The driving factors of ecological speciation and their interactions

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2010-04-22

A thesis submitted to McGill University in partial
fulfilment of the requirements of the degree of Doctor of Philosophy

DEDICATION

À mes parents

ACKNOWLEDGEMENTS

First, I would like to thank my supervisor, Andrew Hendry, for his continuous support. He has believed in my capacity to become an evolutionary biologist, despite my non-conventional background of physics, computer science and geography. He always provided meaningful comments and relevant ideas about scientific research.

I am also thankful to the professor with who I interact, in particular Professor Daniel J. Schoen and Professor Frédéric Guichard, both members of my supervisory committee.

I would like to thank the members of my defense committee: Professor Bernard Shapiro, Professor Donald Kramer, Professor Claire de Mazancourt, Professor Brian Leung, Professor Gregor Fussmann and Professor Sabin Lessard.

I am grateful to the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Redpath Museum for funding.

I am thankful to the Fields Institute, The National Evolutionary Synthesis Center (NESCent), Graduate training committee of the Biology Department at McGill University, Delise Alisson, Complexity Science Group at the University of Calgary (Maya Paczuski, in particular), Marie Curie Speciation training network, The Biology Graduate Students Association (BGSA) at McGill University, and The Society for the Study of Evolution (SSE) for various travel grant.

I am indebted to my colleagues in the Hendry lab for their help and support. They were always there to teach me some biology or to proofread my manuscripts.

In alphabetical order they are: Matthieu Amalric, Daniel Berner, Maryse Boisjoly, Cristián Correa, Erika Crispo, Lari DeLaire, Joey DiBattista, Luis Fernando De León, Swanne Gordon, Kiyoko Gotanda, Anne-Catherine Grandchamp, Ben Haller, Kate Hudson, Istvan Imre, Renaud Kaeuffer, Jacques Labonne, Caroline LeBlond, Camille Macnaughton, Ann McKellar, Jean-Sebastien Moore, Joost Raeymaekers, Katja Räsänen and Amy Schwartz.

I am grateful to those who work behind the scene to make my graduate student life easier. Those people include and are not limited to Susan Bocti (Department of Biology) and Marie LaRicca (Redpath Museum).

Je ne peux passer sous silence toute l'aide que j'ai reçue de ma partenaire de vie, Valérie Hudon, afin de passer à travers trois cycles universitaires et les diverses épreuves de la vie.

Finalement, je tiens à remercier mes parents, Hélène Plante et Jean Thibert, pour l'éducation qu'ils m'ont donnée. Sans leur inconditionnel amour et leur support, cette thèse n'existerait pas.

CONTRIBUTION OF AUTHORS

I am the principal author of all the chapters included in this thesis. This means that I organized and executed the experiments, the analyses, and that I wrote most of the text. Professor Andrew P. Hendry, is coauthor on all papers included in this thesis and he gave me the permission to include them in this thesis.

ABSTRACT

The process by which one species becomes many (speciation) was considered by Darwin to be the *mystery of mysteries*. Speciation is no longer a complete mystery, but we still have a lot to discover about its mechanisms. Of particular interest is the process where reproductive isolation evolves as a byproduct of local adaptation, called ecological speciation. Ecological speciation is of interest to theoretician who can look at the condition that allow local adaptation in face of gene flow, but it is also interesting to field biologist because they can study the process as it is happening. Many factors, such as natural selection, sexual selection, environmental difference between the environments, can influence the evolution of reproductive isolation. In this thesis, I used individual-based numerical simulation to address the relative contribution of natural selection, sexual selection, environmental difference between the environments on the progression of the ecological speciation process. I then evaluated the effect of phenotypic plasticity on a similar system. To inform field biologist about a method to infer ecological speciation using neutral markers, I ran a power analysis to determine in what conditions the method was accurate. Finally, I looked at the relative role competition, sexual selection and the shape of the resource distribution in a sympatric scenario. I found that natural selection greatly influence progress toward ecological speciation, but without the added contribution of sexual selection, speciation could not be achieved. Phenotypic plasticity can either promote or constrain progress toward ecological speciation,

depending on the timing of migration relative to the expression of the plasticity. Using neutral markers to infer ecological speciation is reliable if migration rate is intermediate, i.e. about one migrant per generation per population. In sympatry, sexual selection was the most important promoter of speciation, but could not complete the process if competition was strong. In this thesis, I model different scenarios and identify the role, and interaction, among various promoter of ecological speciation. I also contribute the study of ecological speciation *in the field* by providing a power analysis to determine if the method to infer ecological speciation using neutral markers is accurate.

RÉSUMÉ

Le processus par lequel une espèce devient plusieurs (spéciation) était considéré par Darwin comme le *mystère des mystères*. La spéciation n'est plus un mystère complet, mais nous avons encore beaucoup à expliquer à propos de ses mécanismes. En particulier, comment l'isolement reproductif peut évoluer en tant que sous-produit de l'adaptation locale. Ce processus est connu sous le nom de spéciation écologique. La spéciation écologique est intéressante pour les théoriciens qui peuvent étudier les conditions dans lesquelles l'adaptation locale peut évoluer face au flux génique. Les biologistes de terrain s'intéressent aussi à la spéciation écologique, car ils peuvent l'étudier pendant qu'elle progresse. Plusieurs facteurs peuvent influencer l'évolution de l'isolement reproductif : la sélection naturelle, la sélection sexuelle et l'environnement, entre autres. Dans cette thèse, j'utilise des simulations numériques orientées-individus afin de déterminer la contribution relative de la sélection naturelle, de la sélection sexuelle et des différences entre les environnements dans la progression du processus de spéciation écologique. J'ai ensuite étudié l'effet de la plasticité phénotypique sur un système similaire. Afin d'assister les biologistes de terrain, j'ai fait une étude sur la puissance statistique d'une méthode largement utilisée afin d'inférer la spéciation écologique à partir de marqueurs génétiques neutres. Finalement, j'ai examiné le rôle relatif de la compétition, de la sélection sexuelle et de la forme de la distribution des ressources sur la spéciation sympatrique. J'ai trouvé que la sélection naturelle influence grandement le progrès vers la spéciation écologique, mais sans l'ajout

de la sélection sexuelle, la spéciation ne pouvait pas être complète. La plasticité phénotypique peut soit promouvoir, soit contraindre la progression vers la spéciation écologique, tout dépend si l'expression de la plasticité se fait avant ou après la migration. L'utilisation des marqueurs génétiques neutres pour inférer la spéciation écologique est précise si le taux de migration est intermédiaire, i.e. environ un immigrant par population par génération. En sympatrie, la sélection sexuelle est le plus important promoteur de la spéciation, par contre, elle ne peut être complétée si la compétition est forte. Dans cette thèse, je modélise différents scénarios et j'identifie le rôle de divers promoteurs de la spéciation écologique ainsi que leurs interactions. Je contribue aussi à l'étude de la spéciation écologique *sur le terrain* en fournissant une analyse de puissance afin de déterminer l'applicabilité d'une méthode largement employée pour inférer la spéciation écologique en utilisant des marqueurs génétiques neutres.

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CHAPTER 1
Introduction

The diversity of life today is the result of speciation that occurred in the past (Darwin 1859). To understand diversity, it is therefore essential to understand the process of speciation (Hutchinson 1959; Felsenstein 1981). This understanding will also be critical for the future. We are probably witnessing the sixth wave of mass extinction on our planet (Wake & Vredenburg 2008), largely driven by human influences (Thomas et al. 2004). Because of those changes, the biodiversity is at great risk (Parmesan 2006) and if we want to help maintain and promote biodiversity, we again need to better understand the process of speciation.

In order to answer questions about the origin of species, we need a definition of species. There is no absolute consensus (Coyne & Orr 2004; Hey 2009), but the biological species concept is widely adopted. This concept proposes that species are "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups" (Mayr 1963). This definition contains two essential elements: free reproduction within a group and reduced reproduction between groups. The study of speciation examines how this isolation arises and is maintained. Speciation is the focus of my thesis and so I will first outline some basic background information on our current understanding of the process.

Speciation modes and mechanisms

Speciation *modes* are often characterized by their geographical context (Mayr 1963; Coyne & Orr 2004; Gavrilets 2004): allopatric, parapatric, or sympatric. In allopatric speciation, isolated populations will diverge due to physical separation, resulting in two species occupying non-overlapping locations separated by barriers to dispersal. By contrast sympatric speciation occurs when populations diverge in the same location, with no physical barrier to dispersal. Between these two extremes, parapatric speciation represents a case where an absolute physical barrier to dispersal is absent, but some partial spatial separation is nevertheless present. An example would be fishes living in lakes and their adjacent streams. Technically, the migration rate between environments is zero in allopatry, 0.5 in sympatry, and everywhere in between for parapatry. A more recent view of speciation considers geographic modes of speciation only as one form of assortative mating among others, such as mating preferences (Kirkpatrick & Ravigné 2002). As a result, the classification of speciation now refers less often to geographical contexts and more commonly to the "mechanisms" of divergence and reproductive isolation.

The potential mechanisms driving speciation are many and can be divided in several ways: speciation under uniform selection, speciation by genetic drift, polyploid speciation, and ecological speciation (Schluter 2001). Speciation under uniform selection is the result of isolated populations experiencing similar selection that fix different alleles, because different beneficial mutations arise in each population simply by chance. Subsequently, each population culminates with the

fixation of alleles that are incompatible among populations (Turelli et al. 2001). Genetic drift can also lead to incompatibility between populations by random fixation of different alleles (Schluter 2001), while polyploid speciation occurs when offspring do not have the same number of chromosomes as their parents, which commonly occurs, though not exclusively in plants (Thomson & Lumaret 1992), although not exclusively (Coyne & Orr 2004). My study focuses on speciation initiated by divergent natural selection, namely "ecological speciation" (Schluter 2001).

Ecological speciation

Ecological speciation is a process by which barriers to gene flow evolve as a result of ecologically-based divergent selection (Schluter 2000; Rundle & Nosil 2005). This divergent selection is often based on alternative resources, such as different food types (Grant & Grant 2006) or host plants (Feder et al. 1994; Nosil et al. 2002). Other factors contributing to ecological speciation include sexual selection and ecological interactions (Rundle & Nosil 2005). With respect to competition, it is possible that a single population can diverge into two as a result of competition for a shared resource (Dieckmann & Doebeli 1999; Drossel & McKane 2000; Doebeli et al. 2007).

Two main types of reproductive isolation can result from divergent selection owing to different environments or intra-specific competition: pre-zygotic or post-zygotic. Pre-zygotic isolation prevents gametes from encountering each other, either pre- or post-mating. Pre-mating isolation is a particularly important form of pre-zygotic isolation because the gametes never come into contact. The causes of

pre-mating isolation are many, such as when populations prefer different habitats (Feder et al. 1994; Nosil et al. 2002), mate at different times (Hendry & Day 2005; Savolainen et al. 2006), or survive poorly when moving between habitats (Nosil 2004). Pre-mating isolation can also arise if a female chooses its mate according to traits, such as color pattern (Houde 1987) or song type (Podos et al. 2004), that diverge owing to adaptation to the different environments.

Post-zygotic isolation occurs when male and female gametes encounter each other, but cannot form a fertile viable organism. This inviability can be due to the epistatic effect of incompatibility between alleles at different loci, therefore not an ecological mechanism, as in the Bateson-Dobzhansky-Muller model (Bateson 1909; Dobzhansky 1936, 1937; Muller 1940, 1942). It can also be due to ecological-based hybrid inferiority, where hybrids do not have intrinsic genetic incompatibilities, but rather are poorly adapted for any parental environment (Hatfield & Schluter 1999; Rundle 2002; Schluter 2000, 2001). This latter situation is a potentially important part of ecological speciation.

The genetic architecture can also play a role in speciation. Speciation is more likely if the trait that is under natural selection is the same as the one under sexual selection. Such traits are called "magic traits" and can be found in Darwin's finches (Grant & Grant 1997) and *Hypoplectrus* coral reef fishes (Puebla et al. 2007). The presence of a magic trait facilitates progress toward speciation compared to speciation that involves different traits (Fry 2003). The number of loci coding for a trait also influence speciation. The more loci coding for a trait, the weaker is the selection of each locus for a given strength of selection on a trait.

This reduces the likeliness of speciation (Gourbiere 2004; Gavrilets & Vose 2005; Bürger et al. 2006; Gavrilets et al. 2007; Gavrilets & Vose 2007, 2009).

1.1 Some illustrative examples of ecological speciation

Ecological speciation can be observed in many natural systems that can inform on the different factors influencing ecological speciation. Describing these systems will help to illustrate the empirical motivation for my research and the various questions that arise and can be addressed with simulation models.

Darwin's finches

Darwins finches of Galápagos radiated from a single initial colonizing species into 14 recognized species in approximately 1.5 million years (Grant 1999). The different species now occupy many different niches ranging from insect eaters to seed eaters, with additional partitioning within those broad classes. This diversity of niches, largely unoccupied before finches arrived on the archipelago, has provided ecological opportunities that have driven phenotypic divergence in beak size and shape. This divergence has then led to the evolution of reproductive isolation that is intimately linked to beak size and shape. Darwin finches mate according to their songs, which are determined in part by functional constraints stemming from beak size and shape (Podos et al. 2004). In addition, young females and males imprint on their fathers song type and use these during mating (Grant 1999). In short, both adaptive divergence and mating isolation are linked to beak size and shape, making Darwins finches a prime candidate for so-called "magic trait" speciation (Gavrilets 2004). That this isolation has an ecological component is clear because intrinsic genetic incompatibilities are lacking and a

change in environmental conditions leads to a breakdown of reproductive isolation (Grant et al. 2004; Hendry et al. 2006).

Stickleback

Following the most recent retreat of Pleistocene glaciers, marine three-spined stickleback (*Gasterosteus aculeatus*) colonized fresh water and formed large number of natural replicates in divergent environments, such as lake versus stream, benthic versus limnetic, and anadromous versus freshwater (McKinnon & Rundle 2002). For example, sympatric benthic and limnetic forms in lakes use different, but adjacent resources, namely the benthos and the open water. These different forms have diverged in a large number of characters that aid survival and reproduction in the two environments (McPhail 1992; Nagel & Schluter 1998). These same traits then contribute to mating isolation (Vines & Schluter 2006), as well as natural selection against hybrids that are poorly suited for either environment (Hatfield & Schluter 1999). Again, the ecological basis for this reproductive isolation is clear because intrinsic genetic incompatibilities are lacking and changing conditions in one sympatric benthic-limentic lake led to the fusion of the two species into a single hybrid swarm (Taylor et al. 2006).

Cichlids

More than 500 cichlid species have arisen in Lake Victoria over the past 100000 years, many of them probably in the last 15,000 years (Verheyen et al. 2003). A number of these species are differentiated by diet and morphology, suggesting adaptive divergence has contributed to ecological speciation. Here, however, the main factor determining mating isolation is color, which is less

obviously tied to foraging specialization, with some exceptions (Seehausen et al. 2008). Thus, cichlids have been suggested as a case where sexual selection might be particularly important in speciation, both with or without adaptive divergence. An even more recent example of divergence between benthic and limnetic forms of cichlids comes from a Nicaraguan lake. The reduced complexity of the system allowed Barluenga et al. (2006) to show that the divergence may have occurred in less that 10000 years. Thus, ecological speciation might well occur quite quickly and repeatedly.

Timema **walking sticks**

Timema walking sticks are herbivorous insects that feed and reproduce on different plants (Nosil 2007). In particular, different morphologies (color, body size and shape) have evolved to increase crypsis on the local host plant type (Nosil et al. 2002; Nosil & Crespi 2004, 2006). These morphologies are then linked to reproductive isolation in the form of natural and sexual selection against migrants between plant types and against hybrids, as well as mating isolation (Nosil et al. 2003, 2007).

Palms on an oceanic island

Ecological speciation is not restricted to the animal kingdom. Savolainen et al. (2006) described a potent case in plant kingdom. *Howea belmoreana*, a species of palm tree diverged on a remote island $580km$ of the Australian coast (Lord Howe Island). The higher pH soil triggers reproduction later than the calcarentite substrate. As a result, there is almost no overlap in the reproductive period for palms in the two different substrates. The plasticity of the reproductive period

enables reproductive isolation and *Howea forsteriana* was able to adapt to the calcearenite substrate.

Key questions in ecological speciation

My approach is to study ecological speciation through the implementation of mathematical models. Most models of speciation are designed to calculate the probability of speciation, the average waiting time to speciation, or the average duration of the process (Orr & Orr 1996; Gavrilets 2004). In these genetic models, speciation is often through drift and selection on a "holey" fitness landscape or through a peak shift on a more rugged adaptive landscape (review in Gavrilets 2004). At present, however, no models provide a description of the dynamic process of speciation at the ecological level and on an ecological time frame. That will be my goal.

1.2 Modeling speciation

Gavrilets provides a good compilation of the literature on speciation modeling in his recent book (Gavrilets 2004). The first part of the book is dedicated to adaptive landscapes, the second part looks at an epistatic model (Bateson-Dobzhansky-Muller), and the last part deals with disruptive natural selection and non-random mating. Since this review and integration is well organized and follows a logical progression, I will follow the same plan while surveying existing models. This survey is helpful because it shows how my approach is different, and therefore novel.

Fitness/adaptive landscape

The fitness/adaptive landscape is an unclear concept, especially because Wright and others have used it to define several different concepts (Wright 1931, 1932; Simpson 1953; Schluter 2000). For Wright, the independent variable is genotype space and the dependent variable is fitness. In one Wrightian landscape, the association between the gene and the fitness of an organism is directly made (Wright 1932). Simpson (1953) extended this concept to phenotype, rather than genotype, space as the independent variable, while keeping the mean population fitness as the dependent variable.

Very little is known about the shape of the adaptive landscape in nature: is it a smooth normal distribution, a rugged landscape, a holey landscape, or a flat landscape? This question is critical for how evolution will proceed. On a bell shaped fitness landscape, the population will climb upward until reaching the peak maximizing fitness (Lande & Arnold 1983). On a flat landscape, the population is free to drift. By definition a rugged landscape has multiple peaks. The question here is how a population sitting on one peak can split to occupy two different peaks. Models such as the Russian Roulette and Markov chain are particularly useful to study peak shifts (Gavrilets 2004).

Bateson-Dobzhansky-Muller model

Most speciation models consider the evolution of genetic incompatibilities such as the Bateson-Dobzhansky-Muller model (Bateson 1909; Dobzhansky 1936, 1937; Muller 1940, 1942), or the evolution of the genetic distance between populations or individuals (Gavrilets 2004). The Bateson-Dobzhansky-Muller model is a

two-allele, two-locus model where an incompatibility is present between alleles at different loci. For example, the presence of both a and B in the same individual leads to sterility or inviability. Imagine a homogeneous initial population of $AAbb$ that splits into two habitats favouring different alleles at each locus: $aabb$ in one habitat and $AABB$ on the other. In the event of secondary contact between the two populations, those populations will be reproductively isolated if $AaBb$ hybrids are inviable or infertile. From this model it is possible to calculate the probability of speciation, the average waiting time to speciation, and the average duration of speciation.

These models have yielded many insights into speciation, but they completely ignore ecologically-dependent reproductive isolation, which is thought to be critical (Schluter 2000; Rundle & Nosil 2005). My model focuses specifically on this second context for speciation.

Adaptive dynamics

Another class of speciation models are adaptive dynamics models. This approach is worth mentioning because it is the basis of some well cited theoretical papers about sympatric and parapatric speciation (Dieckmann & Doebeli 1999; Doebeli & Dieckmann 2003). Adaptive dynamics (Dieckmann et al. 2004) is the theoretical study of the inviability of a rare mutant in a population. According to this theory, competition drives the population to a fitness minimum and, at this point, evolutionary branching can occur. This branching is the speciation event (Doebeli & Dieckmann 2000). Adaptive dynamics has been severely criticized in the context of speciation, because of the small effect of mutations (Gavrilets &

Waxman 2002; Gavrilets 2005) and the permanence of the isolation (Polechová & Barton 2005). Moreover, this method usually, though not always, deals with asexual reproduction and a single trait (Gavrilets 2004), whereas the real trick to speciation is to avoid hybridization (Felsenstein 1981; Coyne & Orr 2004).

In this thesis, we will use an explicit genetic model with many loci and different traits. We will use a Simpson version of the adaptive landscape approach, in which a resource distribution available to a certain phenotype of the consumer is used as a "fitness" landscape that allows competition. We will not include any genetic incompatibility to be able to monitor the evolution and adaptation in the face of gene flow.

1.3 Generating or testing hypotheses

Ecologists and evolutionary biologists often proceed by stating and formally discriminating among a series of null and alternative hypotheses. In my study, I can really only state null hypotheses such as A does not influence B, which is already implicit due to the fact that I am examining a series of unknown possible interactions among different factors. The interactions I will investigate are so complex and multifactorial that it is not useful or even helpful to state a large series of alternative hypotheses. In most cases, we do not have direct comparable work to guide our intuition and help generate alternative hypotheses. In the following sections, I will often state particular hypotheses but it is important to recognize that I am only mentioning a few of many possibilities. In general, my work is an exploratory analysis designed to uncover possible patterns and effects (Tukey 1977). Instead of testing alternative hypotheses formally, I therefore view

my study as helping to generate such hypotheses, which might then be subject to future empirical testing.

Divergent environments

Divergent environments, such as differences in host plants or between lake and stream habitats, can induce divergent selection. Different expressions of a trait could be favoured to increase crypsis (Nosil et al. 2002) or swimming performance (Hendry et al. 2002). If intermediate resources are not available, not only will the hybrids, but the production of hybrids be selected against in a process called reinforcement (Kirkpatrick 2001). Reinforcement generates assortative mating that can allow adaptive divergence to proceed further. Environmental differences are required for ecological speciation, but too large differences may prevent the colonization of that other resource, thus preventing ecological speciation.

Sexual selection

Sexual selection plays an important role in ecological speciation by preventing hybridization, i.e. homogenization of the gene pool. Positive assortative mating will reduce the recombination among individuals adapted for different environments. It is possible to have sexual selection on its own, driving sympatric speciation (Higashi et al. 1999), but it is unlikely (Arnegard & Kondrashov 2004; van Doorn et al. 2004). Nevertheless, sexual selection is an important force to progress toward ecological speciation, but in its absence, another mechanism such as habitat choice, must substitute its assortative mating ability in order to progress toward ecological speciation.

Competition

Intra-specific competition is known to regulate population size (Roughgarden 1971) and inter-specific competition to generate competitive exclusion (Hardin 1960). In the study of ecological speciation, competition can thus transition from intra-specific to inter-specific. Intra-specific competition can drive speciation especially when it is strong (Dieckmann & Doebeli 1999). The reason being that phenotypes that differ from those that would optimally exploit the most common resource, can have an advantage because of reduction of competition by foraging on a different resource. For a population with its average phenotype matching the position of the maximum of the resource distribution, disruptive selection will result. In the presence of assortative mating in sexual organisms, this can cause speciation. Then competition will regulate each population size and if those populations are different enough to coexist and reduce inter-specific competition, competitive exclusion will be mitigated. Competition is not required for ecological speciation, but it can be a source of disruptive selection that can initiate the speciation process.

1.4 Thesis subdivision

The thesis is divided in four core chapters. Each chapter uses individual-based numerical simulations to address different questions in ecological speciation.

Chapter 2: Five questions on ecological speciation addressed with individual-based simulations

The first core chapter looks at the emergence of different types of reproductive barriers (natural and sexual selection) in the early stages of speciation; i.e. when gene flow is still possible. The general scenario is one locally adapted

population that generates migrants to a new environment where that species is not already present. We first look at the conditions that allow colonization and local adaptation to this new environment under a constant one-way migration from the old environment into the new environment. By doing so, we explore the positive and negative consequences of migration for local adaptation (Garant et al. 2007). We then explore the emergence and importance of natural selection against migrant and hybrids as a potential reproductive barrier. We also explore the added contributions of sexual selection as a reproductive barrier. Finally, we provide an initial assessment of how neutral genetic markers might be used to infer progress toward ecological speciation, a very common method in the literature.

Chapter 3: The consequences of phenotypic plasticity on ecological speciation

The previous chapter ignored the potential environmental influences on phenotypic development; i.e., phenotypic plasticity. And yet plasticity might be important if it changes the fitness of individuals in the ancestral or new environments. In the present chapter, we therefore address the effects of phenotypic plasticity on the evolution of reproductive barriers during the course of ecological speciation. Using a similar general scenario to that in the previous chapter, we first looked for the conditions under which plasticity was most likely to evolve. We put a strong emphasis on the importance of the timing of migration between environments, relative to the expression of plasticity. That is, plasticity might take place before or after migration between environments. We then investigate the effects of plasticity on the evolution of various reproductive barriers, such as natural and sexual selection against migrants and hybrids. We concluded this

chapter by looking at the effect of phenotypic plasticity on divergence in neutral genetic markers.

Chapter 4: When can ecological speciation be detected with neutral loci?

In the previous two chapters, we were struck by the amount of variation seen in divergence at neutral genetic markers, and this led us to further consider potential consequences for using such markers to infer ecological speciation. We therefore decided to directly test the validity of this particular inferential method. The answer was not clear at the outset. On one side, neutral loci that are not linked to selected loci might flow almost freely among the populations because recombination disassociates them from divergent selection (Emelianov et al. 2004; Gavrilets & Vose 2005). On the other side, divergent selection might cause a generalized barrier to gene flow across the entire genome including unlinked neutral loci (Gavrilets 2004; Grahame et al. 2006; Nosil et al. 2008). The reason is that even unlinked neutral and selected loci are in linkage disequilibrium before being recombined in hybrids. Natural selection against migrants and hybrids might therefore reduce gene flow at even those unlinked neutral markers. We test this intuition in a model with four populations that were paired in two different environments with equal probabilities of migration between all populations. We then examine patterns of neutral genetic divergence under different strengths of selection (various degrees of environmental difference) and different migration rates (movement of individuals among populations). Finally, we examine the statistical power of such an approach with a subsampling procedure coupled to standard population genetic approaches for inferring whether neutral genetic divergence is

greater between populations in different environments than between populations in similar environments.

Chapter 5: Forces influencing progress toward sympatric speciation

Many forces are expected to influence ecological speciation and the importance of these forces is most uncertain in the context of sympatric speciation. First, sexual selection might lead to sympatric speciation on its own (Kondrashov & Shpak 1998; Higashi et al. 1999), although others feel this is unlikely (Arnegard & Kondrashov 2004). Second, competition can lead to disruptive selection that causes speciation on a unimodal resource distribution (Dieckmann & Doebeli 1999) or on a spatial resource gradient (Doebeli & Dieckmann 2003), but see (Polechová & Barton 2005; Leimar et al. 2008). If intra-specific competition is very strong the strength of assortment required to induce speciation is unrealistic, thus an "intermediate" level of competition is more likely to favour sympatric speciation (Bürger et al. 2006). To date, however, the importance of, and interactions among, these forces have not been considered in a single model. We do so by putting a population on an unexploited resource and monitor its evolution. We vary the competition among individuals by changing the size of their foraging range. We vary the sexual selection by changing the ability to discriminate among potential mates. We also vary the shape of the resource, from a single resource to various degrees of divergent environments, modeled by a bimodal resource distribution.

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CHAPTER 2
Five questions on ecological speciation addressed with individual-based simulations

2.1 Abstract

We use an individual-based simulation model to investigate factors influencing progress toward ecological speciation. We find that environmental differences can quickly lead to the evolution of substantial reproductive barriers between a population colonizing a new environment and the ancestral population in the old environment. Natural selection against immigrants and hybrids was a major contributor to this isolation, but the evolution of sexual preference was also important. Increasing dispersal had both positive and negative effects on population size in the new environment and had positive effects on natural selection against immigrants and hybrids. Genetic divergence at unlinked, neutral genetic markers was low, except when environmental differences were large and sexual preference was present. Our results highlight the importance of divergent selection and adaptive divergence for ecological speciation. At the same time, they reveal several

A version of this chapter appears as Thibert-Plante, X. and Hendry, A. P. (2009) Five questions on ecological speciation addressed with individual-based simulations. *Journal of Evolutionary Biology* 22(1):109–123.

interesting non-linearities in interactions between environmental differences, sexual preference, dispersal, and population size.

Keywords: Ecological speciation, individual based modeling, gene flow, selection against hybrids, selection against migrants, sexual preference

2.2 Introduction

Ecological speciation occurs when barriers to gene flow evolve as a result of ecologically-based divergent selection. This process is now well supported by data from natural systems (Schluter 2000; Rundle & Nosil 2005; Barluenga et al. 2006; Savolainen et al. 2006), laboratory experiments (Rice & Hostert 1997, review), and theoretical models (Dieckmann & Doebeli 1999; Doebeli & Dieckmann 2003; Gavrilets & Vose 2007; Gavrilets et al. 2007). Despite this growing body of work, some fundamental aspects of ecological speciation have rarely been formally investigated in theoretical models, leaving some outstanding basic questions. We address five of these questions through individual-based simulations of a scenario where dispersers from one environment colonize a new environment and then evolve in the presence of ongoing gene flow (i.e. "divergence with gene flow", Rice & Hostert 1997).

Our first question relates to the length of time required for a population to completely occupy a new niche, because this event will be critical for progress toward ecological speciation. Several factors may come into play. First, the number of initial colonizers will often be small, thus increasing stochastic extirpation and Allee effects, as well as restricting the genetic variation available for adaptation

(Kinnison & Hairston Jr 2007). However, these negative effects can be circumvented by an increased number of colonizers or ameliorated by reduced density dependence owing to small initial population sizes. Second, if the new environment is too different from the original environment, colonizers may be so poorly adapted that they cannot achieve a positive rate of increase (Gomulkiewicz et al. 1999). In this case, the new population may never adapt and become self-sustaining (Holt & Gomulkiewicz 1997). To address these possibilities, we measured the time to full occupation of a new environment as a function of dispersal rates (influencing the number of colonizers) and the magnitude of environmental differences (influencing the degree of initial maladaptation).

Our second question concerns how ecological speciation is influenced by natural selection against immigrants (Hendry 2004; Nosil 2004; Nosil et al. 2005). This potential barrier to gene flow occurs when individuals that move between environments are less likely to survive owing to maladaptation. Some likely examples include increased predation owing to compromised crypsis (Nosil 2004) and reduced feeding efficiency owing to trait-food mismatches (Schluter 1995). Selection against immigrants may be particularly potent during ecological speciation because it acts before other reproductive barriers; i.e. an immigrant must survive if mate choice or hybrid inviability are to be important. Empirical studies support this suggestion (Via et al. 2000; Nosil 2004; Nosil et al. 2005; Nosil 2007), but no model has examined the importance of selection against immigrants in comparison to other potential reproductive barriers. We address this question

by considering the relative contributions of both natural and sexual selection against immigrants and any resulting hybrids.

Our third question involves the role of mate choice (sexual selection) in ecological speciation. This particular reproductive barrier is thought to be very important, as revealed by empirical studies (Grant & Grant 1997; Boughman 2001; Nosil et al. 2002; Huber et al. 2007) and theoretical models (e.g. Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999). One general conclusion from this previous work is that speciation occurs most easily when the same genes (or physically linked genes) determine both adaptation and mate choice (i.e. "magic trait" models, Gavrilets 2004). It is less clear how easily and rapidly ecological speciation will proceed when mate choice and adaptation are both based on multiple genes that are unlinked across traits. In this latter case, several models have shown that some reproductive isolation can accrue quickly when assortative mating is based on habitat choice (Fry 2003; Gavrilets et al. 2007), but we are here interested in the role of mate choice within a given habitat. To address this situation, we examine the contribution of mate choice to ecological speciation when traits and preferences are both based on multiple, unlinked genes.

Our fourth question focuses on the role of ongoing dispersal and any resulting gene flow between environments. In principle, gene flow can either enhance or constrain adaptive divergence (review: Garant et al. 2007) and therefore positively or negatively influence ecological speciation. On the positive side, dispersal can increase the genetic variation necessary for adaptation (Swindell & Bouzat 2006), reduce inbreeding (Ingvarsson & Whitlock 2000), alleviate Allee effects

(Holt et al. 2005), reduce demographic stochasticity (Alleaume-Benharira et al. 2006), promote "reinforcement" (Servedio & Kirkpatrick 1997), and contribute to competition-driven diversification (Dieckmann & Doebeli 1999; Doebeli & Dieckmann 2003; Gavrilets & Vose 2005; Gavrilets et al. 2007). On the negative side, dispersal can prevent the independent responses of populations to different selective regimes (Slatkin 1987; Hendry et al. 2001; Lenormand 2002) and can increase recombination between genes for adaptation and genes for mate choice (Felsenstein 1981; Fry 2003). We attempt to narrow down these possibilities by examining how different levels of dispersal influence adaptive divergence and reproductive isolation at different levels of natural and sexual selection.

