

Modelling sympatric speciation by means of biologically plausible mechanistic processes as exemplified by threespine stickleback species pairs.

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RRH: MODELS OF SYMPATRIC STICKLEBACK SPECIATION

ABSTRACT

We investigate the plausibility of sympatric speciation through a modelling study. We built up a series of models with increasing complexity while focussing on questioning the realism of model assumptions by critically checking them against a particular biological system, namely the sympatric benthic and limnetic species of threespine stickleback in British Columbia, Canada. These are morphologically adapted to their feeding habits: each performs better in its respective habitat than do the hybrids with intermediate morphology. Ecological character displacement through disruptive selection and competition, and reinforcement through mating preferences may have caused their divergence. Our model assumptions include continuous morphological trait(s) instead of a dimorphic trait, and mating preferences based on the same trait(s) as selected for in food competition. Initially, morphology is intermediate. We apply disruptive selection against intermediates, frequency-dependent resource competition, and one of two alternative mating preference mechanisms. Firstly, preference is based on similarity where mating preference may result from “imprinting” on conspecifics encountered in their preferred foraging habitat. Here, speciation occurs easily – ecological hybrid inferiority is not necessary. Hybrid inferiority reinforces stringency of assortative mating. Secondly, individual preferences exist for different trait values. Here, speciation occurs when linkage disequilibrium between trait and preference develops and some hybrid inferiority is required. Finally, if the morphology subject to disruptive selection, frequency-dependent competition, and mate choice, is coded for by two loci, linkage disequilibrium between the two loci is required for speciation. Speciation and reinforcement of stringency of choosiness are possible in this case too, but rarely. Results demonstrate the contingency of speciation, with the same starting point not necessarily producing the same outcome. The study resulted in flagging issues where models often lack in biological realism and issues where more empirical studies could inform on whether assumptions are likely valid.

keywords: assortative mating, benthic/limnetic species pairs, disruptive selection, frequency-dependent resource competition, reinforcement, sympatric speciation.

INTRODUCTION

Adaptive radiation is the diversification of a clade into a number of species exploiting different resource types and differing in the morphological and physiological traits used to exploit those resources (Huxley 1942; Futuyma 1986; Schluter 1996, 2000). Well-known cases of adaptive radiation include the Galápagos finches (Grant 1986; Grant and Grant, 2008, 2010), the Hawaiian *Drosophila* (Carson & Kaneshiro 1976), the cichlids of East African lakes (Galis & Metz 1998), and the three-spined sticklebacks (*Gasterosteus aculeatus*) in British Columbia, Canada (McPhail 1994). The latter radiation consists of species pairs, which have apparently arisen through independent replications of a similar evolutionary event (Taylor & McPhail 1999; Rundle et al. 2000; Gow et al. 2008).

The forces that drive adaptive radiation are partly understood (Schluter 1996, Gavrillets 2004; Bolnick and Fitzpatrick 2007; Price 2008; Hendry 2009). The major ecological forces are thought to be divergent natural selection and resource competition (Darwin 1959; Fisher 1930; Dobzhansky 1937; Huxley 1942; Mayr 1942; Schluter 1996; Dieckmann & Doebeli 1999, Doebeli et al. 2007; Hendry 2009, Hendry *et al.*, 2009). Sexual selection, by divergent mate preferences, may play a major role as well (Turner & Burrows 1995; Seehausen et al. 1997; Seehausen & van Alphen 1998; van Doorn et al. 1998; 2004; van Doorn & Weissing 2001; Higashi et al. 1999; Wilson et al. 2000, Bolnick and Fitzpatrick 2007; Pfennig & Pfennig, 2009, Puebla, 2009, van Doorn *et al* 2009).

The feasibility of sympatric speciation has long been the subject of debate. Generally, two problems are recognized concerning sympatric speciation. The first is that, if the initial population forms a single entity whose size is regulated regardless of the genotypes of its members, disruptive selection cannot maintain genetic polymorphism but will rather lead to fixation of one of the genotypes (Levine 1953; Maynard Smith 1966, Goldberg & Richardson 1987; Doebeli 1996). If the population inhabits two niches, the population size being regulated separately in the two niches while one genotype has higher fitness in one niche and the other genotype has higher fitness in the other niche, then a stable polymorphism is possible (Levine 1953; Maynard Smith 1966). Hence, the establishment of a stable polymorphism depends on

the existence of separate density-dependent regulation of population size in the two niches, e.g. in the form of frequency-dependent resource competition (e.g. Goldberg & Richardson 1987; Doebeli 1996).

The second problem is that, due to recombination, speciation occurring in a sexually reproducing species without effective geographical isolation appears genetically difficult. Recombination, which randomizes the association between the prezygotic isolating mechanism (assortative mating) and the adaptations to the two environments, acts as a genetic constraint against speciation (Felsenstein 1981; but see Servedio & Sætre 2003; Sæther et al. 2007). Felsenstein (1981) distinguishes between two kinds of speciation: the one-allele model and the two-allele model. The one-allele model supposes that the reproductive isolating mechanism establishes itself by the substitution of the same allele in the two nascent species, for example an allele that promotes mate choice based on similarity. The two-allele model supposes that two different alleles are substituted, for example alleles for different mate preferences. In the first case, recombination between individuals from the two populations does not tend to destroy reproductive isolation (e.g. models by Rice 1987; Kelly & Noor 1996; Fry 2003), whereas in the second case it does (see also the model by Kondrashov & Shpak 1998; Fry 2003; but see Servedio & Sætre 2003; Sæther et al. 2007).

Recently Bolnick and Fitzpatrick (2007) reviewed the empirical evidence for sympatric speciation and gave an overview of the main features of over 70 models. They concluded that sympatric speciation does occur but perhaps rarely and provided three conditions that are widely thought to be necessary for the process to occur. These are that (a) a natural population shows frequency-dependent disruptive selection, (b) natural selection operates directly on assortative mating patterns and (c) assortative mating imposes only weak or negligible costs on the individuals choosing mates this way.

In this paper we develop a series of models of sympatric speciation. We use stochastic simulations of the evolution of a finite population with individual based genetic algorithms (IBGA, Goldberg 1989; Mitchell 1996; Huse *et al.*, 2002). We deploy a more realistic approach to the theoretical study of natural selection than do other theoretical models. This is

because, (1) we model continuous quantitative traits with multiple alleles per locus (as van Doorn & Weissing 2001), instead of only two or three; (2) viability is based on phenotype, which is in turn determined by genotype; therefore, we do not invoke ‘genes for hybrid viability’ (e.g. Kelly & Noor 1996); (3) assortative mating is described in mechanistic terms common in behavioural research; we do not use abstract ‘assortative mating genes’ (e.g. Maynard Smith 1966; Felsenstein 1981; Diehl & Bush 1989). We explore models in which the tendency to mate assortatively is fixed (as in, e.g., Kondrashov & Kondrashov 1999) as well as evolving (as in, e.g., Dieckmann & Doebeli 1999; de Cara et al. 2008) in response to reduced hybrid fitness (Dobzhansky 1940). Assortative mating is often modelled as a preference for phenotypes similar to self (e.g. Rice 1984; Dieckmann & Doebeli 1999), which is equivalent to Felsenstein’s (1981) one-allele model. We argue that this is realistic only under restrictive assumptions that have rarely been tested (e.g. Albert 2005). We simulate the equivalents of both the one-allele and the two-allele model of assortative mating (Felsenstein 1981).

Another feature of our approach is that it captures the contingency of the evolutionary process (Gould 1989). Because we simulate the selection process itself using a stochastic approach, we can run replicates of the starting conditions resulting in parallel evolutionary sequences and so arrive at a relative estimate of how likely speciation is under a given set of conditions. As we show, the same starting conditions will often lead to different outcomes. Using Gould’s (1989) analogy, replaying life’s tape does not always lead to the same result and history is contingent.

In order to check whether our models’ features are relevant to any real biological system, we base our models loosely on information from the stickleback case; we discuss each of the features and parameter values in the light of information known from sticklebacks, and we discuss the implications of our outcomes for the stickleback case. We believe that this approach brings certain questions to the surface, particularly questions on the mechanistics and biological realism of assumptions and processes, that are often left implicitly unanswered in general modeling studies. In no way do we pretend that our models accurately mimic stickleback evolution nor that they provide the ultimate answer to the question of stickleback

speciation. Our model outcomes are meant to show that it is possible for evolutionary processes to occur under the explored conditions and assumptions, not to serve as evidence that evolution actually did occur along those paths. We believe that the relevance of our models extends beyond the stickleback case to the extent to which features of the models resemble reality in other biological systems.

We address the three main conditions necessary for sympatric speciation to occur (Bolnick and Fitzpatrick 2007), such as frequency-dependent competition for resources and disruptive selection, assortative mating based on similarity versus divergent mating preferences, reinforcement of both types of premating reproductive isolation, and the role of sexual selection.

THE BENTHIC AND LIMNETIC STICKLEBACK SPECIES PAIRS

The three-spined stickleback occurs throughout the northern hemisphere in marine and freshwater habitats. This fish consists of a species complex, and presents a striking example of a recent adaptive radiation (Bell & Foster 1994; McPhail 1994). For example, seven species pairs have been known to occur in five unconnected drainages on four adjacent landmasses in the Strait of Georgia region of British Columbia, Canada (for details of the lakes and their species pairs see McPhail 1984, 1992, 1994; Schluter & McPhail 1992; Taylor et al. 1997; Gow et al. 2008). Most comparable lakes in the same region contain only a single species (McPhail 1993; Vamosi 2003). In each of the pairs, one species is called 'benthic' and the other 'limnetic'. Benthics are larger and deeper-bodied, have wider but shorter jaws, smaller eyes, and fewer and shorter gill rakers than do limnetics. Benthics are morphologically and behaviorally adapted for feeding on macrobenthos in littoral habitats, whereas limnetics are adapted for planktivory in the limnetic zone of lakes (Schluter & McPhail 1992; Schluter 1993, 1995). In lakes with a single species, fish show a unimodal variation in morphology. Those at the benthic end of the spectrum feed more in the benthic habitat and those towards the limnetic end feed more in the limnetic habitat (Schluter & McPhail 1992, Hendry *et al.*, 2009). During breeding, the two species both inhabit the littoral

zone, but show a high degree of microhabitat partitioning when there (McPhail 1994; Vamosi & Schluter 1999).

