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LABORATORY EXPERIMENTS ON SPECIATION: WHAT HAVE WE LEARNED IN 40 YEARS?

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Abstract.—We integrate experimental studies attempting to duplicate all or part of the speciation process under controlled laboratory conditions and ask what general conclusions can be made concerning the major models of speciation. Strong support is found for the evolution of reproductive isolation via pleiotropy and/or genetic hitchhiking with or without allopatry. Little or no support is found for the bottleneck and reinforcement models of speciation. We conclude that the role of geographical separation in generating allopatry (i.e., zero gene flow induced by spatial isolation) has been overemphasized in the past, whereas its role in generating *diminished* gene flow in combination with strong, discontinuous, and multifarious divergent selection, has been largely unappreciated.

Key words.—Allopatric speciation, genetic revolutions, nonallopatric speciation, population bottleneck, postzygotic isolation, prezygotic isolation, reinforcement, reproductive isolation, speciation.

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Beginning in the 1950s and continuing to the present, many researchers have set out to duplicate all or part of the speciation process under controlled laboratory conditions. Here we attempt to integrate these studies and ask what we can conclude about the major models of speciation. Speciation via polyploidy, which appears to be common in plants (White 1978), and other chromosomal mechanisms are not discussed here. The extensive body of purely theoretical work on speciation is deemphasized. Instead we focus on inferences deduced from experimental studies. Throughout we define species via the biological species concept, that is, “groups of interbreeding natural populations that are reproductively isolated from other such groups” (Mayr 1963, p. 19).

To define and integrate the major models of speciation, we begin with the “basic allopatry” or geographical model of speciation (summarized in Mayr 1963). In this model, a species range becomes dissected into two parts by a

physical barrier (mountain range, river, etc.), which prevents gene flow between them. The populations are presumed to evolve independently because of the allopatry induced by their physical isolation. Genetic divergence accrues as a result of adaptation to the prevailing environmental conditions and by means of sampling drift. Prezygotic (i.e., positive assortative mating that reduces the production of hybrids) and postzygotic (i.e., reduced viability and/or fertility of hybrids) reproductive isolation develop between the physically isolated populations as an incidental byproduct of genetic differences that gradually accrue between them. Once pre- and/or postzygotic isolation is complete, speciation has occurred.

The three other major modes of speciation include the reinforcement, divergence-with-gene-flow, and bottleneck models. All of these can be expressed as simple modifications of the basic allopatry model.

In the *reinforcement model*, articulated in large

part by Dobzhansky (1937), it is presumed that the physical barrier breaks down before complete reproductive isolation has evolved in allopatry. Heterotypic matings between previously separated subpopulations are presumed to produce low-fitness hybrid offspring, and this selects for positive assortative mating. If this selection is successful and leads to complete prezygotic isolation, then the speciation that began in allopatry is completed despite renewed gene flow between subpopulations.

In the *divergence-with-gene-flow model* (reviewed in Maynard Smith 1966; Endler 1977; Felsenstein 1981), the physical barrier to gene flow is incomplete or absent altogether. When different regions, habitats, or niches within the species range have sufficiently different selection regimes, local, habitat-, or niche-specific adaptation can develop despite substantial gene flow (e.g., Endler 1973, 1977). Reproductive isolation is hypothesized to develop gradually between the genetically differentiated subpopulations and ultimately lead to speciation.

The divergence-with-gene-flow model is actually a family of models that can be viewed as a spectrum between two extremes. At the left extreme of the spectrum (sympatry), there is a single population in a homogeneous environment with simultaneous selection for two opposing phenotypes. At the right extreme of the spectrum (parapatry), there is a sharp discontinuity in selection between a pair of divergently selected, geographically separated subpopulations. Here selection is directional within each subpopulation but disruptive when both subpopulations are viewed as a whole. At the center of the spectrum (cline), there is a gradual change in selection along a geographical gradient, and opposing phenotypes are favored at each end. Endler (1977) has extensively reviewed this context. Almost all of the experimental work on the evolution of reproductive isolation examines the two extreme portions of the spectrum (sympatry and parapatry), and here we focus exclusively on these forms of the divergence-with-gene-flow model.