Our fifth question examines one potential method for inferring ecological speciation. Specifically, some authors use neutral genetic markers to test whether gene flow is lower between populations in different environments than between populations in similar environments (e.g. Lu & Bernatchez 1999; Ogden & Thorpe 2002; Crispo et al. 2006). This method of inference has recently been brought into question by the realization that alleles at neutral markers unlinked to selected loci might flow almost freely between populations in different environments (Emelianov et al. 2004; Gavrilets & Vose 2005). But ambiguity remains because a generalized barrier to gene flow (Gavrilets 2004; Grahame et al. 2006; Nosil et al. 2008, 2009) might arise if selection acts against the whole genome of migrants and first-generation hybrids (i.e. before recombination between parental genomes). We inform this topic by examining how differentiation at unlinked, neutral markers is

related to rates of dispersal and to the magnitude of ecological differences between environments.

Ecological speciation involves a complex interplay among natural and sexual selection, gene flow, adaptive divergence, and reproductive isolation. Our overall goal is to examine the factors that influence these interactions and thus gain insight into the conditions that promote or constrain ecological speciation. The present paper introduces the modeling framework that we have developed to work in this broad area, and then uses this framework to address the above five questions on ecological speciation.

2.3 Modeling framework

For this individual-based model, we first describe the individuals, then their environment, and lastly their interactions. This description also provides a framework to outline the basic assumptions we make in this modeling exercise.

Individuals are diploid with two possible allelic states (0 and 1) at each locus, and each locus may be coding or non-coding with respect to phenotypic traits. A specific set of loci contributes additively to a given phenotypic trait. Random noise can also affect the transcription between alleles and traits, thus leading to heritabilities that are less than unity. We generate this noise around an individual's expected phenotype by drawing from a normal distribution with a variance of σ_N^2. We here consider two phenotypic traits: one that determines the resources an individual can use (the "foraging trait", F), and another that determines female mating preferences for individuals with different foraging trait values (the "target of sexual preference", T). This situation, where mate choice

directly targets the phenotypic trait that influences foraging, does occur in the context of ecological speciation. Examples include beak size in Darwin's finches (Huber et al. 2007; Grant & Grant 2008) and colour in aggressive mimics (Puebla et al. 2007). Although the traits under selection thus influence mate choice, this is not a "magic trait" model, because adaptation and mate choice are based on different, and unlinked sets of loci. The foraging trait was influenced by 256 diploid loci located to an equivalent of 20 cM apart (recombination rate 0.2 with crossover points determined randomly). The target trait had a similar genetic structure but was located on a different chromosome, and so was unlinked to the foraging trait. We also tracked eight neutral unlinked diploid loci, each located on its own chromosome.

Some parameters are common to all individuals and are fixed in all simulations (Table 2–1). One of these parameters is mutation rate: the probability of an allele mutating from one allelic state to the other. Note that in our model each locus has only two possible allelic states and mutation occurs between them. We here allow mutation rates to be higher for non-coding loci than for coding loci, as often seems to be the case when using biologically realistic values (Gavrilets & Vose 2005). Another fixed parameter is the energy cost of offspring production: i.e. individuals accumulate resources prior to reproduction and then use those resources to produce offspring. We specify this energy cost as the amount of accumulated resources needed to produce one individual offspring.

Parameters	Symbol (if any)	Values
Mutation Rate coding		10^{-5}
Mutation Rate non-coding		10^{-3}
Quantity of resource in each environment	R_{tot}	40
Cost to produce an offspring		0.1
Width of the resource distribution	Ω	10
Width of the resource acquisition function	σ	5
Number of immigrants	Nm	$\{10, 20, 30, 40, 50, 60\}$
Strength of sexual selection	a	$\{0, 0.001, 0.005, 0.01, 0.05, 0.1\}$
Noise in gene transcription	σ_N	$\{0, 2, 4, 6\}$
Heredity (measure)	h^2	$\{1.00 \pm 0.06, 0.97 \pm 0.06,$ $0.87 \pm 0.06, 0.72 \pm 0.08\}$

Table 2–1: Parameter space explored in the simulations. The heredity was measured on the simulation with only one environment and without sexual preferences, the measure reported is the mean ± the standard error. Each heredity measure (h^2) corresponds to the amount of noise in gene transcription (σ_N).

The "environment" determines the distribution of resources available to individuals having particular foraging trait values. We here assume two spatially-discrete environments, the "old" environment being the source of colonists for the "new" environment (see below). Within each environment, the total amount of resources is limited in a given generation but is renewed each generation so that the pre-foraging resource distribution remains constant over time. This distribution is a Gaussian function and is defined by three parameters: the position of the resource peak with respect to foraging trait values (Υ), the width of the resource distribution with respect to trait values (Ω), and the total amount of resources (R_{tot}):

$$R(x) = \frac{R_{tot}}{\sqrt{2\pi}\Omega} exp\left(\frac{-(x-\Upsilon)^2}{2\Omega^2}\right). \tag{2.1}$$

An individual's acquisition of resources (energy) depends on the initial distribution of resources with respect to different foraging trait values and the degree of competition from other individuals according to their foraging trait values. The range and efficiency of resource use as defined by an individual's foraging trait is specified by a Gaussian distribution as in Ackermann & Doebeli (2004):

$$f(x) = \frac{1}{\sqrt{2\pi}\sigma} \exp^{\frac{-(x-F)^2}{2\sigma^2}}. \tag{2.2}$$

Each range of resources x is then split among all the individuals with $f(x) > 0.01$ present in the environment. This $f(x)$ is a modified Gaussian function that is truncated when it falls below 0.01. In particular, each individual receives an amount p_i of the resources x proportional to its $f(x)$. The total amount of resources acquired by an individual is the integral across the resource range:

$$p_i = \int R(x) \frac{f_i(x)}{\sum_j f_j(x)} dx. \tag{2.3}$$

For the main simulations, we did not assume any maximum limit on the number of offspring that an individual could produce as a female. This decision meant that, in some colonization situations, individuals could acquire a large amount of resources and produce many offspring, which might not be realistic. We therefore evaluated the effect of this simplification by running some additional simulations after specifying that females could produce a maximum of only five offspring. All results were similar to the model without this restriction, except that colonization of a new environment became slightly more difficult with the restriction (results not shown).

After resource acquisition according to the above procedure, foraging stops and reproduction begins. Every individual is a hermaphrodite (for simplicity and simulation efficiency, Gavrilets & Vose 2005) but self-fertilization is not possible. Individuals can reproduce as males irrespective of their energy stores (resources acquired) but can reproduce as females only when they have more resources than the minimum required to produce a single offspring. If an individual can thus act as a female, it will choose another individual from the population to act as its male mate and will then produce a single offspring, with energy resources decreasing accordingly. The specific individual that a given female selected as a mate was based on a probability distribution (across all individuals in the entire population) that depended on the difference between the target preference of the "female" and the foraging trait of each possible "male" (see below). After mating as a female and producing one offspring, an individual would again act as a female, according to the above procedure, only if she had enough resource to produce another individual offspring. This procedure continued until none of the individuals in the population had enough resources to reproduce as a female. Note that resources are depleted only during offspring production, and only when an individual acts as a female. After reproduction ceased, all parents died (i.e. semelparity with non-overlapping generations).

As noted above, mating probabilities depend on the difference between the female target preference (T) and the male foraging trait (F), as well as the importance of that difference (a). This idea is similar to the Bush (1975) approach described in Fry (2003), except that the preference is not directed toward

an environment, but rather toward a phenotypic trait. Our specific preference function follows Bürger et al. (2006):

$$\Pi_u(T - F) = e^{-a(T-F)^2}, \tag{2.4}$$

where Π_u is the unweighted mating probability. This probability is then weighted according to the distribution of unweighted mating probabilities across all "males" in the population:

$$\Pi(T - F) = \frac{\Pi_u(T - F)}{\sum_i \Pi_u(T - F_i)}. \tag{2.5}$$

At $a = 0$, sexual selection is absent because the mating probability is independent of the foraging trait. As a increases, the unweighted probability of mating decreases as shown in figure 2–1.

The range of possible trait values can be normalized to range from zero to one. Under such normalization $\{F, T, \sigma, \sigma_N, \Omega, \Upsilon\}$ are divided by 512, and a is multiplied by 512^2.

2.4 Simulations, data collection, and presentation

Each simulation starts with a base population in the "old" environment, initially established by individuals with a uniform distribution of trait values across the 99% range of available resources. The population then evolves in this environment for 1000 generations, which was sufficient for stabilization. We define stabilization as occurring when selection on the traits remains constant across generations, which becomes essentially zero in the absence of immigration. After this initialization phase, we add a "new environment" without any resident individuals. The two environments have identical resource distributions except for

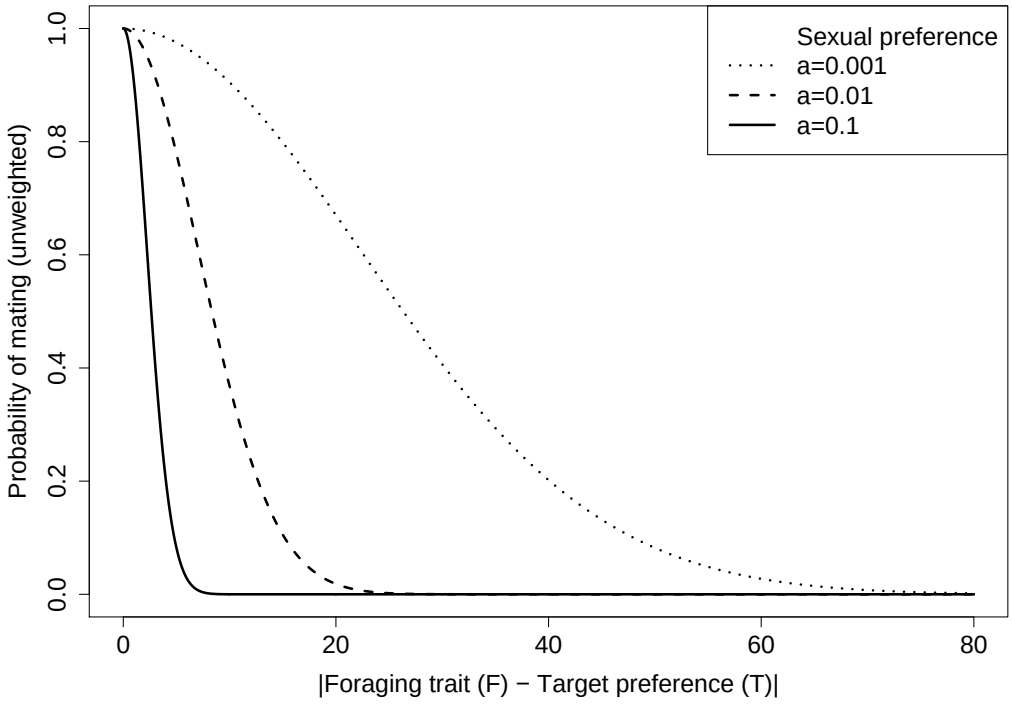


Figure 2–1: Unweighted probability of mating as a function of the difference between the foraging trait F and the target of sexual preference T for different strengths of sexual preference (a).

the position of the peak (Υ). In short, the same amount of resource is available in each environment but the resources are of different types, accessible to individuals with different foraging trait values. An example might be two islands with different distributions of seed sizes, which are therefore most accessible to birds with different beak sizes (e.g. Schluter & Grant 1984). To continue this analogy, we simulate the situation thought to be important in the evolution of this group (Lack 1947; Grant & Grant 2008), where individuals adapted to conditions on one island colonize another island. Each generation, a certain number of individuals, Nm, disperse from the old environment to the new environment. This is a one-way dispersal only - there is no dispersal back from the new environment to the old environment. This number of dispersers then remains constant for the rest of the simulation (1000 generations). The corresponding immigration rate (proportion of the new population composed of immigrants) will decrease until the resident population achieves its final size and then remains constant thereafter (and at a level that was virtually the same across all the simulations). One thousand generations following the appearance of the new environment was chosen because it was a sufficient length of time for stabilization of population and evolutionary dynamics. Simulations were run eight times for each range of parameter values shown in Table 2–1.

For each simulation, we recorded several variables. Time to full occupation was the number of generations from the time when the new environment appears to when it is occupied by the same number of individuals as in the old environment. We judge this to have occurred when population size in the new

environment remains within one standard error of that in the old environment for at least ten subsequent and consecutive generations. The average number of offspring produced by immigrants, hybrids, or residents in the new environment is akin to mean absolute fitness. This metric is calculated as the total number of offspring produced by all individuals in the group divided by the number of potential parents in that group. When two parents came from different groups (e.g. one resident and one immigrant), a contribution of 0.5 offspring was assigned to each parental group. Hybridization rate is the number of matings between immigrants and residents in the new environment relative to that expected under random mating and assuming equal resource acquisition. The random expectation was determined as:

$$\frac{2xy}{(x+y)(x+y+1)}, \tag{2.6}$$

where x is the number of residents and y is the number of immigrants. Hybridization rates estimated in this manner were averaged over the last 100 generations of the simulation (i.e. 900 generations after the new environment appeared). Neutral genetic divergence was indexed as F_{ST} according to Weir & Cockerham (1984). We here present the median value across non-coding loci of the mean F_{ST} values within a locus over the last 100 generations of the simulation.

2.5 Results

We first discuss general evolutionary patterns within a given simulation, then present results that address each of the five questions raised in the introduction. Where relevant, the magnitude of environmental differences is given perspective by standardizing the distance between the two peaks by the width of the resource

acquisition function of an individual:

$$\frac{\Upsilon_{new} - \Upsilon_{old}}{\sigma}. \tag{2.7}$$

Time series of the distribution of foraging traits in the new environment typically showed a short initial phase when maladapted dispersers predominate (Fig. 2–2) and phenotypic divergence between environments is minimal. This was followed by a transition period when the distribution of phenotypes shifted rapidly toward that expected to be adaptive in the new environment (Fig. 2–2). During this period, we sometimes see signs of evolutionary branching into two different modes, perhaps as a result of competition (see Discussion). With increasing time, phenotypes slowly asymptote toward those expected for the new environment and also tend to converge from the bimodal distribution back into a single, broad distribution of phenotypes. This distribution roughly matches the distribution of available resources (Fig. 2–3). Time series of the evolution of female target preferences in the new population initially lag those for the foraging trait (Fig. 2–4). As this mismatch increases, however, selection increasingly favors females who choose males with adaptive foraging traits in the new environment. For this reason, and because evolution of the foraging trait eventually slows down, female target preferences ultimately catch up and match the locally-adapted foraging trait value.

Q1. Time to full occupation: In the absence of sexual preference ($a = 0$), the time required for the new population to become fully established depended on the difference in foraging trait optima between the old and new environments,

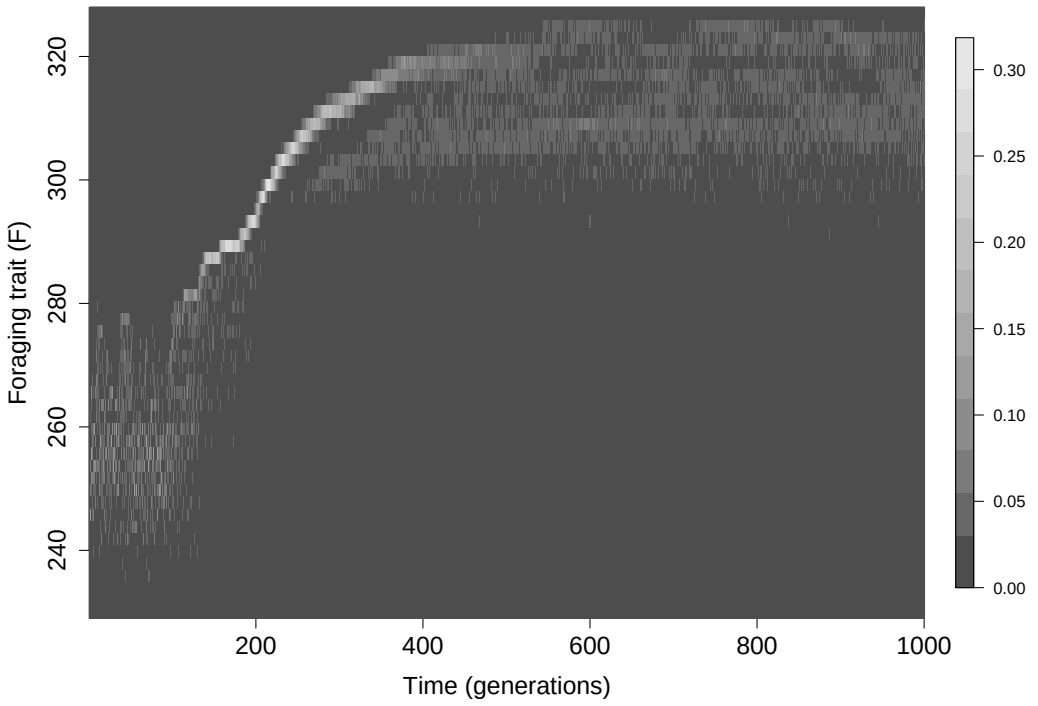


Figure 2–2: Time series of the evolution of the distribution of the foraging trait (F) when there is no sexual preference. The grey scale represents the fraction of the population with a specific trait value at each iteration. Here the position of the optimum in the old environment is 255 and the new is 310, i.e. a standardized environmental difference of 11. The number of immigrants is $Nm = 20$.

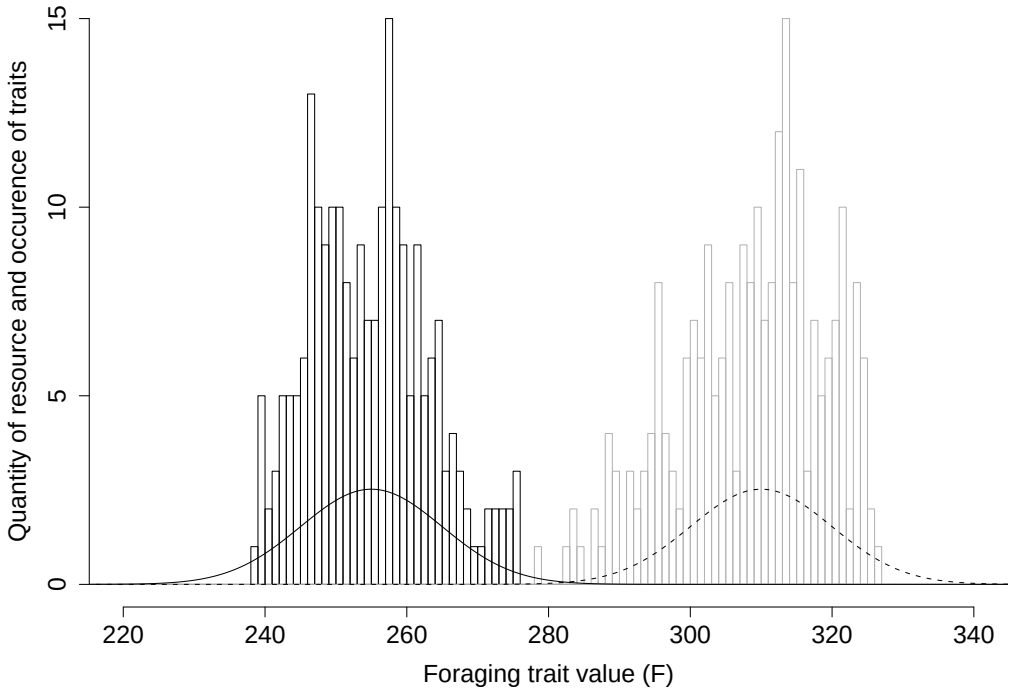


Figure 2–3: Snapshot of the histograms of the foraging traits in the old (black) and new environment (gray) for the resident population only (no dispersers and no hybrids) at the last iteration. As can be seen in the distribution of the resource, the Gaussian distribution, the peak of the old (continuous line) and new (dashed line) environments are 255 and 310 respectively. There is no sexual preference ($a = 0$) and the number of immigrants is $Nm = 20$.

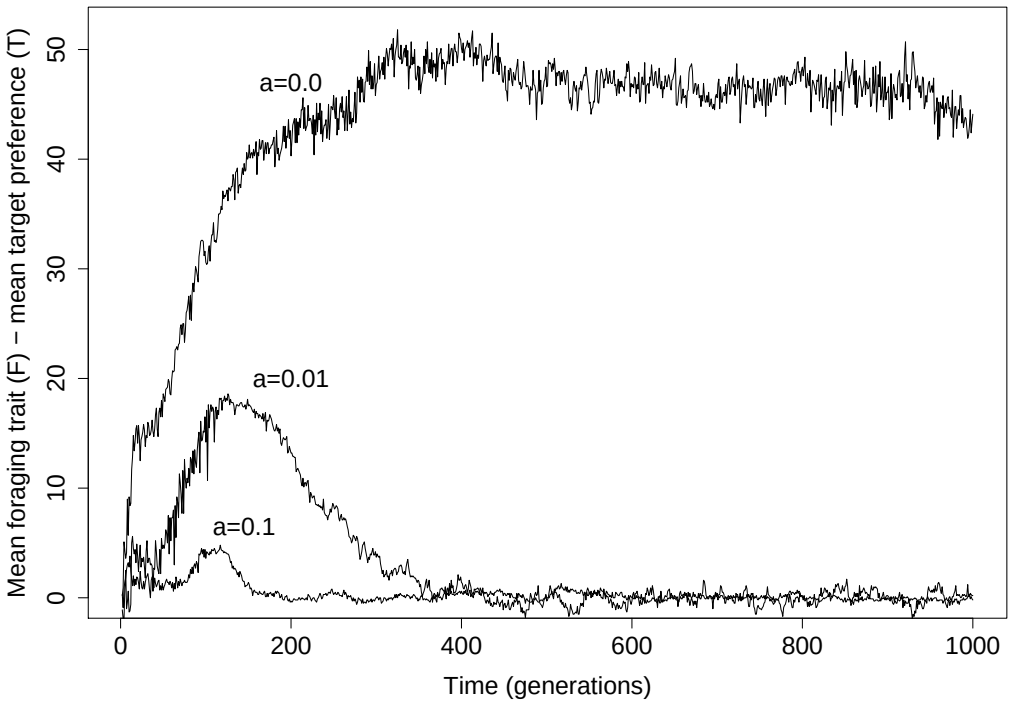


Figure 2–4: Time series of the evolution of the difference between the foraging trait (F) and the target of sexual preference (T) for different strengths of sexual preference (a) for the population in the new environment. Here the position of the optimum in the old environment is 255 and the new is 310, i.e. a standardized environmental difference of 11. The number of immigrants is $Nm = 60$.

and also on the number of dispersers (Fig. 2–5(a)). When the new environment was reasonably similar to the old environment (standardized environmental difference = 0 to 10), full occupation was achieved in less than 100 generations, regardless of the level of dispersal ($10 <= Nm <= 60$, which corresponded roughly to a migration rate (m) between 3 and 20% at full occupation). For greater environmental differences, however, full occupation took considerably longer (Fig. 2–5(a)) and dispersal became important (see below). At large environmental differences (standardized difference > 13), colonization typically did not take place over the 1000 generation interval of our simulations. In these cases, dispersers were too poorly adapted to gain a foothold in the new environment. Adding sexual preference ($a > 0$) to the simulations (Fig. 2–5(b)) had little effect on time to full occupation when the new and old environments were similar (standardized environmental difference < 8). It did, however, decrease the threshold environmental difference at which full occupation became delayed. The reason was that females initially colonizing the new environment still preferred foraging trait values typical of the old environment (where their preference had evolved), and so maladapted immigrants were initially more likely than adapting residents to be chosen by both resident and immigrant females. This slows the process of adaptation until female target preferences (T) evolve substantially toward the locally-adapted foraging traits values (see above).

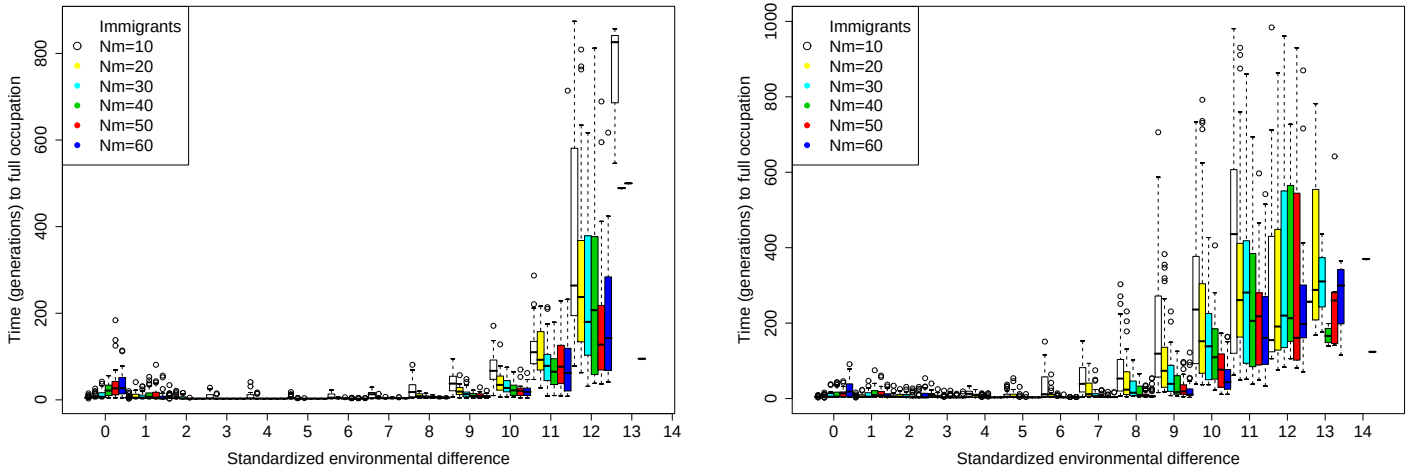
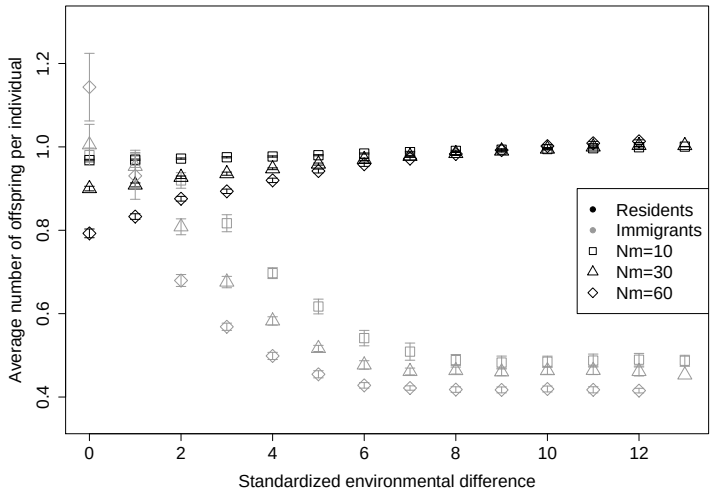


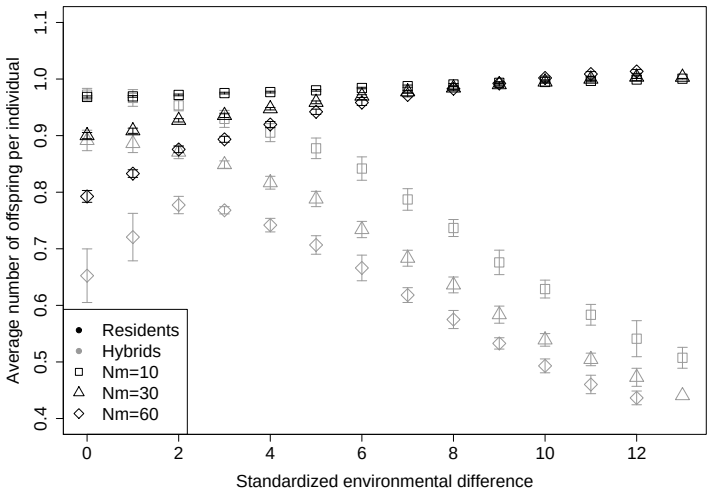
Figure 2–5: Time to full occupation in number of generations as a function of the standardized environmental difference for different levels of immigration (Nm) increasing from left to right for each environmental difference. Without sexual preference ($a = 0$) (panel a) and with sexual preference ($a = 0.1$) (panel b). The box is bounded by the first and third quartile. The line inside the box is the second quartile (median). The whisker extends to 1.5 times the interquartile range (third quartile minus first quartile) above the third quartile or to the maximum value, whichever is the smallest. The same rule applies for the lower part, but considering the minimum values between the 1.5 times the interquartile range below the first quartile and the minimum value. All data outside the whisker range are considered outliers and are represented by open circles.

Q2. Selection against immigrants and hybrids: We found strong natural selection against immigrants that was the direct result of adaptive divergence. This causal association was clearly seen as an increase in this component of reproductive isolation (difference in fitness between residents and immigrants) with an increase in the environmental difference (Fig. 2–6(a)). In the absence of sexual preference, the average fitness (number of offspring produced per individual) of immigrants relative to residents had a lower limit of approximately 0.5. This particular limit arose because immigrants could still act as males (no energy required) even if maladaptation prevented them from acquiring enough energy to act as a female. If we had also assumed energy constraints on male reproduction, or if we had also included viability selection, then natural selection against immigrants would certainly have increased. The above patterns for selection against immigrants were largely mirrored when considering selection against hybrids (Fig. 2–6(b)). The latter was weaker, however, owing to the phenotypic (and therefore adaptive) intermediacy of hybrids relative to immigrants and residents (as a result of additive gene action). In the absence of environmental differences, populations drift in asynchrony around their resource peak, thus the dispersers can have a fitness advantage of being rare/slightly different. As dispersal increases so does competition for the resource, and this leads to a reduction in the average number of offspring per individual.

Q3. Sexual preference: The addition of sexual preference considerably increased reproductive isolation over that achieved solely by natural selection against immigrants. This can be shown most clearly by examining the number of hybrids



(a)

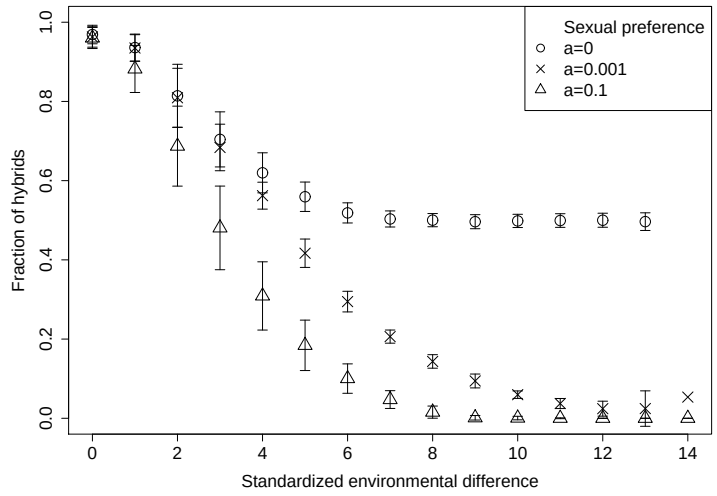


(b)

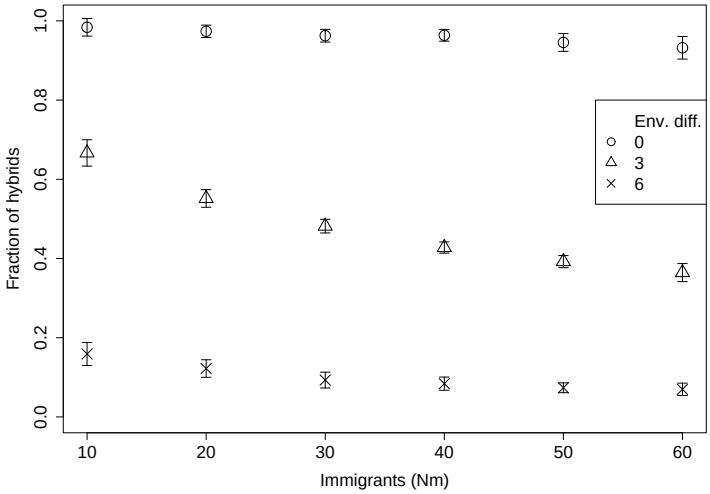
Figure 2–6: Average number of offspring per individual in the new environment as a function of the standardized environmental difference and the number of immigrants (Nm) without sexual preference ($a = 0$). For each simulation we group the pedigree for the last 100 iterations. The vertical bars are the standard errors among the simulations with the same parameters. The immigrants (top panel) and hybrids (bottom panel) are compared to the residents.

produced relative to the random expectation (Fig. 2–7). Without sexual preference ($a = 0$), an increase in the environmental difference decreases hybrid production to a minimum of 0.5 (for the reasons explained above). Increasing levels of sexual preference ($a > 0$) then further decreased hybrid production for a given environmental difference (Fig. 2–7). This result is driven by adaptive divergence because it increased with increasing environmental differences. Indeed, essentially no hybrids were produced when environmental differences were large and when sexual preference was present. In short, adaptive divergence dramatically reduced hybrid production through the joint effects of natural and sexual selection against immigrants (Fig. 2–7).