As discussed by Hendry *et al.*, (2009) stickleback pairs fulfill the first two conditions necessary for sympatric speciation as defined by Bolnick and Fitzpatrick (2007). There is frequency dependent disruptive selection through specialization on different feeding niches and experiments have demonstrated assortative mating between species, based in part on the adaptive morphological traits that distinguish the species, especially body size but probably also shape (Nagel & Schluter 1998; Albert & Schluter 2004).

Significant differences in allozyme frequencies are found between the species (McPhail 1984, 1992) and there are diagnostic microsatellite markers as well (Gow *et al.* 2006, 2007). In nature some hybrids occur (McPhail 1984, 1992, 1994; Kraak *et al.* 2001). Hybrids are of intermediate morphology, and tend to perform intermediately in both habitats: they do worse than limnetics in the limnetic habitat (where benthics do even worse), and they do worse than benthics in the benthic habitat (where limnetics do worst) (Schluter 1993, 1995; Gow *et al.* 2007). Since fitness of hybrids is high in the laboratory, hybrid inferiority is ecological, and does not result from intrinsic genetic incompatibilities (Hatfield & Schluter 1999, Vamosi *et al.* 2000).

THE MODELS AND THEIR RESULTS

The series of eight models (Table 1) represents a build-up of complexity along four dimensions: (1) disruptive selection, (2) frequency dependence through resource competition, (3) assortative mating, and (4) one versus two morphological traits subject to selection. We vary the strengths of disruptive selection and resource competition. We consider (a) the absence of assortative mating; assortative mating based on similarity with (b) fixed and (c) evolving stringency (cf. one-allele case, Felsenstein 1981); assortative mating based on divergent preferences (cf. two-allele case, Felsenstein 1981) with (d) fixed and (e) evolving stringency, and with (f) only females or (g) both sexes being choosy. We do not systematically

explore all combinations of parameter values, because our intention is to find whether speciation is at all possible under different structural model assumptions; for example, if speciation would already be found to be difficult under less stringent ones (e.g., under fixed assortative mating; or under strong divergent selection) we would not explore simulations under even more stringent assumptions (e.g. respectively, under evolving assortative mating; or weak divergent selection). All model parameters are explained in the text and can be found in Table 2. A flow diagram of the modelled process is presented in Figure 1; explanation can be found in the text.

Model 1.

The Basic Model: Disruptive Selection, Resource Competition, No Assortative Mating.

The simulated population consists of fixed and equal numbers of diploid males and females ($N_m = N_f$; here we report results of simulations with $N_m = 100$, but results are similar when we use higher values of N_m , up to 1000). Generations are non-overlapping. In the simplest version of the model, only one locus is assumed. This locus determines the value of a continuous quantitative morphological trait T (e.g. body depth: body length ratio or a body shape index as in Schluter & McPhail 1992). There is no environmental influence on the phenotypic value of the trait. The trait is scaled such that its values range between 0 and 1 inclusive. We locate the optimal benthic shape at $T = 0.25$ and the optimal limnetic shape at $T = 0.75$. Alleles of this locus can have n equidistant discrete values from 0 to 1. We ran simulations with $n = 64$ and with $n = 256$ (these particular values were chosen for programming convenience). Most theoretical studies model loci as having two possible alternative alleles. This implies a dichotomous trait. In the case of quantitative traits, modellers sometimes assume multiple loci, but again with only two, or sometimes three, alleles per locus. While in reality quantitative traits are usually determined by multiple loci, which is particularly true for some well-studied ecological traits in sticklebacks (Peichel *et al.* 2001), for simplicity we prefer to explore the dynamics when one locus has many possible alternative alleles coding quantitatively for a given trait. This is not unrealistic, since DNA

sequences may vary at any of hundreds of bases, which might influence the function of an enzyme to a certain, but not crucial, extent. The trait is expressed additively: the phenotypic value T of an individual is the average of the two allelic values.

A mating between a male and a female produces exactly one son and one daughter. These offspring independently inherit parental alleles randomly, according to Mendelian laws. We incorporate a stochastic mutation rate of μ , which is the probability of an allele changing its value. We ran simulations with $\mu = 1\%$ and $\mu = 0.01\%$. The amount of change in allelic value is random, up to a maximum change in value of 0.08.

Fitness is a function of phenotype T . We choose a sinusoid function (see equation in Fig. 2), with peaks at $T = 0.25$ (benthics) and $T = 0.75$ (limnetics), and valleys at $T = 0.5$ (intermediates) and $T = 0$ and $T = 1$ (extremes). In this way we mimic the situation where benthics forage in the benthic habitat and perform optimally there, and limnetics forage in the limnetic habitat where they also perform optimally. Intermediates cannot compete with either in either habitat, so they do worse. Individuals of extreme phenotype have a lower fitness, because there must be certain optima for body shape. By varying the selection differentials we investigate their influence on the likelihood of speciation. We usually ran our simulations with maximum fitness twice as high as minimum fitness (see Fig. 2). Selection differentials up to fivefold are certainly realistic; some male sticklebacks may have a season's reproductive output of only one hundred or fewer hatched fry whereas others may output several thousands of hatched fry (Kraak et al. 1999); individual females may spawn one or several clutches per season of 70 eggs up to 350 eggs (Kraak & Bakker 1998). On top of this divergent selection pressure illustrated in Figure 2, we incorporated frequency dependence in the following way (following Goldberg & Richardson 1987). We divided the fitness of each individual by the number of competitors for the same resource in a given habitat. An individual counts as a full competitor when it has exactly the same phenotype T . The more its phenotype deviates, the less it counts as a competitor (by a linear decrease), until the phenotypic difference reaches a value of σ (see equation in Table 2). At this value individuals are different enough not to compete. This is because differently shaped individuals will tend

to forage in the alternative habitat. We usually ran simulations with $\sigma = 0.5$ and $\sigma = 0.25$. Such resource sharing gives rise to frequency dependence, because if most individuals are limnetic-like, for example, a rare benthic-like individual will do much better in terms of resource competition, because it has the benthic resource all to itself.

Natural selection operates stochastically. The fitness of an individual after resource sharing is the probability that it contributes offspring to the next generation before sexual selection takes place. The procedure can be viewed as the turning of a roulette wheel (Goldberg 1989; Mitchell 1996) in which the size of an individual's slice of the wheel is proportional to its fitness. In our model without mate choice, the roulette wheel procedure picks a male and a female – males and females play in separate roulettes! – who then produce exactly one son and one daughter and go back to the mating pool. Individuals can mate more than once: some individuals may have many offspring, while others get zero. This procedure is repeated until the numbers of sons and daughters of the next generation equal the numbers of male and female parents of the current generation. Then all individuals of the current generation die, and are replaced by the individuals of the next generation. This process, in turn, is repeated for G generations. We acknowledge that sticklebacks lay clutches of several hundreds of eggs; however, most embryos will die and only few will reach adulthood: in a stable population on average two per parent. We modelled a shortcut of this process.

We assume that the ancestral population consists of intermediates. Therefore, simulations started with individuals' alleles randomly drawn from a narrow ($SD = 0.08$) normal distribution of allelic values around the intermediate value of 0.5 (rounded to the nearest discrete allelic value). Figure 3a shows the typical distribution of allelic values of such an initial population.

A typical outcome of running this simulation model is that after a number of generations of natural selection brought about by disruptive selection and resource competition, the intermediate alleles are eliminated, and under some conditions a polymorphism is often established. The latter outcome is shown in Figure 3b, which

represents the typical distribution of allelic values after $G = 100$ generations of the simulated population ($\sigma = 0.25$). The distribution is bimodal: only benthic-like and limnetic-like alleles are present. However, since nothing stops individuals from interbreeding, intermediate phenotypes are formed in each generation by random mating. Therefore, after $G = 100$ generations the distribution of phenotypic values is trimodal (Fig. 3c), consisting of intermediate, benthic and limnetic individuals. With $\sigma = 0.25$, this result was obtained in 20 out of 20 replicate simulations. With $\sigma = 0.5$ where resource competition drops off less steeply with decreasing similarity, polymorphism was obtained in only 13 out of 20 replicate simulations. In the remaining simulations either benthic alleles (6 replicates) or limnetic alleles (1 replicate) went to fixation. With $\sigma = 1$, fixation of either benthic alleles (5 out of 10 replicates) or limnetic alleles (5 out of 10 replicates) occurred, and a stable polymorphism was never reached. The results above refer to simulations with $n = 256$ alleles and $\mu = 1\%$; similar outcomes were obtained at $G = 500$ with $\mu = 0.01\%$ with $n = 256$ or $n = 64$ alleles. Hence, the establishment of a stable polymorphism appears to depend on the existence of frequency-dependent resource competition, as theoretical studies found before (e.g. Goldberg & Richardson 1987; cf. Maynard Smith 1966; Doebeli et al 2007).

Model 2.

Fixed Assortative Mating Based on Similarity, Disruptive Selection, Resource Competition.

The next model adds assortative mating to the model outlined above. Here assortative mating is based on morphological similarity in such a way that the probability that a female accepts a male as a mate decreases linearly from 1 to 0 along with decreasing phenotypic similarity. If the potential mate has exactly the same phenotypic value T as her own, the female will accept him with a probability of 1. As the phenotypic difference between potential mates grows, the probability of acceptance diminishes until the difference is τ . With a phenotypic difference of τ or more, potential mates will be rejected (see equation in Table 2). We usually ran simulations with $\tau = 0.5$. Note that assortative mating is based on the same morphological trait as is subject to disruptive selection and selection caused by competition.