The *bottleneck speciation model*, summarized in Mayr (1970) and Carson and Templeton (1984), actually represents three mechanistically distinct models that share the feature that speciation is initiated when a population passes through a major reduction in population size (bottleneck), such as would occur when a single inseminated female colonizes an isolated island

and gives rise to a new population. One of several processes are hypothesized to lead to a "genetic revolution" (i.e., a major reorganization of the genome), which in and of itself leads to complete or virtually complete reproductive isolation from the original population.

Below we integrate laboratory studies that attempt to duplicate all or part of these proposed speciation mechanisms. We make no attempt to cite every study germane to each topic, but instead we use representative examples and cite any cases that we are aware of that contradict the generalizations we make. In the discussion, we ask if there is a strong consensus among experiments concerning the feasibility of each proposed speciation model.

THE BASIC ALLOPATRY MODEL

The major prediction to be tested concerning the basic allopatry model is that sampling drift and/or adaptation to different environments can lead to genetic differentiation that produces incidental reproductive isolation. Substantial experimental evidence bears on this prediction.

Sampling Drift.—One simple way to determine the potential for sampling drift to generate reproductive isolation among isolated populations is to look for pre- and postzygotic isolation among inbred lines. We have found no reports of hybrid inviability or sterility in crosses between different inbred lines of *Drosophila* species; however, prezygotic isolation has been observed. For example Keref-Santibanez and Waddington (1958) inbred six lines of *D. melanogaster* by repeated brother-sister matings for 57 generations. They observed weak positive assortative mating in two lines, weak negative assortative mating in one line, and no statistically significant, consistent trend in the remaining three lines. Powell and Morton (1979) inbred (brother-sister mated) 13 lines of *D. pseudoobscura* for up to 12 generations and found no statistically significant prezygotic isolation among them. In contrast, Auerhoff and Richardson (1974) found that increased levels of inbreeding led to increased levels of negative assortative mating in *D. melanogaster*. Both positive and negative assortative mating were found among inbred lines of laboratory mice (Yamazaki et al. 1978). Ahearn (1980) claimed to have found prezygotic isolation between two isofemale lines of *D. silvestris*, one of which had undergone two major bottlenecks under laboratory culture. Reanalysis of the data, however, indicates that no statistically significant

positive assortative mating developed ($P = 0.12$; 2×2 contingency test). Overall studies of mating among inbred strains suggest that sampling drift can both contribute to or detract from isolation among populations.

Divergent Selection and Prezygotic Isolation.—Besides sampling drift, genetic differentiation in response to divergent selection among allopatric populations can lead to reproductive isolation as a correlated response via incidental pleiotropy or genetic hitchhiking, that is, sampling error-induced linkage disequilibrium between alleles affecting the divergently selected character(s) and alleles affecting positive assortative mating. In practice, it is usually impossible to differentiate between pleiotropy and genetic hitchhiking, thus we pool these two causative factors and refer to them collectively as “pleiotropy/hitchhiking.”

Many experimental studies have looked for isolation as a correlated response to divergent selection. For example, Burnet and Connolly (1974) divided a founder stock of *D. melanogaster* into three groups. The first and second were selected for increased and decreased locomotor activity, respectively, and the third was an unselected control. After 112 generations, the selected groups manifest markedly divergent locomotor activity, in the selected directions, whereas the controls remained unchanged. When the lines selected for increased or decreased activity were tested for nonrandom mating, a 50% excess of homotypic mating was observed (i.e., the percentage of homotypic matings was about 75 instead of the random-mating expectation of 50). In a similar type of study using a *Musca domestica* (common house fly) model system, Hurd and Eisenberg (1975) selected for positive and negative geotaxis. After 16 generations of divergent selection under allopatric conditions, a response to selection in the appropriate direction was found in both the positive and negative selection lines. When positively and negatively selected lines were tested for prezygotic isolation, a 60% excess of homotypic mating was observed.