Q4. Dispersal rate: All of the above conclusions are robust to the level of dispersal, which nevertheless caused some interesting nuances. First, when colonization is slowed by large environmental differences, increasing dispersal reduces this delay (Fig. 2–5), probably by providing more variation on which selection can act. Second, increasing dispersal reduces the average relative fitness (number of offspring produced) of immigrants and hybrids (Fig. 2–6) and the number of hybrids produced (Fig. 2–7). The reason is that dispersers (and hybrids) compete amongst themselves for an already scarce resource in the new environment (one tail of the resource distribution) and so more dispersers (and hybrids) reduce the amount of energy available to each. Third, dispersal influences final population sizes depending on the environmental difference and sexual preference. In the first scenario (new and old environments are reasonably similar), increasing dispersal reduces resident population sizes (Fig. 2–8), because



(a)

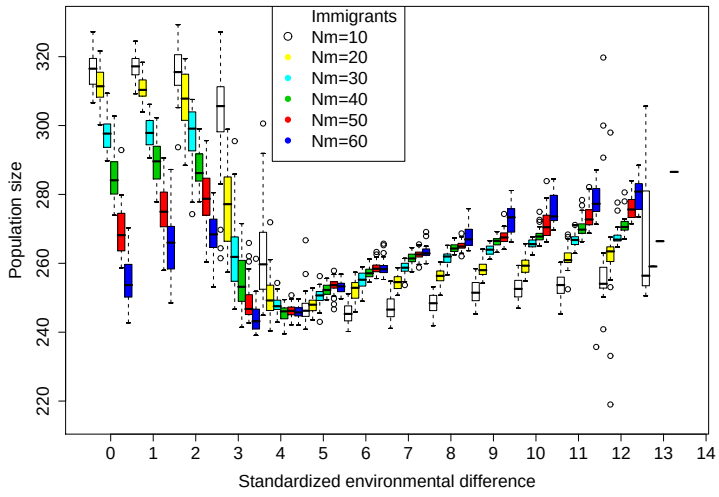


(b)

Figure 2–7: Fraction of hybrids (number of hybrids produced relative to random mating expectation) as a function of the standardized environmental difference and the strength of sexual preference (a) for all levels of immigration (Nm) grouped (top panel). Fraction of hybrids as a function of the number of immigrants for different environmental differences for simulation with sexual preference ($a = 0.1$) (bottom panel). In both cases, vertical bars represent the standard error among the the fraction of hybrids for last 100 iterations.

increasing competition reduces the number of individuals that obtain enough energy for reproduction. In the second scenario (new and old environments are quite different and sexual preference is absent), increasing dispersal increases resident population sizes (Fig. 2–8). This occurs because more residents mate with immigrants and produce maladapted offspring that obtain few resources. The remaining residents can therefore obtain more resources, thus increasing their offspring production. In the third scenario (new and old environments are quite different and sexual preference is present), immigrants do not contribute as females (not enough resources) or as males (resident females disfavor them), and so they have no measurable effect on resident population size.

Q5. Neutral genetic divergence: When sexual preference was absent, average genetic differentiation (F_{ST}) at neutral, unlinked loci was very low - although the number of simulations with outlying large F_{ST} values increased somewhat at the highest environmental differences (Fig. 2–9(a)). When sexual preference was present, F_{ST} was low for small environmental differences, increased over the range of moderate environmental differences, and decreased again for the very highest environmental differences (Fig. 2–9(b)). The main reason for the increase in mean F_{ST} over part of this range was that large environmental differences, coupled with sexual preference, can lead to very few hybrids (Fig. 2–7). Gene flow thus becomes very low and drift can cause substantial neutral divergence. The minor decreases for large environmental differences can be explain by time to full occupation: since it takes longer to adapt, the populations drift for a smaller amount of time. In short, divergent selection can dramatically reduce gene flow



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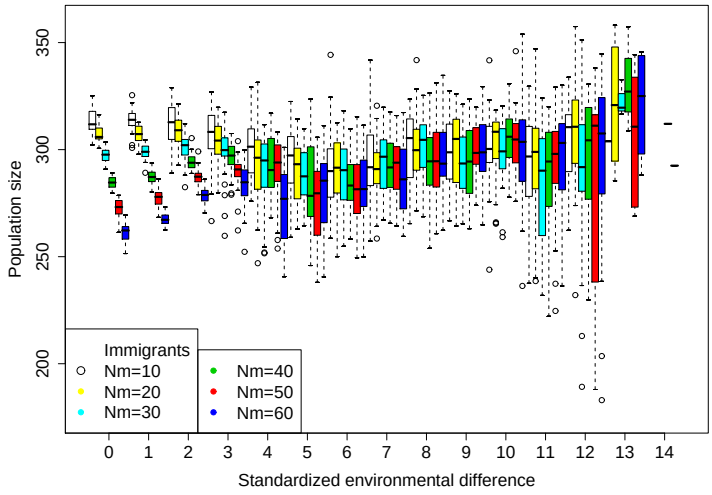


(b)

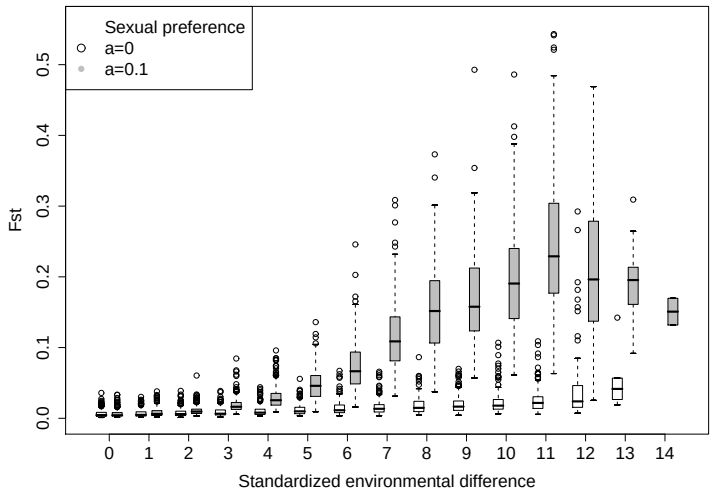
Figure 2–8: Population size as a function of the standardized environmental difference and the number of immigrants. Panel (a) represents simulations without sexual preference ($a = 0$) and panel (b) represents simulations with sexual preference ($a = 0.1$). See figure 2–5 caption for explanation of the boxplot.

at unlinked, neutral markers, but perhaps only under some conditions and for some populations. Dispersal had relatively little effect on F_{ST}, except perhaps that increasing dispersal decreased genetic divergence when sexual preference was absent (Fig. 2–9(b)). The reason was that the numbers of hybrids produced was affected much more strongly by variation in environmental differences and sexual preference than it was by variation in dispersal.

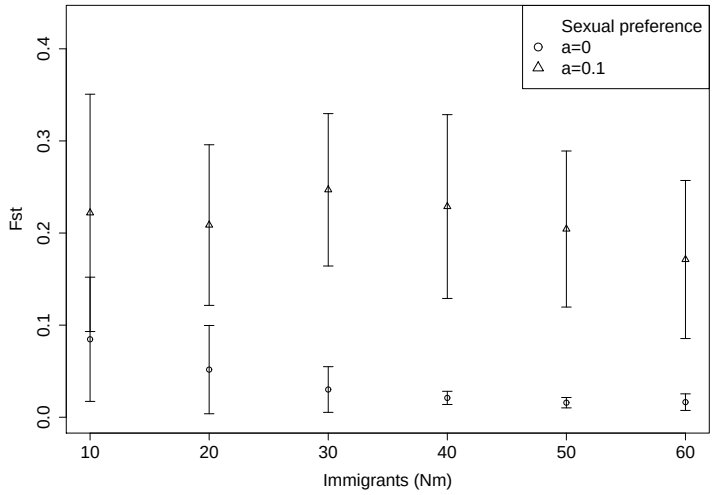
2.6 Discussion

Our model complements previous theoretical work on ecological speciation. First, it was an individual-based simulation that can tackle complex systems where analytical solutions cannot yet be found (Grimm & Railsback 2005). Second, it was specifically designed to address the importance of particular reproductive barriers (natural selection against immigrants, natural selection against hybrids, sexual preference) across a range of conditions (environmental differences, dispersal). Third, it allowed us to directly evaluate one of the common empirical methods for inferring ecological speciation (neutral genetic differentiation). In the following paragraphs, we discuss how our results inform each of the questions about ecological speciation that were raised in the introduction. We place these findings in the context of previous work, and we discuss implications for the understanding and study of ecological speciation.

Q1. Time to full occupation: Environmental differences are a pre-requisite for ecological speciation (Schluter 2000; Rundle & Nosil 2005), but we here formally demonstrate that environmental differences can be a double-edged sword. On the one hand, increasing environmental differences increase adaptive divergence,



(a)



(b)

Figure 2–9: Boxplot of the F_{ST} as a function of environmental difference with and without sexual preferences (panel a). F_{ST} as a function of the number of immigrants (Nm) for a standardized environmental difference of 12 with and without sexual preference (panel b). See figure 2–5 caption for explanation of the boxplot.

and thereby promote ecological speciation. On the other hand, particularly large environmental differences can constrain colonization and adaptation, thereby also constraining ecological speciation (Fig. 2–2). This result is obvious if one looks at adaptive radiations in nature. Darwin's finches of the Galápagos, for example, have radiated into a diverse array of different feeding niches (Lack 1947; Grant & Grant 2008), but none are scavengers, or cave dwelling, or marine, despite the continued availability of these and many other niches.

Several factors influenced the transition between the above promoting and constraining effects of environmental differences. First, when adaptation to the new environment was difficult because of large environmental differences, increasing dispersal made it easier. This result is not particularly novel given that several models have already argued for positive effects of dispersal on adaptive potential, particularly in the presence of Allee effects (e.g. Holt et al. 2004), inbreeding (e.g. Ingvarsson & Whitlock 2000), or low genetic variation (e.g. Gomulkiewicz et al. 1999). Of these effects, the last was most relevant to our model. The reason is that adaptation to a dramatically different environment requires that colonizers include at least some individuals capable of reproducing in the new environment. These individuals with extreme phenotypes would be rare in the old environment. The chance of their inclusion with colonists therefore increases with the number of dispersers, although this effect might be less important if emigration was phenotype-dependent (not considered here). It is also likely that the initially low level of competition in the new environment facilitates resource acquisition by these otherwise marginal individuals. Indeed, the success

of immigrants dramatically decreased as the new population became increasingly adapted to its environment (Fig. 2–6).

A second factor influencing the transition between the promoting and constraining effects of environmental differences was sexual preference. In particular, we found that when environmental differences were so large that they hamper colonization, stronger sexual preferences exacerbated this problem (Fig. 2–5). The reason was that evolution of the female target preference lagged behind evolution of the male trait (Fig. 2–4), because colonizing females carry mate preferences that were adaptive in the old, but not new, environment. That is, females in the new population initially prefer trait values typical of the old population, and thus tend to mate with maladapted immigrant males rather than adapting resident males. This initial trait-preference mismatch slows adaptation in the new environment, and thus limits population growth. Eventually, however, partial adaptation of the male trait leads to selection on females to prefer adapted trait values (offspring survival is thereby higher), and the female target preference begins to shift in the appropriate direction. This "reinforcement" of mating preferences (Servedio & Kirkpatrick 1997; Servedio 2004) then allows local adaptation to accelerate.

Q2. Selection against immigrants (and hybrids): Many empirical studies provide evidence that natural selection against maladapted immigrants can dramatically reduce gene flow between populations adapted to different environments (e.g. Via et al. 2000; Nosil 2004; Nosil et al. 2005, review). The only model (Hendry 2004) to explicitly examine this phenomenon (separate from other reproductive barriers) found that it could evolve very quickly, but that model had

rather restrictive assumptions. Nevertheless, our individual-based simulations were consistent with Hendry (2004) in finding that natural selection against immigrants can, by itself, substantially reduce hybrid production when environmental differences are large (Fig. 2–7). The maximum reduction in our model (one-half of the random expectation) was a product of our specific mechanism of selection: maladapted immigrants could not obtain enough energy to reproduce as females but could still reproduce as males. The effectiveness of selection against immigrants would thus have been greater had we also included other selective factors, such as viability selection or energy constraints on male reproduction. It thus seems clear that selection against immigrants can sometimes be so strong as to render other potential reproductive barriers redundant, as suggested by others (Hendry 2004; Nosil et al. 2005).

Supporting empirical work (e.g. Schluter 1995; Via et al. 2000), selection against hybrids acted similarly to selection against immigrants, but was weaker. The main reason for this weaker effect was the assumption of additive gene action, which made hybrids phenotypically intermediate between residents and immigrants and therefore at a lower fitness disadvantage than immigrants. This formal confirmation of previous assertions (Hendry 2004; Nosil et al. 2005) further highlights the importance of directing more effort to the study of ecological selection against immigrants rather than hybrids. Of course, the relative importance of these two types of barriers in nature will depend on the type of selection (inviability, energy constraints on reproduction), non-additive genetic effects, and the life stage at

which dispersal occurs. Finally, it is important to remember that ecologically-based selection against immigrants and hybrids can be sensitive to environmental change (Grant & Grant 1996; Taylor et al. 2006), and so other reproductive barriers might be needed if speciation is to be irreversible.

Q3. Sexual preference: The importance of mate choice during ecological speciation is supported by many empirical studies (e.g. Seehausen & van Alphen 1999; Boughman 2001; Huber et al. 2007) and theoretical models (e.g. Kondrashov & Shpak 1998; Higashi et al. 1999; Kondrashov & Kondrashov 1999; Gourbiere 2004). Many of these earlier analyses deal with one of the easiest situations for speciation with gene flow: i.e. "magic trait" models, where the same trait determines both adaptation and assortative mating (Gavrilets 2004). When these two phenomena are instead encoded by different genes, speciation becomes more difficult, although sometimes still possible (e.g. Kondrashov & Kondrashov 1999; Fry 2003). Many of these nonmagic trait models, however, dealt with a situation where assortative mating is based on habitat selection rather than mate preferences within a given habitat. Our model was designed to inform this latter situation, albeit in a spatial context.

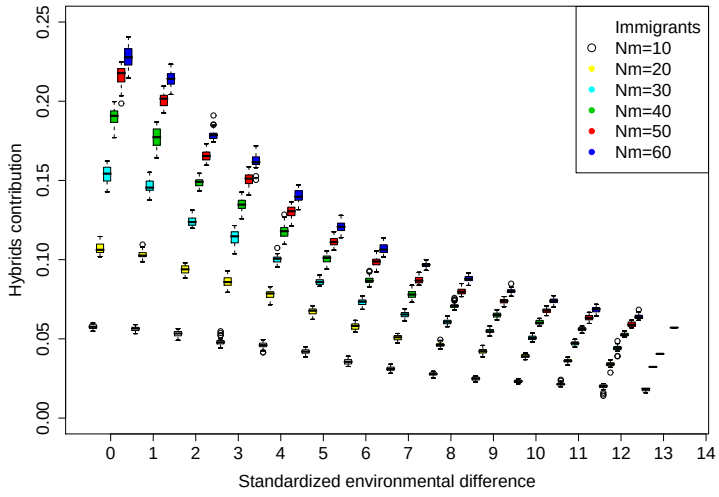
We found that the addition of sexual preference leads to a strong decrease in the reproduction of immigrants, thus dramatically reducing the production of hybrids relative to random expectations. Indeed, the combined effects of natural and sexual selection essentially eliminated the production of hybrid offspring (Fig. 2–7) and allowed some divergence even at unlinked neutral genetic markers (see below). Note that these results really do reflect the interaction of natural and

sexual selection because hybrid production was not reduced when environmental differences were absent but sexual preferences were present. We therefore agree with Arnegard & Kondrashov (2004) that speciation by sexual selection alone is difficult. We also agree with other authors (Kondrashov & Shpak 1998; Higashi et al. 1999; Gourbiere 2004) that sexual selection can make a very important contribution to speciation that is initiated through divergent natural selection.

Q4. Dispersal rate: Numerous theoretical models, experiments, and correlative analyses have argued that dispersal can have either positive or negative effects on adaptive divergence (see Introduction). We here illustrate how the balance between these effects depended on other parameters. When sexual preference was absent, increasing dispersal (1) decreased resident population size when environmental differences were small (because of increased competition with residents) but (2) increased resident population sizes when environmental differences were large (because interbreeding with residents reduced competition among resident offspring). The latter effect then disappeared when sexual preference was added, because immigrants did not acquire many resources and did not reproduce, thus becoming irrelevant to the resident population. Another interesting nonlinearity was that the time to full occupation of a new environment was not influenced by dispersal when environmental differences were small (because occupation was very fast) but decreased with increasing dispersal when environmental differences were large (because colonizers were more likely to include a few individuals capable of reproducing in the new environment). These results further highlight the multifarious effects of dispersal on adaptive divergence (Garant et al. 2007).

We also examined how the above effects of dispersal on adaptive divergence might cascade to ecological speciation. Here, one might initially predict negative effects of dispersal because of increased recombination (Felsenstein 1981; Coyne & Orr 2004), but we instead found mainly positive effects. In particular, increasing dispersal enhanced several reproductive barriers, including selection against immigrants, hybrid production relative to random expectations, and selection against hybrids. The reason for all of these positive effects of dispersal was competition. In particular, increasing dispersal meant that fewer immigrants were able to obtain sufficient resources for reproduction, which thus caused the reduction in hybrids relative to random expectations. Despite these effects, increasing dispersal nevertheless increased the absolute number of hybrids and their absolute effect on reproductive isolation (i.e. proportion of hybrids multiplied by their relative fitness, Fig. 2–10, which increased competition among them and thereby decreased their ability to acquire resources and therefore reproduce. Our results thus further highlight the need to consider both positive and negative effects of dispersal on ecological speciation, particularly in the case of competition for limited resources (Dieckmann & Doebeli 1999; Day & Young 2004)

Q5. Neutral genetic divergence: Divergence at neutral genetic makers has frequently been used to test for the onset of ecological speciation (e.g. Lu & Bernatchez 1999; Ogden & Thorpe 2002; Crispo et al. 2006; Räsänen & Hendry 2008). At present, however, the power and generality of this approach remains uncertain. The reason is that unlinked neutral genetic markers might flow almost freely between selective environments (Emelianov et al. 2004; Gavrilets & Vose



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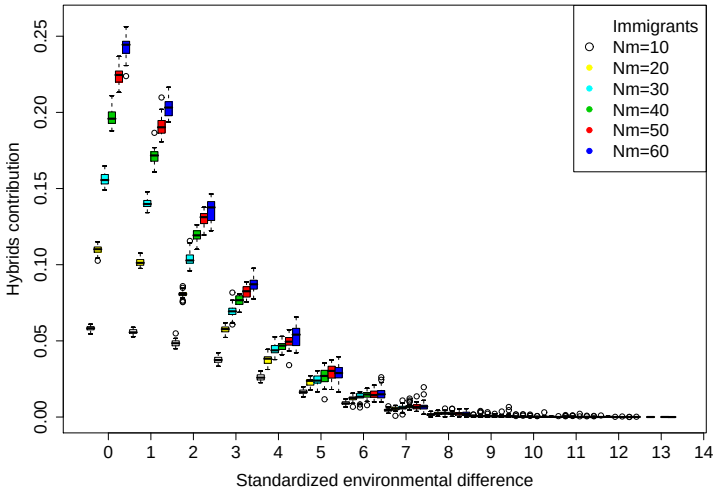

(b)

Figure 2–10: Hybrids' contribution (proportion of hybrids multiplied by their relative fitness) as a function of the standardized environmental difference and the number of immigrants. Panel a represents simulations without sexual preference ($a = 0$) and panel b represents simulations with sexual preference ($a = 0.1$). See figure 2–5 caption for explanation of the boxplot.

2005), although a generalized barrier to gene flow is also possible (Gavrilets 2004; Grahame et al. 2006; Nosil et al. 2009). Our results suggest that neutral genetic markers might provide some indication of ecological speciation, but only under certain conditions. Specifically, environmental differences must be so strong that very few immigrants can successfully breed, which in our model required both large environmental differences and strong female preferences. In such cases, gene flow was very low and so neutral genetic divergence could proceed through genetic drift. It is therefore likely that greater neutral divergence would have been seen if we had simulated smaller populations (our populations usually attained sizes of more than 250 individuals).

Our results also provide a strong reminder of the stochastic effects of genetic drift. This random aspect of evolution lead to a large range in F_{ST} values among independent simulations that used a common set of parameters (Fig. 2–9). This variation was evident across independent loci within a simulation (results not shown) and also across simulations when loci were averaged. The implications are that only a fraction of loci might show substantial differences between only a fraction of populations experiencing divergent selection. Indeed, this result fits well with recent empirical findings that only a few neutral, unlinked loci may differ between populations in different environments and that the specific loci showing this effect differ among different population comparison (Nosil et al. 2008). We suggest that when investigators find the expected pattern (lower gene flow between than within environments), it probably does reflect ecological speciation. When they don't find this pattern, however, ecological speciation might still be occurring.

For these reasons, neutral genetic markers are not a very reliable way to infer ecological speciation, or the lack thereof.

2.7 The speed of ecological speciation

The speed of ecological speciation has received little attention until only recently (Hendry 2004). Indeed, Hendry et al. (2007) could find few relevant empirical studies, although those few did hint that substantial reproductive isolation can evolve on very short time scales (< 100 generations). This interpretation was, however, challenged by Gavrilets et al. (2007) and Gavrilets & Vose (2007), whose simulations were interpreted as showing the evolution of only limited reproductive isolation over such time frames. Indeed, their simulations required on the order of 10000 generations to complete speciation. We suggest that this apparent difference of opinion is illusory because the different studies examined different spatial contexts (essentially parapatry *versus* sympatry) and different degrees of reproductive isolation (partial *versus* nearly complete). Although the present study was not specifically designed to examine this question, the observed dynamics are nonetheless relevant. Our simulations showed that strong divergent natural selection (standardized environmental differences of $10 - 12$), coupled with strong sexual preferences ($a = 0.1$), and high immigration rates ($Nm = 60$) often substantially reduced hybrid production and hybrid fitness (to less than 0.7) after only 50 generations. By 100 generations, essentially zero hybrids were formed and were able to reproduce. In short, ecologically-based reproductive isolation can arise very rapidly, consistent with the deterministic simulations (Hendry 2004) and existing empirical studies (Hendry et al. 2007).

2.8 Acknowledgments

XTP and APH were sponsored by the Natural Sciences and Engineering Research Council (NSERC) of Canada. We thank Cristian Correa for helpful discussions. We also thank Erika Crispo, Claire de Mazancourt, Sergey Gavrilets, Renaud Kaeuffer, Ann McKellar, Amy Schwartz, and Sam Yeaman for their comments on the manuscript. Thanks to McGill University (Department of Biology) and S. Bunnell for help using the bioinformatics cluster for some simulations and two anonymous referees for their valuable suggestions. XTP thanks the Delise Allison Redpath Museum Graduate Student Development Award that allowed him to present the results of this study at Evolution 2008 at the University of Minnesota, Minneapolis.

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Connecting statement

In the previous chapter, we saw how environmental differences and natural and sexual selection influence the progression toward ecological speciation. The expression of phenotype in that chapter was entirely determined by genetic variation, but in reality, environmental features can influence the expression of genes through "phenotypic plasticity". In the next chapter, we add phenotypic plasticity to the model described in the previous chapter. This will allow us to infer how incorporating plasticity might alter forces that influence progress toward ecological speciation.

CHAPTER 3
The consequences of phenotypic plasticity on ecological speciation

3.1 Abstract

We use an individual-based numerical simulation to study the effects of phenotypic plasticity on ecological speciation. Consistent with previous models, adaptive plasticity evolves readily in the presence of migration between populations from different ecological environments and it also aids in the colonization of new environments. We further show that this plasticity can either enhance or degrade the potential for divergent selection to cause reproductive barriers. Of particular importance is the timing of plasticity in relation to the timing of migration between environments. If plasticity is expressed after migration, reproductive barriers are generally weaker, because plasticity allows immigrants to be better suited for their new environment. If plasticity is expressed before migration, reproductive barriers are either unaffected or are enhanced. Among the potential reproductive barriers we considered (natural and sexual selection against migrants and hybrids), natural selection against migrants was the most important, primarily because it was the earliest acting potential barrier. Accordingly, plasticity had its greatest

A version of this chapter is submitted to *Journal of Evolutionary Biology* as Thibert-Plante, X. and Hendry, A. P. The consequences of phenotypic plasticity on ecological speciation

effect on natural selection against migrants. Plasticity had particularly little effect on natural and sexual selection against hybrids, because hybrids could adjust their phenotypes to the environment in which they were produced. In general, phenotypic plasticity modifies the process of ecological speciation and should be taken into account when studying the evolution of reproductive barriers.

Keywords: Ecological speciation, phenotypic plasticity, selection against migrants, individual-based modelling

3.2 Introduction

Ecological speciation occurs when divergent natural selection causes adaptive divergence, which then causes reproductive isolation (Schluter 2000; Rundle & Nosil 2005). The process of ecological speciation now has considerable support from a broad array of theoretical models (Dieckmann & Doebeli 1999; Doebeli & Dieckmann 2003; Fry 2003; Gavrilets & Vose 2005, 2007; Gavrilets et al. 2007; Thibert-Plante & Hendry 2009), meta-analyses (Funk et al. 2006), and empirical studies of natural systems (Rundle et al. 2000; Barluenga et al. 2006; Nosil 2007; Grant & Grant 2008a). At the same time, it has become increasingly apparent that divergent selection does not always cause substantial progress toward ecological speciation, because the ultimate outcome depends on the genetic architecture, the dimensionality of selection, the nature of mating systems, and the extent of migration between populations (Berner et al. 2009; Hendry 2009; Nosil et al. 2009; Nosil & Harmon 2009). Our goal is to explicitly examine one additional factor (phenotypic plasticity) that might influence progress toward ecological speciation.

Phenotypic plasticity is the tendency of a particular genotype to produce different phenotypes under different environmental conditions. Phenotypic plasticity is extremely common in nature (DeWitt & Scheiner 2004) and often appears to be adaptive (Sultan 2000; Pigliucci 2001). That is, phenotypic plasticity allows organisms to deal with unpredictable environments by altering their phenotype to suit local conditions, as opposed to genetic canalization which would eliminate this option. In particular, theoretical models have shown that phenotypic plasticity is favored over local adaptation for organisms in spatially heterogeneous environments (Zhivotovsky et al. 1996; Alpert & Simms 2002; Richter-Boix et al. 2006; Lind & Johansson 2007; Hollander 2008) or in environments that vary through time, whether gradually (Ernande & Dieckmann 2004; Gabriel 2005; Stomp et al. 2008; Svanbäck et al. 2009) or abruptly (Lande 2009). And yet, phenotypic plasticity is not a panacea for adaptive responses to changing environments because plasticity has limits (information reliability, lag time, developmental range and epiphenotype) and costs (maintenance, production, information acquisition, developmental instability, pleiotropy and epistasis) (rewiewed in DeWitt et al. 1998; van Buskirk & Steiner 2009). It is also possible that phenotypic plasticity can be maladaptive under certain circumstances (Langerhans & DeWitt 2002; Grether 2005). All of these variables may also be important in the context of ecological speciation.

How might phenotypic plasticity influence progress toward ecological speciation? By progress toward ecological speciation we mean the evolution of reproductive barriers between populations in different environments. We here

organize some of the possibilities around previous conclusions regarding ecological speciation.

1. *Intermediate levels of divergent selection are most conducive to ecological speciation.* This is a frequent outcome of theoretical models (Gavrilets et al. 2007; Thibert-Plante & Hendry 2009) and results from an intersection of two effects. First, an increase in divergent selection increases adaptive divergence, which should result in stronger ecologically-based reproductive isolation. Second, increasing divergent selection reduces the chance that a new environment will be colonized, thus reducing the opportunity for ecological speciation (Crispo 2008). We propose that plasticity might have two potentially opposing effects here (Baldwin 1896; Price et al. 2003; Ghalambor et al. 2007). First, it might reduce progress toward ecological speciation, because adaptive plasticity should reduce divergent selection on the genetic component of the trait. Second, it might increase progress toward ecological speciation, because plasticity should enhance colonization of new environments. These two effects are not mutually exclusive.

2. *Adaptive divergence leads to natural selection against migrants between ecological environments.* This is one of the most obvious, general, and widely supported predictions of ecological speciation (Hendry 2004; Nosil et al. 2005; Thibert-Plante & Hendry 2009). The reason is that organisms adapted to local environments should perform better in those environments than should individuals adapted to alternate environments (Schluter 2000; Hereford 2009). We suspect that any modifying influence of plasticity will depend on whether the plastic response occurs before or after migration. If plasticity mainly occurs before

migration between environments (e.g., migration of adults), then plasticity might increase selection against migrants, because plasticity has tailored phenotypes to the original environment. If plasticity mainly occurs after migration (e.g., migration of juveniles), then it might decrease selection against migrants because individuals can change their phenotypes to suit the new environment.

3. *Adaptive divergence can lead to the evolution of female preferences for local males: i.e., sexual selection against migrants.* This process is expected to occur most easily when the trait under divergent selection also pleiotropically influences mate choice, such as in "magic trait" models (Dieckmann & Doebeli 1999; Fry 2003; Gavrilets 2005). The process is also sometimes possible if marker traits in males can be used by females to detect locally adapted males (Felsenstein 1981; Doebeli 2005). In this case, female preferences tend to evolve as a result of direct or indirect selection to avoid mating with maladapted immigrants (Kirkpatrick 2001). Empirical work on natural populations suggests that adaptive divergence does often lead to positive assortative mating (Grant & Grant 2008b; Boughman 2001), but not always (e.g. Ellers & Boggs 2003). We suspect that the influence of plasticity on the evolution of this assortative mating will depend on when plasticity occurs relative to migration, for the same reasons as described in the above section. Sexual selection against migrants might be strengthened by plasticity that occurs before migration but weakened by plasticity that occurs after migration.

4. *Adaptive divergence often leads to natural (and sexual) selection against hybrids.* The reason for this prediction is that hybrids are often phenotypically

intermediate between parental forms and should therefore not be well adapted for either parental environment. With respect to reproductive barriers, selection against hybrids also appears to be a common part of ecological speciation (Schluter 2000; Rundle & Nosil 2005). We suspect that plasticity will weaken this effect because hybrids produced in a given environment might be able to shift their phenotypes closer to locally adapted phenotypes.

5. *Ecological speciation should be detectable by reduced gene flow at neutral genetic markers, even if those markers are not linked to genes under selection.* This idea has received mixed support from empirical studies, with some finding that divergent selection (or adaptive divergence) reduces neutral gene flow (Grahame et al. 2006; Nosil et al. 2008; Berner et al. 2009) and others not finding such a pattern (Emelianov et al. 2004; Gavrilets & Vose 2005; Crispo et al. 2006). Theoretical models have helped to understand this variation by revealing that (1) divergent selection can cause a generalized barrier to gene flow when selection acts against the whole genome of migrants and first-generation hybrids (i.e., before recombination between parental genomes), but (2) that this effect is relatively weak, inconsistent, and difficult to detect empirically because selected genes and neutral markers become decoupled after recombination (Thibert-Plante & Hendry 2010). We suspect that the effect of plasticity on neutral gene flow will be to further weaken any patterns because phenotypic divergence is less closely tied to genetic divergence.

We study these topics by incorporating phenotypic plasticity into individual-based models of ecological speciation. We define the process of ecological speciation as the build up of reproductive barriers between populations in different environments. We focus on two spatially-discrete environments with different ecological conditions (different optimal trait values). We start with a population adapted to one of the environments, with some individuals migrating to the other environment. The second environment can be colonized and a local population can become established and adapt to the new environment under continuing migration. Given that the two environments impose divergent selection that causes adaptive divergence, ecological speciation is expected. Onto this framework, we add plasticity to the ecological trait, which can occur before or after migration, has different limits, and may or may not be costly. Importantly, we do not impose a particular level of plasticity but rather allow it to evolve. With this model we ask several basic questions that inform the above expectations.