This corresponds to the situation in the sticklebacks (Nagel & Schluter 1998). Other models often assume two different traits (Kondrashov & Kondrashov 1999; see Dieckmann & Doebeli 1999), probably corresponding to the situation in cichlids (Wilson et al. 2000).

As before, a female and a male are picked by the roulette wheel procedure. Subsequently, a check is made as to whether the female accepts the male as a mate, according to the probability calculated as above. If he is accepted, the pair produces their two offspring. If not, another male is picked by the roulette wheel procedure and he is checked to see if he is acceptable as a mate. This is repeated no more than 10 times, after which the female has to accept the last encountered male, and produce offspring with him.

If a male is acceptable to a female, the female is automatically acceptable to the male as well. However, the way we modelled mate choice, leads to an asymmetry between males and females. In the model, as is common in nature, the females are the choosy sex. It is reasonable to assume that a stickleback female who has a batch of eggs ready for oviposition, will search for a suitable mate until she has found one, or she will oviposit her eggs with the last encountered male assuming that the male accepts her. By contrast, a male stickleback tries to attract females to oviposit in his nest, but may be rejected. Hence, a female is always able to get her eggs fertilised, whereas a male is not always able to use his sperm to fertilise eggs. In the model this means that, as soon as a female has been selected due to viability and competition (through the roulette wheel), her probability of reproducing is 1, although perhaps with a non-preferred male. But for the males, sexual selection has been added as a selection pressure. With any given distribution of phenotypes, some males will have higher mating chances, and hence higher ultimate fitness, than others, based on their phenotype T .

Simulations run with this model show that the selection pressures caused by disruptive selection, resource competition, and female mate preference for similar males can be sufficient to bring about speciation. With $\tau = 0.5$, that is when mates are increasingly acceptable only when they differ less than 0.5, polymorphism is reached in 10 out of 20 replicate simulations ($\sigma = 0.5$). In these cases, after $G = 100$ generations intermediate alleles have been eliminated, and since benthics and limnetics rarely mate with each other, hybrids

(intermediate phenotypes) are rarely produced (Fig. 4). Reproductive isolation is not complete: about 4% of hybrids are produced at generation 100 (among 9 replicates the range is 2%-8%, mean = 4%, SD = 2%). In nature reproductive isolation between benthics and limnetics is not complete either. Current estimates are that about 1-2% of adult fish are hybrids (McPhail 1992; Schluter & McPhail 1992, but see Kraak et al. 2001); this is partly after selection (Gow et al. 2007) and might well correspond to the production of about 4% hybrid offspring per generation. Of course, a value of $\tau = 0.5$ allows for matings between benthics and limnetics, although rarely. With a more stringent choosiness of $\tau = 0.25$, that is potential mates who differ more than 0.25 are rejected, speciation with complete reproductive isolation was reached in 10 out of 10 replicate simulations. The results above come from simulations with $n = 256$ alleles and $\mu = 1\%$; similar outcomes were obtained at $G = 500$ with $\mu = 0.01\%$ with $n = 256$ or $n = 64$ alleles.

Model 3.

No Disruptive Selection, Resource Competition, Fixed Assortative Mating Based on Similarity.

Running simulations of the model above, but with a flat fitness function instead of the sinusoidal pattern in Figure 2, also leads easily to speciation. Although with $\sigma = 0.5$ or $\sigma = 0.25$ results were not so clear, when competition between similar phenotypes is moderately strong ($\sigma = 0.33$) and assortative mating between similar phenotypes is stringent ($\tau = 0.25$) all replicate simulations resulted in two reproductively isolated species (at $G = 100$ in 10 replicate runs with $\mu = 1\%$ and $n = 256$ alleles, and at $G = 500$ in 20 replicate runs with $\mu = 0.01\%$ and $n = 64$ alleles). Apparently, disruptive selection in the form of ecological hybrid inferiority is not even necessary; resource competition and assortative mating are sufficient for sympatric speciation. Similar results were obtained by some other modellers (Koeslag 1995; Doebeli 1996). By contrast, others have found and claimed that some postzygotic isolation (hybrid inferiority) is necessary for speciation to happen (Liou & Price 1994; Kelly & Noor 1996; Hostert 1997; Servedio & Noor 2003).

Model 4.

*Reinforcement of the Stringency of Assortative Mating Based on Similarity, Disruptive Selection,
Resource Competition.*

In the next model assortative mating evolves and is not imposed by us. Models in which assortative mating is already fixed from the beginning (as in, e.g., Kondrashov & Kondrashov 1999) do not differ much from allopatric speciation models (Maynard Smith 1966), except in that isolation is behavioural rather than geographic. We want to model reinforcement, the mechanism put forward by Dobzhansky (1937), whereby partial postmating isolation arising from environmental selection pressures directly favours the evolution of premating isolation. Our next model assumes a tendency for assortative mating based on morphological similarity, but the strength of this tendency (S , the stringency of assortative mating) is variable among individuals, and is genetically coded and subject to mutation.

We therefore added a second locus: a locus for S , stringency of choosiness. This locus segregates independently from the morphology locus. The alleles of this locus again have values between 0 and 1 inclusive with 64 or 256 possible equidistant discrete values. The locus is expressed additively: the stringency of choosiness phenotype (S) is the average of the result of both allelic values. The morphological difference between a female and her potential mate where probability of acceptance becomes zero, is $\tau = S + 0.25$ (Fig. 5). As before, more similar males will be accepted with a linearly increasing probability, such that a male with identical morphology T will be accepted with a probability of 1. Thus, a female with maximal stringency of choosiness, $S = 0$, will reject any male who morphologically differs from her by more than 0.25 (Fig. 5). A female with minimal stringency of choosiness, $S = 1$, will accept any male up to a difference of 1.25 (which is non-existent), meaning that all males have a non-zero probability of being accepted, and a male who is maximally different from her (with a difference of 1) will be accepted with a probability of 0.2 (Fig. 5). For the initial population, stringency alleles are randomly drawn from a normal distribution of allelic values, rounded to the nearest discrete allelic value, with a mean of 0.5 and $SD = 0.24$. This implies that

initially all phenotypes S are present, with modal individuals (with $S = 0.5$) rejecting potential mates differing more than 0.75 ($\tau = S + 0.25 = 0.75$, Fig. 5). The mutation rate at this locus is again μ , with values of either $\mu = 1\%$ or $\mu = 0.01\%$.

A typical outcome of the process of reinforcement in this model is illustrated in Figure 6. As before, disruptive selection in combination with competition soon selects against morphology alleles with intermediate values. Elimination of intermediate alleles is complete after 30 generations (Fig. 6, first column, third row). However, due to interbreeding, intermediate morphological phenotypes are still being produced in large quantities (Fig. 6, second column, third row). As to the stringency of choosiness (Fig. 6, third column), the alleles coding for the most stringent choosiness (of low S value) are being favoured, and their proportion increases steadily in the population throughout the generations. As a result, fewer matings occur between the two different morphotypes, until reproductive isolation is complete at generation 100 (Fig. 6, row 6; no hybrid production, and 90% of stringency of choosiness alleles are in the lowest class of values). With $\sigma = 0.5$, speciation with reinforcement occurred in 9 out of 20 replicate simulations; in the other replicates fixation of either benthic or limnetic alleles occurred while the stringency of choosiness did not change much. The results above refer to simulations with $n = 256$ alleles and $\mu = 1\%$. Similar outcomes were obtained at $G = 500$ with $\mu = 0.01\%$ with $n = 256$ or $n = 64$ alleles, but speciation was slightly less likely to occur. Reinforcement of stringency of choosiness has also been obtained by Kelly & Noor (1996).

Intermezzo:

a mechanistic view of the one-allele model

Up to this point we have modelled assortative mating according to Felsenstein's (1981) notion of the one-allele case. In both morphs the same allele for more stringent choosiness is favoured, and, therefore, recombination is not antagonising the process of speciation. The individuals have a mate preference rule that instructs them to mate with an

individual that has similar morphology to themselves. Sticklebacks are able to differentiate between fish of similar and different sizes to themselves (Ward *et al.*, 2004a). Another, more indirect and therefore more noisy, mechanism may be a rule that instructs an individual to mate with individuals living in the same habitat (Rice 1987). Since individuals feeding in the same habitat are likely to be of similar morphology, mating within the preferred habitat would result in mating with morphologically similar individuals. In the stickleback case, however, benthics as well as limnetics both breed in the benthic habitat, although microhabitat partitioning is known, (McPhail 1994; Vamosi & Schluter 1999). A further possibility, albeit even more indirect and noisy, is imprinting: that the individuals imprint on conspecifics they meet in the preferred habitat during feeding, and that the preference rule instructs them to mate with an individual with a familiar body shape, even if different individuals are encountered on the breeding grounds. Sticklebacks indeed have been shown to be able to use olfaction and vision to differentiate between familiar and unfamiliar individuals (Ward *et al.*, 2004b).

Model 5.

Reinforcement of Assortative Mating Based on Divergent Mate Preferences, Disruptive Selection, Resource Competition.

In the next model we focus on an equivalent of Felsenstein's (1981) two-allele model, in which divergent mate preferences exist. Different preference alleles are favoured in different morphs. We assume that individuals have a genetically coded preferred mate morphology P (cf. the image of an ideal partner). Instead of the stringency of choosiness locus used before, we now add a locus that specifies the individual's ideal mate morphology P , in the same units as morphology itself T . An individual may itself have a morphology of $T = 0.4$, for example, yet prefer a mate morphology of $P = 0.6$. We choose to set $P = 0.25$ to indicate a preference for an optimal benthic body shape, and $P = 0.75$ to indicate a preference for an optimal limnetic body shape. The stringency of choosiness is fixed. A potential mate of precisely the preferred morphotype P is accepted with a probability of 1. This probability

decreases linearly as the potential mate deviates more from the preferred morphotype P , and reaches 0 when the deviation is τ ($\tau = 0.5$ or $\tau = 0.25$). The locus for preferred mate morphotype P segregates independently from the morphology locus. The alleles again have values between 0 and 1 inclusive (with 64 or 256 possible equidistant discrete values). The locus is expressed additively: the preferred morphotype P is the average of the two allelic values. The initial population has alleles randomly drawn from a normal distribution of allelic values rounded to the nearest discrete allelic value, with a mean of 0.5 and $SD = 0.08$ (as the morphology locus), hence, individuals of the ancestral population tend to prefer intermediates as mates. The mutation rate at this locus is again $\mu = 1\%$ or 0.01% .

