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SPECIATION DESPITE GENE FLOW WHEN DEVELOPMENTAL PATHWAYS EVOLVE

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Abstract.—Evolutionary biologists assume that species formation requires a drastic reduction in gene exchange between populations, but the rate sufficient to prevent speciation is unknown. To study speciation, we use a new class of population genetic models that incorporate simple developmental genetic rules, likely present in all organisms, to construct the phenotype. When we allow replicate populations to evolve in parallel to a new, shared optimal phenotype, often their hybrids acquire poorly regulated phenotypes: Dobzhansky-Muller incompatibilities arise and postzygotic reproductive isolation evolves. Here we show that, although gene exchange does inhibit this process, it is the proportion of migrants exchanged (m) rather than the number of migrants (Nm) that is critical, and rates as high as 16 individuals exchanged per generation still permit the evolution of postzygotic isolation. Stronger directional selection counters the inhibitory effect of gene flow, increasing the speciation probability. We see similar results when populations in a standard two-locus, two-allele Dobzhansky-Muller model are subject to simultaneous directional selection and gene flow. However, in developmental pathway models with more than two loci, gene flow is more able to impede speciation. Genetic incompatibilities arise as frequent by-products of adaptive evolution of traits determined by regulatory pathways, something that does not occur when phenotypes are modeled using the standard, additive genetic framework. Development therefore not only constrains the microevolutionary process, it also facilitates the interactions among genes and gene products that make speciation more likely—even in the face of strong gene flow.

Key words.—Adaptation, development, epistasis, gene regulation, population genetics, reproductive isolation.

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Hybrids between species often have reduced viability, fecundity, and mating success (Dobzhansky 1937; Mayr 1963; Coyne 1992; Arnold 1997). The genetic basis of low hybrid fitness, or postzygotic isolation, typically involves interactions among alleles at different loci, alleles that yield high fitness in their usual genetic backgrounds (Dobzhansky 1937; Muller 1942; Mayr 1963; Orr 1995; Gavrilets 1999; Johnson 2000; Johnson and Porter 2000; Turelli and Orr 2000). Dobzhansky (1937) and Muller (1942) both saw that postzygotic reproductive isolation could evolve via incompatibilities among genetic loci. Despite the long history of these models, little attention has been paid to the physiological bases of these incompatibilities or how they might evolve. However, regulated developmental pathways are a ubiquitous mechanism for translating the genotype into the phenotype (Raff 1996; Gerhart and Kirschner 1997; Lewin 1997) and are potentially a rich source of these genetic interactions (Johnson and Porter 2000). We believe the study of the evolutionary dynamics of regulated developmental pathways can provide important insights linking models of speciation with more general models of adaptation (Johnson and Porter 2001). Here we use these models to explore the extent to which speciation can occur despite gene flow.

The evolution of postzygotic isolation has long been presumed to require complete, or virtually complete, isolation of populations (Dobzhansky 1937; Mayr 1963). For neutral loci, even one individual exchanged between populations each generation is sufficient to prevent fixation of different alleles, and more than about five individuals per generation can effectively homogenize neutral allele frequencies (Wright 1931, 1969; Slatkin 1987). In the simplest, best-studied Dobzhansky-Muller models (Dobzhansky 1937; Muller 1942; Turelli and Orr 2000), an ancestral population with two-locus genotype *AABB* divides and diverges to genotypes *aaBB* and *AAbb* in separate populations, and postzygotic isolation occurs if genotypes having *a* and *b* allelic combinations (e.g., *AaBb*) have low fitness. Initial divergence from the *AABB* state presumably involves some form of genetic drift in the simplest Dobzhansky-Muller models because *AABB*, *aaBB*, and *AAbb* all have high fitness, making them effectively neutral to one another. Even minimal gene flow between such populations is expected to act against *a* and *b* alleles that occur simultaneously, uniting the populations' genomes and impeding divergence. Perhaps for this reason, prezygotic isolation is assumed to precede postzygotic isolation in most

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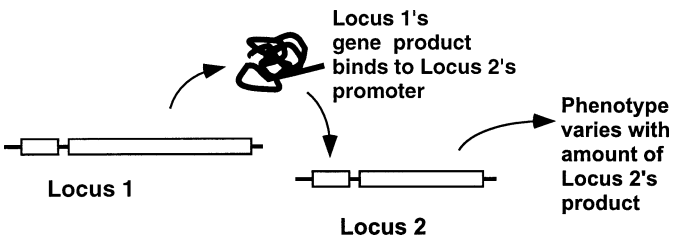

FIG. 1. Phenotype from a regulatory interaction. In the simplest, two-locus pathway, the gene product of the first locus binds to the promoter site of the second locus. The strength of binding determines the amount of gene product at the second locus, and this is determined algorithmically by matching decimal numbers that represent the two loci. In longer pathways, the gene product of the second locus then binds to the promoter of the third, and so on down the pathway. The phenotype is proportional to the amount of gene product made by the last locus.

sympatric and parapatric speciation models (e.g., Endler 1977; Higasi et al. 1999; Kondrashov and Kondrashov 1999). Here we show that, given selection favoring identical but new phenotypes in two populations, postzygotic isolation can evolve even in the face of substantial rates of gene exchange between populations. This process occurs in simple Dobzhansky-Muller models with this type of selection, but more importantly, it also occurs in models of developmental genetic regulation, which have the fundamental epistatic properties of Dobzhansky-Muller models but capture more effectively the mechanisms of physiological gene action.