3.3 Modelling framework

This model combines features of the Gavrilets & Vose (2005); Gavrilets et al. (2007); Gavrilets & Vose (2009) and Thibert-Plante & Hendry (2009) models. The former models focus on adaptive radiation, whereas the latter focuses more specifically on the mechanisms of ecological speciation. These models are reasonably realistic by using hard selection (Christiansen 1975) rather than soft selection (Kisdi & Geritz 1999; Spichtig & Kawecki 2004), and by employing the same realistic mutation rates as Gavrilets & Vose (2005); Gavrilets et al. (2007);

Gavrilets & Vose (2007, 2009). Yet they remain general enough to inform the basic process of speciation.

Two major differences from previous models are important to recognize. First, we do not here, nor in Thibert-Plante & Hendry (2009), model the evolution of habitat choice. The main reason is that this situation has been frequently modeled (Gavrilets & Vose 2005; Gavrilets et al. 2007; Gavrilets & Vose 2009), and we are more interested in what happens when individuals from different environments actually encounter each other. Second, the optimal conditions in our model are not at the phenotypic extremes (as they are in (Gavrilets & Vose 2005; Gavrilets et al. 2007; Gavrilets & Vose 2009)). The reason is that we wish to allow the potential for phenotypes to overshoot the optima reducing bias toward the optimal phenotypes.

The code is written in Fortran and is available upon request.

Environment and migration

The environment is represented by an optimal trait value θ and has a maximum carrying capacity of K_0. The actual carrying capacity (K) is function of the level of adaptation of the population (see below). Two environments (two θ) are present and they are not spatially overlapping, such as lake and stream environments for fishes (Hendry et al. 2002) or different host plant patches for insects (Nosil et al. 2002).

Migration between the populations occurs as a fixed proportion (m) of the population size (N). That is, each population contributes on average Nm randomly chosen individuals to the other population. This migration is random

with respect to phenotype, although the influence of phenotype-biased migration would be useful to consider in future work. In addition, individuals do not have a particular habitat preference, although this could be considered implicit in the sense that migration occurs with a specific probability. We nevertheless do not allow the evolution of habitat preference and therefore the evolution of migration rate.

Individuals

The individuals are diploid hermaphrodites (monoecious). They have different characters that are each controlled by L additive loci with three possible alleles at each locus ($\{-1, 0, 1\}$). At reproduction, the offspring receives, at each loci, one allele from each of the two parents. Individuals have an ecological character (x), a male signalling trait (m), a female preference trait (f), a strength and direction of female preference (c) and a level of plasticity (r). f and c are only expressed when the individual acts as a female, and m is only expressed when the individual acts as male. All traits are genetically unlinked and are scaled to be between zero and one. In addition to these traits, there are 16 neutral unlinked loci that behave like microsatellite markers (see below).

Life cycle

The life cycle of individuals is as follows: birth, development, density-dependent viability selection, mating and death. Random migration between environments occurs either just before development or just after. Since plasticity might have different effects if development occurs before or after migration, we look at the two scenarios independently.

Development and plasticity

In the absence of plasticity ($r = 0$), individuals develop their ecological character x as coded in their genotype. If plasticity is present ($r\alpha_r > 0$), the environment modifies x in the following way

$$x = \begin{cases} x', & \text{if no plasticity: } r\alpha_r = 0 \\ x' + sign(\theta' - x') * r * \alpha_r, & \text{if } |\theta' - x'| \text{ exceeds the maximum plasticity: } |\theta' - x'| > r\alpha_r \\ x' + (\theta' - x') = \theta', & \text{if } \theta' \text{ is within the range of possible plasticity values} \end{cases} \tag{3.1}$$

where $sign()$ is the function that returns the sign of a number, x' is the genotypic value for the ecological character, x is the phenotype of the ecological character, α_r controls the limit of plasticity and θ' is the perceived optimum of an individual value drawn from a normal distribution with mean θ and standard deviation of θ_{err}.

Viability selection

The viability of an individual is a function of its ecological character, the environment, and the population density. Specifically, the fitness of an individual (ω) is a function of the distance to the optimal phenotype in that environment (θ) and the strength of stabilizing selection around that optimum (σ_s):

$$\omega' = \exp\left[-\frac{(x-\theta)^2}{2\sigma_s^2}\right]. \tag{3.2}$$

where ω' represents fitness before the cost of plasticity is added. As σ_s decreases, the strength of selection increases, the virtual valley between the peaks gets

deeper. With a cost to plasticity (C_r), fitness becomes:

$$\omega = \omega'(1 - C_r|r|). \tag{3.3}$$

Finally, the probability that an individual survives to the reproduction stage (ν) is given by the Beverton-Holt model (Kot 2001):

$$\nu = \frac{K}{K + N(b-1)}, \tag{3.4}$$

where b is the average number of offspring produced by a female, K is the carrying capacity adjusted by individual fitness ($K = K_0 w$) and K_0 is the maximum carrying capacity.

Mating preference

Individuals who survive past the viability selection stage can mate. Every individual is chosen once as a female and will produce on average b offspring drawn from a Poisson distribution. Every surviving individual is also a potential father, but there is no self-fertilization. Potential fathers have a probability Ψ of being chosen by a given female with target preference trait (f) and preference direction and strength (c) for the male signaling trait (m) (modified from Bolnick (2004, 2006); Doebeli (2005) by Gavrilets et al. 2007):

$$\Psi(m, f, c) = \begin{cases} \exp\left[-(2c-1)^2 \frac{(f-m)^2}{2\sigma_a^2}\right], & \text{if } c \geq 0.5 \\ \exp\left[-(2c-1)^2 \frac{(f-(1-m))^2}{2\sigma_a^2}\right], & \text{if } c < 0.5. \end{cases} \tag{3.5}$$

At $c = 0.5$ every living male has the same probability of being chosen, and mating is therefore random. At $c > 0.5$, positive assortative mating is present with respect

to the male signaling trait (m) and the female target preference trait (f). At $c < 0.5$, negative assortative mating is present. We also consider a magic trait model (Gavrilets 2004) for which we replace m and f with the ecological character x. The parameter σ_a controls the width of the mating probability distribution as a function of male trait, and as σ_a decreases females become choosier.

Neutral loci

The neutral loci act like microsatellites, with high mutation rates (Weber & Wong 1993) that change the number of repeats in a sequence (Di Rienzo et al. 1994; Valdes et al. 1993). Mutations are stepwise and consist of an increase or decrease in the number of repeats (Kimura & Ohta 1975). A mutation that occurs at the boundary of the possible allele range changes the allele value to the closest other possible value (-1 where the allele is at the maximum of 15 repeats and $+1$ when the allele is at the minimum of one repeat).

The neutral loci are unlinked to each other and unlinked to selected loci, equivalent to a recombination rate of 0.5 between all loci.

Initial conditions

Initially, all of the loci controlling the ecological trait have a value of -1, thus $x = 0$ for all individuals. The same initial conditions apply to the loci controlling the plasticity trait r. The male (m), female (f) and preference and direction trait (c) loci are all at 0, and so $m = f = c = 0.5$ for all individuals. All of these loci have the same realistic mutation probability (10^{-3}) (Dallas 1992; Weber & Wong 1993; Brinkmann et al. 1998). A mutation changes the allele by a value of plus one or minus one, with the same probability, relative to the current allele. Mutation

at the boundaries of the allele range are designed to keep the value within the possible range, by changing the sign of the mutation if necessary.

Both environments have the same carrying capacity (K_0), but they have different optima: $\theta_1 = 0.2$ and $\theta_2 = 0.8$. Initially only one environment is colonized by 10 individuals (θ_1) and the other remains empty until immigration takes place as in Gavrilets et al. (2007).

Parameters

Table 3–1 lists the parameter space explored. All combinations were simulated except for the cost of plasticity ($C_r > 0$) and migration after plasticity when there is no plasticity $\alpha_r = 0$ to avoid redundancy. All simulations were run for 20000 generations with 10 replicates for each parameter combination. The values of the parameters were chosen to be comparable to those simulated in previous models (Gavrilets et al. 2007). Note that to directly estimate the effect of selection on an adapted migrant, we need to divide our σ_s by 0.6 in order to directly compare to the results of Gavrilets et al. (2007), because of differences in the position of the adaptive peaks (see above).

Data tracking

Genetic measures and statistical tests (see below) were performed after reproduction but before migration. During the simulations, we tracked the means and standard deviations of all traits (x', x, m, f, c, r). We also tracked these parameters in the migrants and hybrids. A resident is an individual that is neither a hybrid nor a migrant. A migrant is a first generation immigrant in an environment. A hybrid is the offspring of a cross between a migrant and a resident.

Parameter	symbol(if any)	values
Natural selection	σ_s	$\{0.24, 0.30\}$
Sexual selection	σ_a	$\{0.05, 0.1\}$
Maximum carrying capacity	K_0	$\{2048, 4096\}$
Number of loci	L	$\{4, 8\}$
Cost of plasticity	C_r	$\{0, 0.1\}$
Error plasticity	θ_r	$\{0.05\}$
Maximum Plasticity	α_r	$\{0, 0.3, 0.6\}$
Average number of offspring	b	$\{4\}$
Time of migration		{before, after}
Magic trait		{yes, no}
Migration	m	$\{0.01, 0.1, 0.2\}$

Table 3–1: Parameter space explored

Those three categories, migrant, hybrid and resident, are mutually exclusive. This is an approximation, because for instance, a cross between two migrants will be considered a resident, but the probability of such events is so low that we can sefely ignore them, especially in the presence of natural selection. The viability and the contribution to the next generation (number of offspring they produce) is tracked for the residents, migrants, and hybrids.

At each generation after reproduction, we calculated F_{ST} using neutral markers betwen the two populations following Weir (1996).

3.4 Results

Before discussing the effects on ecological speciation, we first summarize results for the evolution of plasticity. We considered plasticity to have evolved if the mean plasticity value (r) was substantially greater than zero, here defined as $\bar{r} - SE(r) > 0$, where SE is the standard error. Plasticity evolved in most cases when the maximum plasticity was allowed to be greater than zero ($\alpha_r > 0$). Of the

exceptions (158 out of 7680 simulations), all except one occured when plasticity was costly ($C_r > 0$). Several factors influenced the evolution of plasticity. Using generalized linear model we found that plasticity evolved less often when (1) the number of loci (L) controling plasticity were large ($p < 0.01$), (2) selection (s) was strong ($p < 0.001$), (3) migration occurred after plasticity ($p < 0.001$), (4) maximum plasticity (α_r) was small ($p < 0.001$), and (5) plasticity was costly ($C_r > 0$) ($p < 0.001$). When plasticity did evolve, its magnitude (r) was most dependent on the maximum plasticity possible (α_r). For instance, the mean values of plasticity were $r = 0.17$ and $r = 0.26$ for $\alpha = 0.3$ and $\alpha = 0.6$ respectively. Also, a higher cost of plasticity (C_r) reduced the magnitude of plasticity (r) that evolved ($\bar{r} = 0.26$ whitout cost and $\bar{r} = 0.26$ with cost). Finally, plasticity was greater on average when migration occured after plasticity than when it occured before plasticity ($\bar{r} = 0.24$ and $\bar{r} = 0.19$).

Colonization

We considered colonization to have occured when the number of offspring produced in a given environment was greater than 60% of the carrying capacity (K) of that environment. This threshold was high enough to eliminate cases where only immigrants were in the environment, while also being low enough to not exclude established population subject to a maladaptive migration load. Time to colonization is then the number of generations from the beginning of the simulation until the first occurrence of the previous criterion.

Colonization of the second environment ($\theta_2 = 0.8$) occurred 67% of the time without plasticity ($\alpha_r = 0$) , and 96% of the time with plasticity. At a

low migration rate ($m = 0.01$), colonization was not strongly influenced by selection (it took only slightly longer under stronger selection = higher s) and was not influenced by the plasticity scenario: either no plasticity, migration after plasticity, or migration before plasticity (Fig. 3–2 in supplemental materials). At high migration rate ($m = 0.2$ and $m = 0.1$), colonization generally took longer, particularly when selection was stonger (Fig. 3–1). Specifically, when selection was weak ($s = 0.3$), plasticity had little influence on the colonization rate. When selection was strong ($s = 0.24$), plasticity increased the likelihood of colonization, as well as its speed (Fig. 3–1). This effect was strongest when migration occurred before plasticity.

To evaluate the level of overall adaptation (i.e. mean population fitness), we measured the population size relative to its carrying capacity. When the migration rate was low, population size was greater when selection was weaker, but did not depend appreciably on the plasticity scenario (without plasticity, migration before or after plasticity) (Fig. 3–3). At higher migration rates ($m \geq 0.1$), population size depended on the plasticity scenario. Here, population size was lowest without plasticity, when migration occurred after plasticity, and was highest when migration occurred before plasticity. In short, plasticity often promotes the colonization and local adaptation to new environments.

Natural selection against immigrants

Without plasticity, the average survival of immigrants was always lower than that of residents (Fig. 3–4). When migration occurred before plasticity, survival did not differ appreciably between immigrants and residents. When

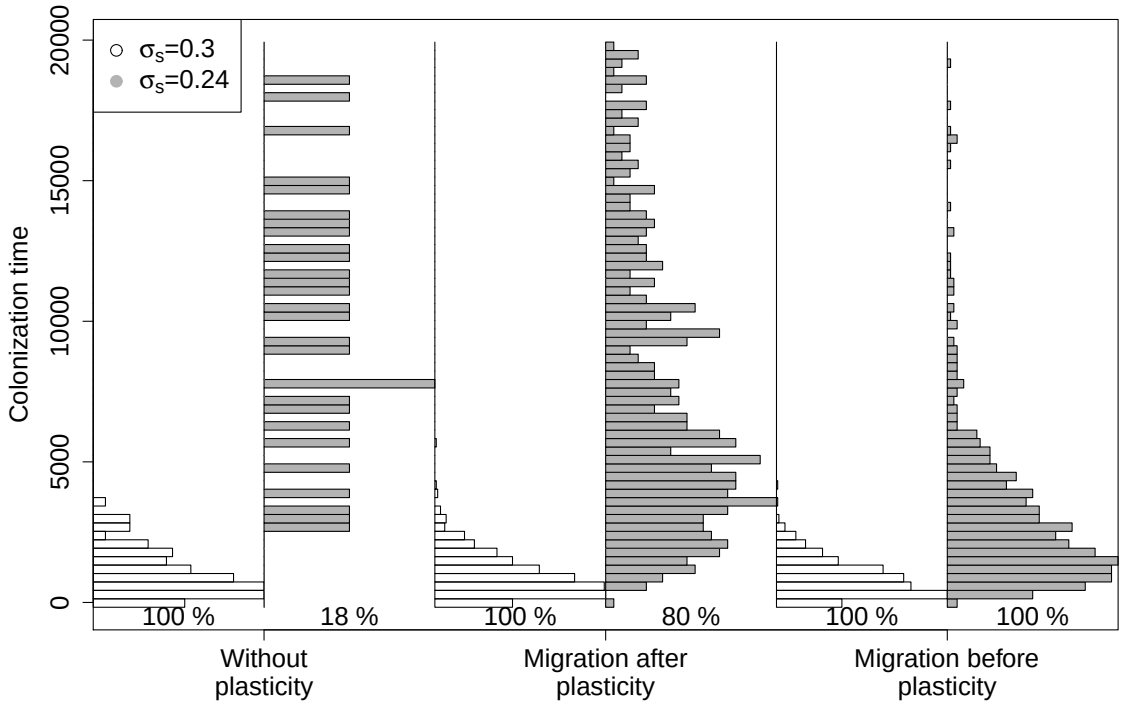


Figure 3–1: Time required to colonize the second environment ($\theta_2 = 0.8$) as a function of the plasticity scenario and the strength of selection when the migration rate is high ($m = 0.2$). The percentage shown at the bottom of each pannel represents the frequency of colonization over the number of simulations.

migration occurred after plasticity, immigrants had the lowest survival relative to residents. All of these effects were stronger when selection was stronger (results not shown). In short, plasticity increased selection against migrants between ecological environments if migration occurred after plasticity but not if it occurred before plasticity.

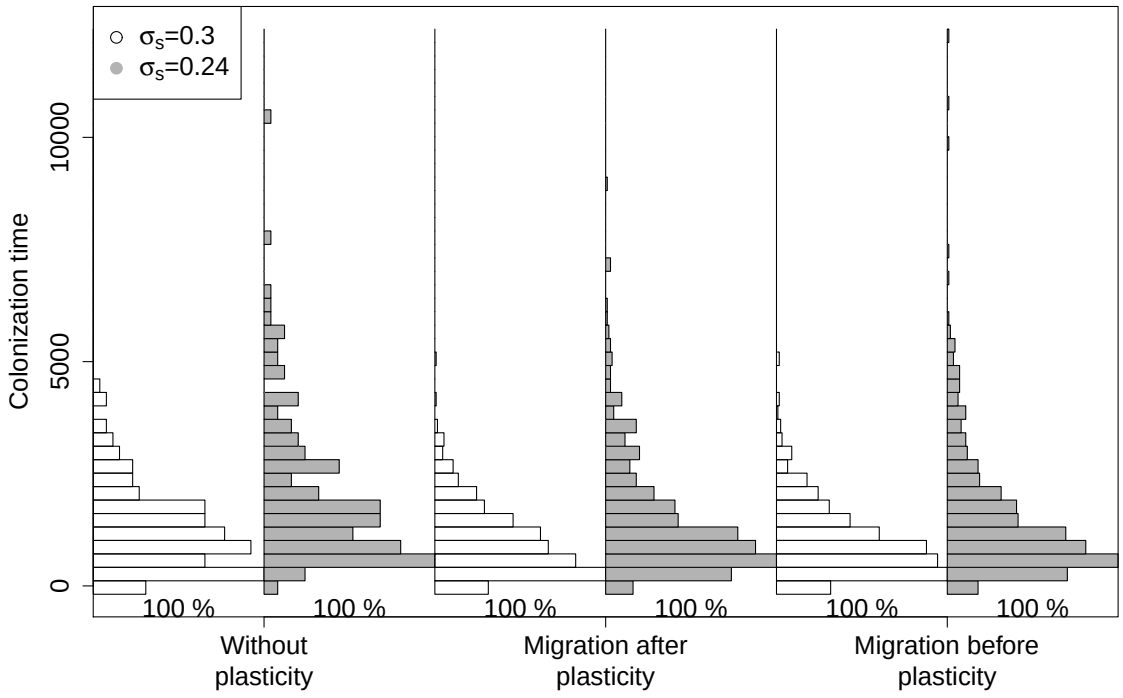


Figure 3–2: Time required to colonize the second environment ($\theta_2 = 0.8$) as a function of the plasticity scenario and the strength of selection when migration is low ($m = 0.01$). The percentage shown at the bottom of each panel represents the frequency of colonization over the number of simulations.

Sexual selection against immigrants

When sexual preference evolved (i.e., $\bar{c} \pm SE(c)$ did not overlap with zero), immigrants and residents produced approximately the same number of offspring on average (Fig. 3–5). Some important variation around this average, however, did emerge which depended on the particular parameter values and plasticity scenarios. Specifically, a number of simulations showed sexual selection against immigrants when plasticity was absent and when plasticity was present with

migration occurring after (but not before) plasticity. This variation was due entirely to magic trait models (Fig. 3–6): i.e., sexual selection against immigrants was not evident in any other situation. Interestingly, in magic trait models, sexual selection favored immigrants in some rare situations, because of the drifting of preference strength and direction (c) (Fig. 3–7).

Natural and sexual selection against hybrids

The survival (Fig. 3–4) and reproductive output (Fig. 3–5) of hybrids was lower than that of residents only when plasticity was absent. The decrease in the reproductive output was due to the magic trait models (Fig. 3–6). The presence of plasticity thus helped to increase the viability of the hybrids in all cases. This was because hybrids developed in the environment in which they are selected and thus developed an appropriate phenotype for that environment.

Unlinked neutral markers

Divergence in unlinked neutral markers was mostly affected by migration rate (Fig. 3–8). As expected, increasing migration rates reduced genetic divergence. Also, genetic divergence in the presence of plasticity was lower when plasticity occurred before, but not after, migration.

3.5 Discussion

Plasticity is expected to evolve when environments fluctuate in time (Gabriel 2005; Stomp et al. 2008; Svanbäck et al. 2009) or space (Zhivotovsky et al. 1996; Alpert & Simms 2002; Lind & Johansson 2007). We modeled the latter situation based on migration between two ecologically different environments. We found that plasticity evolved in 98% of our simulations, confirming once

again the evolutionary advantage of plasticity when selection varies in space. The rare occurrences where plasticity did not evolve were characterized by strong selection, high costs of plasticity, and small numbers of loci controlling the genetic component of the trait. These are the same conditions expected to favor (and ease) genetic evolution (strong selection, fewer loci (Gourbiere 2004; Gavrilets et al. 2007)) and disfavor the evolution of plasticity (high costs, (van Tienderen 1997)). In short, the evolution of plasticity in our model was consistent with previous models, allowing us to then turn our attention to how plasticity evolution influences ecological speciation.

Colonization and overall adaptation

Ecological speciation requires the colonization of divergent environments and the establishment of self-sustaining populations. Therein lies the rub, because divergent environments are expected to reduce the ease of colonization and to increase the potential for migration load. Indeed, a number of modelling studies, including ours, have found that colonization success is low for extreme environments, even if populations could survive in those environments when adapted to them (Holt & Gomulkiewicz 1997; Gomulkiewicz et al. 1999; Thibert-Plante & Hendry 2009). One way around this conundrum might be when plasticity allows organisms to better match new environments, thus facilitating colonization and future adaptation. This possibility was suggested by Baldwin (1896), and has been argued to be important in a growing number of recent publications (Price et al. 2003; Ghalambor et al. 2007; Crispo 2007, 2008).

Our results confirm these expectations in that colonization occurred more quickly when plasticity was present, particularly when migration occurred before plasticity (Fig. 3–1). This makes sense because adaptive plasticity is then expressed in the environment where selection occurs. This property improves the fitness of immigrants even without genetic adaptation and thus facilitates the formation of a resident population in the new environment (which might then adapt genetically). Another benefit of plasticity, again particularly when migration occurs before plasticity, is that it enables larger population sizes (reflective of overall adaptation) in the face of otherwise maladaptive gene flow (Fig. 3–3). The reason is that the migration load is reduced when immigrants can adjust their phenotypes toward locally-adaptive conditions. Of course, increasing costs of plasticity should reduce these two benefits (speed of colonization and population size) because individuals that are more plastic now show a fitness reduction. This was also confirmed by our simulations (not shown).

Another situation where plasticity can be beneficial for populations is in the case of *in situ* environmental change (Lande 2009; Crispo et al. 2010) but a fundamental difference remains between that situation and the colonization scenario modeled here. In particular, the benefits of plasticity should be transitory following sudden environmental change, because stabilization of the new conditions will favor genetic adaptation that should then lead to reduced plasticity (canalization) (Lande 2009). In our simulations, however, plasticity remained high following colonization because ongoing migration meant that variable environments were still present. This has also been shown in previous situations (e.g. Via & Lande

1985). Temporal and spatial variation should, however, be more similar in the case of continuous environmental fluctuations.

Natural selection against immigrants

A number of studies have argued that natural selection against immigrants will be one of the most important and effective barriers to gene flow early in the course of ecological speciation (Via 1999; Hendry 2004; Nosil et al. 2005; Thibert-Plante & Hendry 2009). One reason is that this barrier will act early in the life cycle, before many other reproductive barriers, and so those later barriers can make only incremental reductions in gene flow. Another reason is that adaptive divergence necessarily implies that individuals moving between environments will be maladapted and therefore have lower fitness than residents. In this sense, the many reciprocal transplant studies showing evidence for local adaptation (Schluter 2000; Hereford 2009) indirectly provide evidence for selection against migrants. All of this might change, however, if plasticity allows immigrants to alter their phenotypes to match local conditions.

Our simulations first confirmed that selection against immigrants is likely to be one of the most important barriers during the early stages of ecological speciation (Fig. 3–4). Note that we did not here consider the evolution of habitat preference, which might act even earlier in the life cycle and thereby be even more important (Gavrilets & Vose 2005; Gavrilets et al. 2007). We then confirmed the above idea that plasticity, at least when migration occurs before plasticity, could largely alleviate selection against immigrants. On the other hand, when migration occurs after plasticity, it can further reduce the fitness of immigrants, because

plasticity pushes the phenotypes in the wrong direction relative to the environment to which individuals will migrate. In this sense, plasticity might even be able to initiate reductions in gene flow even without any genetic evolution. It therefore seems critical for studies of ecologically-based reproductive barriers to consider the plasticity of potentially important traits and when that plasticity manifests itself relative to the timing of migration between environments.

Sexual selection against immigrants

In most of our simulations, females did not evolve a strong preference for the locally-adaptive male type, a result similar to that obtained by Gavrilets & Vose (2009). One reason might be that natural selection against immigrants is already an efficient filter (as above) when environments are dramatically different. In this case, so few migrants survive to reproduce that direct and indirect selection for mating with local males is quite weak. On average, therefore, sexual selection against immigrants was a minor contributor to ecological speciation in our model (Fig. 3–5). Other models have shown, however, that if sexual selection is strong, it can cause sexual selection against migrants and thereby contribute to ecological speciation.

An important nuance, however, is that sexual selection against immigrants was variable among replicate simulations when plasticity was absent and when migration occurred after plasticity (Fig. 3–5). This variation was the result of differences among replicates in the magic trait model only (Fig. 3–6), wherein the ecological trait was also the trait on which mate choice was based. In this case, divergence in the signaling trait occurs as a pleiotropic consequence of

divergence in the (same) ecological trait. The finding that mate choice evolves much more easily in magic traits situations is a common finding of theoretical models (Gavrilets 2005). As we surmised in the introduction, this effect was alleviated when migration occurs before plasticity, because immigrants are no longer maladaptive and have similar ecological traits to residents.

In those situations where assortative mating did evolve, it was usually positive (leading to sexual selection against immigrants) but was also sometimes negative. That is, resident females sometimes preferred immigrant males (Fig. 3–7). Similar results have been obtained by Gavrilets & Vose (2009). One reason for this seemingly counter-intuitive result is that, in absence of costs for female preference, the strength of the preference (c) and the marker trait (f) can drift if natural selection is sufficient to largely prevent hybridization (no reinforcement). In these situations, however, so few immigrant individuals survive to reproduce that the result is largely irrelevant to ecological speciation.

The above finding was modified to some extent by the plasticity scenario. When migration occurs after plasticity, the rarity of immigrants during reproduction increased the effects of drift and therefore the frequency of negative, relative to positive, assortative mating. When migration occurs before plasticity, assortative mating with respect to phenotype was higher, because plasticity increased phenotypic similarity. However, this also increased the frequency of negative assortative mating with respect to site of origin (i.e., residents versus immigrants). In short, sexual selection against immigrants was only present in magic trait scenarios when either there was no plasticity or when migration occurred after plasticity.

Selection against hybrids

Many studies have argued that natural and sexual selection against hybrids should be an important contributor to ecological speciation (Vamosi & Schluter 1999; Schluter 2000; Via et al. 2000; Rundle & Nosil 2005; Gow et al. 2007). Early in the course of ecological speciation, this selection is expected to have an ecological basis driven by the maladaptation of the phenotypically intermediate hybrids to either parental environment. Theoretical models have supported this intuition (Servedio 2004; Thibert-Plante & Hendry 2009) and studies of natural systems have confirmed its presence (Schluter 1995; Via et al. 2000; Rundle & Whitlock 2001; Rundle 2002). In analogous situations in our model (i.e., no plasticity), natural selection against hybrids was present and about half as strong as natural selection against migrants (Fig. 3–4). This was expected because additive gene action dictated that hybrids were phenotypically intermediate in ecological traits between immigrants and residents.

Plasticity, however, largely eliminated the effect of natural and sexual selection against hybrids (Fig. 3–4). This was expected in the case of migration before plasticity, because plasticity would help tailor phenotypes to the new environment. This might be less expected in the case of migration after plasticity. Here, hybrids are likely developing in the environment where natural selection occurs and so they have the same advantage of plasticity as in the scenario of migration before plasticity. Thus, plasticity reduces the natural selection pressure for hybrids regardless of the time of migration. These results highlight the critical importance of studying plasticity of adaptive traits for hybrids during the course of ecological speciation.

Neutral markers

As has been found in many other theoretical studies, the rate of migration of individuals between populations is the primary determinant of divergence in unlinked neutral markers (Fig. 3–8). Nested within this predominant influence, was an additional role for selection and plasticity. In relation to the absence of plasticity, genetic divergence was lower (gene flow higher) in the case of migration before plasticity but not in the case of migration after plasticity (Fig. 3–8). This result suggests that the role of adaptive divergence between environments in reducing gene flow, which is already weak and inconsistent (Thibert-Plante & Hendry 2009, 2010), is further weakened when immigrant individuals and hybrids can plastically adjust their phenotypes to suit local conditions. This result further brings into question the utility of using unlinked neutral markers to infer progress toward ecological speciation, especially if many loci are under divergent selection (Feder & Nosil 2010).

Perspective

We have shown how several potential ecologically-driven reproductive barriers can be influenced by phenotypic plasticity, as well as by the particulars of its expression and evolution. A remaining question is what happens when integrating across these barriers to infer the effects of plasticity on overall reproductive isolation. We first consider this effect in populations that have successfully colonized different environments. We have shown that neutral genetic markers are not a perfect guide (Thibert-Plante & Hendry 2010, and Fig. 3–8), and so one must consider the overall fitness of migrants through the proportion of migrant

genes in the second generation (Fig. 3–9, supplemental). When doing so, nearly all effects were driven by selection against migrants, because this was the earliest acting potential barrier and the one for which plasticity was most relevant. Here, when plasticity occurs after migration it can strongly reduce progress toward ecological speciation, and when plasticity occurs before migration it can modestly increase progress toward ecological speciation. These results further highlight the importance of considering plasticity in studies of ecological speciation (Crispo 2008; Svanbäck et al. 2009).

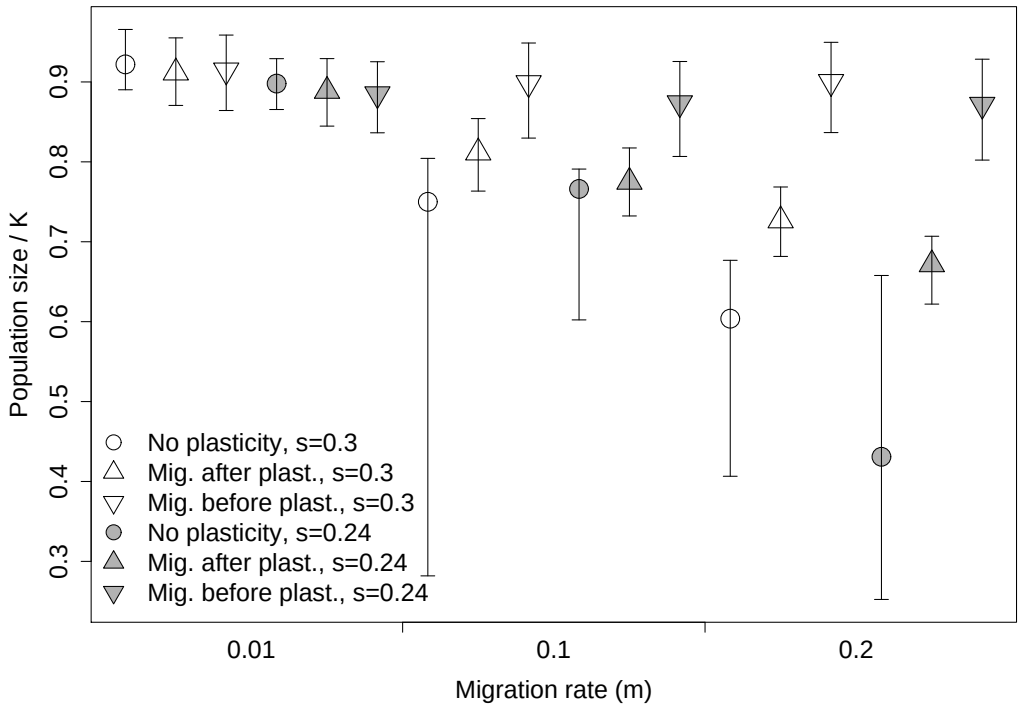


Figure 3–3: Population size over the carrying capacity (a surrogate the population fitness) in the second environment ($\theta_2 = 0.8$), as a function of migration rate, the plasticity scenario and the strength of selection after 20000 generations. The symbols represent the median of the distribution and the bars delemit the range where 68.2% of the values are located (equivalent to one standard deviation if the distribution was normal).