Until now, our disruptive selection pressure was such that optimal benthics and limnetics had a fitness that was twice that of the intermediates and the extremes (Fig. 2). Anticipating that a stronger selection pressure may be required in this model, we also ran simulations with stronger disruptive selection with up to a fivefold difference in fitness between optimal and sub-optimal phenotypes rather than the twofold difference used in previous runs (Fig. 2).

We distinguish between two cases: the case where only females are discriminating and the case where both males and females are discriminating. In the first case, a pair mates if the female accepts the male. This happens if the male's morphology suits the female's preference. Here, the preference is only expressed in females, and only males are sexually selected on the basis of their morphology. In the second case, a pair mates only if the male is accepted by the female and the female is accepted by the male as well; each partner's morphology is required to suit the other's preference. Here, the preference is expressed in both sexes, and males are sexually selected on the basis of their morphology and their preference. Although female mate choice is much more common in nature, stickleback males have been found to express mate choice, often based on size and body shape (Rowland 1982, 1989; Sargent et al. 1986; Bakker & Rowland 1995; Kraak & Bakker 1998). Note also that in the second case, the asymmetry still exists in that all females selected by the roulette wheel procedure reproduce, regardless of their morphology and preference, whereas males may fail

to reproduce due to their morphology and now also due to their preference. A female who prefers a rare male morph will probably end up with a non-preferred average male, whereas a male who prefers a very rare female morph will probably never mate.

Since the preference locus and the morphology locus are initially in linkage equilibrium, individuals exist that are, for example, benthic-shaped but prefer mates with limnetic body shapes. The loci segregate independently; therefore, matings between benthics and limnetics will break up favorable associations between preference alleles and morphology alleles. However, alleles that code for a preference for limnetic mates will be favored in individuals carrying alleles for a limnetic body shape, whereas alleles for a preference for benthics will be favored in individuals carrying alleles for a benthic body shape. The question is, can disruptive selection in combination with resource competition build up and maintain linkage disequilibrium through reinforcement, resulting in reproductive isolation to such an extent that benthics mate only with benthics, and limnetics with limnetics?

The results of 200 simulation runs of this model are shown in Table 3 ($G = 1000$, $\mu = 0.01\%$, $n = 64$ alleles). Three types of outcomes occur contingently, with frequencies as reported in Table 3. Firstly, one of the morphs goes to fixation. In such cases, the population becomes either monomorphic for benthic morphology and benthic preference, or monomorphic for limnetic morphology and limnetic preference. Secondly, the population becomes polymorphic for morphology (T), whereas it remains monomorphic for preference (P). In these cases, preferences usually remain intermediate, but sometimes become benthic or limnetic. Thirdly, polymorphisms arise both of morphology and of preference. In many such cases, linkage disequilibrium between the two loci does not become strong enough to lead to reproductive isolation. But sometimes a linkage disequilibrium maintains reproductive isolation between the two morphs to such an extent that very few (or even 0) hybrids are produced in generation 1000. Speciation, or a high degree of reproductive isolation, is more likely to occur with stronger disruptive selection, tighter competition, more stringent choosiness, or both sexes instead of females only being choosy (Table 3). With higher

mutation rate and more alleles ($\mu = 1\%$, $n = 256$ alleles) results are very similar, but polymorphisms are slightly more likely to occur, and, if they do, linkage disequilibrium tends to be stronger and fewer hybrids tend to be produced.

A typical case of almost complete reproductive isolation with choosy males and females ($\sigma = 0.5$; $\tau = 0.5$; $\mu = 1\%$; $n = 256$ alleles) is depicted in Figure 7. At generation 100, both morphology T and preference P have diverged, meaning that their intermediate alleles have been eliminated. In this replicate, at generation 100 51% of individuals are homozygous for benthic morphology and benthic preference and 41% are homozygous for limnetic morphology and limnetic preference. These percentages are 6% and 6% respectively at generation 0 (expected: 6.25% and 6.25% respectively), and become 47.5% and 46% respectively at generation 200. Apparently, almost complete linkage disequilibrium is reached. At generation 100, 8% hybrids of intermediate morphology T are produced, and at generation 200 only 2%.

Apparently, in our version of the two-allele model, speciation can be attained (as in van Doorn and Weissing 2001). We must note, however, that in contrast to Felsenstein's (1981) notion, in our model an extra selection pressure in the form of sexual selection operates in favor of speciation. Similarly to Felsenstein's (1981) conclusion, speciation occurs under more restrictive conditions in the two-allele case than in the one-allele case (compare the outcomes of this model with the outcomes of model 4). In our version of the two-allele case stronger selection pressures are needed for speciation to result. These pressures may either be stronger disruptive selection, tighter resource competition, more stringently discriminating mate choice, or more sexes being choosy (Table 3).

Model 6.

Reinforcement of the Stringency and the Divergence of Assortative Mate Preferences, Disruptive Selection, Resource Competition.

As a next step, we combined models 4 and 5, by the addition of a third locus to model 5, namely a locus that specifies the stringency of choosiness S , just as before in model 4. In

this case the stringency of choosiness is concerned with the difference between the preferred mate morphology P and the potential mate's morphology, whereas previously it was concerned with the similarity between the two individuals. Now, an individual with a more stringent choosiness requires a mate that resembles the preferred morphology more closely. Again, $\tau = S + 0.25$.

In this model, conditions allowing speciation to occur are even more restrictive than in the previous model. With only females being choosy, a fivefold disruptive selection differential, $\sigma = 0.25$, $\mu = 0.01\%$, and $n = 64$ alleles, polymorphisms of both the morphological trait T and the mate preference P resulted in only 4 out of 20 replicates at $G = 2500$, with a production of on average 26% hybrids (SD = 20%, range 4%-44%; compare with model 5, Table 3). In those cases with low frequencies of hybrids, the stringency of choosiness had evolved to ever lower values in the course of the generations. In the run with only 4% hybrids, the average stringency of choosiness S at generation 2500 was 0.05, whereas it had been initiated at 0.5. In this particular run, at generation 2500, 41.5% of individuals were homozygous for benthic morphology and benthic preference and 44.5% were homozygous for limnetic morphology and limnetic preference. Hence, a high degree of linkage disequilibrium and reproductive isolation was reached. Simultaneous reinforcement of both, divergent mate preferences and stringency of choosiness, seems difficult to attain for the following reason. As long as the preferred mate morphotype P is still the intermediate, there is no selection for more stringent choosiness S , and, conversely, as long as stringency of choosiness S is loose, there is no divergent selection pressure on preferred mate morphotype P .

With both males and females being choosy, speciation was never reached (20 replicates; fivefold disruptive selection differential; $\sigma = 0.25$; $\mu = 0.01\%$; $n = 64$ alleles; $G = 2500$). In all cases stringency of choosiness evolved to higher S values; sometimes most individuals had maximum S values representing loose choosiness as defined earlier, at generation 2500. Due to the asymmetry of our model, selection for more stringent choosiness is opposed in males, because more stringent males have lower mating chances.

Model 7.

Two Morphological Traits that Influence Disruptive Selection and Resource Competition, Fixed Assortative Mating Based on Similarity.

Until now we have assumed that the whole suite of morphologically co-adapted traits is coded for by one locus. This is of course unrealistic (Albert et al. 2008). So we now explore the evolutionary dynamics with two morphological traits, coded for by separate loci that segregate independently. For example, in the sticklebacks not only body shape is important for fitness in either habitat, but also gill raker number and size. Benthics have fewer and shorter gill rakers than do limnetics. Thus, our two morphological traits can be viewed as body shape and gill raker number respectively. In the model the two traits have an epistatic effect on fitness: an individual has a high fitness only if it has both a deep (benthic) body and few (benthic) gill rakers, or a thin (limnetic) body and many (limnetic) gill rakers. Such individuals are called 'pure benthics' and 'pure limnetics' respectively, and individuals that are intermediate in both traits are called 'pure intermediates'. Thin (limnetic) individuals with few (benthic) gill rakers, and deep bodied (benthic) ones with many (limnetic) rakers have low fitness. In such a case, divergence can only take place if it goes hand in hand with the establishment of a linkage disequilibrium between the two traits.

The two morphological traits, $T1$ and $T2$, are coded for as before in an additive way, by loci whose alleles can attain values ranging from 0 to 1. In the initial population alleles for both traits are drawn from a normal distribution (and rounded to the nearest discrete allelic value) with mean 0.5 and $SD = 0.08$. At both loci the optimal values for a benthic forager are set at 0.25, and the optimal values for a limnetic forager at 0.75. At the start they are in linkage equilibrium. The mutation rate at both loci is $\mu = 1\%$ or 0.01% . The relative fitness due to disruptive selection is a function of both traits, in the form of an epistatic fitness landscape (see equations in Fig. 8). Resource competition is based on similarity: in this model similarity is assessed as the difference between each individual's average of its two phenotypic values $T1$ and $T2$.

$$\text{difference} = \left| (T1_{\text{individualA}} + T2_{\text{individualA}}) / 2 - (T1_{\text{individualB}} + T2_{\text{individualB}}) / 2 \right|$$

In this way a pure intermediate is as much competing with a pure intermediate as with an individual that has optimal benthic body shape and optimal limnetic gill raker number, because both individuals will tend to feed 50% of the time in each habitat. Fixed assortative mating is also based on similarity: in contrast to the resource competition case, here we regard the average of the two differences between individuals in both traits.

$$\text{difference} = (|T1_{\text{individualA}} - T1_{\text{individualB}}| + |T2_{\text{individualA}} - T2_{\text{individualB}}|) / 2$$

Accordingly a pure intermediate is only similar to a pure intermediate, and quite different from an individual with benthic body shape and limnetic gill raker number. If one considers it unrealistic that mate choice should be partially based on an invisible internal trait such as gill raker number, one may view the second trait as being mouth shape or eye size.

