littorea*, a relative of *L. saxatilis* with similar habitat, life history, ecology and morphology to the sheltered ecotype, has not established a second ecotype in exposed habitat. Whereas *L. saxatilis* is direct-developing with short dispersal distances, *L. littorea* has long dispersal due to a 4–5 week pelagic larval stage.

Ecotype formation was also unlikely when loci had very small effects and selection was very strong ($\sigma_s \approx 0.02$), as previous theory suggested (Gavrilets & Vose 2007; Gavrilets *et al.* 2007). The natural values of these parameters are unknown, but ecotypes arose within broad regions of the plausible parameter space. More detailed empirical data on the relative fitness of individuals of different sizes in different habitats would allow estimation of σ_s, and thus a better understanding of the relevant dynamics. However, the overall model results suggest that gene flow is unlikely to restrict parapatric ecotype formation in *L. saxatilis*.

Ecotype formation in the current model was swift, usually occurring within 1000 generations. Formation of ecotypes with partial reproductive isolation within a few hundred generations has been documented in a range of empirical and theoretical studies (Gavrilets & Vose 2005; Hendry *et al.* 2007). This agreement among diverse models and systems suggests that in general, ecologically divergent groups will emerge rapidly if at all. However, the timescale and conditions for completion of ecological speciation are less well understood (Hendry *et al.* 2007); within the current model, small changes in ecological conditions could lead to very different predictions for the long-term evolution of reproductive isolation.

Evolution of nonrandom mating

Positive assortative mating evolved in a wide range of scenarios in the model, and often both facilitated ecotype formation and reduced gene flow. However, the relationship between ecotype formation, evolution of nonrandom mating and reproductive isolation was far from straightforward. In agreement with Servedio (2000), evolution of nonrandom mating occurred more commonly with a similarity-based model than with a matching-based model. Ecotype formation, however, occurred most easily in a matching-based model. Assortative mating led to significantly restricted gene flow between ecotypes when mating preferences were open-ended or similarity-based. However, in many conditions, prezygotic isolation appears to have stabilized over time, rather than continuing to strengthen indefinitely, a finding that is congruent with the lack of empirical evidence for speciation via the evolution of increased prezygotic isolation in *L. saxatilis* despite strong assortative mating. This result is also in agreement with theoretical work on single-locus models, which found that disruptive selection did not lead to continuously increasing assortative mating unless selection was strong (Matessi *et al.* 2001).

Exploration of parameter space revealed several unusual theoretical outcomes, highlighting the complex and unpredictable effects of interacting processes during ecological speciation. While these effects have not been seen in *L. saxatilis*, they could be plausible in other species.

Frequently, assortative mating arose during ecotype formation, but later become restricted to the intermediate zone. The pattern of stronger mating preferences at parapatric boundaries thus generated has been commonly reported in nature, and is usually interpreted as evidence for reinforcement contributing to speciation (Jiggins & Mallet 2000); however, our results suggest an alternative mechanism. Recently diverged populations are likely to be predisposed to perceive and respond to similar cues, potentially with either attraction or aversion. Therefore, it would not be surprising if sexual selection ran in opposite directions in different parts of the same population, potentially generating incoherent mate choice systems and exacerbating gene flow between ecotypes over long periods. A recent study of the damselfly *Ischnura elegans* revealed a fine-grained spatial sexual selection mosaic where sexual selection on one trait runs in different directions at different locations (Gosden & Svensson 2008), in keeping with this argument.

Evolution of a mating preference for the other ecotype was seen in one scenario when the intermediate zones were wide and ecological selection was weak. We attribute this effect to selection favouring ecological intermediates in the intermediate zones, while local mating implied that negative assortative mating within habitats had little deleterious effect because most individuals would only encounter individuals of their own ecotype.

Asymmetries between the ecotypes also generated some notable phenomena. Assortative mating commonly evolved asymmetrically, being stronger in the sheltered ecotype than in the exposed. In extreme cases, if the exposed ecotype continued to mate at random, the sheltered ecotype sometimes invaded the exposed habitat through sexual selection and drove the parental ecotype to extinction, despite the larger population and superior ecological adaptation of the latter. We know that *L. saxatilis* ecotypes do mate assortatively and there are no documented cases of sheltered *L. saxatilis* invading exposed habitat, so this outcome appears to be unrealistic for this species. In addition, there is no known asymmetry of mating preferences in *L. saxatilis* ecotypes. Symmetric mating preferences were observed in the current model when mate choice was governed by similarity-based preferences.

In many cases where mate selection involved matching an independent preference trait, assortative mating initially facilitated ecotype formation, but failed to persist after ecotypes evolved. We attribute this finding to the spatial dynamics of the model, which allowed drift at the centre of the exposed and sheltered habitat patches to overwhelm selection in the hybrid zone. This finding provides the first simulation support for a classical verbal objection to the reinforcement hypothesis: that selection on mate choice systems at a narrow population interface may be too weak to influence evolution of mating behaviour in more central locations (Moore 1977). A number of previous models have suggested that local dispersal facilitates parapatric speciation by allowing local populations to evolve semi-independently (Endler 1977; Lande 1981; Kawata 2002; Doebeli & Dieckmann 2003). The current results show that on the contrary, evolution of prezygotic isolation may be inhibited by low gene flow within parental populations preventing the evolution of coherent mate recognition systems.