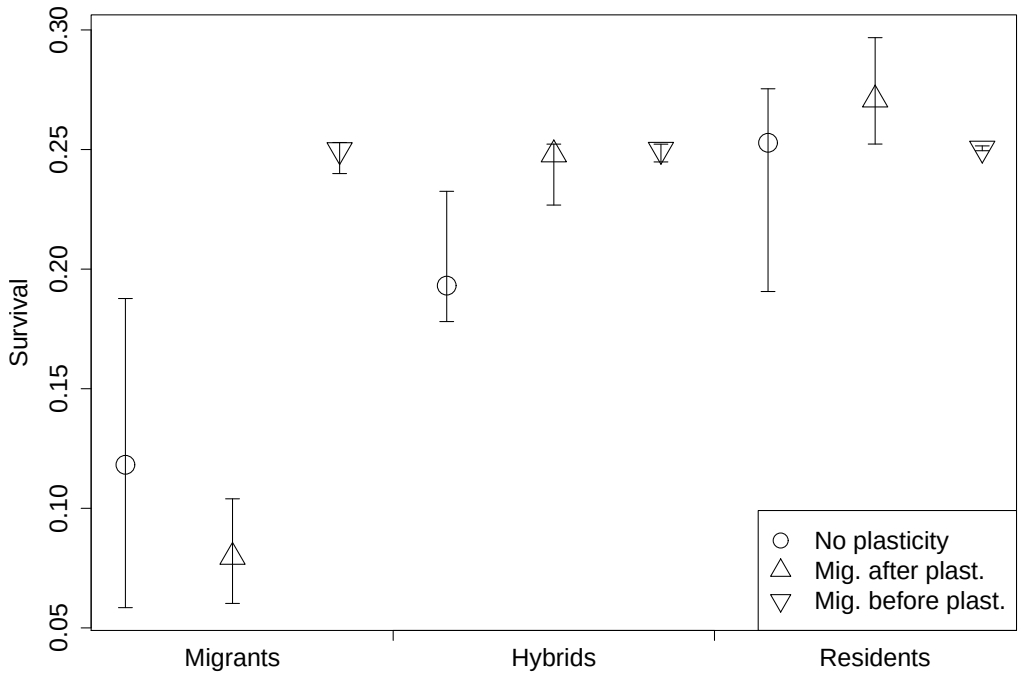


Figure 3–4: Survival probability in the second environment ($\theta_2 = 0.8$) of immigrants, hybrids and residents for different plasticity scenarios after 20000 generations. The symbols represents the median of the distribution and the bars delemit the range where 68.2% of the values are located (equivalent to one standard deviation if the distribution was normal).

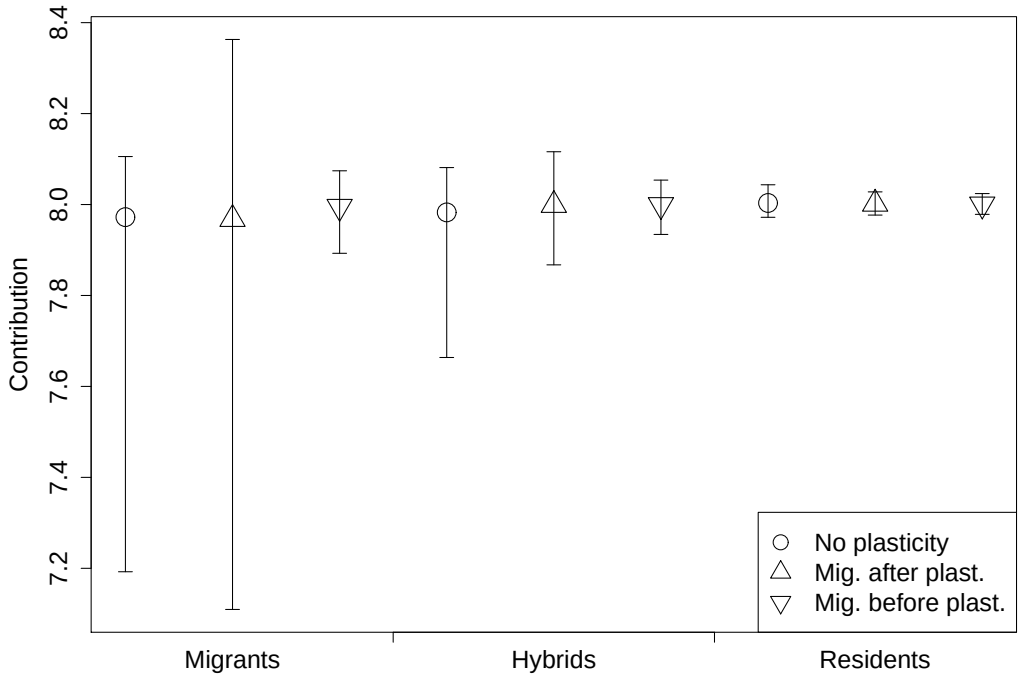


Figure 3–5: Contribution (average number of offspring per parents) in the second environment ($\theta_2 = 0.8$) of the immigrants, hybrids and residents for different plasticity scenarios after 20000 generations. Only the simulation where female preference evolved are shown. The symbols represent the median of the distribution and the bars delemit the range where 68.2% of the values are located (equivalent to one standard deviation if the distribution was normal).

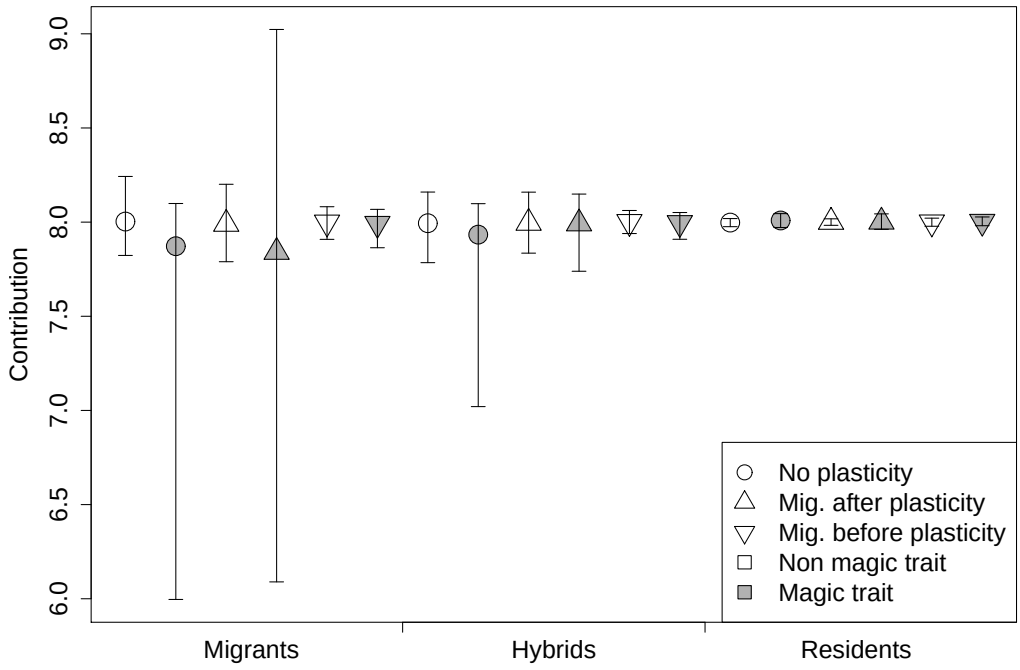


Figure 3–6: Contribution (average number of offspring per parents) in the second environment ($\theta_2 = 0.8$) of the immigrants, hybrids and residents for different plasticity scenarios after 20000 generations. Only the simulation with magic trait and where female preference evolved are shown. The symbols represent the median of the distribution and the bars delemit the range where 68.2% of the values are located (equivalent to one standard deviation if the distribution was normal).

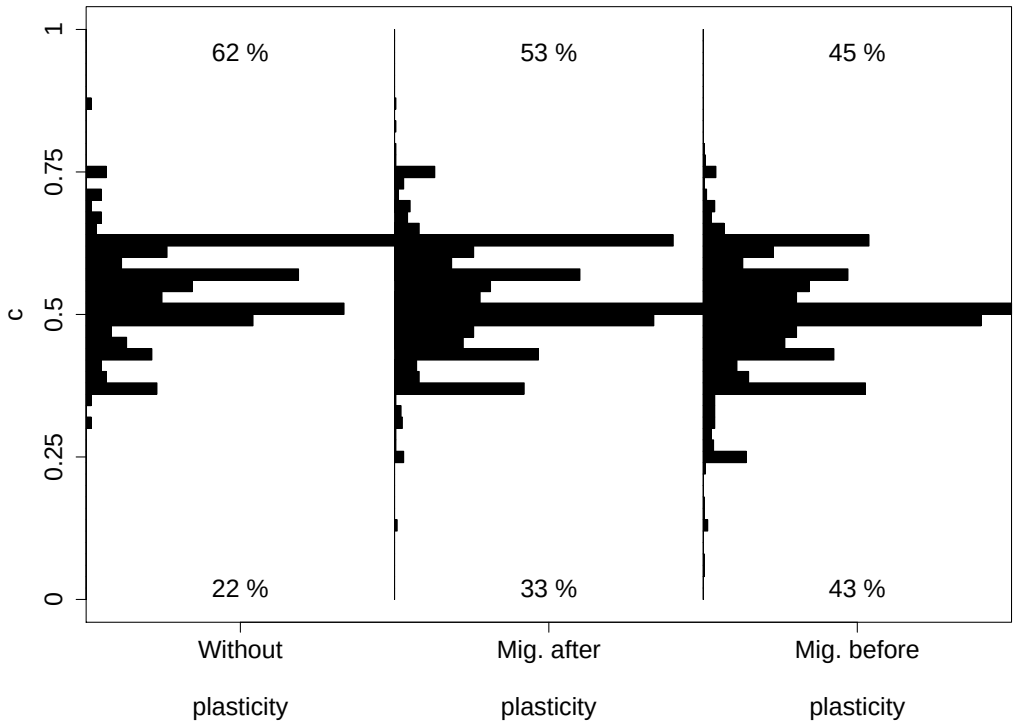


Figure 3–7: The distribution of the measure preference trait (c) after 20000 generations as a function of different plasticity scenarios for magic traits only. We filter out simulations where preference did not evolve ($\bar{c} \pm SE(c)$ overlap with 0.5). The numbers below the panels are the percent of simulations where preference evolved negative assortative ($c < 0.5$) mating and the number above the panels are the percent of simulations where preference evolved assortative mating ($c > 0.5$).

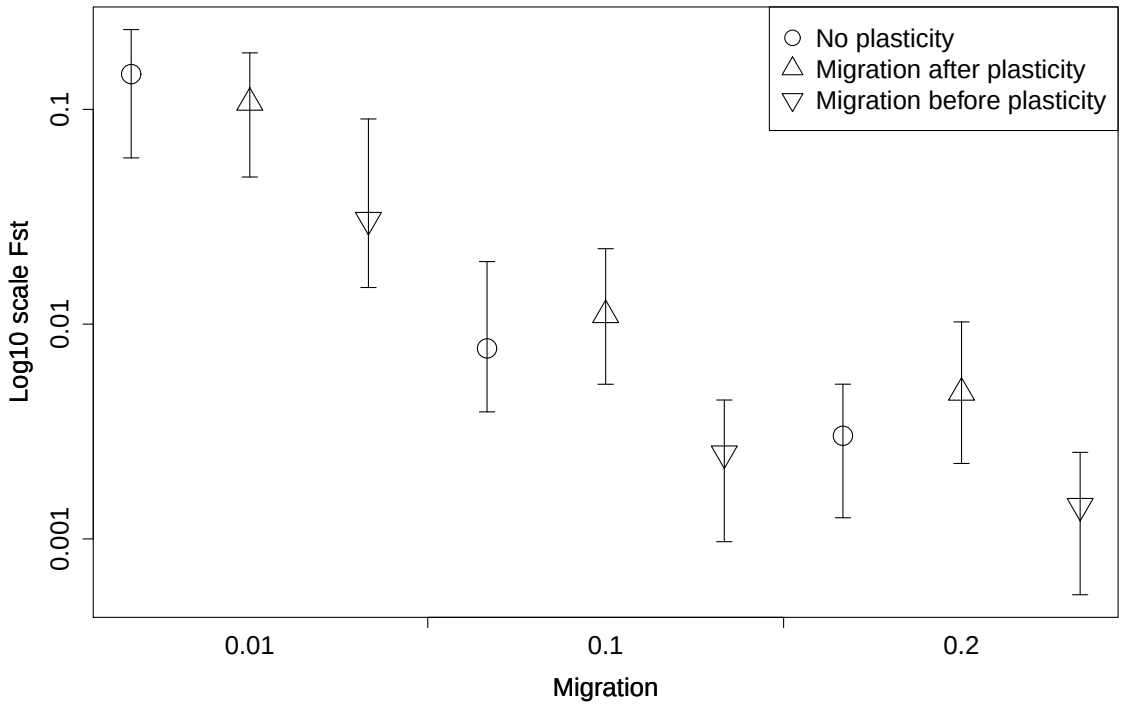
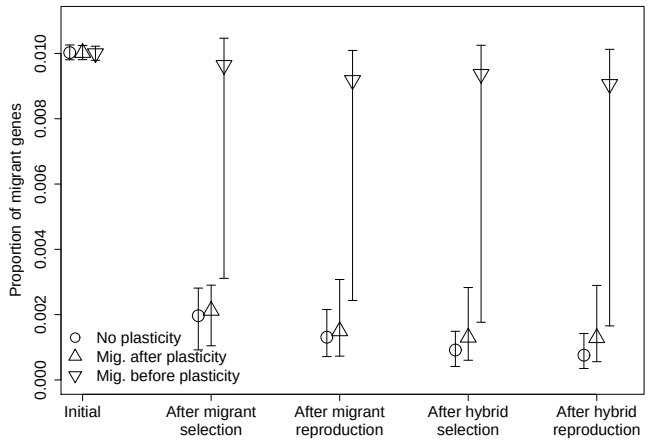
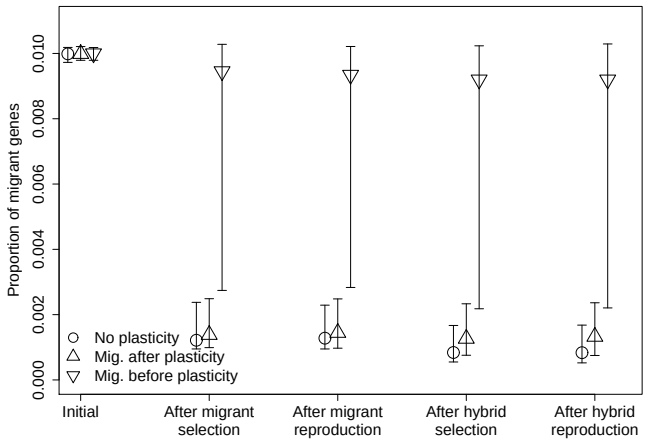


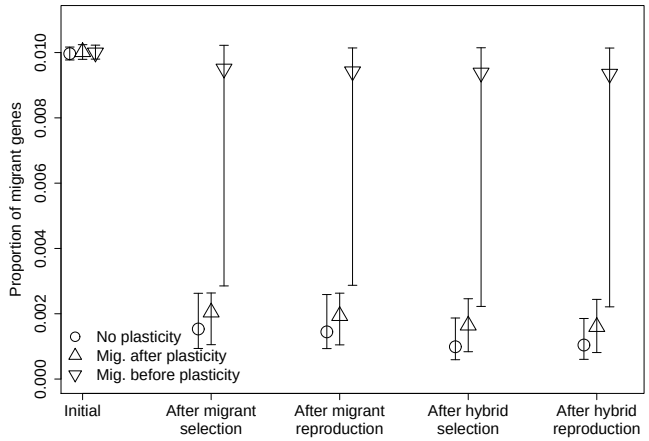
Figure 3–8: F_{ST} as a function of migration rate and plasticity scenario after 20000 generations. Logarithmic scale (base ten) is used to better visualize values across different orders of magnitude. The symbols represent the median of the distribution and the bars delemit the range where 68.2% of the values are located (equivalent to one standard deviation if the distribution was normal).



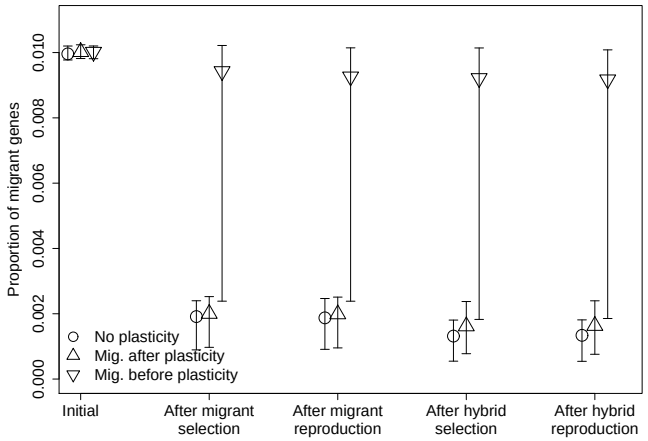
(a) Magic trait and the evolution of preference



(b) Non-magic trait and the evolution of preference



(c) Magic trait and no evolution of preference



(d) Non-magic trait and no evolution of preference

Figure 3–9: Supplemental: The proportion of migrant genes over two generations when migration is low ($m = 0.01$). The symbols represent the median of the distribution and the bars delemit the range where 68.2% of the values are located (equivalent to one standard deviation if the distribution was normal).

We next consider the overall effect of plasticity during colonization and immediately thereafter. First, plasticity allows the colonization of more diverse environments (Baldwin effect), which should therefore increase the frequency of colonization events and thereby promote ecological speciation. Second, adaptive plasticity that occurs before migration can reduce the fitness of migrants even without any genetic divergence between populations. In this case, reproductive isolation owing to divergent environments can actually commence before adaptive genetic divergence. This inverts the causal pathway assumed in most studies of ecological speciation where adaptive divergence must be present in order to cause reproductive isolation. Moreover, this plasticity can then aid adaptive genetic divergence in cases where migration rates would otherwise be too high. That is, adaptive plasticity can cause initial reductions in gene flow, which can then allow adaptive divergence, which can then further reduce gene flow. In short, phenotypic plasticity might sometimes be an important catalyst in progress toward ecological speciation.

3.6 Acknowledgments

XTP and APH were sponsored by the Natural Sciences and Engineering Research Council (NSERC) of Canada. Thanks to E. Crispo for fruitful discussion. A. Schwartz for proof reading. D. Bolnick, P. Nosil and R. Svanbäck for constructive criticism of the manuscript. Thanks to McGill University (Department of Biology) and S. Bunnell for help using the bioinformatics cluster for some simulations.

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Connecting statement

In the previous two chapters, we tracked genetic population divergence using neutral genetic markers. Large variation in the outcome was observed in both chapters. Therefore, the next chapter formally tests the use and reliability of neutral genetic markers as a tool, inferring the progress toward ecological speciation.

CHAPTER 4
When can ecological speciation be detected with neutral loci?

4.1 Abstract

It is not clear under what conditions empirical studies can reliably detect progress toward ecological speciation through the analysis of allelic variation at neutral loci. We use a simulation approach to investigate the range of parameter space under which such detection is, and is not, likely. We test for the conditions under which divergent natural selection can cause a "generalized barrier to gene flow" across the genome. Our individual-based numerical simulations focus on how population divergence at neutral loci varies in relation to recombination rate with a selected locus, divergent selection, migration rate, and population size. We specifically test whether genetic differences at neutral markers are greater between populations in *different* environments than between populations in *similar* environments. We find that this expected signature of ecological speciation can be detected under part of the parameter range, most consistently when divergent selection is strong and migration is intermediate. In contrast, the expected signature is not reliably detected when divergent selection is weak or migration is

A version of this chapter is in press at *Molecular Ecology* as Thibert-Plante, X. and Hendry, A. P. When can ecological speciation be detected with neutral loci?

low or high. These findings provide insight into the strengths and weaknesses of using neutral markers to infer ecological speciation in empirical studies.

Keywords: divergent selection, ecological speciation, F_{ST}, heterogeneous genome, individuals-based simulation, neutral gene flow, neutral marker

4.2 Introduction

Ecological speciation is a process whereby divergent selection causes the evolution of reproductive barriers between populations adapting to different environments (Schluter 2000). This process has been confirmed in a number of natural systems (Rundle & Nosil 2005), but recent work has sometimes failed to detect its presence (review: Hendry 2009). This apparent variation in progress toward ecological speciation could be the result of biological factors that promote or constrain adaptive divergence and reproductive isolation (Berner et al. 2009; Hendry 2009; Nosil et al. 2009a), or it could be the result of methodological limitations (Räsänen & Hendry 2008; Hendry 2009). We here use numerical simulations to consider methodological limitations that might attend one common method for inferring progress toward ecological speciation. Our main motivation is to provide information relevant to empiricists studying ecological speciation.

We specifically evaluate the use of neutral genetic markers to test the prediction that gene flow is lower between populations in *different* environments than between populations in *similar* environments (e.g., Smith et al. 1997; Gíslason et al. 1999; Lu & Bernatchez 1999; Ogden & Thorpe 2002; Crispo et al. 2006; Nosil et al. 2008; Berner et al. 2009). This prediction has its origin in expectations that stronger ecological reproductive barriers should reduce gene flow between

environments and thereby allow greater genetic difference even at neutral markers (Barton & Bengtsson 1986). This basis for inference has, however, been brought into question by the realization that alleles at neutral markers unlinked to selected loci might flow almost freely between populations even when those populations are adapted to different environments (Emelianov et al. 2004; Gavrilets & Vose 2005; Thibert-Plante & Hendry 2009). And yet, ambiguity remains because a generalized barrier to gene flow might be possible (Gavrilets 2004; Grahame et al. 2006; Nosil et al. 2008, 2009a) if selection acts against the whole genome of migrants and hybrids. That is, linkage disequilibrium before recombination between genomes of individuals from different populations will potentially reduce gene flow even at unlinked neutral markers.

We must here make a distinction between our analysis and another recent approach to studying ecological speciation. The other approach involves the use of genome scans to identify outlier loci associated with ecological differences between populations (Emelianov et al. 2004; Grahame et al. 2006; Via & West 2008; Via 2009; Nosil et al. 2009a; Feder & Nosil 2010). This method is particularly useful for identifying regions of the genome that show low gene flow between populations in different environments, and are therefore under divergent selection. The method we address here is different and complementary. That is, we ask whether a generalized barrier to gene flow can be detected at non-outlier loci that are presumably "far" from the selected loci: i.e. neutral loci not physically linked to loci under divergent selection. Insight into divergence at these loci will help

(1) the interpretation of non-outlier loci in genome scans, and (2) inferences from classic population genetic studies that focus only on such loci.

Our specific goal is to evaluate the conditions under which ecological differences between the environment of different populations can cause detectable divergence at neutral loci. We examine the influence of several potentially important parameters, including migration rate, the strength of divergent selection, recombination rate, and population size. First, we expect that intermediate migration rates will allow the greatest increase in neutral genetic divergence between populations in different environments as compared to populations in similar environments (henceforth, the "expected signature" of ecological speciation). The reason for this prediction is that, under very low migration rates, all populations will diverge approximately equally owing to drift (because gene flow is too low to constrain divergence in any case), whereas under very high migration rates, divergent selection will not be effective at reducing divergence at neutral markers (because gene flow can remain high even after any selection against migrants and hybrids). Second, greater divergent selection will cause greater ecologically-based reproductive barriers (Schluter 2000; Thibert-Plante & Hendry 2009; Nosil et al. 2008, 2009a,b), and so we expect it will also allow greater divergence at neutral loci. Third, we expect that lower recombination (e.g., closer physical linkage) between neutral and selected loci will cause greater divergence, through the process of genetic hitchhiking (Maynard Smith & Haigh 1974; Charlesworth et al. 1997). Related to this, we also expect that selection needs to be much stronger than recombination to reduce gene flow (Spirito et al. 1983; Bengtsson 1985). Fourth,

we expect that larger population sizes will more reliably generate the expected signature, because selection tends to overwhelm drift in larger populations (Whitlock & Phillips 2000).

The above theoretical expectations have not been thoroughly and systematically evaluated in the context of ecological speciation. Previously, Thibert-Plante & Hendry (2009) used a simulation model to consider these issues, but the present analysis is much more comprehensive and more closely tied to empirical situations (e.g., the neutral markers used here are similar to microsatellites). We also more comprehensively explore the parameter ranges under which the expected signature can be most reliably detected using common statistical methods. This latter question is important because, even when a generalized barrier exists, it might be so weak and variable as to elude detection in a typical empirical study.

4.3 Model

We simulate diploid individuals in a multi-locus, multi-allele model, in which one locus is under selection and the other 40 are neutral. The locus under selection has only two possible alleles, and the selectively-favored allele differs between the two environments. Four populations exchanging migrants are modeled, with two populations per environment type (Fig. 4–1 and Table 4–1). Other types of population structure could have been modeled but this is the simplest for examining patterns across a large range of parameter space. A single selected locus with only two alleles was chosen for simplicity; future work might profitably examine the effects of distributing selection across multiple loci (Feder & Nosil 2010).

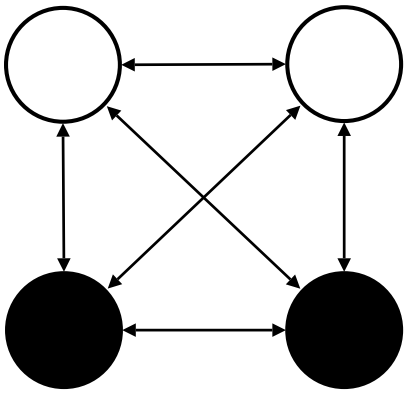


Figure 4–1: Population structure in our simulations: four different populations exchanging migrants (arrows) in similar and different environments (colors).

Selected locus	Fitness in environment 1	Fitness in environment 2
aa	1	$1 - s$
aA or Aa	$1 - \sigma$	$1 - \sigma$
AA	$1 - s$	1

Table 4–1: Absolute fitness of each genotype possible in the two different environments, where $0 \leq \sigma \leq s \leq 1$.

Life cycle

The simulated life cycle begins with migration and then proceeds to reproduction. Selection occurs during reproduction and is manifest as differences in reproductive output according to the relative fitness of individuals (see below). Explicit viability selection is thus absent from our model, but the lack of reproduction by an individual is evolutionarily equivalent to its death. Migration occurs evenly across all populations (the same number of individuals migrating from each population is distributed among the other populations), a given individual can move only once during the life cycle, and the individuals that move from each population are chosen randomly.

Mating proceeds by randomly selecting two individuals from a population with probabilities according to their relative fitness within that population. Fitness is dependent on the interaction between an individual's genotype and its environment ($G \times E$), according to the rules stated in Table 4–1. The probability of an individual i being selected for reproduction at any time is

$$p_i = \frac{f_i}{\sum_j f_j} \tag{4.1}$$

where f_i is the fitness of individual i and $\sum_j f_j$ is the sum of the fitnesses of all individuals in the population. A selected pair produces one offspring and then returns to the potential pool of parents. This procedure is repeated until the offspring population reaches the size of the parental population. Reproduction then stops and the life cycle starts over again with migration. Generations are thus non-overlapping.

Neutral genes

The neutral loci act like microsatellites: i.e., high mutation rates (Weber & Wong 1993) (on the order of 10^{-3}) that change the number of repeats in a sequence (Di Rienzo et al. 1994; Valdes et al. 1993). Alleles are identified by their number of repeats. The number of repeats is limited to be between one and 999 which is larger than the number of alleles that our population should carry at equilibrium (Kimura & Ohta 1975). Mutations are stepwise and consist of an increase, or decrease, of one in the number of repeats (Kimura & Ohta 1975). Stepwise mutations at the boundaries (one and 999) always produce the only permitted adjacent value (two or 998, respectively), but are unlikely to ever be

reached. We did not add a complex parameterizable distribution for multi-step mutation to reduce the number of parameters in the model, but we ran simulations with multi-step mutations (Di Rienzo et al. 1994) and it did not change the conclusions (results not shown).

We vary the recombination rate of neutral loci with the selected locus. The recombination rate is a parameter that represents the probability that recombination occurs between the selected and neutral loci, in any reproduction event. A recombination rate of zero implies that the neutral locus and the selected locus will never be separated by recombination. For a recombination rate of 0.5, the loci are unlinked and can be considered to be on different chromosomes. For each neutral locus, the rate of recombination with the selected locus is independent of the other neutral loci. This unintuitive pattern is achieve by having a single recombination point between the selected and neutral locus. We then control the recombination rate of each of the locus independently, i.e. the recombination rate between two loci with the same recombination rate with the selected locus is 0.5. Genetic measures and statistical tests (see below) are performed after reproduction but before migration.

Simulation set up

At the start of a simulation, two random alleles are allocated to each individual at the selected locus. This leads to Hardy-Weinberg equilibrium with allele frequencies of 0.5 at the selected locus in each population. The strength of natural selection on a locus is set at s, the proportional decrease in fitness for the disfavored allele (relative to unity for the favored allele) in each environment. Allelic

effects are additive, such that heterozygotes have intermediate fitness ($1 - \sigma$, where $\sigma = \frac{s}{2}$) (Table 4–1). In each life cycle iteration thereafter, migration between the populations occurs as a fixed proportion m of the population. That is, each population contributes (and receives) on average Nm individuals split equally among (and from) the other populations. Population size (N) is the same in each population and is maintained at a constant level (as described above). We use a binomial random number generator to get Nm at each generation (Kachitvichyanukul & Schmeiser 1988). With this technique, we achieve a non null number of migrant on average, even at low migration rate while respecting the average Nm number of migrant.

Simulations are run for 5000 iterations (i.e., generations) and F_{ST}, according to Weir (1996), is tracked for each locus over the entire simulation. We used 10 replicate simulations for each parameter set: strength of selection ($s = 0$ to 1.0, in increments of 0.1), migration rate ($m = \{0.5, 0.3, 0.2, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}\}$), recombination rate ($r = \{0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5\}$), and population size ($N = \{100, 1000\}$). All the above parameter combinations are explored and are independent, except for the different recombination rates. Here different neutral loci are linked to the same selected locus but with different recombination rates. Initially, the 400 loci (ten different recombination rates with 40 loci each) of each individual are set at 500 number of repeats, the middle point in the possible number of repeat. Each of the 40 loci have exactly the same probability of recombination. The mutation rate of the locus under selection is 10^{-5}, while for neutral loci it is 10^{-3}. These mutation rates are the same as those used in other numerical

studies (Gavrilets & Vose 2005, 2007; Gavrilets et al. 2007) and are similar to some estimates from real organisms (Dallas 1992; Brinkmann et al. 1998). We chose per patch population sizes of 100 and 1000 to bracket those used in related numerical simulations (Gavrilets & Vose 2005, 2009).

We also ran the same analysis, but instead of using the entire population, we randomly sampled 20 individuals in each population. Theoreticians might want to know if a particular method has adequate statistical power, but empiricists might be interested in knowing if a particular level of power can be achieved via subsampling populations. We thus used 20 individuals per population so that our results can be compared to other theoretical studies, (e.g., Balloux & Goudet 2002).

Statistical tests

Many statistical analyses of genetic differences are computationally intensive, such as bootstrapping F_{ST} 10000 times to get confidence limits or implementing Bayesian analyses in STRUCTURE 2.2 (Pritchard et al. 2000). We estimated that applying such analyses to all of our simulations would take more than a century of computation on a 2GHz computer. We therefore implemented several different short-cuts.

Our first short-cut was simply to use only 100 bootstrap replicates for comparisons of F_{ST}. Specifically, we calculate F_{ST} 100 times based on independent random samples with replacement of individuals (N = population size) from each population. We then conclude that F_{ST} is larger among populations in similar environments than among populations in different environments if the 95%

confidence intervals do not overlap (one-tailed test). Given our four populations, this generated six comparisons: two for populations in similar environments and four for populations in different environments. We treated these as independent estimates to calculate the proportion of times, in each replicate simulation, where comparisons of populations in different environments yielded a higher F_{ST} than comparisons of populations in similar environments.