Simulations of this model often result in linkage disequilibrium between the two morphological traits, and in divergence of two morphotypes until they are reproductively isolated species. In 17 out of 20 replicates ($\sigma = 0.25$; $\tau = 0.25$; $\mu = 0.01\%$; $n = 64$ alleles; $G = 1000$) a polymorphism in both traits resulted, and in the remaining 3 replicates fixation of either limnetic or benthic alleles at both loci was the outcome. In 9 of the replicates with polymorphisms in both traits, 0% hybrids were produced at $G = 1000$ and in the other cases 1% to 7% hybrids were produced.

Model 8.

Reinforcement of the Stringency of Assortative Mating Based on Similarity, Two Morphological Traits that Influence Disruptive Selection and Resource Competition.

Now we explore a model with two morphological traits, as above (model 7) with evolving stringency of choosiness as in model 4.

With $\mu = 0.01\%$ ($n = 64$ alleles; $\sigma = 0.25$; $N_m = N_f = 500$; $G = 2000$) a polymorphism in both traits occurred and linkage disequilibrium was established at generation 2000 in all of 20 replicates, but many hybrids were still produced (on average 19%, SD = 3%, range 13%-25%). In the case with only 13% of hybrids, choosiness had only become moderately more stringent as S had evolved to moderately lower values compared to the initial values: the allelic values at generation 2000 ranged between 0.25 and 0.30 (initiated at 0.5). In this generation 40% of individuals were homozygous benthics for both traits and 35% were homozygous limnetics for both traits. With $\mu = 1\%$ ($n = 64$ alleles; $\sigma = 0.25$; $N_m = N_f = 500$; $G = 2000$) reinforcement produced a higher degree of reproductive isolation (on average 12% hybrids were produced at generation 2000, SD = 2%, range 10%-16%). Here S often evolved to the lowest values (= most stringent choosiness). In one case with 10% hybrids two thirds of choosiness alleles had values < 0.05 at generation 2000 (initiated at 0.5). In this generation 41% of individuals were homozygous benthics for both traits and 43% were homozygous limnetics for both traits.

Apparently, if morphology is coded for by more than one locus, reinforcement of stringency of choice for similar mates can lead to strong linkage disequilibrium and some degree of reproductive isolation.

DISCUSSION

Our explorations of sympatric speciation with various types of models confirm what other modellers have found: sympatric speciation is possible, but is less likely when linkage disequilibrium is required. Our models allow us to examine in some detail the validity of the three conditions for sympatric speciation identified by Bolnick and Fitzpatrick (2007) particularly the first two, that natural populations must show frequency-dependent disruptive selection, and that natural selection operates directly on assortative mating patterns. We did not evaluate explicitly in the model the third element, the cost of assortative

mating, although a cost is incorporated in models where the female has to choose males sequentially; she has up to 10 males to pass up before mating has to occur. This delay introduces an opportunity cost to the female; moreover, the female may end up with a non-preferred male. Because of the detail in our models, it is possible to determine under what conditions, the three preconditions necessary for sympatric speciation to occur (Bolnick and Fitzpatrick 2007) are likely to break down, particularly with respect to assortative mating.

We found that speciation is easily obtained under frequency-dependent resource competition and fixed assortative mating based on similarity; hybrid inferiority is not required. This latter result has not been reached by all modellers. It corresponds with findings by Koeslag (1995) and Doebeli (1996) and see Bolnick and Fitzpatrick (2007), while others have found that some postzygotic isolation is necessary for speciation to happen and be maintained (Liou & Price 1994; Kelly & Noor 1996; Hostert 1997; Bolnick and Fitzpatrick 2007). Recent empirical work has also shown that the collapse of the species pair in Enos Lake, British Columbia (Kraak et al., 2001; Taylor et al. 2006) has been accompanied by a loss of postmating isolation (Behm *et al.*, 2010).

In the case where the stringency of assortative mating is not fixed, but subject to evolution (cf. one-allele case, Felsenstein 1981), reinforcement will lead to reproductive isolation in the face of disruptive selection and resource competition. In the case where assortative mating is not based on similarity, but on divergent mate preferences instead (cf. two-allele case, Felsenstein 1981), reinforcement and reproductive isolation require that linkage disequilibrium be established. Speciation is still feasible, but conditions are more restrictive: stronger disruptive selection, tighter competition, more stringent choosiness, or both sexes being choosy rather than just the females.

Conditions that allow speciation are even more restrictive in the case where both stringency of choosiness and divergent mate preferences are involved. Finally, if the morphology that is subject to disruptive selection, frequency-dependent competition, and mate choice, is coded for by two loci, instead of one, linkage disequilibrium between the two loci is required to reach speciation. Also in this case, speciation is feasible under fixed

assortative mating, and reinforcement of stringency of choosiness can lead to linkage disequilibrium and some degree of reproductive isolation.

In the models reported here, the phenotypes are entirely determined by the genotype. We did not consider environmental influences, such as phenotypic plasticity, or the complexities of developmental effects on phenotypic expression. For example, slow growth rate could influence mortality by causing individuals to spend a longer time at a size, which is more vulnerable to predation. For Models 2 to 7 we also ran replicates in which the phenotype is determined by the genotype and random environmental variation, and this did not influence the outcomes significantly. Furthermore, we implicitly assumed perfect habitat choice as follows: an individual of optimal benthic morphology ($T = 0.25$) forages always on benthos, and an individual of optimal limnetic morphology ($T = 0.75$) forages always on plankton. An individual of exactly intermediate morphology ($T = 0.5$) spends half of its time foraging on benthos and half of its time foraging on plankton, and similar principles apply to other proportions. The rationale behind this is derived from experiments on the ideal free distribution, which have shown that sticklebacks are able to choose food patches on the basis of profitability (e.g. Milinski 1984). However, it is unlikely that they will do this without making errors. Mate choice, too, is prone to errors and environmental circumstances. For example, it is well known that fish become less choosy when faced with predation risk (e.g. Forsgren 1992). We need to simplify in order to understand the processes that shape nature, although it would be interesting to monitor the outcomes of simulations when more and more complexities are added.

A few other caveats of our modelling study need to be mentioned. To limit computing time to reasonable lengths, we set population size at unrealistically low values and mutation rates at unrealistically high values. Indeed, we found that varying these values affected the feasibility of speciation: in general, speciation occurred more easily in larger populations and less easily with lower mutation rates. Another issue is that the initial settings of our simulation runs, where most individuals are initially at the fitness-minimum, can be seen as a special case that is likely to favour divergence. We did not explore how likely it

would be for divergence to occur when the population is initiated at either mostly limnetic or mostly benthic morphology, but it may well be less likely. Similarly, the fact that the initial populations always had at least some degree of assortative mating present from the start, and in fact, random mating was not possible except in model 1, will have increased the likelihood of speciation occurring. In a strictly randomly mating population the evolution of assortative mating is likely to be most difficult in the early stages. And this will especially be the case under the assumption (models 5 and 6) where assortative mating would cause males to refuse to mate at all if they do not encounter their preferred type. For all these reasons, we cannot use our outcomes as quantitative predictions of the likelihood of occurrence of speciation under the mimicked conditions. We merely show what kind of assumptions make it more likely to occur than others in relative terms.

The results of this study have relevance to our understanding of the evolution of the stickleback species pairs. Notwithstanding the fact that the stickleback speciation events are now thought to have been initiated in allopatry (Hendry *et al.* 2009), our results show that sympatric speciation in this system would have been possible in theory. And, the fact that in some of our models speciation occurred with rather low probabilities might explain the rarity of two-species lakes in British Columbia. If, under certain conditions, the probability that speciation occurs is only about 15%, then the presence of species pairs in seven lakes and the presence of solitary species in 30 similar lakes is a very likely finding. Chance might have led to the situation that, for example, on Texada island four lakes contain species pairs, whereas four other lakes do not (McPhail 1993). However, Vamosi (2003) explored how the lakes with and without species pairs differed ecologically, and suggests that these ecological factors rather than chance played a role in the causation of speciation. Our results also show that because of the contingent nature of evolution, a given set of ecological conditions in a lake will not necessarily lead to the evolution of species pairs. The same lake conditions can lead to different outcomes purely as a result of the stochasticity of the process. Some conditions can give rise to an array of different end results: polymorphism does or does not occur, and, if

it does, various levels of reproductive isolation may be achieved (ranging from complete, via sufficient to consider the morphotypes to be true species, to not at all).

Recent work and commentary has emphasised the fragility of the species pairs and their vulnerability to ecological change (Kraak *et al.*, 2001; Taylor *et al.* 2006; Gow *et al.*, 2006; Wootton 2009; Hendry *et al.*, 2009; Behm *et al.*, 2010). This could mean that either the species pairs have not yet established irreversible barriers to mating, which is probably the consensus right now, or the pairs are not the result of true speciation (Wootton 2009; Hendry *et al.*, 2009). Wootton (2009) points out that some work has led to the suggestion that the limnetic form in British Columbian lakes is a late invader that is in the process of being absorbed into the resident benthic form. Hendry *et al.* (2009) suggest that the species pairs may never get beyond the easily reversible separation achieved so far. This echoes work on Darwin's finches and African cichlids. As abundant rain fell in 1982-83 on Daphne Major in the Galapagos archipelago, as a result of El Niño, postmating isolation was lost for a while between *Geospiza fortis* and *G. fuliginosa* because hybrids no longer had beak dimensions that disadvantaged them during feeding (Grant and Grant 2008). Similarly, in case of the African cichlids eutrophication of the lakes has caused the water to become murkier, thereby breaking down the premating isolation barriers (Seehausen *et al.* 1997). Our models confirm the fragility of the speciation process and would need to be developed further to explore how permanent barriers to gene flow could evolve over longer time spans.