Developmental genetic pathways can be viewed as rules for translating the genotype into the phenotype. They are present in all organisms but have not yet been well studied by population geneticists (but see Stern 2000; Johnson and Porter 2001; True and Haag 2001). In developmental pathways, genes have regulatory and product sites, and the product of one locus in the pathway interacts with a regulatory site of the next. The final phenotype is a consequence of the entire set of interactions along the pathway and pathways may branch and interconnect in various, almost arbitrary, ways. Here we study the simplest case of linear pathways with simple binding interactions (Fig. 1). Phenotypes with more complex binding interactions have been analyzed in Johnson and Porter (2000). We present these regulatory interactions as protein-DNA binding and use a simple matching function because *cis*-regulatory interaction is so fundamental to the relationship between genotype and phenotype. However, the model generalizes to physiochemical interactions among most types of genetically encoded molecules. These include protein-protein interactions, such as among enzymes and their cofactors, or among signaling molecules and membrane-bound receptors, and protein-RNA interactions such as occur during post-transcriptional processing.

In contrast to this developmental genetic model, most models used in population and quantitative genetics are additive or can be reduced to additivity by scaling. In these models, each gene contributes a small portion of the phenotype and these portions sum or multiply to the total. Developmental genetic models are derived from mechanistic rules discovered by developmental geneticists (Gerhart and Kirschner 1997), whereas additive models are descriptive (Falconer and MacKay 1996; Lynch and Walsh 1998), inspired by Fisher's (1918) statistical decomposition of the phenotype. Developmental genetic models also differ from holey adaptive landscape models (Gavrilets 1997, 1999; Gavrilets and Gravner 1997; Gavrilets et al. 1998, 2000a,b), where hybrid fitness drops from one to zero when an arbitrary threshold number of allelic differences between parents is crossed. However, as we discuss later (see Discussion), these models do share some interesting properties. In contrast, models of speciation via postzygotic isolation explicitly invoke epistatic interactions (Dobzhansky 1937; Muller 1942; Orr 1995; Turelli and Orr 2000). These models are designed to be general, so they are not explicit about how genetic incompatibilities evolve, and because they focus on speciation per se, they have stood largely independent of the mainstream literature on microevolutionary processes. Developmental genetic models provide a plausible context for Dobzhansky-Muller incompatibilities to occur and bridge this gap to the microevolutionary models used by population and quantitative geneticists.

We have shown (Johnson and Porter 2000) that low hybrid fitness ($w_{F1} < 0.001$) can evolve rapidly between two populations when the phenotype is constructed using developmental genetic pathways, but not when the phenotype is constructed using additive genetic models. Without gene flow in the simplest two-locus pathway, speciation occurs 50% of the time regardless of population size, and the probability increases to about 80% in the four-locus pathway (Johnson and Porter 2000). We found that rapid evolution of low hybrid fitness requires phenotypic evolution in the two populations, and it does not evolve when the populations are held under stabilizing selection. Selection, rather than genetic drift, is therefore the primary factor responsible for the differentiation that generated divergence in underlying genetic pathways. We have studied the more telling case where the two populations evolve in parallel to the same end phenotype, so that fitnesses in the two populations are comparable and hybrid incompatibilities are a result of gene interaction alone. Hybrids between populations adapting to different ecological conditions are expected to have the added fitness cost of deviating from both parental environments, thus increasing the strength of postzygotic isolation (Schluter 1998, 2000).

DEVELOPMENTAL PATHWAYS MODEL

We used the developmental genetic model in Johnson and Porter (2000), described below, extended to include the effects of gene flow on speciation. We analyzed the model using individual-based simulation. Two identical populations were established with two-, three- or four-locus genotypes that produce developmentally regulated phenotypes (Fig. 1). These represent the simplest pathways, where the promoter and product site of each allele is represented by a single number. Mutation changed these numbers with probability μ.

The strength of regulatory binding is modeled as a function of how closely the numbers match between product and promoter sites of adjacent loci in the pathway. The binding strength between adjacent loci i and j is $B_{ij} = \exp[-(product_i - promoter_j)^2]$. The binding strength is taken as the amount of locus j's gene product that is generated by the regulatory interaction; stronger binding yields more gene product. This generates a bell-shaped binding function with maximum

binding of 1.0 when the match is perfect. Not much is known quantitatively about how the distribution of possible mutations in the product or promoter site will affect binding. We believe it is reasonable as a first approximation to expect that small changes in the molecular structures will have small effects, and increasingly larger changes will have increasingly larger effects. The bell-shaped binding function captures this effectively and is mathematically convenient. Our conjecture is that the details of the shape will not have much qualitative effect on the evolutionary outcome as long as the binding function is unimodal and not too asymmetrical. We can make the allelic interactions more complex if we represent the promoter and product sites with more numbers. We find that including these extra dimensions has an effect similar to increasing the number of loci in the pathway (Johnson and Porter 2000), so we did not study the effects of this type of complexity here.

The final phenotype (P) from the pathway of n loci is found by multiplying the effects of each interaction, giving $P = \Pi_{i=1}^{n-1} B_{ij}$, where $j = i + 1$ is the next locus in the pathway. (Regulatory genetic pathways are different from metabolic flux pathways, where the amount of final product is limited by the slowest metabolic rate in the pathway.) The relationship is multiplicative because we expect the amount of product at each step of the pathway to proportionally affect the number of promoter sites bound at the next step. Each individual therefore can have a unique phenotype determined by its genotype. Under this model, phenotypic scores range from zero to one, which we interpret as the range from no expression of the phenotype to its maximal expression. This scale can readily be transformed to other measurement scales, such as sizes, rates, or counts, in which real traits are measured.