Negative results also have been reported. For example, a 45-generation study by van Dijken and Scharloo (1979) on locomotor activity in *D. melanogaster* (i.e., the same trait selected by Burnet and Connolly above) found no persistent deviation from random mating between the populations selected for high activity or low activity. Negative results in *Drosophila* studies were also found by Koref-Santibanez and Waddington

(1958), Ehrman (1964, 1969), and Barker and Cummins (1969), where divergent selection in allopatry was applied to bristle number, temperature, and bristle number, respectively.

When we surveyed 14 studies from the literature in which divergent selection was applied to allopatric populations and then a measure was taken for the development of prezygotic isolation, we were surprised to find such a large excess of positive results (10 positive to 4 negative; part A of table 1). While allowing for the fact that negative results are less likely to be published, it still remains clear that it is not unusual to find prezygotic isolation as a fortuitous byproduct of adaptation to divergent selection regimes.

One issue in studies such as those outlined above is the degree to which isolation is the result of sampling drift that occurred while the populations were being selected in allopatry, versus isolation, which is a byproduct of genetic difference built up because of divergent selection among populations. In most of the studies that we surveyed, it was impossible to tease these two factors apart, but in two studies it was possible (part B of table 1).

Kilias et al. (1980) collected two base populations from different geographical localities in Greece. Each of these was split into two allopatric populations, one of which was reared under cold-dry-dark conditions, the other under warm-moist-light conditions. After 5 yr of adaptation under allopatry, divergently selected populations derived from the same or different original base populations showed prezygotic isolation (about a 50% excess of homotypic matings relative to the random mating expectation) but parallel-selected populations experiencing the same environmental conditions showed no isolation. If sampling drift were a major factor leading to prezygotic isolation, then prezygotic isolation should have accrued between allopatric populations experiencing both divergent and parallel selection.

Because isolation was found only among divergently selected populations, this study supports the idea that pleiotropy of the selected variation itself, or tightly linked variation, was responsible for the development of prezygotic isolation. A similar finding of prezygotic isolation among divergently selected but not among parallel-selected lines was found by Dodd (1989) for *D. pseudoobscura* populations adapted to high-starch or high-maltose environments. These two studies suggest that the prezygotic isolation that

TABLE 1. Prezygotic isolation experiments grouped by method.

Study	Prezygotic reproductive isolation?
Part A: divergent selection in allopatry	
Koref-Santibanez and Waddington 1958	No
Ehrman 1964, 1969	Yes/No, inconsistent across samples
del Solar 1966	Yes
Kessler 1966	Yes, but asymmetrical
Barker and Cummins 1969	No
Grant and Mettler 1969	Yes
Burnet and Connolly 1974	Yes
Soans et al. 1974	Yes
Hurd and Eisenberg 1975	Yes
van Dijken and Scharloo 1979	No
de Oliveira and Cordeiro 1980	Yes
Kilias et al. 1980	Yes
Koepfer 1987	Yes, but asymmetrical
Dodd 1989	Yes
Part B: parallel selection in allopatry	
Kilias et al. 1980	No
Dodd 1989	No
Part C: divergent selection with hybrid inviability in sympatry (destroy hybrids experiments)	
Koopman 1950	Yes
Wallace 1953	Yes, but transient
Knight et al. 1956	Yes
Kessler 1966	Yes
Paterniani 1969	Yes
Ehrman 1971, 1973, 1979	Yes, but complex pattern across years
Barker and Karlsson 1974	Yes
Crossley 1974	Yes
Dobzhansky et al. 1976	Yes
Part D: divergent selection with hybrid viability in sympatry	
Thoday and Gibson 1962	Yes
Grant and Mettler 1969	No
References (18 experiments) cited in Thoday and Gibson 1970 and Scharloo 1971	No, 18 of 18 experiments
Spiess and Wilke 1984	No
Part E: divergent selection with hybrid viability in sympatry and with isolation via pleiotropy	
Coyne and Grant 1972	Yes, in one of two replicates
Soans et al. 1974	Yes
Hurd and Eisenberg 1975	Yes
Rice 1985	Yes
Rice and Salt 1988, 1990	Yes

develops between divergently selected allopatric populations is largely due to the pleiotropy of genes built up directly via selection or indirectly via tight linkage and genetic hitchhiking.

