Ecological and Mutation-Order Speciation in Digital Organisms

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abstract: Reproductive isolation between populations often evolves as a by-product of independent adaptation to new environments, but the selective pressures of these environments may be divergent ("ecological speciation" or uniform ("mutation-order speciation." In this study, we use an artificial life platform to directly compare the strength of reproductive isolation (specifically, postzygotic) generated by ecological and mutation-order processes. We also tested the effect of gene flow as well as the dimensionality (i.e., number of selective pressures) of the environments on the strength of postzygotic isolation. We found that ecological speciation generally formed stronger isolation than mutation-order speciation, mutation-order speciation was more sensitive to gene flow than ecological speciation, and environments with high dimensionality formed stronger reproductive isolation than those with low dimensionality. How various factors affect the strength of reproductive isolation has been difficult to test in biological organisms, but the use of artificial life, which provides its own genetic system that evolves, allowed us to computationally test the effect of these factors more easily.

Keywords: postzygotic, reproductive isolation, hybridization, dimensionality, gene flow, Avida.

Introduction

Reproductive isolation between populations often evolves as a byproduct of independent adaptation to new environments (Coyne and Orr 2004; Schluter 2009; Sobel et al. 2010). When these environments' selective pressures are different, divergent selection can cause populations to acquire different, often incompatible, alleles. This divergent process can generate reproductive isolation both in nature (reviewed in Rundle and Nosil 2005; Schluter 2009) and in laboratory experiments (Dettman et al. 2007, 2008; reviewed in Rice and Hostert 1993 and Fry 2009). Complete reproductive isolation due to this process is known as "ecological speciation" (Schluter 2009). On the other hand, if the environments' selective pressures are similar or identical (parallel or uniform selection), populations may diverge genetically by the chance fixation of different alleles. Although laboratory experiments and theoretical studies suggest that such process may lead to "mutation-order speciation" (Schluter 2009; Nosil and Flaxman 2011), its effectiveness in generating reproductive isolation compared to ecological speciation is unknown.

The main purpose of this study is to directly compare the strength of reproductive isolation generated by ecological and mutation-order processes. Specifically, we measure both the degree of postzygotic isolation (i.e., hybrid inviability) as well as the amount of genetic divergence between populations evolved under either different environments or the same environments. Because there is a higher chance of parallel evolution when environments are similar (Schluter and Conte 2009), we expect that postzygotic isolation and genetic divergence under a mutation-order process will be weaker than under an ecological process.

We also examine the effect of migration on both ecological and mutation-order processes. Migration between populations increases the chance of gene flow, which often slows genetic (and thus adaptive) divergence, although gene flow can also promote divergence (Garant et al. 2006; Räsänen and Hendry 2008). We vary the amount of migration between populations under both ecological and mutation-order processes, from allopatry to sympatry. We expect that migration will have a stronger negative effect on the evolution of reproductive isolation under mutation-order scenarios because, under uniform selection, an adaptive mutation that arises in one population is also selectively favored in the other Nosil and Harmon 2009; (Schluter 2009; Nosil and Flaxman 2011).

We also examine how the environments' dimensionality (i.e., number of selective pressures) affects the strength of reproductive isolation for both ecological and mutation-order processes. In high-dimensional environments, there are more opportunities for populations to adapt in different ways (Rice and Hostert 1993; Nosil et al. 2009), which may lead to stronger reproductive iso-

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lation for both ecological and mutation-order processes. High dimensionality, however, may also constrain speciation when trade-offs among adaptive traits hinder adaptation and therefore decrease the probability of reproductive isolation.

Finally, we examine one possible cause for differences in hybrid fitness between ecological and mutation-order processes. As populations adapt to their local environments independently, there is no guarantee that alleles acquired in one population will interact positively, or even neutrally, in the hybrid with alleles acquired in the other population (Coyne and Orr 2004). Negative interactions between alleles from two populations are known as Dobzhansky-Muller incompatibilities (DMIs), and both ecological and mutation-order process may cause them to form and thus lead to postzygotic isolation (Schluter 2009). It is unknown, however, whether ecological or mutation-order speciation differ in their propensity to produce DMIs. Although a mutation-order process may be expected to have fewer DMIs initially than an ecological process because uniform selection selects for the same alleles, such expectation diminishes the more populations diverge (Schluter 2009).

In this study, we use the software Avida to carry out our experiments. Avida (Ofria and Wilke 2004) is an artificial life research platform where digital organisms evolve due to genetic variation, inheritance, and differential reproduction (see "Methods"). Avida has been used previously in a wide range of ecological and evolutionary studies (e.g., Lenski et al. 2003; Chow et al. 2004; Elena et al. 2007; Ostrowski et al. 2007; Misevic et al. 2010; Anderson 2012). There are several reasons for using digital organisms to study evolution: we can observe millions of generations in a few days, conduct hundreds of replicate experiments, easily manipulate genomes, and accurately measure fitness. Digital organisms in Avida are not meant to specifically mimic the details of real biological organisms. Instead, digital organisms have a unique genetic system (see "Methods"). Despite these differences, the general principles that make evolution possible are still the same, which allows Avida to be used to test the generality of evolutionary theories and hypotheses. Indeed, several evolutionary properties have been found to be remarkably similar to that of biological organisms (Wilke and Adami 2002; Adami 2006; e.g., the distribution of mutational effects, the types of epistasis, and the genetic architecture of sexual organisms). Digital organisms improve on simple two-locus models of speciation because in Avida, traits are complex, involving multiple loci and epistatic interactions among alleles (Lenski et al. 1999).

Methods

Avida

Experiments with digital organisms were carried out using Avida (ver. 2.9.0), freely available at http://avida.devosoft.org. In this section, we provide a brief overview of Avida; for a full description, see Ofria and Wilke (2004). Avida is highly configurable, so unless otherwise noted, the following description applies to the default settings. In Avida, each digital organism consists of several components: a linear sequence of instructions (akin to a haploid genome), memory space in the form of registers and stacks, pointers to memory locations, and a central processing unit (CPU) that executes instructions. The instruction set makes up an assembly-like programming language, consisting of instructions for arithmetic operations, memory manipulation (e.g., swap registers or push into a stack), conditional execution (i.e., "if" statements), iteration (looping), input/output operations, and allocation and copying of memory. Organisms execute their instructions sequentially, sometimes skipping instructions for conditional statements or repeating the same instructions inside a loop; when the last instruction is executed, execution starts again at the first instruction. By executing instructions in their genomes, organisms are able to (1) replicate and (2) perform computational "tasks" that affect the speed at which they replicate and thus increase fitness.