Gene exchange was implemented by giving each individual the probability m to move to the other population. Viability selection followed gene flow. We modeled fitness as a Gaussian function with optimum P_{opt} and variance Ω, using $w(P) = \exp[-(P - P_{opt})^2/2\Omega]$. Each individual i survived viability selection with probability $w(P_i)$ before joining the breeding pool. We allowed selection to operate identically in the two populations by changing the optimal phenotype by the amount ΔP_{opt} each generation. This produced gradual, directional selection, and the two populations were selected for identical new phenotypic optima. The value of Ω has little effect on the outcome as long as the mutation rate and effect size are scaled so that sufficient new mutants are available to track the changing phenotypic optimum. Otherwise, the failure to respond to directional selection can produce extinction in one or both populations.

We scored mean hybrid fitness as low when $\bar{w}_{F1} < 0.1$, and we interpreted this as a species boundary. This threshold accommodates the effect on mean hybrid fitness of occasional crosses involving new immigrants ''hybridized'' with members of their source population, which produce high-fitness progeny. We did not consider the cases where the populations evolved to different optimal phenotypes. We would expect more frequent speciation in these cases because low hybrid fitness could also be due to the absence of an appropriately intermediate environment. Our goal, however, was to study the effects of gene flow on speciation due to developmental genetic incompatibility alone.

TABLE 1. Mutation rate μ in the simulations with two loci in the developmental pathway. Rates were scaled to a genomic rate of 1.5 mutations·population^{-1}·generation^{-1} for each 1% change in optimal phenotype and a mutation effect size of 0.1 (SD), so that sufficient mutants were available to keep the populations from reaching extinction. N is the population size. The simulations with three loci, and therefore two pairwise interactions among loci, were scaled to one half these mutation rates, and the simulations with four loci used one third these rates.

ΔP_{opt}	$N = 125$	$N = 250$	$N = 500$
0.5%	0.006	0.003	0.0015
1%	0.012	0.006	0.003
2%	0.024	0.012	0.006

SIMULATION METHODS

We use an individual-based simulation with diploid, hermaphroditic, randomly mating individuals. Generations were discrete and the loci unlinked. Mutations changed promoter or product values to new decimal numbers with mutation effect sizes that followed a Gaussian distribution (mean = 0; SD = 0.1). We varied the population size (N) and probability (m) that an individual would migrate to the other population, using a pattern that allowed us to differentiate these from the effects of their product Nm, the number of migrants exchanged, which is another measure of gene flow. We studied the effects of two-, three- and four-locus, linear developmental pathways, initialized with allelic values yielding the maximal phenotypic score. We moved P_{opt} a total of 10 standard deviations of fitness at rate ΔP_{opt} in each run, so that each run lasted 500, 1000, or 2000 generations depending on ΔP_{opt}. In one set of runs, we held mutation rate and ΔP_{opt} constant and varied N and m. These runs were complicated by extinction, so in another set, we scaled the mutation rate so that there were about 1.5 mutations·population^{-1}·generation^{-1} for each 1% change in P_{opt} (Table 1). This held constant the ratio of genomic mutation rate to selection and still provided sufficient new mutants to avoid extinction as the optimal phenotype changed. See Johnson and Porter (2000) for further details and discussion of the role of mutation. Fifty F_1 hybrids were constructed in the last generation by randomly choosing one parent from each population, and hybrid phenotypes were assessed against the fitness function shared by the parents. From 150 to 500 replicates were run for each condition, with larger samples used to estimate lower rates of hybrid fitness reduction.

DOBZHANSKY-MULLER MODEL

We also studied the effect of gene flow on speciation probability in the Dobzhansky-Muller model. To facilitate comparison with the developmental pathways model, we made the fitness surfaces in the two models analogous. For the Dobzhansky-Muller model, we set initial fitnesses of $w(AABB) = w(AaBB) = w(aaBB) = w(AABb) = w(AAbb) = 1$, and the fitnesses of the remaining four genotypes were set to zero. To mimic the directional selection we used in the developmental pathways model, we reduced the fitness

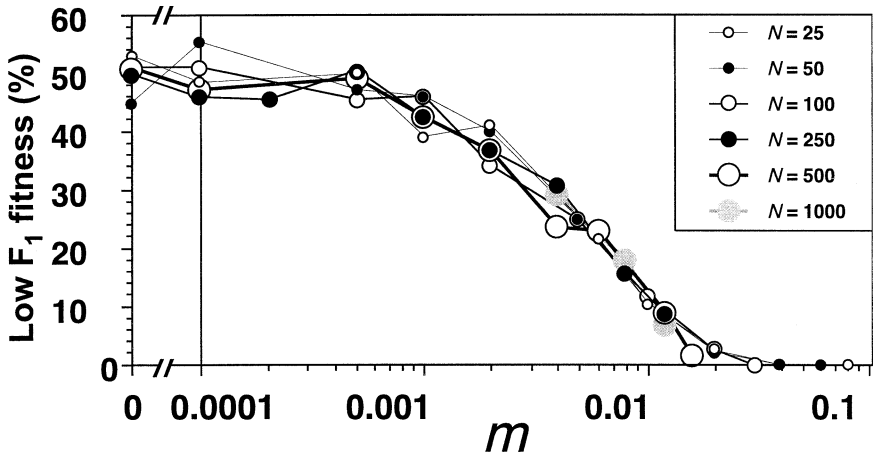

FIG. 2. Speciation in the face of significant gene flow in the Dobzhansky-Muller model. Percentage of 200 replicates showing low F_1 hybrid fitness ($\bar{w}_{F1} \leq 0.1$), equivalent to a species boundary, after 2000 generations. Standard deviations range from 3% to 5% throughout. There is no effect of *N* or *Nm*, but *m* is highly significant.