This result also implies that the failure to observe assortative mating in ecotypes does not indicate that assortative mating played no role in their divergence. Again, this finding is not applicable to *L. saxatilis*, which mates assortatively, but it could be relevant in other systems (see review by Jiggins & Mallet 2000). We found that when ecotypes exist in parapatry, short-term evolution of assortative mating may greatly facilitate ecotype formation, but vanish without leaving any genetic signature. This result complements the results of Gavrilets *et al.* (2007), who found that the initial evolution of sexual morphs was followed by ecological divergence and then speciation by reinforcement. In the current model, the early stages of this process were observed, but speciation failed to occur after ecotype formation. The past model used higher dispersal rates and smaller spatial environments, so that selection against hybridization was presumably stronger throughout the populations. Either scenario could be plausible in some systems.

What conditions would favour speciation of L. saxatilis *ecotypes?*

Rather than progressing inexorably to speciation, ecotypes in our model often persisted indefinitely with moderate genetic differentiation. Moreover, the scenarios that favoured ecotype formation were not necessarily those that favoured speciation. In particular, the zone of hybrid superiority greatly facilitated ecotype formation but was an equally powerful inhibitor of speciation. Although significant barriers to gene flow between ecotypes evolved, these barriers usually plateaued rather than increasing indefinitely. Completion of speciation in *L. saxatilis* may be inhibited by selection favouring hybridization in the zone of hybrid superiority, combined with low gene flow reducing selection against hybridization in exposed and sheltered habitats.

Nonetheless, nonrandom mating favouring ecologically similar individuals evolved under a wider range of conditions and usually contributed to restricted gene flow. In some scenarios, positive assortative mating strengthened with time, suggesting that effective prezygotic isolation may eventually evolve and lead to speciation in *L. saxatilis*. It is unknown which, if any, of the mate choice systems explored here is employed by *L. saxatilis*; current data suggest a combination of chemical and mechanical cues are involved (Hollander *et al.* 2005; Johannesson *et al.* 2008). Empirical observation of consistent and symmetric evolution of ecotype-based mating preference (Hollander *et al.* 2005) is consistent with simulation results for similarity-based mating preferences. Physiological mechanisms for similarity-based mating can be easily envisaged when size is a relevant display trait, as it is in *L. saxatilis*. Our results suggest that if mate choice is based on matching an independent trait, prezygotic isolation would consistently evolve only if selection is strong and the number of loci governing mate choice is large, or if selection is weak and the number of loci governing mate choice is intermediate. In these cases, assortative mating would often be asymmetric.

A key difference between the current model and previous ones where speciation by reinforcement was observed (e.g. Gavrilets *et al.* (2007)) is that the interface between populations was relatively narrow. This suggests that uniform patch size (for both ecotype habitats and intermediate zones) may restrict speciation in the current model. In reality, habitat patch sizes vary for Swedish *L. saxatilis*. Larger patches may facilitate ecotype formation, and at the same time smaller patches could provide a wide interface for population interaction, creating selection for reinforcement. Thus, variation in patch size may make speciation more likely for *L. saxatilis* ecotypes. The existence of numerous similar islands with differing patch sizes in Swedish *L. saxatilis* may allow these predictions to be tested empirically. While variation in assortative mating index has not been assessed in the Swedish ecotypes, values ranging from 0.55 to 1 were obtained for similar Spanish ecotypes at 12 sites (Rolan-Alvarez *et al.* 1999).

Our results reveal unanticipated tensions between the processes driving ecotype formation and speciation. For example, the zone of bounded hybrid superiority not only facilitated ecotype formation but also increased gene flow between ecotypes, and could even lead to the evolution of negative assortative mating. Frequent long-distance dispersal could inhibit ecotype formation, however, when ecotypes did form, they evolved more rapidly and with enhanced prezygotic isolation. Simple genetics, potential for nonrandom mating and weak selection all favoured ecotype evolution, but could ultimately either reduce or enhance gene flow, depending on the specific combination of conditions in question, and on the initial population state. Indeed, even with identical initial parameters, chance could lead to qualitatively different outcomes. These results highlight the distinction between ecological divergence and the evolution of reproductive isolation; while both phenomena are crucial to ecological speciation, they are driven by potentially independent and even conflicting forces. Thus, our results imply that predictions about ecological speciation within specific systems are hazardous without both quantitative modelling and highly detailed data.

Acknowledgements

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This project was completed during SS's postdoc with SG at the University of Tennessee. SS is now research fellow at Monash University modeling spatial gene flow. KJ is running a long-term project on ecological and genetic mechanisms of speciation in Littorina saxatilis. MP and JH both graduated in this project. MP is now postdoc at the University of Gothenburg. JH is Marie Curie postdoctoral fellow at the University of Sheffield studying speciation and phenotypic plasticity. SG researches theoretical evolution as Distinguished Professor of Ecology and Evolutionary Biology and Mathematics, and Associate Director of NIMBIOS at the University of Tennessee.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Analysis of variance for F_{ST} and mating preference

Fig. S1 Ecological fitness function and the meaning of parameter σ_s.

Fig. S2 Mating probability distributions for male and female phenotypes.

Fig. S3 The proportion of runs with ecotypes over time.

Fig. S4 The effect of varying parameter mate choice strength parameter σ_a.

Fig. S5 Final-state relationship between F_{ST} and mating discrimination in different conditions.

Fig. S6 Mating discrimination (mean deviation of the c trait from 0.5) in each condition over time.

Fig. S7 Mean F_{ST} values in each condition over time.

Fig. S8 Effect of initial value of mating discrimination trait i_c.

Fig. S9 Effect of varying the probability of long-distance dispersal, p_l.

Fig. S10 Strength of mating discrimination in sheltered and exposed ecotypes.