To replicate, an allocation instruction creates the memory space required by the organism's offspring, and a copy instruction inside a loop allows the organism to copy itself into the new memory space. The copy instruction that allows organisms to replicate has a configurable probability of making mistakes, therefore introducing various kinds of mutations (e.g., point mutations, indel mutations, and slip mutations). By default, replication is asexual. However, Avida may be configured to perform sexual replication, in which the genomes of two asexually produced offspring are recombined by exchanging two randomly sized regions of their genomes. The offspring (whether clonal or two recombinants) are put into the population in random locations, replacing whatever organisms were already there. Generations are therefore overlapping, as offspring are born continuously, replacing older individuals who are likely not their parents.

In addition to replication, genomic instructions allow organisms to acquire 32-bit input values and use them to perform computational tasks. Tasks are Boolean operations, such as NOT, AND, and OR, and are applied to input values bit by bit. For example, if input values were 8 bits, the operation 10011101 AND 11101011 would produce 10001001 because 0 AND 0 is 0, 0 AND 1 is 0, 1 AND 0 is 0, and 1 AND 1 is 1. In Avida, however, there is no AND operation nor any other Boolean operation

except for NAND, from which all other boolean operations may be built, a property of NAND known as "functional completeness" in Boolean algebra. For example, P AND Q, where P and Q are input values, is equivalent to (P NAND Q) NAND (P NAND Q), and P OR Q is equivalent to (P NAND P) NAND (Q NAND Q). Because tasks are made up of the same building blocks (NANDs), evolved tasks may share the same pieces of code and therefore may not be completely independent.

When an organism performs a task, the organism's "merit" is increased by a specific amount, specified in a configuration file, for that task. The merit of an organism is a unitless value used by Avida to determine the number of instructions an organism may execute each time step. If two organisms had the same merit, they would execute the same number of instructions at each time step; however, if one organism had twice the merit as another, the first organism would execute twice the number of instructions compared to the second in a single time step. Thus, an organism with twice the merit as another, would replicate twice as fast. Organisms initially inherit the merit of their parents; otherwise, new organisms would be at a disadvantage compared to the rest of the population. The default environment rewards for nine binary (i.e., two-input) tasks, but the environment can be configured to reward for an additional 68 three-input tasks.

Adaptation in Avida occurs naturally (i.e., it is not simulated), as a result of the three ingredients required for natural selection: inheritance, variation, and differential reproduction. Inheritance comes from replication (sexual or asexual), variation comes from mutation and recombination, and differential reproduction comes from their rate of replication (determined by their replication code and performance of tasks). The ability to perform tasks evolves as organisms with the right mutations replicate faster than others and therefore take over the population. There are many ways in which to perform any one task, and independently evolved organisms often evolve the same task in different ways and with different degrees of efficiency.

Experimental Design

The Avida configuration files used to run our experiments are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.b87rp (Anderson and Harmon 2013) To generate the ancestral population, we founded a population with an organism that could replicate but could not perform any tasks. We then let this population evolve under the default nine-task environment for 500,000 updates (~42,000 generations). An "update" is a measurement of time in Avida, increasing by one each time organisms execute 60 instructions (on average). For the evolution of the ancestral population, we set the maximum population size to 10,000 individuals. The length of the genome was set to 200 instructions, and to ensure homologous recombination during sexual reproduction, the genome length was fixed. The mutation rate was set to 0.1 mutations per genome per generation.

We then set up four treatments (described below), which we call "drift," "ecological," "mutation-order 1," and "mutation-order 2." For each treatment, the population size was set to 2,000 individuals and divided into two demes, each of size 1,000. In the drift treatment, both demes' environments were the same as the ancestral (environment A). For each remaining treatments, we set up two subtreatments (described below): low dimensionality and high dimensionality. For this study, dimensionality refers to the number of tasks for which that environment rewards organisms for performing such tasks. In the ecological treatment, the demes' environments were different from each other and different from the ancestral (environments 1L and 2L for low dimensionality and environments 1H and 2H for high dimensionality). In the mutation-order 1 treatment, the demes' environments were the same as each other but different from the ancestral (environment 1L for low dimensionality and environment 1H for high dimensionality). Similarly, in the mutation-order 2 treatment, the demes' environments were the same as each other but different from the ancestral (environment 2L for low dimensionality and environment 2H for high dimensionality).

The specific tasks that were rewarded in each environment remained the same for the rest of this study. The number of tasks for the low and high environments were chosen as two extremes: two tasks for low dimensionality and the maximum of 34 (i.e., 68 possible tasks divided randomly into two demes) for high dimensionality. The specific tasks for environments 1L and 2L were chosen at random from tasks that were known to evolve within 10,000 updates in preliminary runs. Environments 1L and 2L shared no tasks; similarly, environments 1H and 2H shared no tasks. The specific tasks rewarded in each environment are part of the Avida configuration files, which are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.b87rp (Anderson and Harmon 2013).

Each treatment was replicated 20 times with a different random sample of 2,000 organisms (1,000 per deme) from the ancestral population. Successive random samples were reused for each treatment, so that the genotypes in replicate *n* of a treatment were the same as the genotypes in replicate *n* of another treatment. We ran each replicate for 10,000 updates (~850 generations). For each run, the entire population of organisms was saved every 100 updates (~8.5 generations). To examine the effect of gene flow, each replicate was run under eight migration rates (for the entire length of the run): 0.0 (allopatry), 0.00001, 0.0001, 0.001, 0.01, 0.05, 0.1, and 0.5 (sympatry). Migration rate

is the probability of an offspring being born in a deme different from its parents' deme; the migrating offspring was placed in a random location in the other deme (i.e., there were no hybrid zones), but the parents remained in their own demes. In all, there were 1,120 runs.