w(*AABB*) by subtracting a constant Δs each generation, and we reduced the fitnesses *w*(*AaBB*) and *w*(*AABb*) by subtracting $\Delta s/2$. This had the effect of gradually intensifying selection that favored *AAbb* and *aaBB* (phenotypically identical in that they had identical fitnesses). We initialized our simulations with populations fixed for *AABB* and allowed mutation $A \leftrightarrow a$ and $B \leftrightarrow b$ with probability $\mu = 0.003$. We set $\Delta s = 0.001$ and ran the simulations for 2000 generations at varying *N* and *m*. As above, we sampled 50 hybrid offspring and counted an outcome as a speciation event when hybrid fitness was $\bar{w}_{F1} \leq 0.1$.

RESULTS

In both models, postzygotic isolation evolved frequently even in the face of substantial gene flow. The Dobzhansky-Muller model yielded the simplest patterns so we present them first.

Dobzhansky-Muller model.—The proportion of migrants between populations, *m*, has a major influence on speciation probability. As *m* increases, the frequency of speciation decreases (Fig. 2). However, *N* has no effect alone or in its interaction with migration (logistic regression with three variables assessed simultaneously; *m*: $\chi^2_1 = 487.7$, $P < 10^{-107}$; *N*: $P = 0.81$; *Nm*: $P = 0.92$). The results are almost identical when counting speciation using $\bar{w}_{F1} \leq 0.001$ (not shown). Regardless of the threshold hybrid fitness used to define speciation, we see speciation at gene flow rates much higher than $Nm \approx 1$, the threshold rate sufficient to homogenize the neutral genome under a variety of migration patterns (Slatkin 1987). The highest rate shown here is $Nm = 12$ (Fig. 2; $N = 1000$, $m = 0.012$). This rate is considered virtually panmictic for neutral loci (Wright 1978), but it still permits speciation to occur 6.8% of the time under these Dobzhansky-Muller conditions. Because the Dobzhansky-Muller outcome depends only on *m*, speciation will result under arbitrarily high *Nm* if a large enough *N* is considered. Here the fitness of *AaBB* and *AABb* genotypes was reduced $\Delta s/2$ each generation, but we see similar outcomes when these heterozygous genotypes retain fitnesses of 1.0 throughout. In these cases the *A* and *B* alleles are sheltered in heterozygotes and remain frequent after 2000 generations and fewer cases of $\bar{w}_{F1} \leq 0.1$ are seen. However, the results appear almost identical to Figure 2 in these cases if outcomes with $\bar{w}_{F1} \leq 0.5$ are counted.

Developmental pathways model.—In the two-locus cases where mutation rate was held at $\mu = 0.003$ and $\Delta P_{opt} = 0.0025$ and selection was run for 2000 generations, significant effects of *m*, *N*, and *Nm* were found (logistic regression; *m*: $\chi^2_1 = 260.8$, $P < 10^{-57}$; *N*: $\chi^2_1 = 31.48$, $P < 10^{-7}$; *Nm*: $\chi^2_1 = 21.60$, $P = 3.36 \times 10^{-7}$). Speciation occurred less frequently in the smaller populations for a given gene flow rate *m* (Fig. 3a). These smaller populations were also under appreciable duress from the selection regime and limited by the availability of new favorable mutants (mutations·population^{-1}·generation^{-1} ranged from 0.075 to 0.75 across population sizes), as shown by their higher extinction probabilities (Fig. 3b). In these cases higher *m* generated a rescue effect by introducing favorable new genotypes. This forestalled extinction but also reduced speciation by making it more likely that the populations would share compatible genotypes. Regardless, as in the Dobzhansky-Muller model, the gene flow parameter *m* played the dominant role and speciation occurred with high *Nm* if the population size was large.

To control against the effect of extinction, we ran the two-locus case scaling the mutation rate to population size, so that there were about 1.5 mutations·population^{-1}·generation^{-1}. This kept the mean fitnesses of the populations above $w = 0.8$ for the great majority of generations and no extinctions occurred. Here both types of gene flow, *m* and *Nm*, had important effects but population size alone did not (logistic regression; *N*: $P = 0.59$; *m*: $P < 0.0001$; *Nm*: $P = 0.0011$). As *m* increased, the frequency of speciation decreased, and increasing *Nm* magnified the effect (Fig. 4a). Speciation was less frequent when the optimal phenotype changed more slowly (Fig. 4b). We again observed speciation at gene flow rates much higher than $Nm \approx 1$. The highest rate shown here is $Nm = 16$ (Fig. 4a; $N = 500$, $m = 3.2\%$), which still yielded speciation 4.4% of the time in the developmental pathways model. We have obtained similar outcomes at much lower mutation rates when we used larger populations or slower rates of phenotypic change, as long as sufficient new mutants are available to