Our explorations suggest that both empiricists and modellers should be more explicit about mechanisms of mate choice. In our model 4 (equivalent to a one-allele model), the gene for assortative mating appears to be a conditional gene that instructs, "if sitting in a deep-bodied individual, then prefer deep-bodied mates; if sitting in a thin-bodied individual, then prefer thin-bodied mates". As mentioned before, this requires that individuals have knowledge of their own morphology compared to a potential partner just encountered. Alternatively, one might suppose that the gene causes mating to occur within the preferred habitat and the different phenotypes have different habitat preferences (Rice 1987). Or a mechanism of preferred mating with familiar individuals after imprinting has taken place

during feeding in the preferred habitat might be in place. Filial imprinting while fry reside in the nest of the father might also result in mate preferences for types similar to self (Albert 2005). Rice (1984) speaks of assortative mating alleles “that increase the likelihood of mating between individuals carrying the same allelomorph”. Unless mating in the preferred habitat is meant (or imprinting implied), this appears to be similar to the ‘green beard’ phenomenon in the literature on altruism (Dawkins 1976). There, an allele is proposed that instructs the organism to “behave altruistically to anyone who carries the same allele”. In that case the problem is, how can an individual know whether another individual carries the same allele for this type of altruistic behaviour? The solution is the unrealistic assumption of an allele that pleiotropically codes for, for example, a green beard, and, at the same time, for altruistic behaviour towards individuals with green beards. In the case of assortative mating, most theorists (e.g. Maynard Smith 1996; Felsenstein 1981; Diehl & Bush 1989; see Bolnick and Fitzpatrick 2007) have assumed assortative mating genes, with, for example, individuals carrying allele *A* preferring to mate with individuals carrying allele *A*, and individuals with allele *a* preferring to mate with individuals with allele *a*. This seems rather unrealistic: It supposes that the same gene pleiotropically codes for a phenotype, which is a variant of a physical trait that can be assessed by a conspecific, as well as for a mating preference for that variant of the trait. In other words, the gene codes for the ‘colour of the beard’ as well as for the preference for that particular ‘beard colour’; it is hard to imagine a biological mechanism for this. Van Doorn et al. (2009) also recognized the unrealistic assumptions of many models regarding divergent mate preferences and proposed, as we do, a model where mate choice is based on a trait that indicates adaptation to the habitat. However, while in their model the link between the trait and local adaptation is indirect, via an ornament with condition-dependent expression, in our model the trait subject to mate choice is itself the ecologically adapted trait. While their model may bear relevance to speciation in ornamented birds diverging in resource utilization, the assumptions of our model clearly seem more realistic in the case of the stickleback, where assortative mating has been shown to be partly based on the same trait as the ecological adaptation (Nagel & Schluter 1998; Albert & Schluter 2004).

If it is difficult to envisage a biological mechanism resulting in the one-allele model, it may be more plausible to suppose two loci that segregate independently: one locus for the trait and one locus for the preference, not a preference for similarity, but a preference for a particular variant (i.e. the two-allele model), as in models 5 and 6. Perhaps assortative preferences for similar mates (i.e. of the one-allele type) found in nature have actually arisen from the two-allele situation, in the sense that selection has led to such tight linkage between the two loci that it now appears to be one. It is not at all implausible that mate preferences are at first independent of the individuals' own morphology: the study of mate preferences has revealed cases in which a preference for a certain variant of a trait existed before that variant of the trait itself had evolved in that lineage. For example, female platyfish (*Xiphophorus variatus*) prefer sword tails, even if such tails do not exist in their species and have never been present in their ancestors (Basolo 1995; see review in Ryan 1997). Boughman (2001) has shown that benthic and limnetic females have pre-existing sensory biases based on the spectral characteristics of the water in their respective habitats. In Enos Lake, this has resulted in divergent female preferences based on male nuptial colour. Clearly, in this case the preference has initially evolved independently of the trait.

It would be very interesting to know whether limnetic and benthic sticklebacks have their mate preferences as innately fixed preferences, or whether they are shaped by the phenotype of the conspecifics that they encounter. Preference based on similarity would be possible either if individuals can directly compare their own morphology with that of the prospective mate, or if preferred mating occurs with a familiar type after imprinting on companions during foraging in the preferred habitat or after filial imprinting on the father in the fry stage. The latter has been tested experimentally (Albert 2005) but filial imprinting does not appear to occur. Imprinting during foraging as adults could easily be tested in housing experiments. Breeding experiments should be done to distinguish between the one-allele case (preference for similar mates) and the two-allele case (preference for particular variants). If the one-allele model applies, then all F2 individuals should have an assortative preference for mates similar to self, whereas if the two-allele model applies, the association between own

morphology and preference is broken down in the F2 (Felsenstein 1981). Empiricists can also test whether the stringency of choosiness varies among individuals, and whether this is inherited. Finally, we would like to know whether males and females similarly express assortative mate preferences and stringency of choosiness, or whether they differ. All these aspects of mate choice are shown to influence the likelihood of sympatric speciation occurring, and hence, they bear significantly on the issue of the evolution of the species pairs.

If the details of mate choice in the stickleback would be known more precisely, we could fine-tune the details of simulation models to resemble reality more precisely. Perhaps it is possible to use models to gain insight into why some lakes have species pairs whereas others have not, e.g. by including additional ecological selection pressures that differ between the preferred habitats (Vamosi 2003). Variation among lakes certainly exists with respect to population size and density (and its effects on resource competition), availability of both food types, predation risk in either habitat, and parasites in either habitat. The species pairs occur in lakes with fewer competing species and predators than are present in similar lakes with no species pairs (Vamosi 2003). The next step is to construct models in which we can vary such parameters, to test whether they possibly have played a causal role in the pattern of presence/absence of sympatric species pairs.

In conclusion, our investigation confirms that some general findings of the more abstract models hold true under more mechanistic assumptions. Most importantly, the more naturalistic approach has yielded explicit recommendations that certain model assumptions should be tested before final conclusions can be reached about particular cases of speciation. Finally, the results demonstrate the contingency of speciation, with the same starting point not necessarily producing the same outcome.

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Figure 1. Flow diagram of the individual based genetic algorithm.

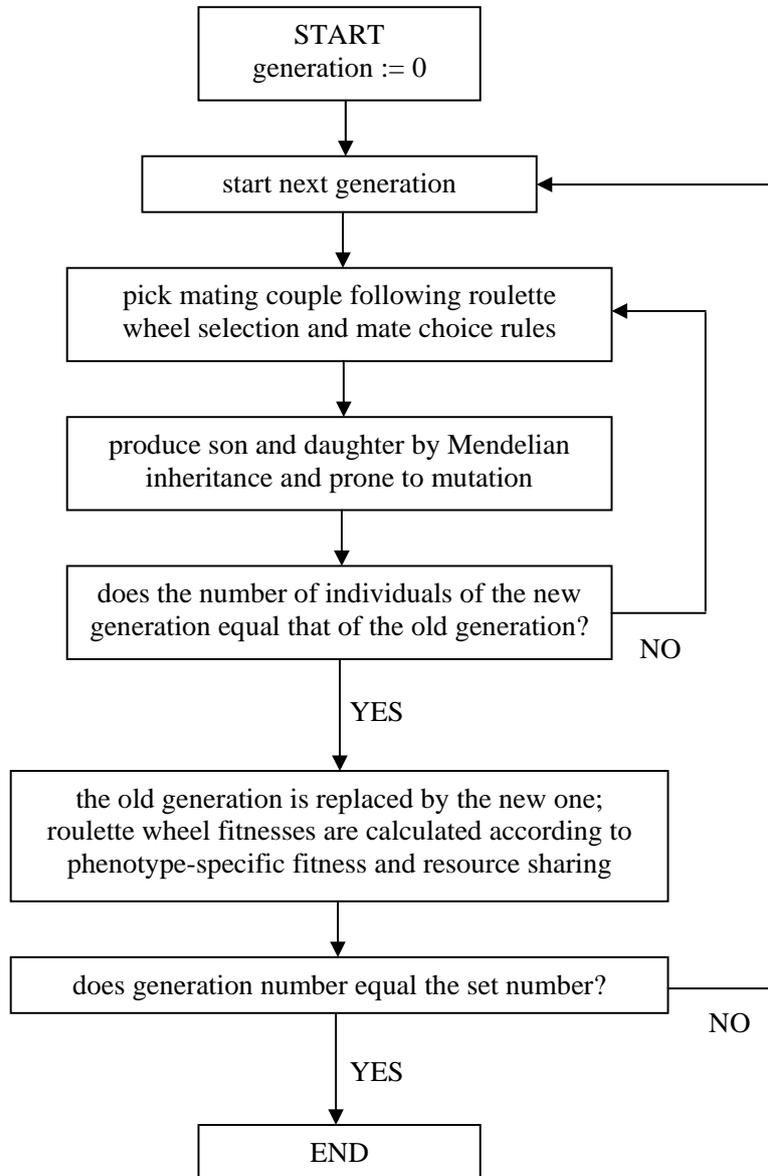


Figure 2. Relative fitness is a function of morphology T . Here $\text{fitness} = \sin(2\pi * T)^2 + 1$ (relative fitness varies between 1 and 2, i.e. twofold). In model 3 we used a flat fitness function: $\text{fitness} = 1$. In model 5, we used the above fitness function, as well as: $\text{fitness} = \sin(2\pi * T)^2 + 0.5$ (relative fitness varies between 0.5 and 1.5, i.e. a threefold selection differential), and $\text{fitness} = \sin(2\pi * T)^2 + 0.25$ (relative fitness varies between 0.25 and 1.25, i.e. a fivefold selection differential).