We measured the overall strength of selection in each environment (1L, 2L, 1H, and 2H) at the end of each "ecological" replicate run. First, for every organism in a population that could perform at least one task, we counted the number of tasks it could perform and calculated its fitness relative to the mean. We then used linear regression on the relationship between the number of tasks an organism could perform and its relative fitness. The slope of this line is the strength of selection (Conner and Hartl 2004). We report these results here, as they are part of the environment in which populations evolved. For environments 1L and 2L, the mean strengths of selection were 0.3440 (0.3375–0.3562, 95% bootstrap confidence interval [CI]) and 0.3353 (0.3341–0.3366), respectively. For environments 1H and 2H, the mean strengths of selection were 0.2045 (0.1852–0.2248) and 0.1899 (0.1762–0.2041), respectively. We discuss the strength of selection in "Discussion."

Postzygotic Reproductive Isolation

In this study, we focus on the evolution of postzygotic reproductive isolation. To measure the strength of postzygotic isolation for each treatment, we first selected 1,000 random pairs of organisms (one from each deme) and created one hybrid per pair at the end of each replicate run. We then calculated the fitness of each hybrid as the mean fitness relative to each parent. Finally, we compared the mean hybrid fitnesses for each treatment—the lower this fitness, the stronger the isolation. Note that hybrids were created after the experiments were finished; no hybrids were put back into the population.

Two types of hybridizations were performed for creating hybrids after the populations had evolved. The first followed the method used in Avida for sexual reproduction (and the way in which all our experimental populations experienced): a randomly sized genomic region starting at a random locus was chosen (both random numbers came from a uniform distribution), and two recombinant offspring were created by exchanging the genetic region of one parent with the other (two-point crossover). We randomly chose one of the two offspring as the hybrid. We also performed a more fine-scaled hybridization method where each locus of a hybrid had the same probability (0.5) of it coming from either one or the other parent. This method effectively increased the number of crossover points up to 200 and the number of regions that can be exchanged up to 100. We used this multiple-point crossover method to break apart coadapted gene complexes, following the same logic that researchers use when carrying out parental backcrosses or intercrosses between hybrids (e.g., Li et al. 1997; Burton et al. 1999). Multiple crossover points can expose incompatible gene complexes between species, revealing patterns of divergence that would be difficult to detect with recombination at only two crossover points.

Genetic Divergence

To quantify the homogenizing effects of gene flow, we calculated the genetic divergence between each replicate pair of demes under 0.0 and 0.01 migration for each treatment. Genetic divergence was measured as the fixation index $F_{ST} = 1 - H_S/H_T$, where H_S is the mean heterozygosity of each deme and H_T is the heterozygosity of both demes treated as one population (Hartl and Clark 1997, p. 118). The heterozygosity of a deme is the mean heterozygosity at all loci. The heterozygosity at a locus is $H = 1 - \sum_{i=1}^{n} x_i^2$, where n is the number of alleles segregating at that locus and x_i is the frequency of the ith allele (Gillespie 2004, p. 15). Values of F_{ST} between 0 and 0.05 would indicate little or no genetic divergence between two demes (Hartl and Clark 1997, p. 118). We expect that under zero migration F_{ST} values will be significantly higher than those under the 0.01 migration rate. Significance among treatments was determined by comparing their 95% confidence intervals of the mean F_{ST}. Each confidence interval was estimated by calculating 10,000 means of random samples (with replacement) of the F_{ST} values from the 20 replicates (i.e., each sample contained 20 F_{ST} values). The interval between 2.5% and 97.5% of means defined the confidence interval.

To test whether gene flow causes the same mutations—specifically those involved in performing a task—to fix under mutation-order speciation, we carried out a two-step process to identify and map such mutations. First, to determine whether the fixed mutations in a deme were necessary to perform a task, we reverted each locus, one by one, of the deme's consensus sequence to the ancestral state. If any reversion eliminated the ability to perform a task, then the allele at that locus must be important for that task. We ignored loci in which a reversion caused complete inviability of the organism, as these loci were involved in more than just task performance. Second, we aligned the consensus sequences of each pair of demes under 0.0 and 0.01 migration and highlighted the mutations we found above.

Hybrid Phenotypes

To examine a possible cause for differences in hybrid fitness between ecological and mutation-order processes, we counted the number of times that hybrids had low fitness

due to DMIs. Under two-point crossover recombination, a hybrid is made up of two parental components, which we call C1 and C2. If C1 or C2 contains the instructions to perform a task but the full hybrid cannot perform that task, C1 and C2 must interfere with one another through at least one DMI. To determine whether a hybrid had low fitness due to DMIs, we constructed two genotypes by making two copies of the hybrid, where we replaced C2 in the first copy and C1 in the second copy with the corresponding ancestral genetic region. In this way, we constructed two "component" genotypes, where each parental component was isolated in the genetic background of the ancestor. We then determined the tasks that these component genotypes as well as the original hybrid could perform. If either component genotype could perform a task but the hybrid could not, then at least one DMI was present (fig. 1). We performed this analysis on 1,000 hybrids per replicate in both the ecological and mutation-order treatments under zero migration, low dimensionality, and two-point crossover recombination.