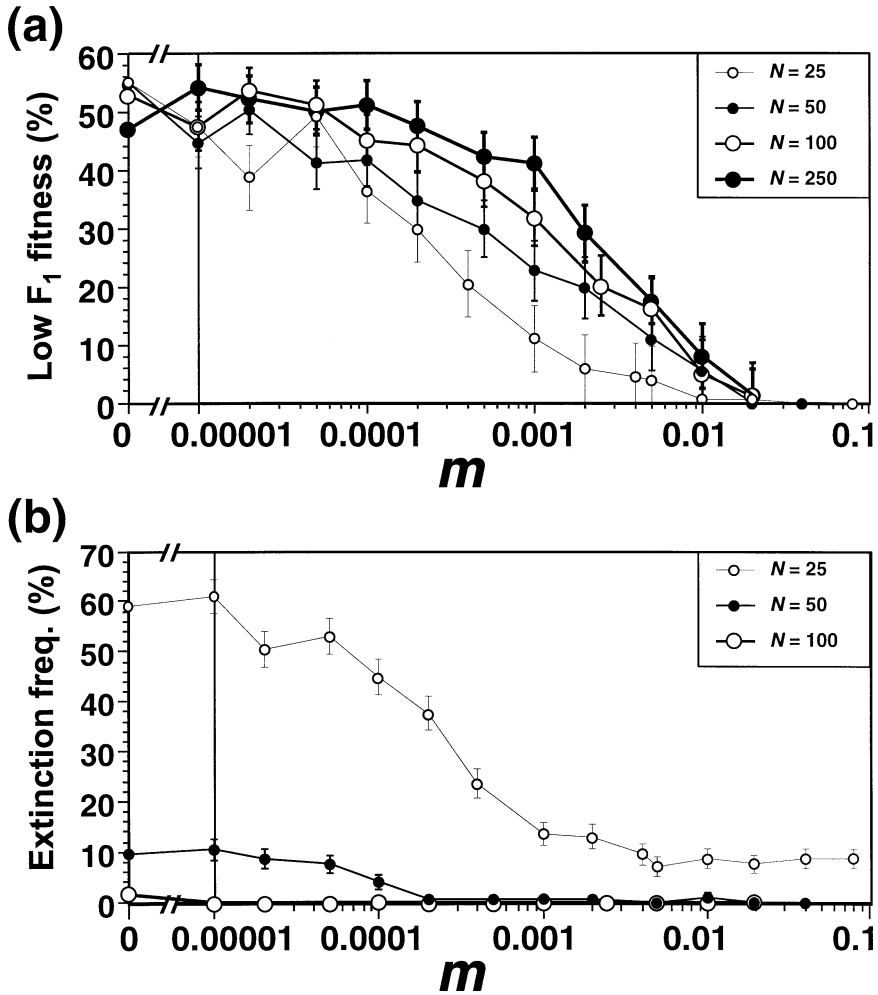

FIG. 3. Speciation in the face of significant gene flow in the two-locus developmental pathway. Percentage (SD) of replicates showing low F_1 hybrid fitness ($\bar{w}_{F1} \leq 0.1$), equivalent to a species boundary, after P_{opt} changed 10 standard deviations at rate =0.0025 and μ = 0.003. (a) Speciation as a function of the gene flow rate (*m*) for different population sizes (*N*). *N*, *m*, and *Nm* had significant effects. (b) Extinction occurred in the smaller populations and higher *m* rescued a greater proportion of the populations from extinction.

permit adaptation to the changing optimal phenotype (Johnson and Porter 2000).

If more loci were included in the developmental pathway, then whether the probability of speciation increased or decreased depended on the gene flow rate *m*. Without gene flow, the speciation rate was higher with more loci (Johnson and Porter 2000). However, with gene flow above the range of $m \approx 0.2$–0.5%, the speciation rate dropped to below that of the two-locus case as more loci were added to the pathway (Fig. 5) and increasing the number of loci magnified the effect. With three loci, speciation still occurred 6% of the time with $Nm = 3.0$ (Fig. 3; $N = 250$; $m = 1.2\%$), and with four loci it occurred in 2.4% of the trials when $Nm = 2.0$ (Fig. 3; $N = 250$; $m = 0.8\%$). Here again, gene flow reduced the probability of speciation. Even so, speciation still occurred with significant probability even at gene flow rates high enough to substantially mix the neutral genome.

DISCUSSION

In regulated genetic pathways, phenotypic and underlying genetic changes are not necessarily congruent. This provides a mechanism for generating evolutionary divergence that resists the homogenizing effects of gene flow. Examination of the underlying dynamics of our model shows that in it, replicate, isolated populations, evolving in parallel to identical new phenotypes, often adapt by changing their regulatory genotypic interactions in different ways (Johnson and Porter 2000). In hybrids, this genotypic divergence causes the genetic incompatibilities and low fitnesses that are characteristic of natural and laboratory crosses between separate species. Gene flow inhibits this divergence in proportion to the migration rate *m* by increasing the probability that the populations evolve to the same underlying genotypes.

When phenotypes are determined by developmental pathways, the interplay between gene flow, mutation, and selection has a strong effect on the probability of speciation. Capturing this relationship analytically is difficult because the relationship between ΔP_{opt} and selection strength is dynamic. The selection coefficient on any given allele is a function that depends on the explicit allelic interactions (Hansen and Wagner 2001a,b; Wade 2002) and the genetic makeup of the population, as much as it depends on the changing phenotypic