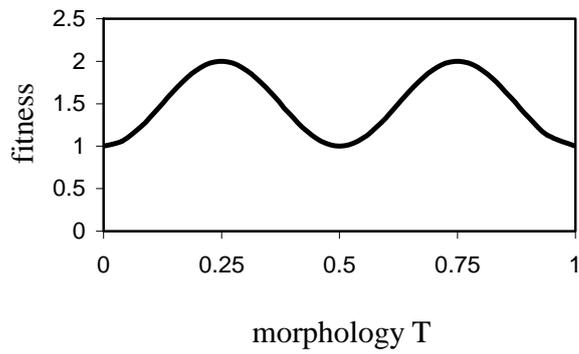
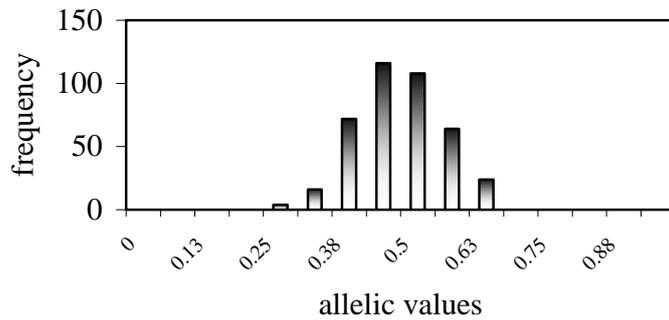
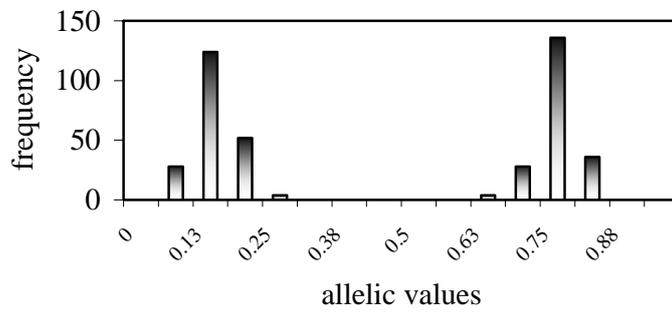


Figure 3. Model 1. A. Typical initial distribution of the allelic values at generation 0. B. Typical distribution of the allelic values at generation 100. C. Typical distribution of T, the phenotypic values, at generation 100. $Nm = Nf = 100$; $\sigma = 0.25$; $\mu = 1\%$; $n = 256$ alleles. Similar results were obtained in 20 out of 20 replicate simulations with $\sigma = 0.25$, in 13 out of 20 replicate simulations $\sigma = 0.5$, and in 0 out of 10 replicate simulations with $\sigma = 1$.

A.



B.



C.

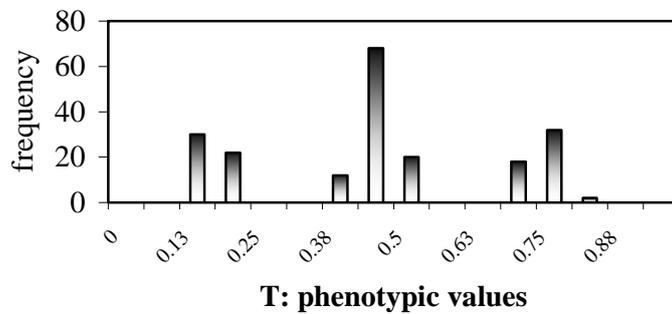


Figure 4. Model 2. Typical distribution of T , the phenotypic values, at generation 100. $N_m = N_f = 100$; $\sigma = 0.5$; $\tau = 0.5$; $\mu = 1\%$; $n = 256$ alleles. Only 10 out of 200 (5%) individuals are hybrids. Similar results were obtained in 10 out of 20 replicate simulations with $\tau = 0.5$, and in 10 out of 10 replicate simulations with $\tau = 0.25$.

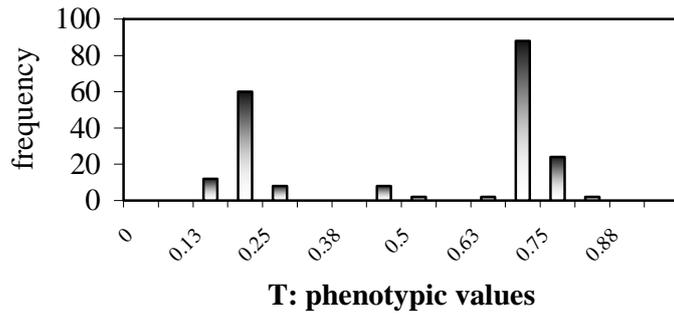


Figure 5. The probability that a female accepts a male as a mate is a function of the morphological difference between them, and her stringency of choosiness S (here $\tau = S + 0.25$), as in model 4 (variants applied in models 6 and 8). In the figure, 3 values of S are shown; S can have all values that are averages of two allelic values (from 64 or 256 equidistant values from 0 to 1).

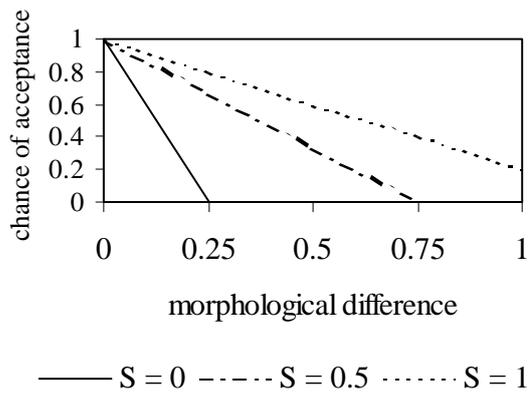


Figure 6. Model 4: Reinforcement of stringency of assortative mating. First column: typical distribution of morphology alleles. Second column: typical distribution of morphology phenotypes T. Third column: typical distribution of stringency of choosiness alleles. First row: at generation 0. Second row: at generation 15. Third row: at generation 30. Fourth row: at generation 50. Fifth row: at generation 80. Sixth row: at generation 100. Values on x-axis are the same as in Fig. 2 and Fig. 3. $N_m = N_f = 100$; $\sigma = 0.5$; $\tau = S + 0.25$; $\mu = 1\%$; $n = 256$ alleles. Similar results were obtained in 9 out of 20 replicate simulations with the same settings.

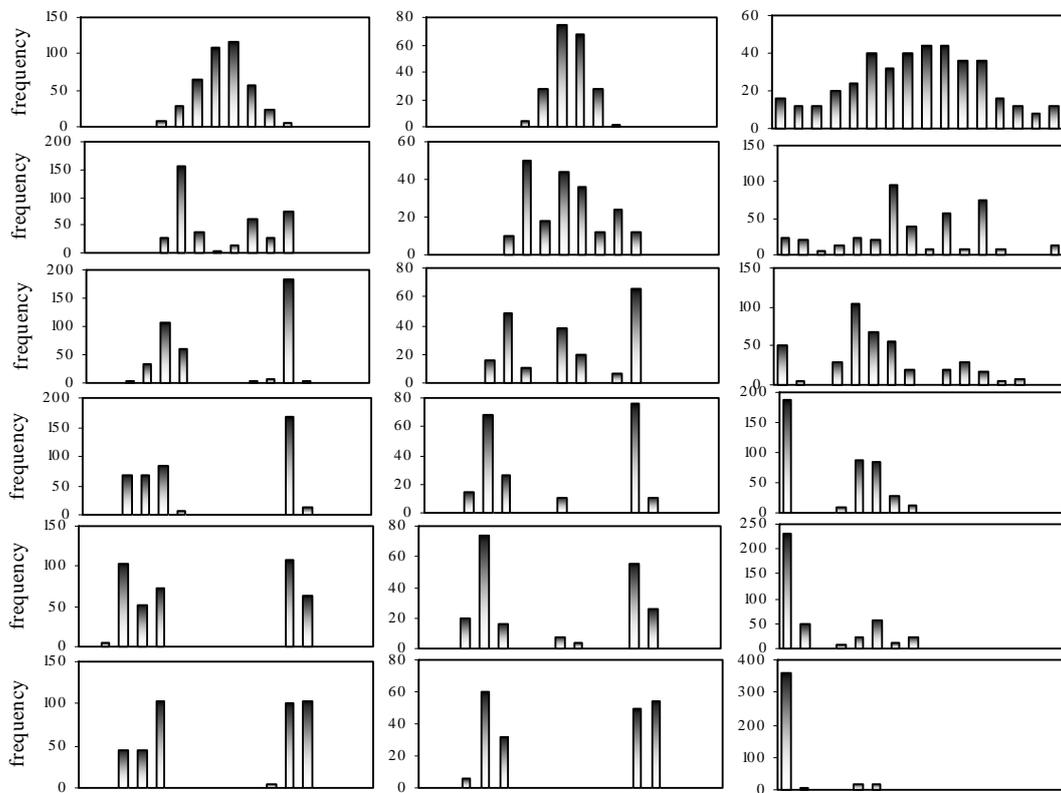


Figure 7. Model 5: Reinforcement of divergent mating preferences. First column: typical distribution of morphology alleles. Second column: typical distribution of preference alleles. Third column: typical distribution of morphology phenotypes T. First row: at generation 0. Second row: at generation 100. Third row: at generation 200. At generation 100, 92% of individuals have either all benthic alleles at both loci, or all limnetic alleles at both loci. At generation 200 this percentage is 93.5%. Values on x-axis are the same as in Fig. 2 and Fig. 3. Both males and females are choosy; $N_m = N_f = 100$; $\sigma = 0.5$; $\tau = 0.5$; $\mu = 1\%$; $n = 256$ alleles. The frequencies with which similar results were obtained under various configurations can be found in Table 3.