Because in this system an organism's fitness is largely determined by the number and type of tasks it can perform (i.e., its phenotype), we identified the tasks that could be performed by each hybrid for both ecological and mutation-order processes. Note that this analysis is independent of the environment because an organism may have the ability to perform a task even if the environment does not reward for it. For simplicity, we focused only on the zero-migration, low-dimensionality set of treatments that were hybridized with a two-point crossover. For the ecological treatment, hybrids were categorized by the number of tasks they could perform: no tasks in either environment ("0-0"), one task in one environment but none in the other ("1-0"), one task in each environment ("1-1"), two tasks in one environment but none in the other ("2-0"), two tasks in one environment and one in the other ("2-1"), and two tasks in both environments ("2-2"). For the mutation-order treatment, hybrids were categorized by the tasks they could perform: no tasks ("None"), task 1 ("1"), task 2 ("2"), and both tasks ("1 and 2"). For those hybrids that could perform both tasks in the mutation-order treatment, we determined the tasks that each hybrid's parental components could perform. We categorized these parental components as no parental component performs any task ("0,0"), one parental component performs one task but the other none ("1,0"), each parental component performs a different task ("1,1"), and at least one parental component performs both tasks ("2,*"). This analysis will reveal the reason, at the phenotypic level, for differences in hybrid performance between ecological and mutation-order processes. Four replicates from the ecological treatment, one replicate from the mutation-order 1 treatment, and six replicates from the mutation-order 2 treatment were removed from the analysis above. In the ecological treatment, the removed replicates contained parents that could fortuitously perform a task of the other environment (even though there was no selective pressure for that task), and thus it would be unclear from which parent the task was inherited by the hybrids. In the mutation-order treatments, the removed replicates contained parents that could not perform both tasks and, therefore, was the reason that some of the hybrids were unfit.

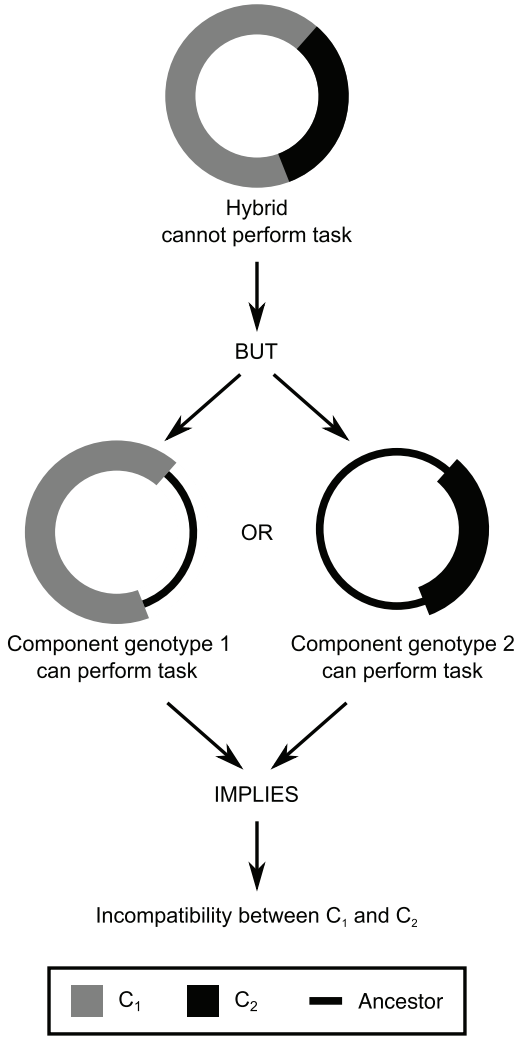

Figure 1: Method to determine whether a hybrid contains genetic incompatibilities. A hybrid is composed of two parental components, C1 and C2. Note that a parental component is only the parental region inherited by the hybrid; it is not the complete parent. If the hybrid cannot perform the task but either parental component can, then there must be at least one incompatibility between the components.

Results

Postzygotic Reproductive Isolation

When hybrids between the evolved demes were created by recombining a single genetic region (two-point crossover), reproductive isolation between demes that adapted to different environments (ecological treatment) was considerably stronger than reproductive isolation between demes that adapted to the same environment (mutation-order treatment; fig. 2*A*, 2*B*). With zero migration, for instance, reproductive isolation in the ecological treatment was more than twice as strong than in the mutation-order treatment. There was no reproductive isolation between demes evolving neutrally in the ancestral environment (drift treatment): the mean hybrid fitness was >0.99 at all migration rates.

Reproductive isolation in the mutation-order treatment was more sensitive to gene flow than in the ecological treatment (fig. 2*A*, 2*B*). At the 0.01 migration rate, for instance, the mean hybrid fitness in the mutation-order treatment was >0.98, but in the ecological treatment reproductive isolation was almost as strong as without migration. The mutation-order 2 treatment was more sensitive to gene flow than the mutation-order 1 treatment (no reproductive isolation at a migration rate of 0.00001).

When the environment rewarded for many tasks (high dimensionality), reproductive isolation was often stronger than when the environment rewarded for only two tasks (low dimensionality; cf. fig. 2*A* and 2*B*, 2*C* and 2*D*). This pattern was most evident in the ecological treatment, even at moderately high migration rates; for example, the mean hybrid fitness in the ecological treatment at 0.1 migration was 0.97 under low dimensionality but only 0.74 under high dimensionality. In the mutation-order treatments, however, reproductive isolation under high dimensionality at migration rates >0 was not always stronger than under low dimensionality, showing again that mutation-order was sensitive to gene flow.

When hybrids between the evolved demes were created by recombining up to 100 genetic regions (multiple-point crossover), reproductive isolation in the ecological and mutation-order treatments was stronger (fig. 2*C*, 2*D*). Note that recombination with multiple crossover points was used only to create hybrids for the calculation of postzygotic isolation; all populations were evolved under two-point crossover recombination. The mean hybrid fitness with multiple-point crossover was significantly lower than that with two-point crossover, dropping 33% and 48% in the ecological treatment for low and high dimensionality (respectively) and 53% and 43% in the mutation-order treatments. The difference in strengths of reproductive isolation between ecological and mutation-order treatments was now smaller than that with two-point crossover. Reproductive isolation in the mutation-order treatment remained more sensitive to gene flow than in the ecological treatment. Reproductive isolation in the genetic drift treatment with little migration was significantly greater than with two-point crossover. Interestingly, reproductive isolation in the genetic drift treatment with 0.00001 migration and high dimensionality was even greater than in the mutation-order 2 treatment.