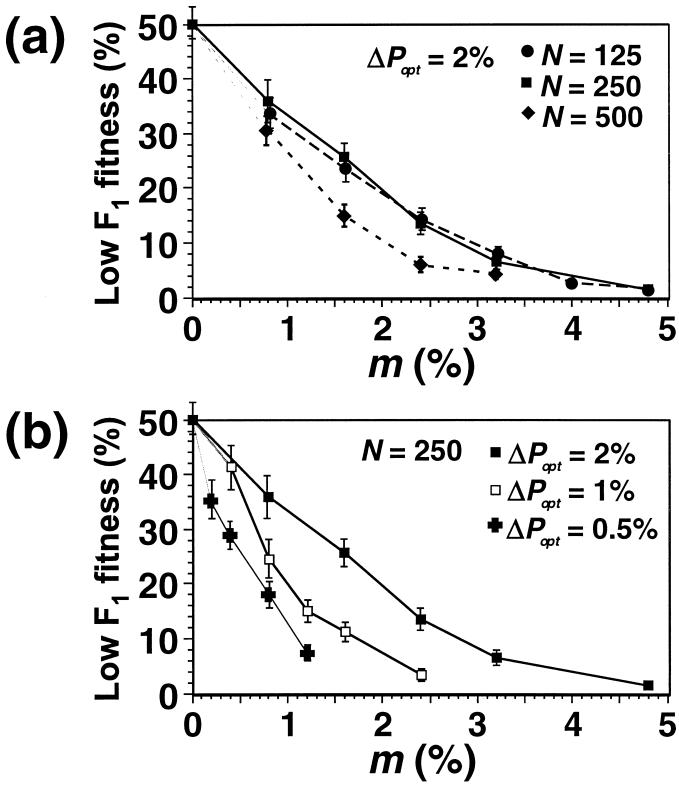

FIG. 4. Speciation in the face of significant gene flow in the two-locus developmental pathway, holding mutations·population^{-1}·generation^{-1} approximately constant to avoid complications with extinction. Percentage (SD) of replicates showing low F_1 hybrid fitness ($\bar{w}_{F1} \leq 0.1$), equivalent to a species boundary, after P_{opt} changed 10 standard deviations. The remaining replicates showed high F_1 hybrid fitness ($\bar{w}_{F1} > 0.8$). See Table 1 for mutation conditions. (a) Speciation as a function of the gene flow rate (m) for different population sizes (N). Low hybrid fitness occurs 50% of the time when gene flow is absent, the theoretical expectation when two loci interact (Johnson and Porter 2000). The effect of population size (N) alone is negligible but the product Nm significantly magnifies the effect of m. (b) Speciation is less frequent when the optimal phenotype changes more slowly.

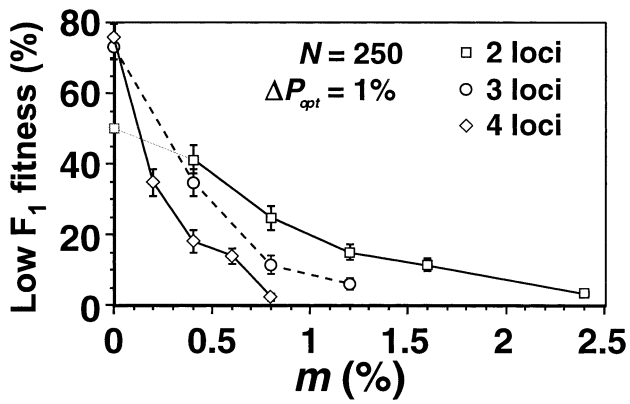

FIG. 5. Speciation in the face of gene flow in pathways of two to four loci. As in Figure 4, but note the different scales on both axes. Increasing the number of loci magnifies the inhibitory effect of gene flow but significant speciation probabilities are still found. The two-locus case is repeated from Figure 4b for comparison.

optimum imposed by the environment. However, some qualitative relationships can be described. In terms of the model's dynamics, the mean phenotype realized each generation depends on the accumulation of stochastically timed mutations of random effect. These mutations affect the phenotype by changing the binding affinities; that is, they regulate the phenotypic expression in a more favorable way. For any given regulatory interaction, there are two ways in the model to reduce the binding strength: The allelic value of the promoter can be reduced below that of the product site or vice versa. The outcome that is realized depends on which mutants arise early in the adaptive process (Johnson and Porter 2000). The genotypic fate of each population therefore ultimately depends on the adaptive value of the new alleles it acquires, either by new mutation or by gene flow from the neighboring population. This type of genetic redundancy—the same phenotype being achieved by different sets of interacting alleles—seems to be inherent in regulatory pathways (Stern 2000; Hansen and Wagner 2001a,b; True and Haag 2001) and has very different consequences from the genetic redundancy in additive genetic models where alleles at different loci have interchangeable effects (e.g., Goldstein and Holsinger 1992; Gavrilets 1997, 1999).

In both Dobzhansky-Muller and developmental pathway models, whether the populations diverge is likely to depend on whether gene flow can spread a new beneficial allele before a phenotypically redundant, but genetically incompatible, allele arises in the opposite population. Higher gene flow increases the probability that populations will share these new alleles, and such populations have a smaller chance to diverge. However, as more loci are added in the developmental pathway model, the contribution of a particular locus to the overall phenotype, via its binding interactions, is proportionally reduced. Selection on each locus is therefore also proportionally reduced, so that gene flow can more easily swamp a phenotypically redundant mutant. For a given selection strength, developmental pathways with more loci therefore have lower probabilities of divergence at high gene flow rates (Fig. 3). However, in those cases where sufficient divergence does accumulate, it is difficult to reduce it. Immigrant alleles then face negative frequency-dependent selection in the new genetic background and the probability of further divergence accelerates. However, if the population size is small enough and the mean fitness is low enough that the risk of extinction is extreme, then new immigrant genotypes may be able to increase in frequency and rescue the population from extinction despite initial frequency-dependent selection against them.