Divergent Selection and Postzygotic Isolation.—Two major contexts for postzygotic isolation exist. The first is unconditional and occurs when hybrids between divergently selected lines have lowered viability and/or fertility under benign conditions. The second is environment-dependent and occurs whenever hybrids have an intermediate phenotype that is selectively inferior in specific environmental contexts.

It is commonplace for hybrids (from the F_1 and many offspring from backcrosses, the F_2 , F_3 , etc.) between divergently selected lines to have an intermediate phenotype (for review, see Falconer 1981), and this will lead to environment-dependent postzygotic isolation whenever populations in different habitats or regions become differentiated because of divergent selection (see below). This type of isolation, though intuitively obvious, is rarely measured in laboratory studies, owing to the difficulty in duplicating divergent, multifarious natural selection. Most laboratory studies measure viability and fecundity only un-

der benign conditions, and therefore will overlook environment-dependent postzygotic isolation and cause this form of isolation to be unappreciated, despite its potential importance in nature.

We found only two studies that looked for both environment-dependent and unconditional postzygotic isolation in response to divergent selection in allopatric populations. de Oliveira and Cordeiro (1980) applied divergent selection for pH tolerance in allopatric populations of *D. willistoni* for 122 generations (high and low treatments with a control maintained at medium pH). As expected from prior studies of quantitative traits (Falconer 1981), hybrids had reduced fitness (as measured by offspring produced per mated pair) under the demanding conditions of high and low pH, and this supports the conclusion that environment-dependent postzygotic isolation evolved. Hybrids also had reduced fitness under the more benign conditions of moderate pH, however, suggesting that unconditional postzygotic isolation also evolved.

In the second study, Robertson (1966a,b) selected for tolerance to the toxin ethylenediaminetetraacetate (EDTA) in a replicated set of six populations while retaining the original unselected stock. F₁ hybrids between selected lines and the control had reduced values for a variety of fitness characters when challenged with EDTA, but these hybrids also had reduced fitness under the more benign conditions of EDTA-free food. Chromosomal substitution analysis indicated that all of the major chromosomes contributed to adaptation to EDTA and that substitution of the third pair of chromosomes into the background of the EDTA-adapted strain caused complete sterility of females on all diets tested and lethality of both sexes at high-EDTA conditions. This study supports the idea that both environment-dependent and unconditional postzygotic isolation can evolve as a correlated response to adaptation to new environmental conditions.

We found additional evidence for unconditional postzygotic isolation when we reanalyzed the results of Ringo et al. (1985), in which *D. simulans* populations were sequentially selected for three different suites of traits. In this work, 25 of 216 tests for postzygotic isolation were individually statistically significant ($P < 0.05$), whereas only 5% (10.8) would be expected by chance. This excess of individually significant tests is collectively statistically significant (binomial P -value of 0.0006).

The previously described study by Kilius et al. (1980) also tested for postzygotic isolation. Recall that in this study highly significant prezygotic isolation was found among allopatric populations that were selected for different moisture-temperature-humidity conditions. No net postzygotic isolation was detected among their allopatric populations, although some components of fitness suggested that low levels of postzygotic isolation may have developed.

As an aside, we point out that positive heterosis is commonly observed between highly inbred lines (Falconer 1981). To the extent that divergent selection reduces effective population size and leads to inbreeding depression, heterosis can reduce postzygotic isolation by at least partially offsetting any reduced level of environment-dependent adaptation of hybrids.

We found no other explicit studies testing for postzygotic isolation among divergently selected allopatric populations. As a result, it is difficult to make any generalizations concerning the relative frequency with which this form of isolation develops in laboratory studies. All we can observe is that in three of the four cases in which unconditional postzygotic isolation was sought, it was found.