Genetic Divergence

The genetic divergence under zero migration was significantly higher than that under 0.01 migration for all treatments (table 1), demonstrating that gene flow between populations had a homogenizing effect. Under 0.01 migration, the mutation-order treatments had little genetic divergence ($F_{ST} < 0.05$), which was significantly lower than the ecological treatments, suggesting that the mutation-order treatments were more sensitive to gene flow than the ecological treatments. Interestingly, the drift treatment under zero migration showed high levels of genetic divergence, as high as the ecological and mutation-order treatments for low dimensionality. Under zero migration, the genetic divergence for each treatment for high dimensionality was significantly higher than those for low dimensionality. Under 0.01 migration, the genetic divergence for the ecological treatment for high dimensionality was significantly higher than that for low dimensionality, but for the mutation-order treatments there was no difference in the genetic divergence between low and high dimensionalities. In agreement with these results, the sequences for each pair of demes for treatments under zero migration did not align as well as those under 0.01 migration (fig. 3). These result suggest that the reason that reproductive isolation was mostly absent under a mutation-order process with gene flow is that the key mutations that allowed organisms to perform tasks were the same (i.e., no genetic divergence for task-related mutations).

Hybrid Phenotypes

In the ecological treatment, we found that each replicate had, on average, 268.3 hybrids (218.0–315.8, 95% bootstrap mean CI) of 1,000 that contained at least one DMI between their parental components. In the mutation-order treatments, this quantity was 77.1 (45.8–111.2) and 128 (83.5–176.5) of 1,000. Therefore, populations that adapted to different environments accumulated more DMIs than populations that adapted to similar environments.

Because hybrids, on average, inherit half the genome of each parent, we expected that hybrids, on average, would inherit half the tasks from each parent (here we focused on the treatments without migration, low dimensionality,

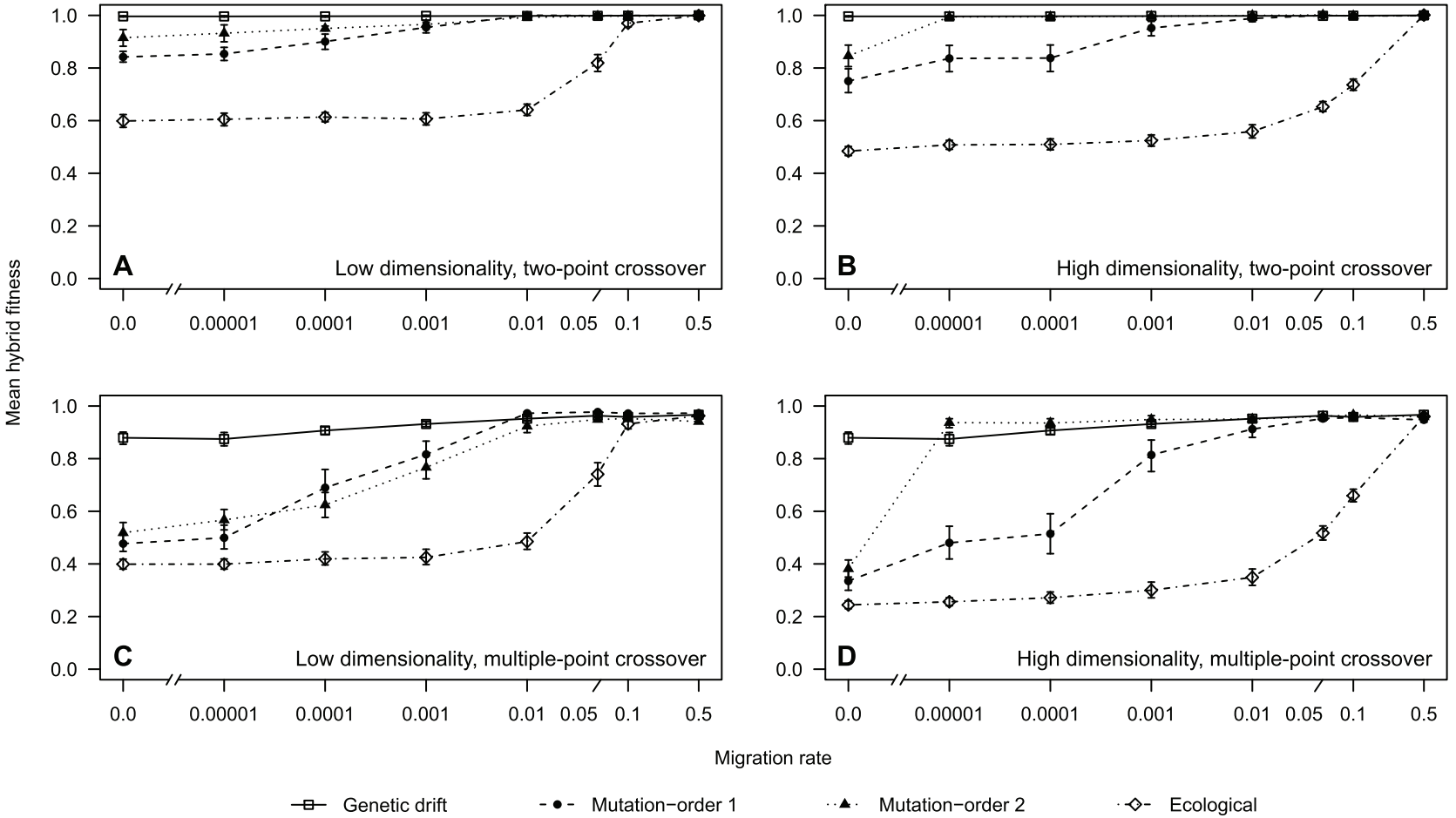

Figure 2: Mean fitness of hybrids (*Y*-axis) between populations evolved under various migration rates (*X*-axis) and different treatments (markers and lines): the ancestral environment (Genetic drift), different new environments (Ecological), or the same new environment (Mutation-order 1 and Mutation-order 2). At low dimensionality (*A*, *C*), the new environments rewarded only two tasks; at high dimensionality (*B*, *D*), the new environments rewarded 34 tasks. With two-point crossover (*A*, *B*), only one region was exchanged when creating hybrids; with multiple-point crossover (*C*, *D*), up to 100 regions were exchanged.

Table 1: Genetic divergence (F_{ST}) between demes for each treatment.