We also evaluated whether our shortcut of using 100 replicate F_{ST} bootstraps (rather than the more typical 10000 replicates) caused any bias in interpretation. For this, we compare confidence intervals from our 100 bootstraps to confidence intervals from 10000 bootstraps in three separate simulations. The three simulations are chosen for their different range of F_{ST}, and their $(selection, migration)$ parameters are: $(0.1, 10^{-3})$, $(0.5, 10^{-3})$ and $(0.5, 10^{-6})$. These comparisons are made during 50 consecutive generations after the first generation, after 1000 generations and after 4950 generations. The results of these different iterations and simulations are combined for analysis.

Our second short-cut was to use STRUCTURE (Pritchard et al. 2000) for only a subset of the simulations. For this, we use the ad hoc criteria of Evanno et al. (2005) to test for how many discrete populations were present in a given simulation. Specifically, we use the admixture model with the degree of admixture (alpha) inferred from the data and with the option of correlated allele frequencies between populations. The distribution of allele frequencies (lambda) is set to one and the length of burn-in is 10000. Twenty replicates are run for each data set and the number of populations evaluated are from one to ten. For this analysis, we

use the same simulations as those for the above bootstrap test. A clear signature of ecological speciation in STRUCTURE implies finding two populations with individuals within a population from a single environment. Failure of one of these two conditions implies a failure to detect ecological speciation.

4.4 Results

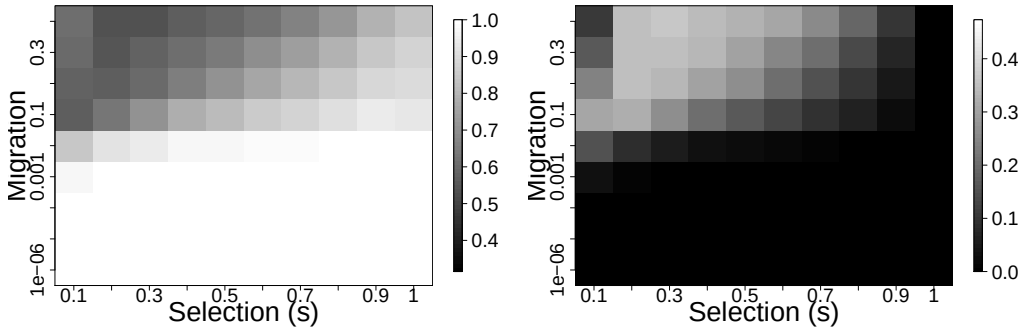
To illustrate general patterns seen at the end of our simulations ($4900 - 5000$ generations), we will focus first on results for larger populations: 1000 individuals in each of the four populations. We later discuss any differences seen in the simulations with 100 individuals per population.

The selected locus

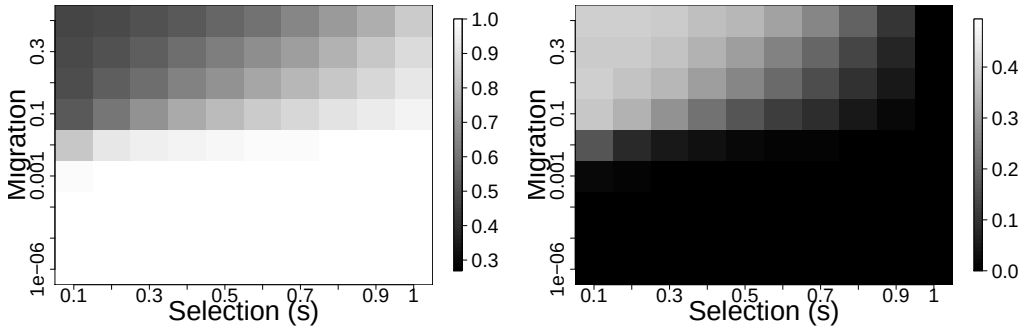
Dynamics at the selected locus are straightforward, showing a migration-selection balance when migration is low (Fig. 4–2). At low selection and high migration, fixation of the positively selected allele is rare and the negatively selected allele can sometimes fix (Fig. 4–2(a)). As selection increases and migration decreases, fixation of the positively selected allele becomes more common, and this essentially always occurs at very low migration rates. When fixation of the positively selected allele occurs, the fitness reduction in the new environment will be s for migrants and σ for hybrids.

Divergence at neutral markers

At low migration rates ($m = 10^{-4}$), genetic divergence is strong ($F_{ST} \approx 0.22$) between all pairs of populations regardless of whether they are in similar or different environments (Fig. 4–3). In fact, this level of divergence is essentially the expected equilibrium ($F_{ST} = 0.18$) from Rousset (1996) (Eq. 6 therein). In this



(a) Homozygote, Population size = 100 (b) Heterozygote, Population size = 100



(c) Homozygote, Population size = 1000 (d) Heterozygote, Population size = 1000

Figure 4–2: Average frequency of individuals who are homozygote for the positively selected locus (a, c) and the average frequency of individuals who are heterozygosity at the selected locus (b, d) averaged for the last iteration averaged across all simulations.

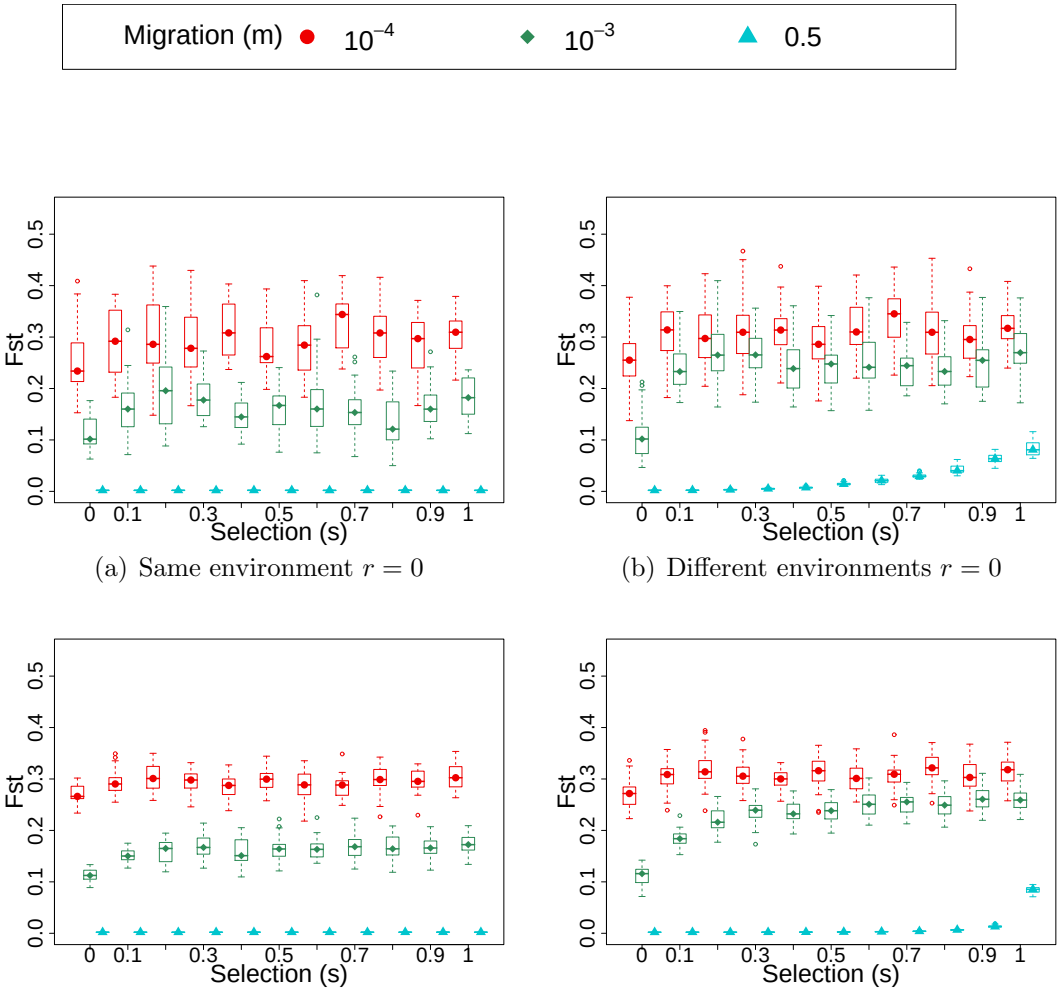
situation of near-complete divergence in all cases, the strength of selection has no apparent influence, but an increase in recombination rate decreases the variation in F_{ST}.

At a higher (here considered "intermediate") migration rate (e.g., $m = 10^{-3}$), genetic divergence decreases and other parameters become influential. (Note that for a population size of $N = 1000$ a value of $m = 10^{-3}$ corresponds to $Nm = 1$). For instance, populations in different environments (Fig. 4–3 b, d, f, h) here show higher neutral genetic divergence than do populations in similar environments (Fig. 4–3 a, c, e and g). This is true across all non-null selection levels when recombination with the selected loci is absent (Fig. 4–3 a,b) and for stronger levels of selection when recombination is present (Fig. 4–3 c-h).

At the highest migration rate ($m = 0.5$), neutral genetic divergence is present in only two instances. The first occurs when recombination with the selected locus is absent and selection is moderately strong (Fig. 4–3 a,b). The second occurs when recombination is present and selection is exceptionally strong (right-hand side of Fig. 4–3 c-h).

Statistical tests for signatures of ecological speciation

We now consider situations in which the above patterns might be statistically distinguishable in a typical empirical study. We will here use the phrase "positive results" when the expected signature of ecological speciation is detected: greater genetic differences between populations in different environments than between populations in similar environments. In all cases, the proportion of comparisons



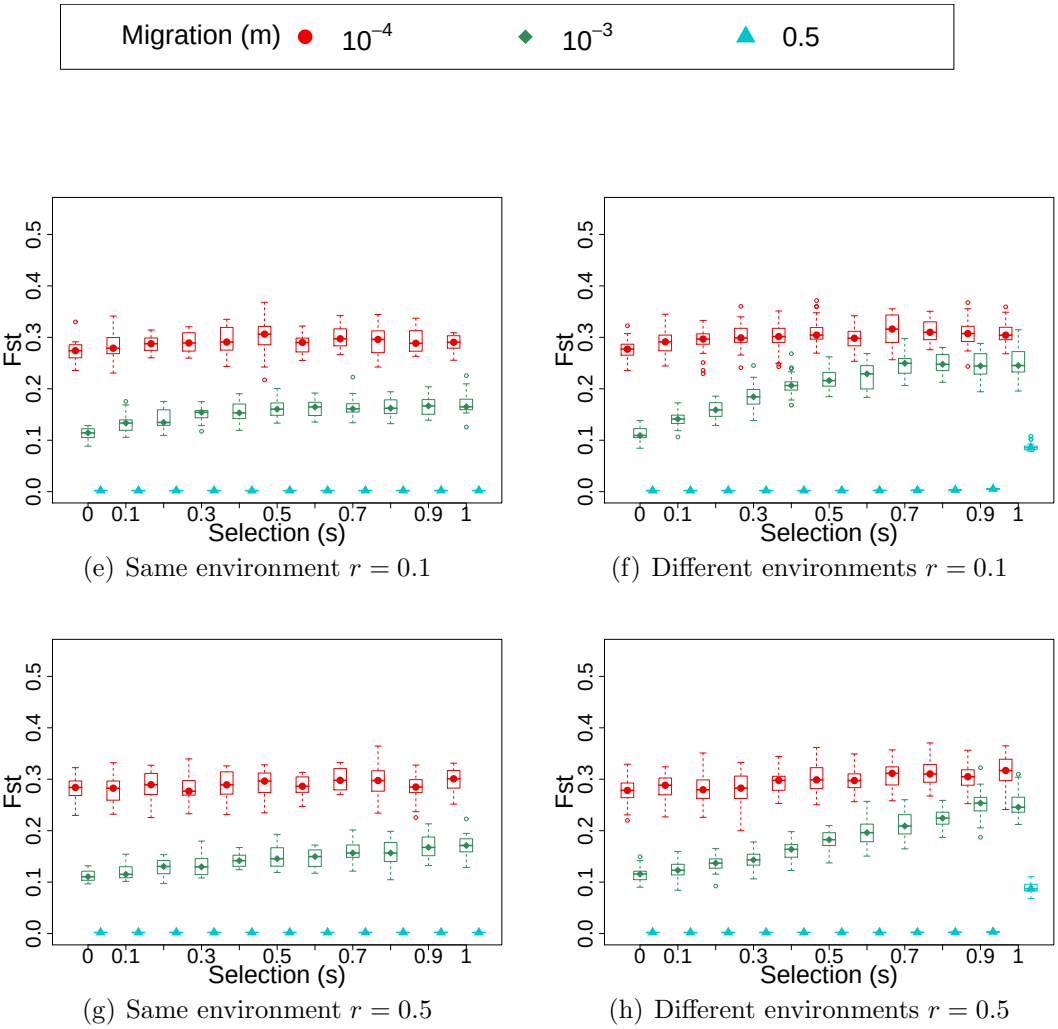
(a) Same environment $r = 0$

(b) Different environments $r = 0$

(c) Same environment $r = 0.02$

(d) Different environments $r = 0.02$

Figure 4–3: (part 1 of 2)



(e) Same environment $r = 0.1$

(f) Different environments $r = 0.1$

(g) Same environment $r = 0.5$

(h) Different environments $r = 0.5$

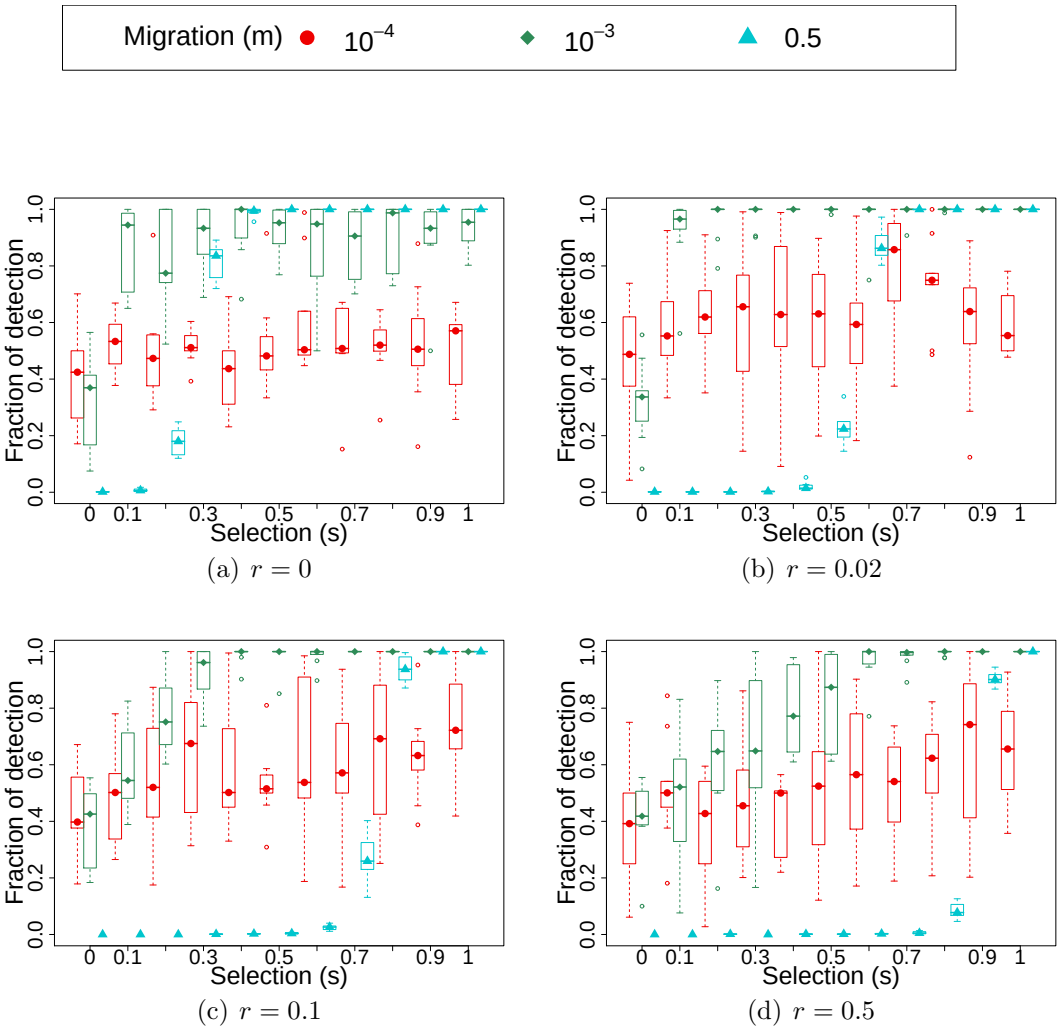
Figure 4–3: (part 2 of 2) Qualitative comparison of F_{ST} between populations in similar environments (left panels) and different environments (right panels). These results are for the last iteration of each simulation with the larger population size (1000). The box is bounded by the first and third quartile, the line inside the box is the second quartile (median), and the whiskers extend to 1.5 times the interquartile range (third quartile minus first quartile) or to the maximum or maximum value, as appropriate. All data outside the whisker range are considered outliers and are represented by open circles.

for a given parameter set that yield a positive result is roughly equivalent to the expected statistical power of the test.

At low migration rates ($m = 10^{-4}$), positive results are obtained approximately half of the time (Fig. 4–4). Positive results become more common when recombination rates are not zero and when selection is reasonably strong. This result is not, however, a reliable cue for ecological speciation. It instead simply reflects random divergence among populations that sometimes by chance leads to the expected signature.

In presence of natural selection ($s > 0$) and intermediate migration rate (e.g., $m = 10^{-3}$), positive results are nearly always obtained when recombination is absent or low (Fig. 4–4(a)). When recombination rates are high, positive results are nearly always obtained when selection is also high (Fig. 4–4(d)). When recombination rates are high and selection is weak to moderate, positive results are highly variable within a given parameter set. Subsampling has virtually no effect when migration rates are low or intermediate (Fig. 4–5).

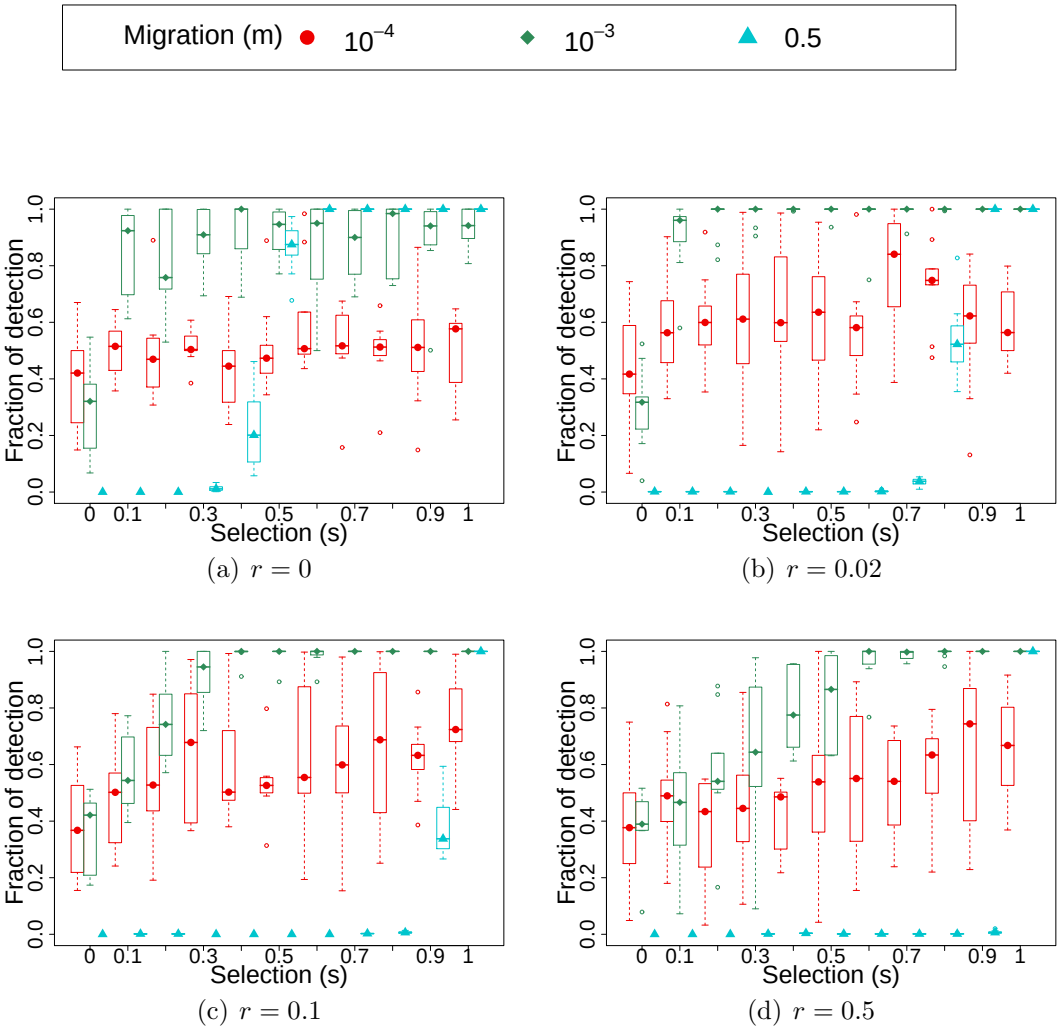
At the highest migration rate ($m = 0.5$), a sharp transition in the frequency of positive results is seen, changing from no positive results at low selection to all positive results at high selection. The location of this transition is influenced by recombination rate, with a lower level of selection required to obtain positive results when recombination rates are lower (Fig. 4–4). The transition point is pushed to stronger strength of selection when the populations are subsampled (Fig. 4–5).



(a) $r = 0$ (b) $r = 0.02$

(c) $r = 0.1$ (d) $r = 0.5$

Figure 4–4: Results of statistical analyses (100 bootstrap comparisons of 95% confidence intervals) for when F_{ST} between populations in different environments is greater than that between populations in similar environments. These results are cumulative for the last 100 iterations of each simulation with the larger population size (1000). See figure 4–3 caption for explanation of boxplot conventions.



(a) $r = 0$ (b) $r = 0.02$

(c) $r = 0.1$ (d) $r = 0.5$

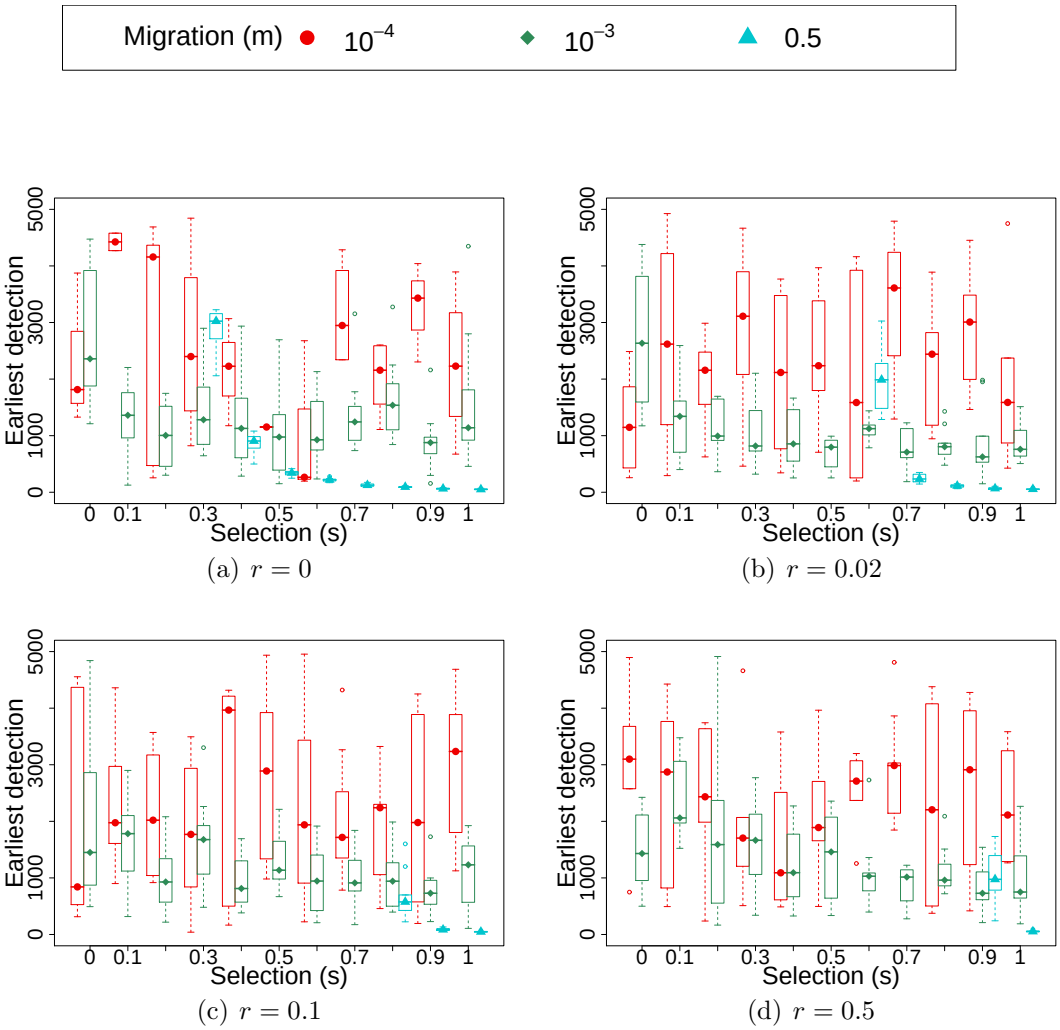
Figure 4–5: Results of statistical analyses (100 bootstrap comparisons of 95% confidence intervals) for when F_{ST} between populations in different environments is greater than that between populations in similar environments. These results are cumulative for the last 100 iterations of each simulation with 20 individuals sampled in each population of size (1000). See figure 4–3 caption for explanation of boxplot conventions.

Earliest divergence

All of the above results are those recorded after 4900 generations, at which time an equilibrium is nearly always evident in our simulations. Many empiricists, however, may be dealing with non-equilibrium conditions. We therefore here briefly consider the time frame at which the expected signature of ecological speciation can be consistently detected following colonization of new environments. We specifically recorded, for a given simulation, the first time (number of generations) at which positive results are obtained for ten successive generations. For intermediate migration rates (the situation where detection was most reliable at equilibrium; see above), consistent positive results are usually obtained in a few hundred to a thousand generations. Consistent detection occurs earliest when selection is strongest (Fig. 4–6). Note also that detection is "sometimes" possible after only a few generations; it just is not always consistent over ten generations. For example, when gene flow is high ($m = 0.1$), selection is high ($s = 0.8$), and recombination is free ($r = 0.5$), 30% of the tests yield a positive results after only 15 generations, a similar time frame to the empirical study of Hendry et al. (2000).

Population size

We now consider how the above results hold, or are modified, when population sizes are small (100) instead of large (1000). The main change here is a general increase in F_{ST} (Fig. 4–7 in supplemental) over the simulations described above. Apart from this numerical difference, trends with respect to the other parameter values are generally not altered, either qualitatively (Fig. 4–7 in supplemental) or statistically (Fig. 4–8 in supplemental). However, when positive results are



(a) $r = 0$ (b) $r = 0.02$

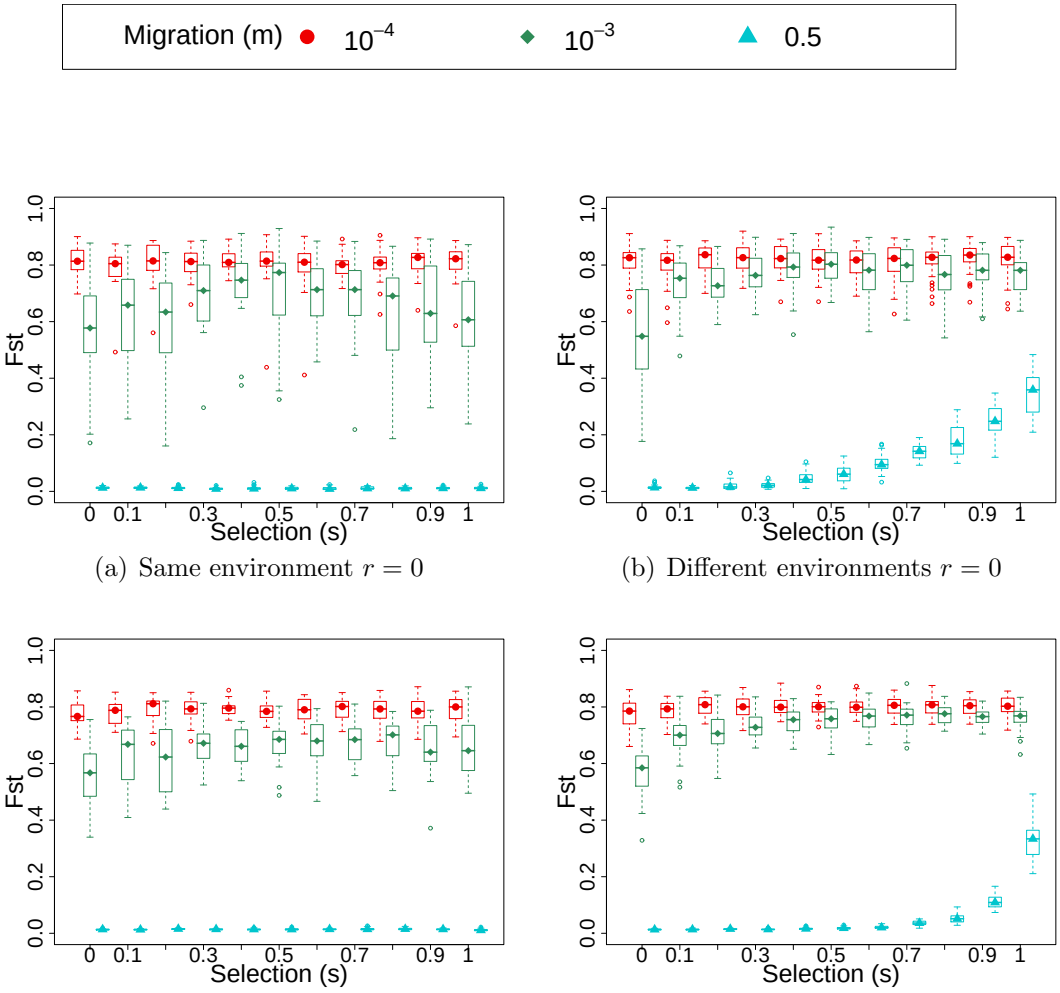
(c) $r = 0.1$ (d) $r = 0.5$

Figure 4–6: Time of the first consistent statistical detection of the expected signature of ecological speciation. These results show the first generation after colonization at which consistent detection (i.e., for at least ten consecutive generations) was achieved in simulations with the larger population size (1000). See figure 4–3 caption for explanation of boxplot conventions.

rare for large populations, they are even rarer for smaller populations. In short, it can sometimes be more difficult to detect the expected signature of ecological speciation when populations are small than when they are large.

Evaluating our statistical shortcuts

In one-tailed comparisons of the lower F_{ST} confidence limit for populations in different environments to the upper F_{ST} confidence limit for populations in similar environments, it makes little difference whether bootstrapping is based on either 100 or 10000 replicates (Fig. 4–9 in supplemental). Specifically, conclusions regarding whether populations in different environments show greater genetic difference than populations in similar environments are equivalent in 98.84% of the comparisons tested. Of those 1.16% differences, 0.89% represented false positives for the expected signature when using 100 replicates and 0.27% represented false negatives under those conditions. Also, there is no trend of those false positives and false negatives with migration rate, strength of selection, number of generations, or recombination rate. In short, our bootstrap shortcut was of no consequence to our conclusions.