Small populations are not required for this mechanism of speciation to operate efficiently. The proportion of migrants m that has the greatest inhibitory effect on the speciation probability and N plays a secondary role when it is important at all. Population size is irrelevant in the Dobzhansky-Muller model when divergence to *aaBB* and *AAbb* genotypes is driven by selection. The importance of N in the developmental pathways model depends on selection strength, mutation rate, and perhaps other conditions as well. When mutation rate and selection strength were held constant, migration interacted with extinction probability to drive down differentiation in smaller populations and speciation was more likely

in larger populations (Fig. 4). When the number of mutants·population^{-1}·generation^{-1} was held constant, population size showed a small but significant inhibitory effect acting indirectly through *Nm*, the number of individuals exchanged between populations, and the speciation probability was somewhat higher in smaller populations. Taking into account the extinction probability when counting the proportion of speciation outcomes, speciation under directional selection is probably somewhat more likely overall in larger populations. This prediction from the developmental pathways model is parallel to predictions (Orr and Orr 1996) from the Dobzhansky-Muller model, but because of the increased extinction probability rather than any increase in the probability that nascent incompatibilities will arise.

We started our developmental pathway simulations at $P = 1.0$, corresponding to complete binding throughout the pathway. Starting at $P < 1.0$ (less than complete binding) substantially reduced the probability of speciation in the situation with no gene flow (Johnson and Porter 2000). This limitation became less important as we increased the complexity of each binding interaction (Johnson and Porter 2000), and it is not clear whether it is a limitation of our modeling strategy or a biologically meaningful constraint. The answer may depend on the physical chemistry of different binding motifs. Note that the key limitation here is whether initial binding in the pathway is complete, not whether the phenotype is maximally expressed. It is equivalent from a modeling standpoint to transform the regulatory system to a pathway of inhibitory interactions starting with complete binding and no expression of the phenotype ($P = 0.0$) and select for an increasing optimum phenotype.

Our model of speciation by microevolutionary divergence of regulated genetic pathways is based on several key observations and principles. First, regulated genetic pathways are a ubiquitous mechanism for building the phenotype from the genotype. Developmental regulation is therefore potentially a continual source of the gene interactions that have been implicated in the low fitness of hybrids (Dobzhansky 1937; Muller 1942; Orr 1995; Johnson 2000; Turelli and Orr 2000). Second, the loci that have been identified as responsible for low hybrid fitness or disruption of the hybrid phenotype are involved in molecular interactions, including the regulatory *Odysseus* (Ting et al. 1998) and *achete-scute* (Skaer and Simpson 2000) loci in *Drosophila* and the *bindin* gene expressed in sea urchin sperm (Palumbi 1999). Third, our results depend on selection to generate the divergence rapidly. We do not expect genetic drift alone to quickly produce significant genetic incompatibilities in regulatory interactions (Johnson and Porter 2000). Continual directional selection is likely to exert an influence on a broad range of traits, particularly those involved in arms-race dynamics that are characteristic of many biotic interactions (Rice 1998; Orr and Presgraves 2000). These include traits involved in sexual behavior and physiology, predatory and pathogenic interactions, and local intraspecific competition. Clearly, parallel directional selection may also lead to rapid change when the physical or biotic conditions change simultaneously over wide geographic areas, such as sustained climatic change or new interactions with invading alien species. It may also be found when peripherally isolated populations are relieved of the swamping effects of gene flow and allowed to adapt to local conditions (García-Ramos and Kirkpatrick 1997). Although directional selection is likely to often be continual under natural conditions, it seems unlikely that it will remain unidirectional for very long, especially where biotic interactions form the basis of selection. In a two-locus, two-allele Dobzhansky-Muller model where the universe of genotypes is so limited, changing the direction of selection often entails a return to the original genotypes and loss of postzygotic isolation. In developmental genetics models, the phenotype depends much less on the underlying allelic values, and it is quite plausible that postzygotic isolation will not be reduced even when the direction of selection is reversed; what matters most for enhancing postzygotic isolation is that selection continually operate to change the optimal phenotypes.

Our developmental genetic model of adaptation is also founded on the assumption that allelic variation in the loci that regulate development produces phenotypic effects that are continuous rather than binary. Clearly, there is variation among loci in the binding affinities of their promoter sites to transcription regulators (Ludwig et al. 2000; Stern 2000). For example, the regulatory Bicoid protein of *Drosophila* binds to a *hunchback* translational (RNA) promoter with high affinity but to the head gap promoter with low affinity (Chan and Struhl 1997). Similar variation in affinity can be expected for DNA binding (Arnone and Davidson 1997). The regulatory effects of binding-site interactions are likely to depend on the physiochemical properties of proteins and nucleotide sequences at and near the binding sites and will therefore be subject to numerous subtle variations in response to nucleotide and amino acid replacements, concentration, pH, temperature, and other factors.

The developmental genetic model captures key properties of the Dobzhansky-Muller model (Dobzhansky 1937; Muller 1942; Orr 1995; Turelli and Orr 2000) of speciation by genetic incompatibility, but there are important differences. First, although developmental genetic models yield speciation in very much the same way that the simplest two-locus, two-allele Dobzhansky-Muller models do, they do not have the same underlying allelic structure. If we use a modified version of the Dobzhansky-Muller syntax for designating alleles in our developmental genetic model, then speciation results when the respective populations diverge from *AABB* to *aabb* and $a'a'b'b'$, and low fitness results when hybrid individuals are produced with $a'b$ and ab' allelic combinations. Genotypes *aabb* and $a'a'b'b'$ had the same phenotypes in our simulations, but this would not be necessary if ecological speciation is considered.