		Migration rate	
Dimensionality	Treatment	.0	.01
...	Drift	.3136[A]	.0187[B]
Low	Ecological	.3173[A]	.1387[C]
Low	Mutation-order 1	.3096[A]	.0186[B]
Low	Mutation-order 2	.3002[A]	.0216[B]
High	Ecological	.4187[D]	.2680[E]
High	Mutation-order 1	.4221[D]	.0304[B]
High	Mutation-order 2	.3758[F]	.0199[B]

Note: Shared superscript letters indicate that those values are not significantly different (95% bootstrap confidence interval).

and two-point crossover). In the ecological treatment, we found that hybrids were more likely to perform zero, one, or two tasks from one parent and none from the other (fig. 4*A*). Less than 10% of hybrids were able to perform all four tasks. For the mutation-order treatments, most hybrids could perform both tasks (fig. 4*B*, 4*C*), but because the parents could also perform both tasks, this information alone did not tell whether hybrids inherited one task from each parent or some other combination. When we analyzed those hybrids that could perform both tasks, we found that most inherited both tasks from just one parent (fig. 5), although for mutation-order 2 the difference between those that performed one task from each parent and those that performed both tasks from one parent was not significant. Surprisingly, for the mutation-order 1 treatment there were many hybrids that were fit even though their parental components could perform no tasks or just one task (fig. 5*A*).

Discussion

In this study, we used experimental evolution of digital organisms to compare the strength of postzygotic reproductive isolation generated by ecological and mutation-order processes. We assessed the strength of postzygotic isolation by measuring the mean hybrid fitness relative to each parent in its native environment. We found that, using a two-point crossover recombination method, the mean hybrid fitness was around 55% under ecological divergence but around 83% under a mutation-order process. Other studies have also found that the mean hybrid fitness is lower under divergent selection than under parallel selection. Dettman et al. (2007) found that the mean relative fitness of hybrids between yeast populations evolved in different environments (high salinity and low glucose) was around 87%, but hybrids from populations evolved under the same environmental conditions were as fit as their parents. Similar patterns were found in a filamentous fungus by Dettman et al. (2008), although in one of the parental environments hybrids between populations under divergent selection performed better than hybrids under parallel selection. Along with these studies, our study supports the view that ecological divergence causes stronger reproductive isolation than a mutation-order process.

It has been suggested that gene flow during speciation may be common (Coyne and Orr 2004, p. 112; Nosil 2008), which requires that genetic divergence with gene flow be possible. We found that a migration rate of 1% was not enough to prevent genetic divergence under an ecological process (table 1). This finding supports the notion that it is possible for populations under divergent

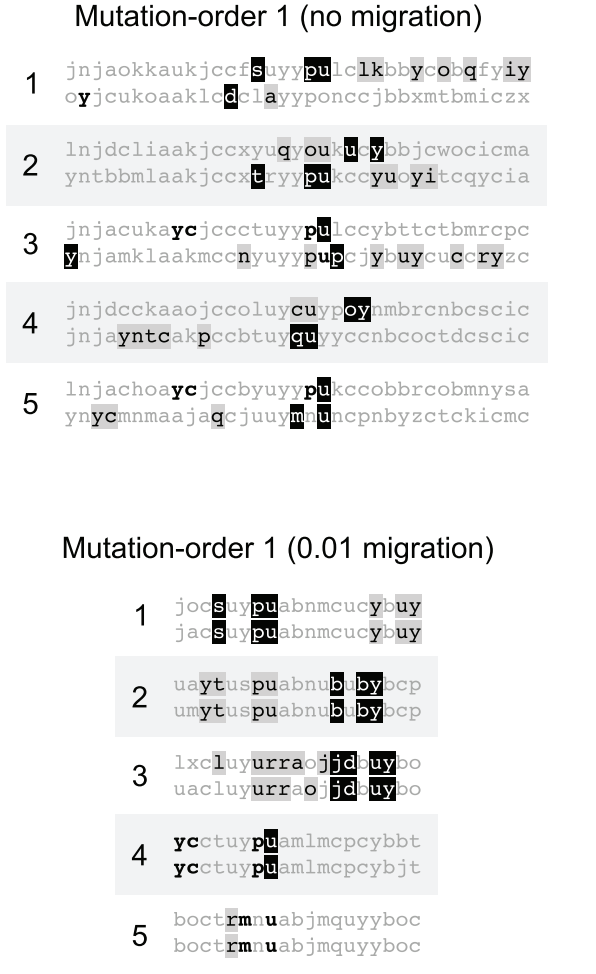

Figure 3: Consensus sequences of the first five evolved replicate pairs of demes in the mutation-order 1 treatment under zero migration (*top*) and 0.01 migration (*bottom*). Similar results were observed for the mutation-order 2 treatment. Sequences were 200 instructions in length, but only the loci that differed among each set of five replicates are shown. Derived alleles involved in performing a task are highlighted (black highlight = task 1, gray highlight = task 2, bold font = both).