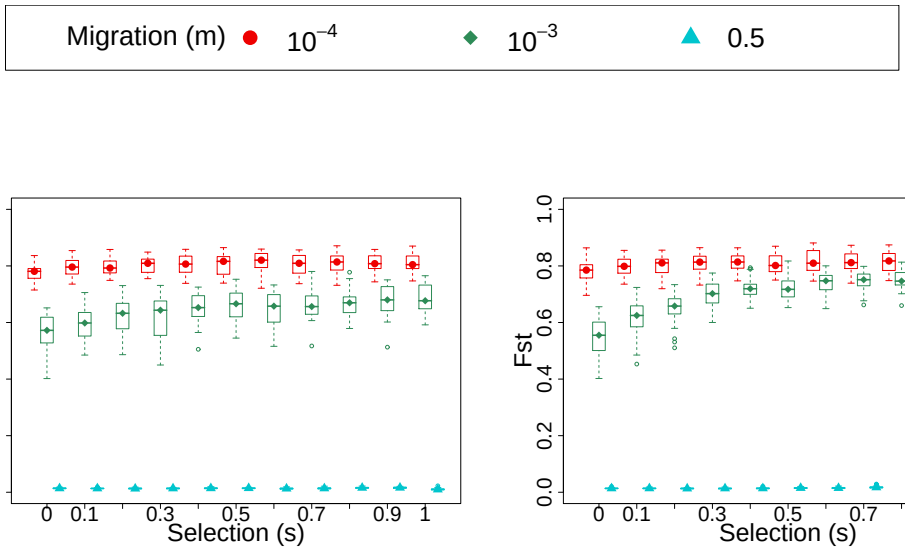
(a) Same environment $r = 0$

(b) Different environments $r = 0$

(c) Same environment $r = 0.02$

(d) Different environments $r = 0.02$

Figure 4–7: Supplemental (part 1 of 2)



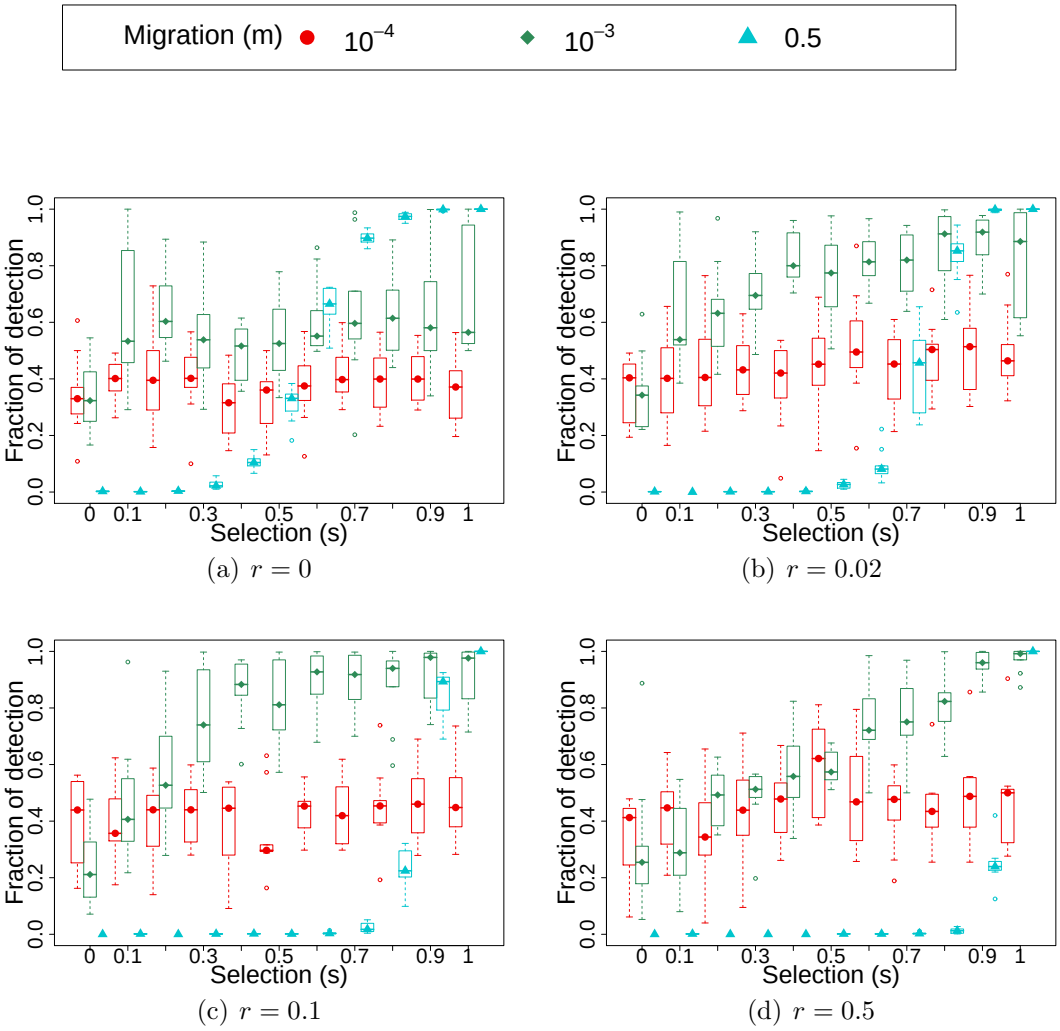
(e) Same environment $r = 0.1$

(f) Different environments $r = 0.1$

(g) Same environment $r = 0.5$

(h) Different environments $r = 0.5$

Figure 4–7: (Supplemental) (part 2 of 2) Qualitative comparison of F_{ST} between populations in similar environments (left panels) and different environments (right panels). These results are for the last iteration of each simulation with the smaller population size (100). See figure 4–3 caption for explanation of boxplot conventions.



(a) $r = 0$ (b) $r = 0.02$

(c) $r = 0.1$ (d) $r = 0.5$

Figure 4–8: (Supplemental) Results of statistical analyses (100 bootstrap comparisons of 95% confidence intervals) for when F_{ST} between populations in different environments is greater than that between populations in similar environments. These results are cumulative for the last 100 iterations of each simulation with the smaller population size (100). See figure 4–3 caption for explanation of boxplot conventions.

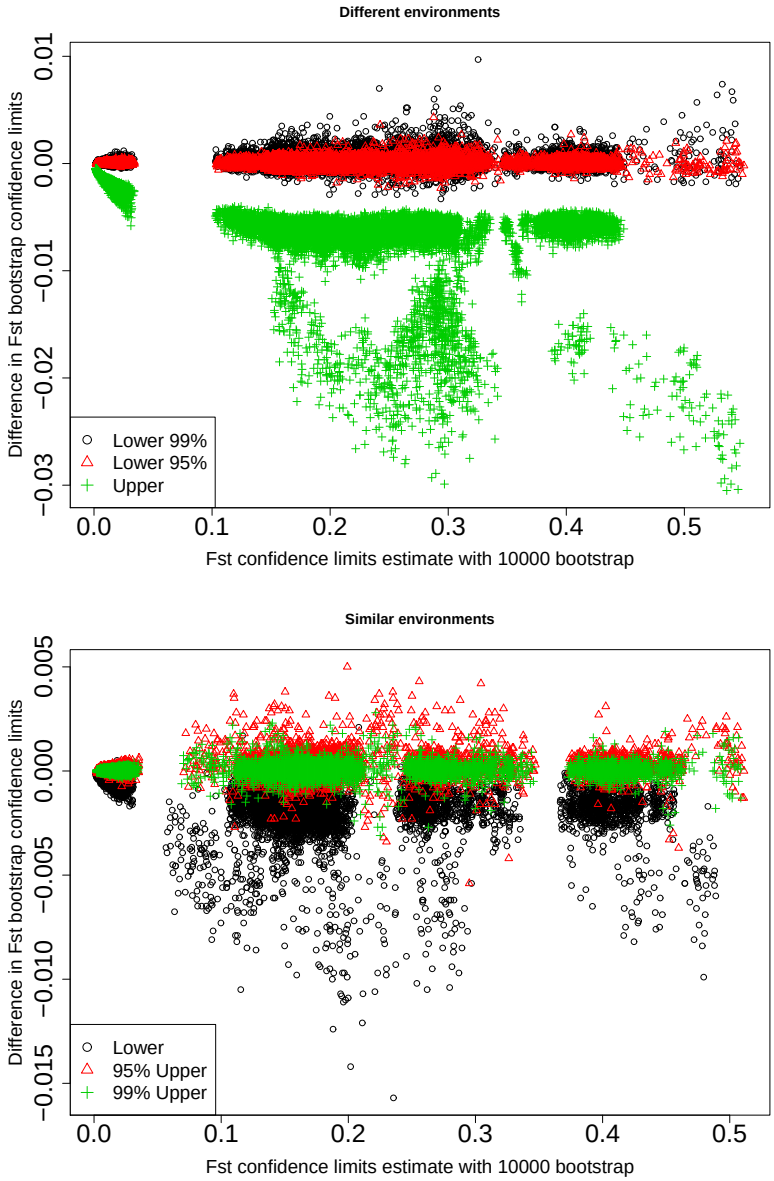


Figure 4–9: (Supplemental) Difference between confidence limits obtained with 100 and 10000 bootstraps (larger minus smaller bootstraps, y-axis) relative to the estimated F_{ST} with 10000 bootstraps (x-axis) for populations in different (top panel) or similar (bottom panel) environments. The key observation is the two bootstrap levels show consistent upper confidence limits for similar environment and consistent lower confidence limits for different environments.

Selection (s)		0.1		0.5		0.8	
Migration (m)		10^{-3}		10^{-6}		10^{-3}	
Iteration	Recombination	Structure	Bootstrap	Structure	Bootstrap	Structure	Bootstrap
	0	2*	25	6	0	2*	50
100	0.08	4	25	4	62.5	4	0.5
	0.5	4	12.5	4	12.5	4	62.5
	0	2	50	4	37.5	2	100
3000	0.08	4	37.5	4	37.5	2	100
	0.5	4	87.5	3	25	2	100
	0	2	100	4	50	2	100
5000	0.08	2*	50	4	50	2	100
	0.5	4	37.5	4	0	2	100

Table 4–2: Comparison between STRUCTURE and the 100 bootstrap F_{ST} comparison method. STRUCTURE gives the number of populations and the bootstrap method gives the percent of comparisons when F_{ST} is greater for populations in different environments than for populations in similar environments. When STRUCTURE finds only two populations, and these are in different environments, then it has detected the expected signature of ecological speciation. A star ($*$) indicates when STRUCTURE found two populations but did not group them by environment type.

For the subset of analyses with STRUCTURE, we find very similar results to those obtained using the above bootstrap F_{ST} comparisons (Table 4–2). That is, when the F_{ST} bootstrap method consistently found positive results, STRUCTURE always found two populations corresponding to the two environments. When the F_{ST} bootstrap method did not find consistent results, neither did STRUCTURE. In these latter cases, STRUCTURE sometimes grouped populations from different environments and sometimes split populations from similar environments. Only one of the 27 test in STRUCTURE identified the signature of ecological speciation when F_{ST} comparisons did not.

4.5 Discussion

Does divergent selection cause a detectable generalized barrier to gene flow? That is, can we use neutral genetic markers to reliably detect ecological speciation? The short answer is that it depends on a variety of parameters, which is the same general conclusion obtained by Thibert-Plante & Hendry (2009). The quantitative results of these two studies should not, however, be directly compared because the two models are very different. Results of the present model are much more appropriate and comprehensive when considering implications for empirical studies. In the following discussion, remember that the "expected signature" of ecological speciation is a reduction in gene flow between populations in different environments relative to that between populations in similar environments.

One important parameter determining detection success (i.e. statistical confirmation at the expected signature) is migration rate. In short, reliable detection is really only possible at intermediate levels of migration. If migration

is too high, detection is difficult simply because divergent selection is often not powerful enough to reduce gene flow to the point where neutral genetic divergence can proceed. If migration is too low, however, detection is inconsistent. That is, genetic divergence at neutral markers is detectable about half of the time, but this occurs simply by chance; i.e., gene flow is so low that all populations drift apart to a similar degree. In this case, detecting greater differences between populations in different environments is a false positive because the result is not caused by divergent selection. Finding the expected signature of ecological speciation in cases of very low gene flow is therefore not a strong indication that ecological speciation is actually occurring.

A second important parameter is the magnitude of the environmental difference between populations, i.e., the strength of divergent selection. Confirming intuition, genetic divergence at even unlinked neutral markers is generally higher when environmental differences are greater, as long as migration rates are intermediate (as above). This effect of selection occurs because greater environmental differences lead to greater natural selection against maladapted migrants and first-generation hybrids. In this case, linkage disequilibrium before recombination of genes between individuals from different environments causes reduced gene flow at neutral loci even if they are not physically linked to loci under divergent selection. Several other parameters influence the level of divergent selection at which the expected signature is apparent. As migration increases, for instance, a larger environmental difference (stronger divergent selection) is required to generate a generalized barrier to gene flow. One might therefore wonder how these critical

levels of divergent selection correspond to those observed in nature. The advantage of local individuals over foreign individuals in reciprocal transplants has an average of 45% , with 68% of the observations falling between −66% and 156% (Hereford 2009). The advantage of local individuals as defined by Hereford (2009) ($s_{Hereford}$) can be compared to our selection strength by using equation 4.2.

$$s_{Hereford} = \frac{2s}{2 - s} \tag{4.2}$$

Most of the levels of divergent selection (s) in our model that first lead to consistent detection of the expected signature were well within this range. For instance, the critical level above which detection is positive 80% of the time is $s = 0.4$ ($s_{Hereford} = 0.5$) for the larger population size at intermediate migration rate ($m = 10^{-3}$) with full recombination ($r = 0.5$).

A third important parameter is recombination rate. As expected, the closer a neutral locus is to a selected locus, the greater the effect of divergent selection on reducing gene flow at the neutral locus. That is, genetic hitchhiking can increase the chance of detection of the expected signature of ecological speciation. This effect can be most clearly seen in the case of high gene flow ($m = 0.5$): as recombination increases, greater environmental differences are required to detect the expected signature (Fig. 4–4). The same pattern is found for lower migration rates, but the transitional strength of selection is less abrupt. But how then does one interpret divergence at the neutral loci that are linked to selected loci? Certainly, this is no longer necessarily indicative of a "generalized barrier" to gene flow (Gavrilets 2004). We will return to this question below.

A fourth important parameter is population size. When the expected signature of ecological speciation is either always or never detected for large populations under a given parameter set, the same is generally true for small populations. For parameter combinations where more variable outcomes are obtained for large populations, however, smaller populations generally made it even more difficult to detect the reduced gene flow that results from environmental differences. The reason appears to be that smaller populations are more sensitive to drift and less responsive to selection (Falconer & Mackay 1996). In other words, when populations are smaller, the signal from selection is more difficult to separate from the noise due to drift.

Even though we have not tested it directly, the number of neutral loci should not have a major influence. An increase in number of loci does not change the variance on F_{ST} most of the time, but when it does, a slight decrease of variance is observed (Balloux & Goudet 2002). Thus increasing the number of loci is not the best way to improve statistical power to detect ecological speciation, effort should be put elsewhere if more statistical power is needed.

Increasing the concentration of selected loci, by increasing their number, would have the effect of decreasing the recombination rate of many neutral loci. Therefore, increasing the conentration of selected loci will have a similar effect to reducing the recombination rate. This is straightforward, but only if each selected locus in the multi-locus model has the same impact on fitness as the single seleted locus had. If the impact of the fitness is diluted for each locus as we increase the number of selected loci, then we do not know if the proximity of many weaker

selected loci will increase divergence at the neutral loci, or if the weaker selection will reduce the range of impact of the selected loci and reduce divergence at the neutral loci.

Note that although our particular model focused on a specific population structure and type of comparison among the populations, other types of population structure and types of comparisons among the populations could have been executed. Examples include analyses of clines across habitat transitions (e.g., Ogden & Thorpe 2002; Berner et al. 2009), comparisons of rate of dispersal to gene flow (e.g., Hendry et al. 2000), comparisons of isolation by distance for populations that are or are not in similar environments (e.g. Smith et al. 1997; Crispo et al. 2006), and correlations between adaptive divergence and neutral genetic divergence (e.g., Gíslason et al. 1999; Lu & Bernatchez 1999).

General implications

We confirm that ecological differences can sometimes cause reduced gene flow at unlinked neutral markers (see also Gavrilets et al. 2007; Gavrilets & Vose 2007; Nosil et al. 2008), and we show that this effect can be statistically detectable within a certain range of parameter space. Consistent and appropriate statistical detection is most likely to occur when divergent selection is strong, migration rates are intermediate, and population sizes are large. Detection is also easier when recombination rates between neutral and selected loci are lower, but in this case we are no longer confirming a generalized barrier to gene flow. Instead, a possible generalized barrier here becomes confounded with what amounts to direct selection on the neutral locus acting through genetic hitchhiking. This issue poses major

practical and conceptual issues for empiricists because the use of truly unlinked neutral markers greatly reduces the range of parameter space under which the expected signature of ecological speciation can be detected. On the other hand, the use of neutral markers that are linked to selected loci means that analyses are sensitive to the direct effects of selection on regions of the genome, rather than just the indirect effects of ecologically driven reproductive barriers.

When unlinked neutral loci are used, several additional points of caution are necessary. First, we uncovered a wide range of parameter space where divergent selection is present but not detectable at neutral genetic markers. This means that a failure to detect the expected signature of ecological speciation does not necessarily mean that divergent selection is absent and ecological speciation is not proceeding (i.e., a false negative). Second, we found a range of parameter space where the expected signature is found but it is driven by chance, not divergent selection. Most notably, these "false positives" are very common when gene flow is low, because divergence among all populations is high but it is largely independent of divergent selection. Third, we found large ranges of parameter space where results are highly variable among replicate simulations. That is, for a given parameter set, the expected signature is sometimes found and sometimes not, presumably owing to the stochastic nature of divergence at neutral markers. As expected, then, more reliable results are obtained when populations are larger.

How then can our results be of assistance to empiricists seeking to detect progress towards ecological speciation: i.e. the ecologically driven evolution of reproductive barriers? One encouraging short answer is that, except when

migration is very low, finding the expected signature of ecological speciation often indicates that ecological speciation really is present. In these case, unlinked neutral markers can be quite useful. This is somewhat of a relief (at least to us) because we have previously used positive results in related assays to infer the presence of ecological speciation (Berner et al. 2009). One discouraging short answer is that when the expected signature is not detected, this does not necessarily mean that ecological speciation is not proceeding. This is not a relief (at least to us) because we have used negative results in related assays to infer minimal progress toward ecological speciation (Crispo et al. 2006). Taken together, these two answers add up to the conclusion that neutral genetic markers are a valuable part of studies of ecological speciation, but that confidence in interpreting a given result often requires additional information. For instance, our analysis confirms the importance of obtaining information about particular neutral loci (possible linkage to selected loci) and particular population parameters (migration, selection, population sizes). These parameters can then tell the investigator whether or not to worry about false positives or false negatives in neutral-marker assays. In addition, the use of neutral genetic markers should be coupled to other methods for inferring ecological speciation, such as the testing of an ecological basis for specific reproductive barriers. Ecological speciation is clearly out there (Rundle & Nosil 2005), but just as clearly not everywhere (Hendry 2009), and so continued improvements to our inferential methods are necessary.

4.6 Acknowledgments

XTP and APH were sponsored by the Natural Sciences and Engineering Research Council (NSERC) of Canada. Thanks to E. Crispo for all the help with the statistics. We also thank Erika Crispo and Ben Haller for their comments on the manuscript. XTP is grateful to the Biology Graduate Student Association (BGSA) of McGill for a travel grant to present this work at the Canadian Society for Ecology and Evolution in Halifax and to The Society for the Study of Evolution (SSE) for an international travel grant to present this work in Moscow, Idaho. Thanks to McGill University (Department of Biology) and S. Bunnell for help using the bioinformatics cluster for the simulations.

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Connecting statement

In the previous three chapters, the presence of two discrete environmental types aided progress toward ecological speciation by generating immediate reproductive barriers without any evolution. A more difficult problem addresses the question of how ecological speciation is affected when such external physical barriers, such as distance or obstacles, are not present. Many models have considered this sympatric ecological speciation, but my goal will be to combine a series of important forces that are usually considered in isolation.

CHAPTER 5
Forces influencing progress toward sympatric speciation.

5.1 Abstract

Many factors influence progress toward sympatric speciation. Some of the important ones include competition, sexual selection, and the degree to which discrete resources are present. What is not well understood is the relative importance of these forces, as well as interactions among them, to sympatric speciation. We use an individual-based numerical model to investigate these uncertainties. Sexual selection is modelled as the degree to which male foraging traits influence female mate choice. Competition is modelled as the degree to which individuals with different phenotypes compete for portions of the resource distribution. Discreteness of the environment is modelled as the degree of bimodality of the underlying resource distribution. We find that strong sexual selection is necessary but not sufficient to cause sympatric speciation. In addition, sympatric speciation is most likely when competition is lower among phenotypes and when the resource distribution is most strongly bimodal. Even under these ideal conditions, however, sympatric speciation only occurs 50% of the time, with the probability increasing when the number of loci coding for the trait is low. In all cases, stochasticity plays an important role in determining progress toward ecological speciation. We argue that an increased understanding of the promoters

and inhibitors of ecological speciation is best achieved with models that include multiple potential forces.

Keywords: competition, individual-based modelling, resource shape, sexual selection, sympatric speciation

5.2 Introduction

Speciation can occur in several different geographic modes (allopatric, parapatric and sympatric) (Gavrilets 2004) and, within each mode, can be influenced by several forces. Some important forces include natural selection resulting from divergent environments (Funk 1998; Via et al. 2000; Schluter 2000; Nosil 2004), natural selection resulting from intra-specific competition (Doebeli 1996; Dieckmann & Doebeli 1999; Bolnick 2004a), and sexual selection (Turner & Burrows 1995; Kondrashov & Shpak 1998; Higashi et al. 1999; Takimoto et al. 2000; Ritchie 2007). The case of sympatric speciation has been of particular interest because, in this case, reproductive barriers must evolve *in situ* to prevent homogenization. Thus far, theoretical models of sympatric speciation have shown the potential for each of the forces listed above to cause diversification under at least some circumstances. No model, however has attempted to infer the relative importance of these different forces, as well as the role of interactions among them. Here we use individual-based numerical simulations to explicitly assess the importance of various combinations of sexual selection, divergent environments (unimodal or increasingly bimodal resource distributions), and intra-specific competition on sympatric speciation.

Sexual selection has been considered in a number of sympatric speciation models, either with competition (Dieckmann & Doebeli 1999; Drossel & McKane 2000) or without competition (Higashi et al. 1999) and with divergent environments (Gavrilets & Vose 2005; Gavrilets et al. 2007) or without divergent environments (Dieckmann & Doebeli 1999; Drossel & McKane 2000). The main finding of this work is that sexual selection can be an important (and often necessary) contributor to sympatric speciation when divergent selection is present (because of either environmental divergence or intra-specific competition). What is more controversial is whether sexual selection can drive sympatric speciation by itself in the absence of divergent natural selection. On the one hand, Higashi et al. (1999) argue that the runaway process of male secondary sexual trait evolution can lead to the evolution of divergent female preferences and sympatric speciation. On the other hand, van Doorn et al. (2004) argue that the conditions that promote and maintain this process are unlikely without some form of disruptive selection.

Competition in sympatric speciation models of sexual organisms has been modelled either with divergent environments (Doebeli 1996; Doebeli & Dieckmann 2003) or without divergent environments (Dieckmann & Doebeli 1999). Here, one conclusion has been that competition can indeed drive sympatric speciation on even a unimodal resource (Dieckmann & Doebeli 1999) or a broad distribution of resources (Drossel & McKane 2000), and that assortative mating seems to be a necessary part of this process. This remains controversial; for instance Polechová & Barton (2005) have argue that phenotypic clustering is only a transitory state

driven by limits to the resource distribution, a conclusion that Doebeli et al. (2007) have since disputed.

Divergent environments are the original force thought to drive sympatric speciation. Many classic models (Levene 1953; Maynard Smith 1962) as well as recent models (Gavrilets & Vose 2005; Gavrilets et al. 2007) have shown that two specialist species can evolve in environments with two discrete resources, such as different host plants or other foraging environments. The question that remains, however, is how strong this force is relative to competition on a unimodal resource. Only one paper has examined this question showing that resource bimodality leads to a more effective resource use at equilibrium (Doebeli 1996).

We address the above issues through simulations in which we independently vary (1) the strength of sexual selection that females exert on male foraging traits, (2) the degree to which individuals with different phenotypes compete for resources, and (3) the extent to which resources are divergent (unimodal versus increasingly bimodal resource distributions). We also consider the effects of the number of loci governing the foraging and mate choice traits. This design is intended to be a more integrated model of sympatric speciation that can consider multiple forces simultaneously.

5.3 Modelling framework

The model is an individual-based simulation that employs biologically a realistic parameters. For instance, it uses hard selection (Christiansen 1975), realistic mutation rate (Dallas 1992; Weber & Wong 1993; Brinkmann et al. 1998),

and the same modelling techniques as Gavrilets et al. (2007) and Gavrilets & Vose (2009). The code is written in Fortran and is available upon request.

Environment

The environment is represented by a resource distribution. This distribution is unimodal to bimodal in shape, with the peaks separated by $\Delta\theta = \theta_2 - \theta_1$ with $\theta_{1,2}$ being the positions of the peaks:

$$R'(x) = \pi \exp\left(-\frac{(x-\theta_1)^2}{2\sigma_R^2}\right) + (1-\pi)\exp\left(-\frac{(x-\theta_2)^2}{2\sigma_R^2}\right). \tag{5.1}$$

$R'(x)$ is normalized such that the total amount of resources is K_0, the carrying capacity, in the discrete form (see below). The peaks are always symmetrically positioned around the center of the possible resource distribution. Thus, an increasing distance between the peaks means an increasing resource "valley" between the two peaks. The resource distribution is replenished at the start of each generation.

Individuals

The individuals are diploid hermaphrodites. They have different characters that are each controlled by L additive loci with three possible alleles at each locus ($\{-1, 0, 1\}$). An individual's foraging ability peaks at their foraging trait value (U) on the resource distribution and they have a strength of mating preference (c) for that trait U. Thus the value of c is used to represent the strength of sexual selection. The foraging trait U and sexual selection c are genetically independent; i.e., this is not a "magic trait" model (Gavrilets 2004). All the trait values are scaled to be between extremes of zero and one. The life cycle of individuals

is: 1. birth, 2. foraging, 3. viability selection, 4. mating, and generations are non-overlapping.

Foraging

The foraging ability ($F'(x)$) of an individual is a Gaussian function with a mean of U and standard deviation of σ_C:

$$F'(x) = \exp\left(-\frac{(x-U)^2}{2\sigma_C^2}\right). \tag{5.2}$$

Because of the genetic architecture described above, U can only take $4L+1$ discrete values. We therefore normalize both the resource and the foraging functions to be non null at those accessible $4L+1$ values. The foraging ability ($F(x)$) of each individual is normalized to sum to one, while the resource distribution R sums to K_0 (maximum carrying capacity). σ_C controls the competition factor among individuals with different foraging trait phenotypes: as σ_C increases, there is more foraging ability overlap for a given trait difference and thus more competition among different phenotypes. Thus, we refer to increasing σ_C as an increasing strength of competition (Table 5–1).

Viability selection

The viability of an individual is a function of its foraging ability and competition for resources. Each of the $4L+1$ portions of the resource is shared among the individuals in the population, proportional to their foraging ability in that portion. The resources acquired on a specific resource type x ($\omega_i'(x)$) by an individual i with foraging ability F_i is function of the number of individuals of all trait values n_j

Parameter	symbol(if any)	values
Strength of preference	c	$\{0.3, 0.5, 0.6, 0.7, 0.8, 0.9, \text{evolvable}\}$
Distance between the peaks	$\Delta\theta$	$\{0, 0.2, 0.4, 0.6, 0.8, 1\}$
Sexual selection	σ_a	$\{0.083, 0.167, 0.24, 0.333\}$
Competition	σ_C	$\{0.001, 0.083, 0.167, 0.24, 0.333, 1000\}$
Resource width	σ_R	$\{0.042, 0.083, 0.167, 0.24, 0.333, 1000\}$
Maximum carrying capacity	K_0	$\{2048, 4096\}$
Number of loci	L	$\{4, 8\}$
Average number of offspring	b	$\{3\}$

Table 5–1: Parameter space explored

including its own n_i:

$$\omega_i'(x) = \frac{F_i(x)R(x)}{\sum_j n_j F_j(x)}. \tag{5.3}$$

This resource acquisition is then summed over the entire resource range to yield the total amount of resources acquired by an individual:

$$w_i = \sum_x w_i'(x). \tag{5.4}$$

The probability that an individual survives to the reproductive stage (ν) is given then by a modified Beverton-Holt model (Kot 2001):

$$\nu_i = \frac{\omega_i}{\omega_i + (b-1)}, \tag{5.5}$$

where b is the average number of offspring produced by a female (Table 5–1).

Mating preference

Individuals who survive can then mate. Each surviving individual is chosen once as a "female" and will produce on average b offspring, the actual number produced being drawn from a Poisson distribution. Every other surviving individual is a potential "father" for that female, and individual males can be chosen by more

than one female. Males have the following probability of being chosen by a given female with U_2 and c (modified from Bolnick (2004b, 2006); Doebeli (2005) by Gavrilets et al. 2007):

$$\Psi(U_1, U_2, c) = \begin{cases} \exp\left[-(2c-1)^2 \frac{(U_1-U_2)^2}{2\sigma_a^2}\right], & \text{if } c \geq 0.5 \\ \exp\left[-(2c-1)^2 \frac{(U_1-(1-U_2))^2}{2\sigma_a^2}\right], & \text{if } c < 0.5. \end{cases} \tag{5.6}$$

At $c = 0.5$, every male has the same probability of being chosen, and so mating is random. At $c > 0.5$, positive assortative mating occurs based on the foraging trait (U). At $c < 0.5$, negative assortative mating occurs based on the same foraging trait. For all simulations, c and σ_a are fixed parameters, except a series of simulation where c evolves (Table 5–1).

Initial conditions

The loci controlling the ecological trait (U) initially have an equal probability of having values $\{-1, 0, 1\}$. As a result, the average individual at the start of the simulation has a phenotype that is at the centre of the resource distribution. All loci have the same mutation probability of 10^{-3}, which is similar to (Dallas 1992; Weber & Wong 1993; Brinkmann et al. 1998; Gavrilets & Vose 2005). Mutations increment or decrement the locus value by one, with equal probability. Mutations at the boundary are designed to keep the value within that range. Initially, the population is at carrying capacity. Table 5–1 lists the parameter space explored in the simulations. All combinations are run for 2000 generations in order to reach stability, with ten replicates for each parameter combination.

Tracking

We use an integrative approach where three main independent axes are considered for their effects on sympatric speciation. The first axis is the degree of competition among individuals with different phenotypes, the standardized measure for this is σ_C. The second axis is the degree of sexual selection. Here we use the standard deviation of the standardized mating preference function ($\frac{1}{M}\int \Psi(U1, x, c)dx = 1$), where M is the normalization factor:

$$sdSex = \left(\frac{1-2c}{\sigma_a}\right)^2. \tag{5.7}$$

As $sdSex$ increases, the strength of assortative mating decreases. The last axis is resource shape. Here we calculate the amount of resources from one peak (θ_1) that are present under the center of the other peak ($U_2 = \theta_2$):

$$shape = \exp\left(-\frac{\Delta\theta^2}{2\sigma_R^2}\right). \tag{5.8}$$

At $shape = 1$ there is no bimodality, and as $shape$ decreases, the bimodality gets stronger.

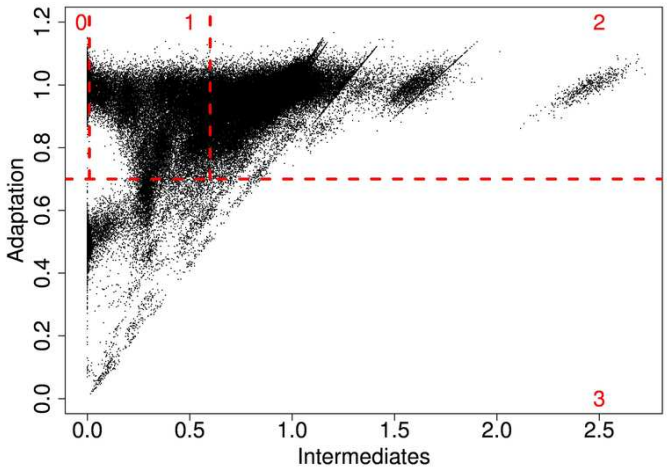
We look at the effect of competition, resource shape and sexual selection on adaptation and the number of intermediate forms (often hybrids, see Results). Adaptation is measured as the population size after reproduction and is normalized by the carrying capacity. For hybridization, we calculate the deviation from the random expectation (no natural selection, no sexual selection, and no competition) of the number of individuals at the centre of the resource distribution

$-\frac{\sigma_C}{2} \leq U \leq \frac{\sigma_C}{2}$, hereafter called "intermediate individuals". The criterion for intermediate individuals is thus normalized by the foraging range of the individuals.