Overall, laboratory studies strongly support the conclusion that prezygotic and environment-dependent postzygotic reproduction isolation can readily develop as a fortuitous byproduct of pleiotropy or hitchhiking associated with genes that adapt populations to different environmental conditions. Limited support for unconditional postzygotic isolation is also present. Drift alone may play a role in the development of such coincidental reproductive isolation, but the experimental evidence for this is quite meager.

THE REINFORCEMENT MODEL

The observational basis for suspecting that reinforcement is an important speciation mechanism is remarkably compelling: it is common to observe stronger levels of prezygotic isolation in areas where a pair of closely related species have overlapping ranges, compared with the same comparison when the species are sampled from nonoverlapping portions of their ranges. We found so many published records of this pattern occurring in species ranging from *Drosophila* (see also Coyne and Orr 1989) to fish that there seems little doubt the pattern is general. Several examples include crickets (Otte 1989), frogs (Blair 1974), fruit flies (Ehrman 1965; Wasserman and

Koepfer 1977), damselflies (Waage 1979), and fish (Hubbs and Delco 1962).

These observations certainly are consistent with the idea that prezygotic isolation has evolved to prevent the production of low-fitness hybrid offspring. The logical jump between the observational data and the conclusion that the reinforcement model of speciation is in fact operating is made tenuous for three reasons: (1) there are other biological explanations for the observed pattern (Butlin 1989), (2) there are strong theoretical objections to the reinforcement models (Felsenstein 1981), and (3) no repeatable laboratory experiments have been able to duplicate even the early stages of the reinforcement model.

One group of laboratory studies that has been used to support the reinforcement model are the numerous "destroy-the-hybrids" experiments, typically carried out with *Drosophila* species (part C of table 1). Many variations of the experimental design exist, but the basic protocol is to collect equal numbers of male and female virgins from each of two genetically marked strains. These are held separately until sexually mature and then mixed in a common mating chamber and finally allowed to produce offspring. Through the use of genetic markers, offspring can be classified as being derived from homotypic or heterotypic matings, and from the former a new set of males and females is collected and treated as described above. Repeated cycles of the protocol generate strong, multigenerational selection for homotypic mating. Almost all of the experiments of this kind that we have located in the literature report the evolution of increased prezygotic isolation between the selected strains (for a notable exception, see Robertson 1966a).

These studies clearly indicate that most *Drosophila* laboratory populations have the requisite additive genetic variation for the evolution of homotypic mating. However, because all of the hybrids are destroyed each generation, these studies do not truly test the reinforcement model. The protocol therefore simulates the case in which speciation *already has been completed* via postzygotic isolation and asks if prezygotic isolation will follow. The key "ingredient" missing is gene flow between the strains.

What happens when gene flow is permitted? One of the most extensive attempts to replicate the reinforcement model in the laboratory is the effort described by Wallace (1982) and Ehrman et al. (1991). Several different subexperiments were nested within their experimental design, but

the main protocol was the following. A base population of *D. melanogaster* was split into two allopatric populations. One population was allowed to adapt to increasing levels of NaCl, whereas the other was allowed to adapt to increasing levels of CuSO₄. After 3 yr in allopatry, the populations showed substantial adaptation to the two environmental toxins. Next, samples from the divergently selected populations were mixed to simulate secondary contact between formerly allopatric populations adapted to different environmental conditions. In the secondary contact cages, two types of food were available: vials with high levels of NaCl and those with high levels of CuSO₄.

After 4 yr of sympatry, no prezygotic isolation was observed between the subpopulations using the NaCl and CuSO₄ food types. Adaptation (i.e., resistance) to the two salts was reduced in the sympatry treatment, perhaps because the divergently adapted lines interbred and/or because females did not preferentially lay eggs on the food type to which they were adapted [oviposition preference was not measured in the experiments (B. Wallace, pers. comm. 1993)]. Although some positive assortative mating was found in one replicate of another related experiment, the collective results led Ehrman et al. (1991, p. 206) to conclude that "there was no clear evidence for incipient reproductive isolation." We found these very complicated experiments difficult to interpret, but they nonetheless represent the most ambitious, long-term attempt to simulate the reinforcement model.