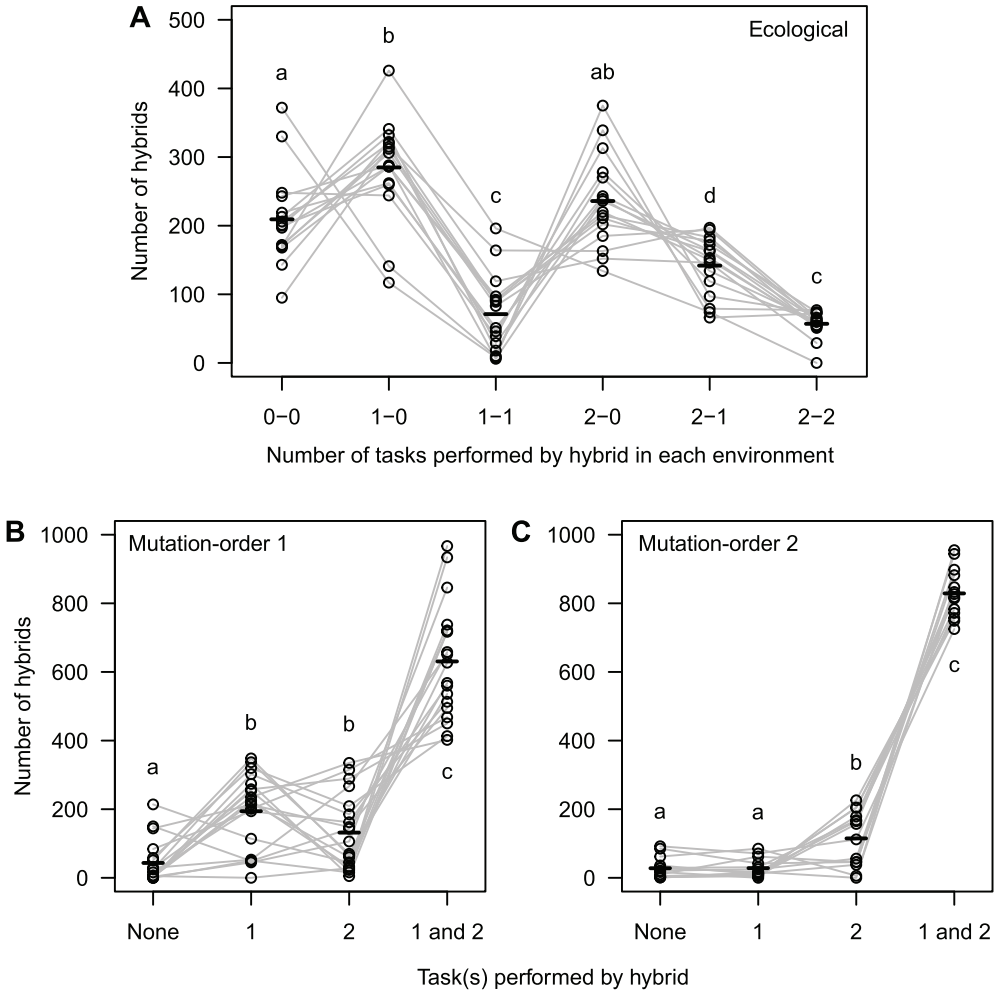

Figure 4: Number of hybrids able to perform certain tasks (see "Methods") for the ecological (*A*), mutation-order 1 (*B*), and mutation-order 2 (*C*) treatments under zero migration, low dimensionality, and two-point crossover. Each point is a hybrid count (out of 1,000) for a single replicate. Counts from the same replicate are connected by gray lines. The mean hybrid count per category among replicates is indicated by a horizontal bar. Nonsignificant differences between the mean hybrid counts share the same letter above (or below) the points in each category.

selection in the face of gene flow to continue to diverge. Under a mutation-order process, however, a migration rate of 1% was enough to prevent genetic divergence, which suggests that mutation-order speciation is more sensitive to gene flow than ecological speciation. Nosil and Flaxman (2011) also found in their computer simulations that genetic divergence under a mutation-order process did not occur >1% gene flow. One of the mutation-order treatments under high dimensionality was even sensitive to a migration rate of 0.00001. We speculate that in this treatment (corresponding to environment 2H), there was one or more large-effect adaptive mutation(s) that, when migrated to the other deme, spread quickly and homogenized the demes. We conclude that different populations under parallel selective pressures probably require almost complete isolation for divergence to occur.

Reproductive isolation between populations evolving in high-dimensional environments has been predicted and observed to be stronger than in single or low-dimensional environments (Rice and Hostert 1993; Nosil and Harmon 2009; Nosil et al. 2009). In *Timema* walking-stick insects, for example, reproductive isolation showed a positive correlation with environmental dimensionality (Nosil and Sandoval 2008; Nosil and Harmon 2009); further examples are reviewed by Nosil et al. (2009). Most empirical studies, however, rely on incomplete measures of dimensionality (imagine the difficulty in accounting for all selective pressures in the field). In this study, we were able to control

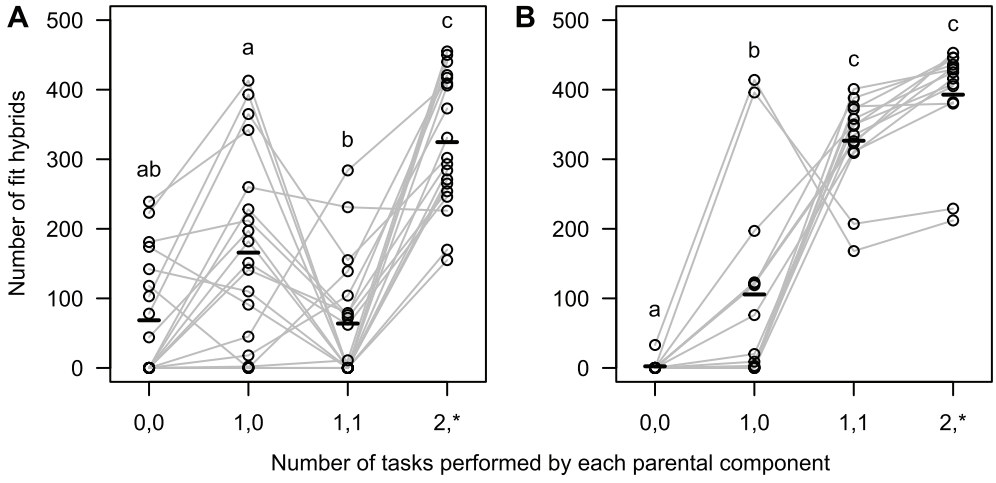

Figure 5: Number of fit hybrids (i.e., can perform two tasks) whose parental components can perform certain tasks (see "Methods") for the mutation-order 1 (*A*) and mutation-order 2 (*B*) treatments under zero migration, low dimensionality, and two-point crossover. Each point is a hybrid count for a single replicate. Counts from the same replicate are connected by gray lines. The mean hybrid count per category among replicates is indicated by a horizontal bar. Nonsignificant differences between the mean hybrid counts share the same letter above the points in each category.

precisely the number of selective pressures for the low- and high-dimensionality treatments. We found that under an ecological process, reproductive isolation was stronger between populations in high-dimensional environments than in low-dimensional environments. Under a mutation-order process, however, this pattern held only when no migration occurred between populations, but when gene flow was allowed this pattern went away. Our results support previous findings that dimensionality matters for ecological speciation but suggests that for mutation-order speciation with gene flow, environmental dimensionality may not be as important.