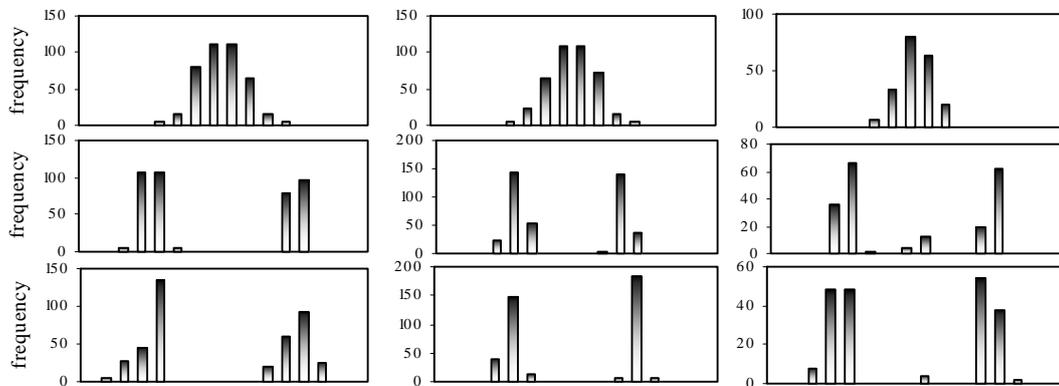


Figure 8. Fitness landscape for models 7 and 8. Relative fitness is a function of trait T1 and trait

T2. Epistasis is modeled as follows:

If $(T1 < 0.5 \text{ and } T2 < 0.5)$ or $(T1 > 0.5 \text{ and } T2 > 0.5)$,

then fitness = $\sin(2\pi * T1)^2 + \sin(2\pi * T2)^2 + 2$.

Else, if $|T1 - T2| < 0.5$,

then fitness = $|\sin(2\pi * T1)^2 - \sin(2\pi * T2)^2| + 2$.

Else, if $(T1 > 0.75 \text{ and } T2 < 0.25)$ or $(T1 < 0.25 \text{ and } T2 > 0.75)$,

then fitness = 1.

Else, fitness = $-\sin(2\pi * T1)^2 - \sin(2\pi * T2)^2 + 2$.

Hence, fitness varies between 1 and 4 inclusive.

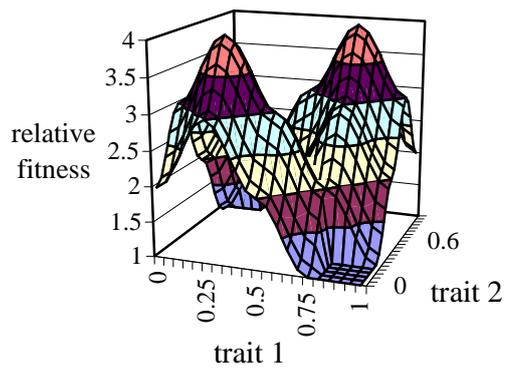


Table 1. The eight models and their build-up of complexity along various dimensions.

Model	Disruptive selection differential	Resource competition strength	Assortative mating	Stringency of preference	Number of choosy sexes	Number of traits
1	Twofold	Varied	No	-	-	1
2	Twofold	Medium	Similarity-based	Fixed	-	1
3	Absent	Moderate	Similarity-based	Fixed	-	1
4	Twofold	Medium	Similarity-based	Evolving	-	1
5	Two- to fivefold	Varied	Divergent	Fixed	Varied (both, or females only)	1
6	Fivefold	Strong	Divergent	Evolving	Varied (both, or females only)	1
7	Fourfold	Strong	Similarity-based	Fixed	-	2
8	Fourfold	Strong	Similarity-based	Evolving	-	2

Table 2. Model parameters.

Parameter	Explanation and values
N_m, N_f (from model 1 onwards)	number of males, number of females, equal and fixed values are set at 100 or at 500
G (from model 1 onwards)	number of generations for which a simulation is run value is set at 100, 200, 1000 or 2500
T (from model 1 onwards)	phenotypic value of a morphological trait, such as body shape, e.g. body depth : body length ratio value evolves and ranges from 0 to 1 inclusive; 0.25 is the optimal benthic body shape, 0.75 is the optimal limnetic body shape, and 0.5 is the intermediate body shape
μ (from model 1 onwards)	mutation rate of allelic values (per allele, per generation) value is set at 1% or at 0.01%
σ (from model 1 onwards)	morphological difference between two individuals above and at which the individuals do not count as competitors anymore; $\text{competitor} = 1 - \text{difference} / \sigma, \quad \text{difference} < \sigma$ $= 0, \quad \text{otherwise}$ value is set at 0.25, 0.33, 0.5, or 1

<p>τ</p> <p>(from model 2 onwards)</p>	<p>difference between two individuals' morphologies, or between one individual's preferred mate morphology and the other individual's own morphology, above and at which a potential mating is refused;</p> <p style="text-align: center;">mating probability = $1 - \text{difference} / \tau$, $\text{difference} < \tau$ = 0, otherwise</p> <p>value is set at 0.25 or 0.5, or evolves as $\tau = S + 0.25$ (see below)</p>
<p>S</p> <p>(from model 4 onwards)</p>	<p>stringency of mate choice; low values represent stringent choice, high values loose choice; S determines τ (see above) as $\tau = S + 0.25$ (see also Fig. 5)</p> <p>value evolves and ranges from 0 to 1 inclusive</p>
<p>P</p> <p>(from model 5 onwards)</p>	<p>phenotypic value of a preference for a particular mate morphology; an individual with value P prefers a mate with morphology $T = P$ (see above, see also τ above)</p> <p>value evolves and ranges from 0 to 1 inclusive; 0.25 indicates a preference for the optimal benthic body shape, 0.75 for the optimal limnetic body shape, and 0.5 for the intermediate body shape</p>
<p>$T1, T2$</p> <p>(from model 7 onwards)</p>	<p>phenotypic values of two different morphological traits, e.g. body shape and gill raker number</p> <p>values evolve and range from 0 to 1 inclusive; for both traits, 0.25 is optimal for benthic feeding, 0.75 is optimal for limnetic feeding, and 0.5 is intermediate</p>

Table 3 A. Female mate choice only.

		Twofold disruptive selection differential	Threefold disruptive selection differential	Fivefold disruptive selection differential
$\sigma = 0.5,$ $\tau = 0.5$	Fixation <i>T</i> polymorph <i>T & P</i> polymorph % hybrids	14 5 1 39	20	20
$\sigma = 0.25,$ $\tau = 0.5$	Fixation <i>T</i> polymorph <i>T & P</i> polymorph % hybrids	14 6 41, SD=3, 36-44	10 10 41, SD=5, 33-50	9 11 38, SD=9, 25-55
$\sigma = 0.5,$ $\tau = 0.25$	Fixation <i>T</i> polymorph <i>T & P</i> polymorph % hybrids	16 4	16 4 6, SD=5, 0-11	12 8 6, SD=6, 0-18

		Twofold disruptive selection differential	Threefold disruptive selection differential	Fivefold disruptive selection differential
$\sigma = 0.25,$ $\tau = 0.25$	Fixation <i>T</i> polymorph <i>T</i> & <i>P</i> polymorph % hybrids	12 8 30, SD=6, 23-39	3 17 28, SD=13, 4-53	3 17 14, SD=11, 4-49

Table 3 B. Male and female mate choice.

		Twofold disruptive selection differential	Threefold disruptive selection differential
$\sigma = 0.5,$ $\tau = 0.5$	Fixation	15	17
	T polymorph	5	
	T & P polymorph		3
	% hybrids		13, SD=3, 10-16
$\sigma = 0.25,$ $\tau = 0.5$	Fixation		
	T polymorph	9	1
	T & P polymorph	11	19
	% hybrids	34, SD=11, 19-49	27, SD=10, 4-43
$\sigma = 0.5,$ $\tau = 0.25$	Fixation	19	16
	T polymorph		
	T & P polymorph	1	4
	% hybrids	11	3, SD=2, 0-5
$\sigma = 0.25,$	Fixation		
	T polymorph	7	

		Twofold disruptive selection differential	Threefold disruptive selection differential
$\tau = 0.25$	<i>T</i> & <i>P</i> polymorph	13	20
	% hybrids	14, SD=11, 0-34	7, SD=8, 0-28

Twenty replicates were run with each of 20 parameter combinations, all with $G = 1000$, $\mu = 0.01\%$; $n = 64$ alleles. Parameters that varied are: only females (*A.*), or both males and females choosy (*B.*); relative fitness differences (see text and Fig. 2); σ , τ . Three possible outcomes occurred: (1) fixation (fixation of either the benthic or the limnetic morph); (2) trait polymorphism (a bimodal distribution of morphology alleles and a unimodal distribution of preference alleles); (3) trait and preference polymorphisms (bimodal distributions of both morphology and preference alleles). The number of times that each type of outcome occurred with each parameter combination is indicated in each cell. The degree to which reproductive isolation between the two morphs is reached in outcomes of type (3) is expressed as % hybrids produced in generation 1000 (mean, standard deviation, range).

Table 4. Results of runs of the eight models with variant levels of parameter value. The frequency of occurrence of a stable polymorphism is given for each of the tabulated conditions. For cases of polymorphism the level of reproductive isolation is given in terms of % hybrids produced after a number (see text) of generations.

Model	Level of varying parameter	Frequency (%) of occurrence of polymorphism	Reproductive isolation: % of hybrids
1 Varying stringency of resource competition	$\sigma = 0.25$ $\sigma = 0.5$ $\sigma = 1$	100 65 0	No isolation
2 Varying stringency of choosiness	$\tau = 0.25$ $\tau = 0.5$	100 50	0% 2-8%
3		100	0%
4		45	0%
5	Results in Table 3		
6 Varying number of choosy sexes	females choosy both sexes choosy	20 0	4-44% -
7		85	0-7%

Model	Level of varying parameter	Frequency (%) of occurrence of polymorphism	Reproductive isolation: % of hybrids
8 Varying mutation rate	$\mu = 1\%$	100	10-16%
	$\mu = 0.01\%$	100	13-25%