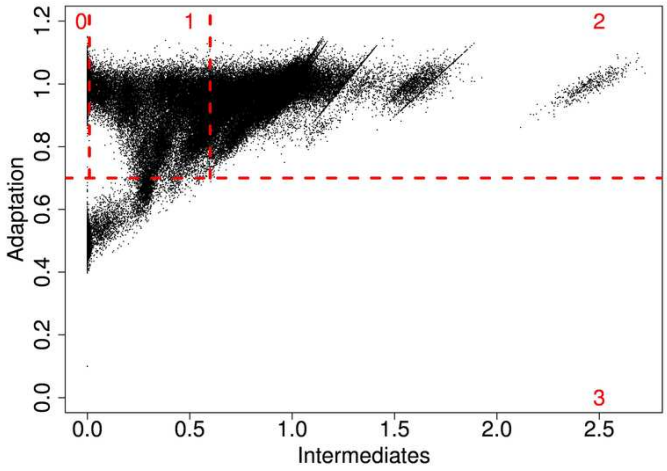
5.4 Results

Extinction occurred in 474 of the 241920 simulations (0.2%). All of these extinctions occurred when both the foraging range of individuals was the smallest ($\sigma_C = 0.001$) and the distance between the peaks was large ($\Delta\theta > 0.6$). In these cases, a wide and deep fitness valley was present between two resource peaks and this valley could not be bridged by an individual, because foraging was too specialized. Because our simulations started with a population having phenotypes in the center of the resource distribution, these conditions sometimes made it impossible to colonize either resource peak, causing system-wide extinction. The simulations leading to extinction were excluded from the following analyses.

The overall results are shown in a phase plane (Fig. 5–1(a), Table 5–2) representing both the level of adaptation (actual population size relative to carrying capacity) and the number of intermediate phenotypes ($-\frac{\sigma_C}{2} \leq U \leq \frac{\sigma_C}{2}$) relative to a null scenario. This null scenario entailed no natural selection, no sexual selection, and no competition; under these conditions each allele occurs with equal frequency. We define four zones on this phase plane.



(a) All



(b) All except $c = 0.3$

Figure 5–1: (a) Phase plane of all the simulations. (b) Phase plane of all simulations except those with negative assortative mating (all except $c = 0.3$). Each simulation is represented by its adaptation (population size divided by the maximum carrying capacity) and its level of hybrids (number of hybrids observed divided by the number expected without competition, natural or sexual selection).

Condition	symbol(if any)	Zone 0 (FSS)	Zone 1 (PTSS)	Zone 2	Zone 3
All		3.44	15.21	75.07	6.28
Random mating	$(c = 0.5)$	0.00	7.85	81.17	10.98
Strong assortative mating	$(sdSex < \frac{1}{3})$	13.76	25.11	60.99	0.14
Strong competition	$(sdComp \geq \frac{1}{3})$	1.14	0.19	98.67	0.00
Weak competition	$(sdComp < 0.1)$	2.75	39.74	39.42	18.10
Unimodality	$(shape > \frac{2}{3})$	0.78	13.20	83.74	2.29
Bimodality	$(shape \leq \frac{1}{3})$	5.41	16.83	68.48	9.29
Evolving c		0.29	9.82	79.23	10.66
Negative assortative mating	$(c = 0.3)$	0.00	3.34	82.56	14.10

Table 5–2: Percentage of simulations ending in each of the four zones. FSS and PTSS represent full sympatric speciation and progress toward sympatric speciation, respectively.

Zone 0 (upper left): adaptation is very high (≥ 0.7) and intermediates are extremely rare (≤ 0.01). This zone represents cases of full sympatric speciation (FSS) where two phenotypic clusters are present and hybridization between them is very rare.

Zone 1 (upper center): adaptation is high (≥ 0.7) and intermediates are more common than in Zone 0 but still notably fewer than expected in the null model (≤ 0.6 and > 0.01). This zone represents cases of progress toward, but not the attainment of full, sympatric speciation (PTSS).

Zone 2 (upper right): adaptation is high (≥ 0.7) and intermediates are common (> 0.6). Here, the entire resource range is occupied by a large number of individual specialists distributed across the resource range, or more rarely, a single generalist population.

Zone 3 (lower): adaptation is low and intermediates are few. This zone includes cases where only one of two existing resource peaks is occupied; the population is specialized on one of the two available resources, and the other is not exploited. This zone also includes cases where adaptation is not achieved because negative assortative mating prevents specialists from efficiently exploiting a resource by strongly homogenizing the gene pool (Fig. 5–1(b)).

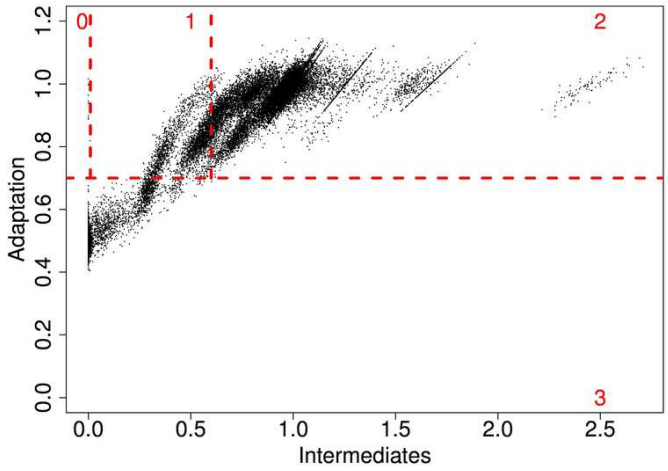
Forces determining progress toward sympatric speciation

With no sexual selection ($c = 0.5$, $sdSex = \infty$), we never recorded an instance of full sympatric speciation (FSS, Zone 0), and only a few instances of progress toward sympatric speciation (PTSS, Zone 1). Although some cases of high adaptation and few intermediates are evident (Fig. 5–2(a), Table 5–2),

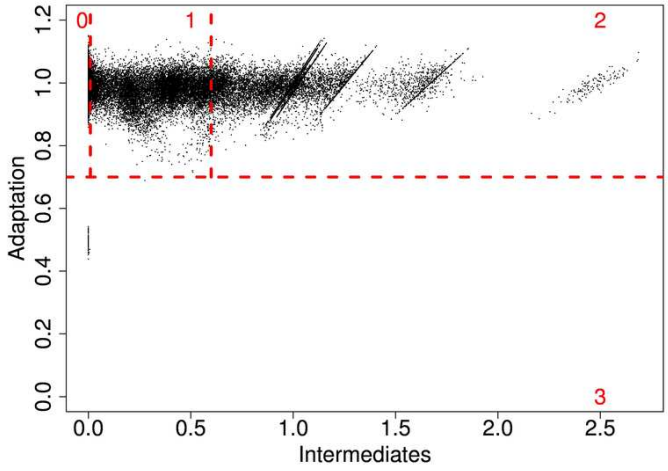
these cases are not accompanied by phenotypic bimodality. Instead, there is a reduction in adaptation as the intermediates decrease. With strong assortative mating ($sdSex < \frac{1}{3}$), FSS and PTSS were much more common (Fig. 5–2(b), Tables 5–2), although they still occured in only about 14% and 25% of the simulations, respectively.

With strong competition among different phenotypes ($sdComp \geq \frac{1}{3}$), strong maladaptation (Zone 3) never occurred (Fig. 5–3(a)) and both FSS and PTSS were rare (Table 5–2). This was because all individuals could, to some extent, use the entire resource range irrespective of their phenotype. With weak competition among phenotypes (i.e. high specialization, $sdComp < 0.1$), cases of maladaptation (Zone 3) were increasingly common (Fig. 5–3(b)). These situations occurred when adaptation to one peak by specialized individuals made subsequent colonization of a second peak unlikely. Zone 3 also includes some cases of negative assortative mating (Fig. 5–1(b)) or random mating (no sexual selection), in which gene flow prevented adaptive divergence. Weak competition also led to some cases of FSS (3%) and PTSS (40%). The reason was that different populations of individuals could here specialize on alternative resources.

With resource unimodality ($shape > \frac{2}{3}$), strong adaptation almost always occurred (Fig. 5–4(a), Table 5–2), because nothing prevented adaptation to the single resource peak. FSS sometimes occurred but these cases were very rare (Table 5–2). With strong resource bimodality ($shape \leq \frac{1}{3}$), FSS (5%) were much more common and PTSS remained about the same (16%) (Table 5–2).

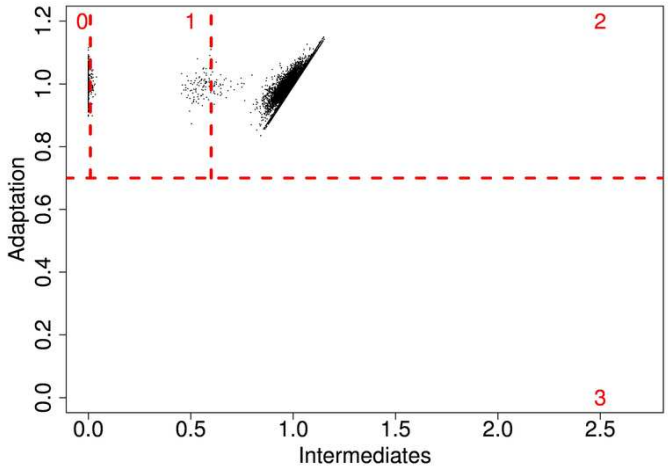


(a) $c = 0.5$

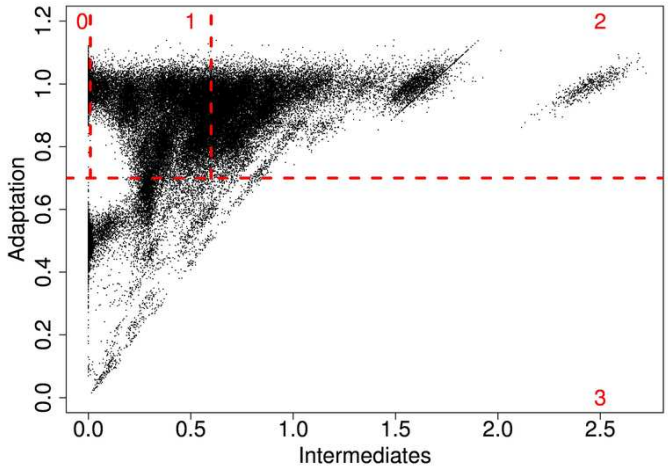


(b) $sdSex < \frac{1}{3}$

Figure 5–2: (a) Phase plane of simulations without sexual selection ($c = 0.5$). (b) Phase plane of simulations with strong sexual selection ($sdSex < \frac{1}{3}$). Each simulation is represented by its adaptation (population size divided by the maximum carrying capacity) and its level of hybrids (number of hybrids observed divided by the number expected without competition, natural or sexual selection).

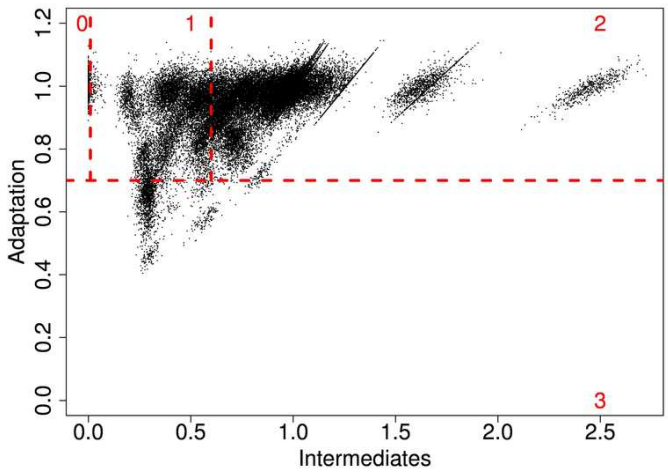


(a) $sdComp > \frac{1}{3}$

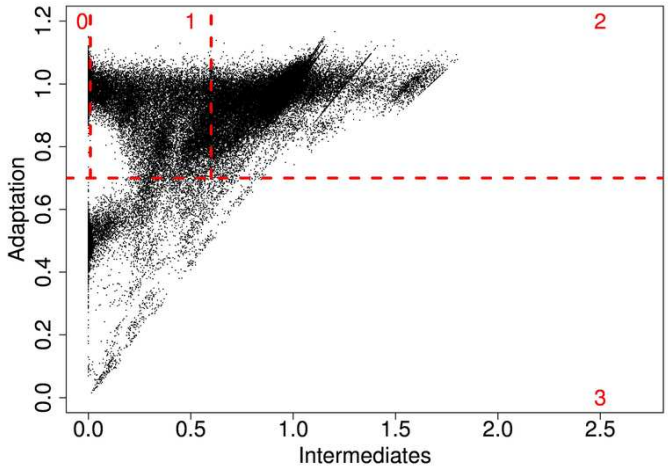


(b) $sdComp < 0.1$

Figure 5–3: (a) Phase plane of simulations with large competition ($sdComp > \frac{1}{3}$). (b) Phase plane of simulations with weak competition ($sdComp < 0.1$). Each simulation is represented by its adaptation (population size divided by the maximum carrying capacity) and its level of hybrids (number of hybrids observed divided by the number expected without competition, natural or sexual selection).



(a) $shape > \frac{2}{3}$

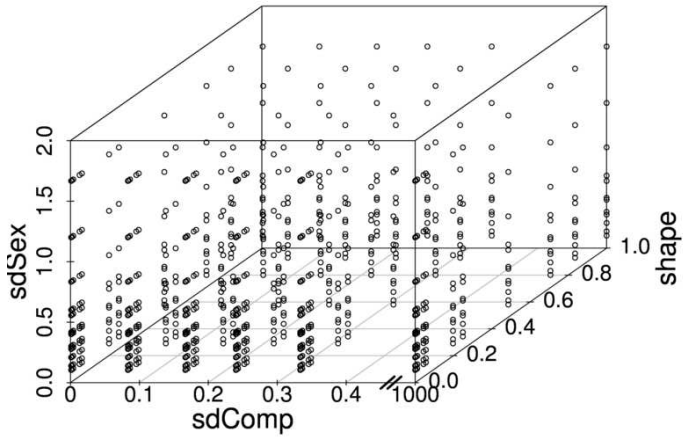


(b) $shape \leq \frac{1}{3}$

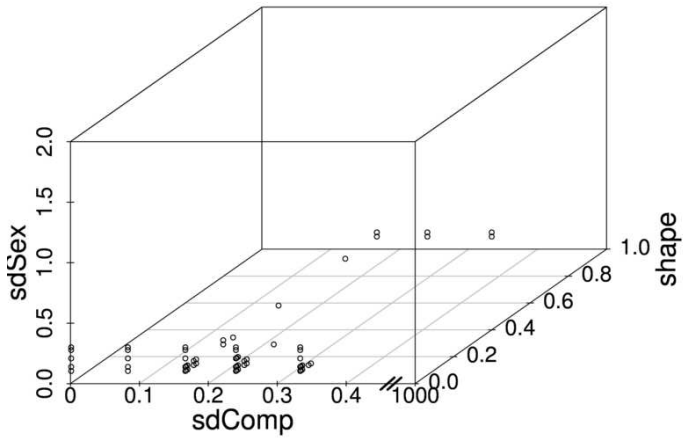
Figure 5–4: (a) Phase plane of all unimodal simulations ($shape > \frac{2}{3}$). (b) Phase plane of simulations with strong bimodality ($shape \leq \frac{1}{3}$). Each point is represented by its adaptation (population size over carrying capacity) and its level of hybrids (number of hybrids over the number expected without competition, natural or sexual selection).

Interactions and other potential influences

As the foregoing summary reveals, sexual selection, competition, and resource distributions all contribute to FSS and PTSS. However, the percentage of simulations that achieved FSS, in even the most favorable case, was less than 14% (Table 5–2). This suggests the presence of other limits on progress toward sympatric speciation. One of these influences is interactions among the three forces we examined. In particular, almost all of the cases of FSS occurred when sexual selection was strong and resources were strongly bimodal (Fig. 5–5, Table 5–3 and 5–4). In these conditions, resource gaps prevent specialist individuals from using alternative resources, which then drives mating isolation between populations using those different resources. The only other condition allowing FSS entailed strong sexual selection, unimodal or weakly bimodal resource distributions, and intermediate levels of competition. Here, competition was strong enough to provide an advantage to individual phenotypes deviating from the peak of the resource distribution, but not so strong as to promote two weakly interacting phenotypes at equilibrium. These situations parallel those described in Dieckmann & Doebeli (1999) and Drossel & McKane (2000).



(a) All



(b) Zone 0

Figure 5–5: All simulations done (a) and those falling in zone 0 FSS (b).

Sexual selection	weak			intermediate			strong		
Bimodality	small	medium	large	small	medium	large	small	medium	large
Weak competition	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.49
Intermediate competition	0.00	0.00	0.00	0.00	0.00	0.00	7.43	0.34	54.67
Strong competition	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.00	10.35

Table 5–3: Percentage of simulations ending in zone 0 (FSS) for different parameters. For sexual selection, we define weak ($sdSex \geq \frac{2}{3}$), intermediate ($\frac{1}{3} \leq sdSex < \frac{2}{3}$) and strong ($sdSex < \frac{1}{3}$). For competition, we define weak ($sdComp < 0.1$), intermediate ($0.1 \geq sdComp < \frac{1}{3}$) and strong ($sdComp \geq \frac{1}{3}$). For bimodality, we define small ($shape > \frac{2}{3}$), medium ($\frac{1}{3} < shape \leq \frac{2}{3}$) and large ($shape \leq \frac{1}{3}$).

Sexual selection	weak			intermediate			strong		
Bimodality	small	medium	large	small	medium	large	small	medium	large
Weak competition	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	18.84
Intermediate competition	0.00	0.00	0.00	0.00	0.00	0.00	8.48	2.50	38.64
Strong competition	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00	7.31

Table 5–4: Percentage of simulations for each parameter combination that their simulation end in zone 0 FSS. Equivalent to Table 5–3, but without normalization. For definition, see the caption of Table 5–3.

In addition to interactions among the three key forces, other forces might influence progress toward sympatric speciation. We found that the maximum carrying capacity (K_0) had no influence, but a larger number of loci controlling the trait slightly reduced the number of cases of FSS (3.2% versus 3.7%, significantly greater using a generalized linear model, $p < 0.001$). This result is consistent with the models of Gourbiere (2004); Gavrilets (2004); Bürger et al. (2006); Gavrilets et al. (2007); Gavrilets & Vose (2007, 2009), who found that fewer loci increase the likelihood of speciation.

The previous scenarios all used a constant value for mating preference (c) throughout each run. The *evolution* of sexual preference (c) from random mating might also be important. We found that sexual preference did not evolve in most of the simulations in which its evolution was permitted. That is, only 13% of the time did the population starting at $c = 0.5$ later evolve a c with a standard deviation that did not overlap the starting point. Of those 13%, most (73%) of the cases represented the evolution of positive assortative mating with respect to phenotype (c significantly greater than 0.5). Stronger positive assortative mating tended to evolve when the preference range (σ_A) was narrow, competition was strong (σ_R larger), and the distance between the peaks ($\Delta\theta$) was large. The strength of preference sometimes evolved to high values (as much as $c = 0.97$), but the average was around $c = 0.6$. Both a smaller carrying capacity and large number of loci controlling each trait led to fewer simulations evolving assortative mating. Only a few simulations where sexual preference evolved from a starting point of $c = 0.5$ led to FSS (Table 5–2).

5.5 Discussion

A large number of theoretical models have examined the conditions that promote and constrain ecologically-based sympatric speciation (Doebeli 1996; Dieckmann & Doebeli 1999; Doebeli & Dieckmann 2000; Drossel & McKane 2000; Doebeli & Dieckmann 2003; Polechová & Barton 2005; Bürger et al. 2006; Doebeli et al. 2007). Few of these studies, however, consider three key effects together: sexual selection, competition, and the shape of the resource distribution. By doing so, we address the likelihood that each force is necessary and/or sufficient, whether individually or in combination, for sympatric speciation.

Sexual selection

Sexual selection has long been considered an important part of sympatric speciation, because it allows for the evolution of assortative mating. Without this populations occupying a diversity of resources become homogenized (Kirkpatrick & Nuismer 2004). We confirm this result: sympatric speciation never occurred when sexual selection was absent, regardless of the nature of competition and the shape of the resource distribution (Tables 5–2, 5–3 and 5–4). In sexual organisms, then, sexual selection is often necessary for sympatric speciation. Exceptions will occur when other means of generating assortative mating are present, such as habitat choice (Feder et al. 1994; Nosil et al. 2002) and differences in mating time (Hendry & Day 2005; Savolainen et al. 2006; Devaux & Lande 2008).

A number of other studies have addressed whether sexual selection is sufficient by itself for sympatric speciation (Higashi et al. 1999), with most arguing that it

isn't (Arnegard & Kondrashov 2004; Gourbiere 2004; van Doorn et al. 2004). Instead, it seems that adaptive divergence must also be present. We also confirm this result because sexual selection, even when strong, never caused speciation unless disruptive selection was present due to either a bimodal resource distribution or competition. We conclude that sexual selection is necessary but not sufficient for sympatric speciation in sexual organisms, at least in the absence of other drivers of assortative mating (see above).

Competition

Competition on a single unimodal resource distribution has been argued by some authors to drive sympatric speciation (Doebeli 1996; Dieckmann & Doebeli 1999; Bürger et al. 2006). Other authors, however, have argued that this situation is extremely rare and only found under a very limited, and potentially unrealistic, parameter range (Gavrilets 2005; Polechová & Barton 2005). Our results support both assertions. First, we found that sympatric speciation can indeed occur on strictly unimodal resource distributions (Fig. 5–4(a)). However, we also found that this occurred only rarely in less than 1% of the simulations with unimodal distributions (Table 5–2). Sympatric speciation under these conditions only occurred when sexual selection was present (as above) and was most likely to occur under an intermediate level of competition (Table 5–4). In those cases, competition was strong enough to induce disruptive selection, but not strong enough to cause competitive exclusion.

Resource distribution

Divergent environment is thought to be an important contributor to sympatric speciation (Doebeli 1996). For example, many radiations of insects involve divergence onto different host plants (Feder et al. 1994; Funk 1998; Drès & Mallet 2002; Nosil et al. 2002) but only rarely within those host plants. Exceptions to the later point occur in the case of specialization on different plant parts or through different reproductive timing (Joy & Crespi 2007). Thus, adaptive radiation in phytophagous insects generally involves specialization on discrete resources, rather than the partitioning of a single unimodal resource. Moreover, classic models confirm the relative ease with which two specialists evolve in an environment with two resources (van Tienderen 1991; Fry 2003; Ackermann & Doebeli 2004). Our results confirm that divergent environments is an important promoter of sympatric speciation. In most previous models, this speciation occurred through the evolution of host preference (Fry 2003; Gavrilets & Vose 2005; Gavrilets et al. 2007) that results in reduced gene flow between classes. Our model, however, involved sexual preference rather than host preference, further extending the conditions under which divergent environment can drive sympatric speciation.

Even strongly bimodal resource distributions, however, did not inevitably cause sympatric speciation. Instead, sexual selection was also necessary (as above) as was some degree of specialization on different resources (weak or intermediate competition). Even under these conditions, however, sympatric speciation occurred less than 40% of the time (Table 5–4). The other simulations led to either incomplete resource use (low overall adaptation) or many individuals spread across the

entire resource range. A small number of loci (L) was also conducive to sympatric speciation, due to stronger selection at each locus (Gourbiere 2004; Gavrilets & Vose 2005; Bürger et al. 2006; Gavrilets et al. 2007; Gavrilets & Vose 2007, 2009). Even here, however, sympatric speciation often did not occur. This reminds us of the stochastic nature of sympatric speciation even under optimal conditions.

It is also important to note that our simulations started with phenotypes in the center of the resource distribution, equidistant between the two peaks. Sympatric speciation would probably be less likely if we had started with a population well adapted to one resource and then introduced a second resource. The reason is that individuals specializing on a single resource peak will have difficulty using, and therefore colonizing, a second resource peak. Here, then, might be a situation where increasing resource bimodality decreases the likelihood of sympatric speciation.

Interactions and extensions

A key feature of our results is that sexual selection, the nature of competition, and the shape of resource distributions all interact to influence progress toward sympatric speciation. Thus, one might say that competition on unimodal distributions can indeed generate speciation, but it is also fair to say that bimodal distributions make it much easier (Doebeli 1996). For instance, as the resources distribution becomes increasingly bimodal, sympatric speciation occurs can more easily at all levels of competition. And yet, neither competition nor resource bimodality resources can drive sympatric speciation on their own; sexual selection that reduces gene flow between diverging groups is also necessary.

We feel that the best insights into the forces influencing sympatric speciation are not derived from studies that examine only one or a few forces. Instead, the relative importance of different forces and their interactions can only be revealed by more inclusive models. To our existing framework, it would therefore seem appropriate to incorporate even more forces, such habitat preference (Fry 2003; Gavrilets et al. 2007; Gavrilets & Vose 2007). Habitat preference might, for example, reinforce assortative mating and ease the evolution of reproductive isolation. At the same time, it might weaken the importance of sexual selection acting toward the same goal.

Finally, we modeled the evolution of a foraging trait by changing only the position of the center of an individual's foraging ability on the resource distribution. It would therefore be useful to also allow the *evolution* of generalist versus specialist foragers (van Tienderen 1991; Ackermann & Doebeli 2004). This would entail allowing the width of the foraging distribution of individuals to evolve independently of its central value. This method was employed in studies of generalist versus specialist models (van Tienderen 1991; Ackermann & Doebeli 2004), but not in the context of comparing the relative strength of various promoters of sympatric speciation on a bimodal resource distribution. In short, many opportunities exist for further exploration, using integrative simulation models, of the forces and interactions that promote sympatric speciation.

Acknowledgments

XTP and APH were sponsored by the Natural Sciences and Engineering Research Council (NSERC) of Canada. We also thank Ben Haller for his comments

on the manuscript. Thanks to McGill University (Department of Biology) and S. Bunnell for help using the bioinformatics cluster for simulations.

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CHAPTER 6
Conclusion

We simulated different scenarios of ecological speciation, each highlighting a series of potententially important forces, and how to detect them. Each chapter yielded several new insights into the phenomenon.

From chapter 2: Five questions on ecological speciation addressed with individual-based simulations

Progress toward ecological speciation, from the colonization of a new environment to the evolution of reproductive barriers and the reduction of gene flow, was considered. We found that a higher migration rate helps the colonization of a new environment by increasing the variation on which selection can act. This represents a formal demonstration of assertions by previous works (Swindell & Bouzat 2006; Garant et al. 2007). Once the population is established, we found that natural selection against migrants plays the greatest role in developing reproductive isolation between the populations. This represents the first theoretical demonstration of the hypothesized pre-eminence of this particular reproductive barrier in many cases of ecological speciation (Via et al. 2000; Hendry 2004; Nosil et al. 2005). At the same time, selection against migrants could not, in our model, generate complete reproductive barriers, with sexual selection needed to finish the job. This result is similar to other models in showing that sexual selection is a critical part

of ecological speciation in many instances. We also showed that, under these conditions, neutral genetic divergence was observed between populations, even early on within the ecological speciation process. Large variation in this result, however, confirmed the need to further consider investigating the conditions under which neutral genetic markers can reliably detect progress toward ecological speciation (Emelianov et al. 2004; Gavrilets & Vose 2005).

From chapter 3: The consequences of phenotypic plasticity on ecological speciation

Here we found that phenotypic plasticity drastically changes progress toward ecological speciation, a possibility that had not been considered in any previous theoretical models. Moreover, we found that the particular timing of plasticity is critically important. If plasticity is expressed before migration, it can promote ecological speciation by strengthening reproductive barriers that result from local adaptation: i.e., natural and sexual selection against migrants. If plasticity is expressed after migration, it can hamper ecological speciation by weakening those same barriers, because adaptive plasticity can better suit migrants and hybrids for their new environments and therefore reduce selection against them. These results show the importance of more seriously considering phenotypic plasticity in studies of ecological speciation.

From chapter 4: When can ecological speciation be detected with neutral loci?

Neutral genetic markers are often used to infer progress toward ecological speciation (Smith et al. 1997; Gíslason et al. 1999; Lu & Bernatchez 1999; Ogden & Thorpe 2002; Crispo et al. 2006; Nosil et al. 2008; Berner et al. 2009) but the

utility of this method is not certain (Emelianov et al. 2004; Gavrilets & Vose 2005). Here we tested the reliability and power of this method under different conditions of migration, recombination, and selection. We found that divergence in neutral markers can be an indicator of progress toward ecological speciation, but only under certain conditions. In particular, the method was powerful and reliable (1) under an intermediate migration rate, with approximately one migrant per population per generation, (2) under environmental conditions sufficient to induce natural selection against migrants, and (3) when population sizes were large. Outside of these conditions, however, the use of these markers yielded a substantial number of false positives (inferring ecological speciation when it was not present) or false negatives (failing to infer ecological speciation when it was present). Our results should provide a very useful guide to empiricists wishing to use neutral genetic markers to infer progress toward ecological speciation.

From chapter 5: Forces influencing progress toward sympatric speciation

By removing the spatial component inherent in the previous models (all essentially parapatric context), a major external and non-evolutionary aid to speciation was also removed. This shift to sympatric speciation allowed us to contribute to this highly controversial area of theoretical (Polechová & Barton 2005; Doebeli et al. 2007) and empirical (Barluenga et al. 2006; Savolainen et al. 2006) research. In particular, we sought to go beyond existing models to evaluate the relative importance of, and interactions, among a number of forces thought to contribute to sympatric speciation. We found that sexual selection was the most important promoter of sympatric speciation because it generated the assortative

mating that was necessary for the evolution of two distinct forms. At the same time, strong assortative mating was not sufficient by itself to induce sympatric speciation, requiring instead some form of disruptive selection. Here we found that intermediate levels of competition among phenotypes could cause sympatric speciation, but that bimodal resource distributions were even more important. We hope that these results highlight the importance of considering multiple forces in the same model, and the importance of underlying bimodal resource distributions rather than competition on a unimodal resource.

Integration, perspective, and general contributions

Speciation can be seen as a bifurcation process, but unlike the traditional study of bifurcation (Strogatz 2000), the branching does not occur on a point, but rather evolves over a gradual transition (Hendry et al. 2007), resulting in an outcome that is not deterministic. An interesting feature of speciation is thus the ambiguity of a clearly defined relevant end point. This characteristic provides us with an impetus to study the factors influencing progress toward and away from speciation. We feel that this thesis is one of the few to start considering this "progress toward" ecological speciation idea in a consistent manner.

Of particular interest in this thesis were the mechanisms of speciation and the conditions by which these mechanisms abide. Considering the presence of a great number of species inhabiting the earth (Hutchinson 1959), many conditions have traditionally been inferred to favor the process of speciation. Though predicting the outcome of this stochastic process is a work in progress, the conditions that may engender speciation are becoming better understood. The proliferation of

species is one possible outcome, but an alternative result involves the increase in the variation within a species without reproductive isolation. This may be referred to as a failure in the process of speciation, but the mechanisms that promote or maintain the level of variation within a species are also of interest in conservation biology.

Within this framework, some conclusions permeated across the different simulations. The emergence of these general observations in all of the simulations, despite their varying structure, suggests they should be general patterns during ecological speciation. First, divergent natural selection was a powerful driver of speciation (Chapters 2-5), reinforcing the arguments that ecological speciation could be an important contributor to the diversity of life (Nosil 2008). Second, divergent natural selection did not always drive speciation (Chapters 2-5), echoing recent calls for a greater focus on the process that do and do not allow substantial progress toward ecological speciation (Nosil et al. 2009; Hendry 2009; Berner et al. 2009). Third, natural selection against migrants (Chapter 2-4) and sexual selection against migrants (positive assortative mating, Chapter 2, 3, and 5) were always important drivers of progress toward ecological speciation. The first result, in particular, confirms previous assertions (Hendry 2004; Nosil et al. 2005) that studies of ecological speciation should focus more clearly on what happens to migrants that move between environments, rather than spending so much attention on what happens to any hybrids thereby produced. Fourth, natural selection can reduce gene flow at neutral markers but the reliability of this signature of ecological speciation was very low (Chapters 2-4). Perhaps this will answer, once

and for all, the arguments over the utility of this method (Emelianov et al. 2004; Gavrilets & Vose 2005).

The conditions that have been explored in this thesis were studied within a static environment. This is a reasonable and inherent starting point in order to understand the results when similar scenarios treat the environment as fluctuating. With the current climate change concerns and the impact that may have on the biodiversification processes and ecological speciation, more accurately and reliably understanding the parameters affecting species abundance and distribution is of great importance. It has been shown that some sister species may come into contact, that some may expand their range and colonize new environments, while others will go extinct. The adaptation of those species will depend on the level of hybridization they will experience with either their sister species or with the population that have not colonized the new environment.

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