This conclusion was also supported by our measurements of genetic divergence: there was no difference in genetic divergence between low and high dimensionality for the mutation-order treatments under some gene flow. For the ecological treatment, however, the genetic divergence in high dimensionality was higher than in low dimensionality and higher than the mutation-order treatments, again showing that mutation-order treatments were more sensitive to gene flow. Interestingly, under zero migration the drift treatment (where mutations fixed neutrally) was as high as the ecological and mutation-order treatments under low dimensionality, suggesting that most of the divergence in the ecological and mutation-order treatments was actually the result of neutral fixations and few adaptive mutations. Indeed, in post hoc analyses we found that about 90% of mutational differences between these treatments were due to neutral fixations, not adaptive mutations. Another result to note is that under zero migration, the ecological and mutation-order treatments had about the same level of genetic divergence, which is closer to our result with multiple-point crossover than two-point crossover, suggesting that in some cases the amount of postzygotic reproductive isolation and genetic divergence are decoupled.

This decoupling between reproductive isolation and genetic divergence has been observed in biological populations (Stelkens and Seehausen 2009; Macías Garcia et al. 2012). In these studies, genetic divergence was not found to be a good predictor of sexual dimorphism or assortative mating (Stelkens and Seehausen 2009; Macías Garcia et al. 2012). In some cases, genetically closely related species were ecologically and phenotypically divergent; in other cases, genetically distant species were phenotypically and ecologically close (Stelkens and Seehausen 2009). One proposed reason for this decoupling is that temporal changes in selection pressures alter the way in which natural and sexual selection interact (Macías Garcia et al. 2012). Although assortative mating was not present in our digital populations—there was no mechanism for mate choice—reproductive isolation could not be predicted solely based on genetic divergence. We speculate that the reason was due to the degree of incompatibility between alleles for the different modes of speciation: alleles between populations were not as incompatible under a mutation-order process than under an ecological process. Our results support the notion that reproductive isolation is not directly caused by genetic divergence but is a by-product of processes that also affect genetic divergence (Pereira et al. 2011). Therefore, in order

to determine reproductive isolation between populations, one cannot rely solely on their genetic divergence; reproductive isolation should be measured directly.

Traits that are physically modular are hardly broken apart by recombination, hiding genetic incompatibilities that may have formed between populations. To determine whether genetic incompatibilities had formed between our populations but were hidden by the modularity of traits, we re-created hybrids through time using multiple-point crossover recombination rather than two-point crossover recombination. In multiple-point crossover recombination, each locus of a hybrid's genome had an equal probability of coming from either parent; in this way, modular traits could be broken apart by recombination. We found that the strength of reproductive isolation decreased for both ecological and mutation-order speciation, such that mutation-order speciation was almost as strong as ecological speciation (fig. 2). We even see some reproductive isolation in the drift treatment, showing that incompatibilities also formed but at a much slower rate than speciation by natural selection. These findings show that genetic incompatibilities were hidden by the modularity of traits. In other words, genetic incompatibilities that formed between populations were not always seen in hybrids because two-point crossover recombination did not break apart coadapted gene complexes coding for a task. We note that the genetic architecture of our populations evolved under two-point crossover recombination, not multiple-point crossover recombination, and thus, the modularity of traits and formation of genetic incompatibilities may be different under a different recombination method.

Part of the reason that hybrids were more unfit in the ecological treatment than in the mutation-order treatment was that in the ecological treatment more genetic incompatibilities (DMIs) formed between populations. This result supports the view that genetic incompatibilities are an important cause of ecological speciation (Rundle and Nosil 2005). Another reason that hybrids were more unfit in the ecological treatment was that for a hybrid to be fully fit it had to inherit both sets of tasks from both parents (i.e., four tasks), whereas for the mutation-order treatment, hybrids required only two tasks to be fit. In the ecological treatment, most hybrids inherited either one or two tasks from one parent and none from the other, and in the mutation-order treatments, most hybrids inherited both tasks from one parent, although in the second mutation-order treatment hybrids often inherited one task from each parent.

We found that the selection coefficients of adaptive alleles in the low-dimensionality environments were higher than those in the high-dimensionality environments. An opposite trend may have made it difficult to know whether it was higher dimensionality or stronger selection that resulted in hybrids being less fit under high dimensionality than low dimensionality. The smaller selection coefficients in the high-dimensionality environments may seem puzzling at first. But given that each additional task an organism could perform gives it an equal amount of merit, the higher the merit, the less an additional task contributes to the total merit. Therefore, the more tasks organisms can perform in the high-dimensionality environment, the less beneficial each one becomes (i.e., diminishing returns). The strengths of selection in either environment are nevertheless high overall, but it is not uncommon for selection to be high in new environments (e.g., Lenski et al. 1991; Dettman et al. 2007). Future studies could investigate how the strength of selection may affect the strength of postzygotic isolation by manipulating the selection coefficients in each environment.

In summary, we used the artificial life platform Avida, which allowed us to precisely control the type of selection (divergent or uniform), to compare the strength of reproductive isolation between ecological and mutation-order speciation. By accurately measuring the fitness of hybrids between populations, we showed that ecological speciation formed stronger postzygotic isolation than mutation-order speciation, although they were not so different when recombination involved crossover at multiple points. In addition, Avida allowed us to test various specific migration rates during the evolution of population pairs, where we found that mutation-order speciation was more sensitive to gene flow than ecological speciation. We were also able to control the number of selection pressures in each population, and we found that environments with high dimensionality formed stronger reproductive isolation than those with low dimensionality. These results support ideas brought up in the literature but which have been difficult to test in biological organisms. Avida provided a platform for us to test these ideas much more easily, and although digital organisms are more simplistic than biological organisms, they are a genetic system that evolves and speciates and therefore allows us to test the generality of hypotheses about speciation, which often do not require the specific details about how biological organisms work.

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