The second important difference from the Dobzhansky-Muller model is that the developmental genetic model is multiallelic and considers gene interactions to be quantitative in their effect rather than qualitative. In our simulations, populations passed through many consecutive allelic substitutions to track changing optimal phenotypes across 10 standard deviations of fitness. The *A* versus *a* or a' and *B* versus *b* or b' alleles of the Dobzhansky-Muller syntax are therefore analogous to beginning and ending allelic states along a continuum of changing states in the developmental genetic model. In this respect, the continuous-state allele form of the developmental genetic model share a property of the holey

landscapes models of Gavrilets et al. (1998), in that it permits a continuum of high-fitness intermediate states between genotypically divergent populations.

Reproductive isolation also evolves in the face of gene flow in the holey adaptive landscape models (Gavrilets et al. 1998), but unlike our developmental genetic models, reproductive isolation also evolves readily in them without directional selection. We suggest some reasons for these similarities and differences. Holey landscape models also capture important properties of the Dobzhansky-Muller model. Here, the mating phenotype has an additive genetic basis and reproductive isolation occurs when the allelic difference between prospective mates crosses an arbitrary threshold. Fitness is a step function: $w = 0$ below the threshold, additive genotype or $w = 1$ above it. This is in contrast to our fitness model, which is bell shaped. The flat-topped fitness function allows a wide variety of neutral mutants to accumulate by drift, provided the populations are initialized with genotypes relatively far from the fitness threshold. The fitness landscape of the multilocus, holey-landscape model is a multidimensional lacework of high-fitness ridges and low-fitness holes. As the threshold level of genetic variation is approached in the holey landscapes model, negative frequency-dependent selection comes into play as many of the multilocus genotypes become incompatible. This frequency dependence acts to preserve and perhaps even magnify nascent incompatibilities among populations, because it purges local populations of their rarer alleles. Our bell-shaped fitness function treats the majority of new mutants as mildly deleterious, so that under stabilizing selection, allelic replacements are much rarer. Mutation and genetic drift alone were insufficient to cause the divergence necessary for reproductive isolation (Johnson and Porter 2000). Under directional selection, the allelic replacements that do occur are the ones favored by selection. This may be the explanation for the difference in the roles of genetic drift in the two models.

The holey adaptive landscape models also embody a nonspecific, diffuse form of epistasis that is very different from the epistasis explicit in the developmental genetic model. In the holey landscape model, any pair of loci can be compatible in particular contexts, and genetic incompatibilities of the sort that deter hybridization are a consequence of a threshold level of genomewide differentiation. Gene flow therefore introduces alleles that, taken individually, are in most cases intrinsically compatible in the new genetic background. Selection acts mainly against the whole immigrant genotype, but if breeding is successful, selection becomes successively weaker on alleles as they recombine into the new genetic background. Partial barriers can be swamped by introgression (Gavrilets et al. 2000b), but this is by no means inevitable. If selection is strong enough to nearly eliminate breeding by the immigrants, then introgression rarely occurs. Population-level divergence can proceed in the face of gene flow rates of $Nm \approx 3$ individuals·generation^{-1} (Gavrilets et al. 1998). This is very similar to the underlying dynamics of prezygotic isolation (e.g., Kondrashov and Kondrashov 1999), even though the holey landscape models are general enough that they can apply to pre- or postzygotic isolation.

In the developmental pathway model of speciation, divergence in the face of gene flow occurs for a different reason. Here, the epistasis is explicit and mostly in the form of direct interactions (some compensatory mutation can occur in different parts of longer pathways, an indirect form of epistasis, but we have not seen much effect of this; Johnson and Porter 2000). Immigrants are as fit as residents phenotypically, but frequency-dependent selection acts increasingly strongly against alleles as they filter by recombination into the new genetic background. If selection against these introgressing alleles is strong enough, then the immigrant lineages go extinct and evolution toward greater genetic incompatibility proceeds in the face of gene flow. This dynamic is difficult to apply to a prezygotic isolation scenario, but it fits well to that expected of postzygotic isolation.

Muller (1942) was very cognizant, almost apologetic, of the fact that the simplest two-locus, two-allele model was likely to be a naive oversimplification of real genetic mechanisms that underlie the evolution of postzygotic isolation; his goal was to merely identify the logically essential elements of this process. Indeed, it is difficult for us to imagine, from a molecular genetic perspective, a realistic system of functioning genes that that would be effectively described by the simplest Dobzhansky-Muller syntax. We expect that developmental genetic models, with increasingly sophisticated mechanisms for translating genotype to phenotype and then to fitness, will continue to capture the fundamental dynamics of Dobzhansky-Muller speciation models (Johnson 2002). However, they will not ultimately be ''Dobzhansky-Muller models'' in any strict sense, because Dobzhansky-Muller models are only of real use in thinking about the evolution of postzygotic isolation. Developmental genetic models will be useful for thinking about how physiologically based systems of interacting genes are affected by microevolutionary processes that cause population differentiation and adaptation, as well as reproductive isolation.

Development has long been known to act as a constraining force on evolutionary change, in that it limits the range of phenotypes that new mutants may exhibit (Wake et al. 1983; Maynard Smith et al. 1985; Raff 1996). Our results show that development can also act, equally importantly, as a creative factor in evolutionary change. Regulatory genetic interactions are the essential feature of developmental systems. When regulated traits evolve, they must certainly do so in large part by modifying the regulatory interactions. There are likely to be several ways to modify a given molecular interaction to get a given phenotypic result, and selection acts only on the outcome. Development therefore provides a context for cryptic divergence in the allelic basis of regulatory interactions, and this divergence creates the Dobzhansky-Muller incompatibilities commonly seen between species. Reproductively isolated populations are free to find their own evolutionary trajectories, thus increasing the overall diversity that evolution produces. Without this regulatory genetic context provided by development, we might see much less biological diversity.

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