A conceptually similar study examining secondary contact between *Drosophila* populations initially adapted to different environmental conditions (EDTA and EDTA-free food) was done by Robertson (1966a). These experiments were much simpler than those described above and simulated parapatry with restricted migration (via narrow tubes connecting large population cages) between adjacent, divergently selected populations. Gene flow reduced, but did not eliminate, environment-specific adaptation compared with allopatric controls. No prezygotic reproductive isolation could be detected after 20 generations of secondary contact. Because a 15-generation "destroy-the hybrids" experiment was done in parallel with these parapatry experiments (with the same divergently selected stocks but with quite small sample sizes), and because no isolation was detected in this zero-gene-flow study, it is not clear if the requisite additive genetic variance for

positive assortative mating was available at the outset of these experiments.

A second, more extensive group of experiments testing the reinforcement model (part D of table 1) are the large collection of "disruptive-selection experiments" that accrued after the remarkable results of Thoday and Gibson (1962). Thoday and Gibson's protocol applied strong disruptive selection to an arbitrary character, bristle number, and then asked if prezygotic isolation would develop to prevent the production of the low-fitness offspring that resulted from heterotypic matings between the extreme types. Remarkably, after only 12 generations of strong disruptive selection on bristle number, complete prezygotic isolation was observed between the high-bristle-number and low-bristle-number selected lines. This experimental outcome would appear to provide strong experimental support for the reinforcement model.

Thoday and Gibson's results were so striking that laboratories around the world set out to repeat them. These results are reviewed in Thoday and Gibson (1970) and Scharloo (1971). They reported that all attempts to repeat the experimental outcome with new stocks have failed. The one thing that is repeatable about Thoday and Gibson's experimental protocol is that it does not lead to prezygotic isolation.

The one apparent exception to this pattern of the nonrepeatability of Thoday and Gibson's experimental outcome is the work of Barker and Karlsson (1974). They applied disruptive selection to bristle number at two levels: moderate, in which the upper and lower 25% of individuals were selected for breeding, and high, in which the upper and lower 1% of individuals were selected. No prezygotic isolation was observed in the moderate selection treatment. Low levels of isolation (statistically significant, although highly erratic) accrued, however, in the high selection treatment throughout 17 generations of disruptive selection.

By carefully screening the distribution of offspring from all females used in their study, Barker and Karlsson could demonstrate that after the second generation all parents used to generate subsequent generations were derived from homotypic matings (i.e., all hybrid offspring were discarded because of the high level of selection applied). The one disruptive selection study that would appear to lend support to Thoday and Gibson's experimental result turns out to be a "destroy-the-hybrids" experiment by virtue of

the extremely strong level of disruptive isolation that was applied. The outcome is therefore fully consistent with prior studies: prezygotic isolation accrues only when gene flow is absent between the selected lines.

As an aside, Barker and Karlsson's study suggests a simple explanation for why Thoday and Gibson's original study (and a subsequent replication with the same starting stock, described in Thoday 1964) may have led to complete prezygotic isolation. If their stock had an unusually high amount of standing genetic variance for both bristle number and positive assortative mating, then their extremely high level of selection could completely prevent gene flow between the high-bristle-number and low-bristle-number lines. This causes their experiments effectively to become a "destroy-the-hybrids" protocol, in which positive assortative mating typically evolves (see above) and is limited only by the amount of standing genetic variation for positive assortative mating in the founding stock. As illustrated below, elimination of all hybrids also eliminates the principal genetic constraint to sympatric speciation.

Overall, the "destroy-the-hybrids" experiments do not provide support for the reinforcement model because they prevent gene flow between the selected populations by imposing complete postzygotic isolation. When gene flow is permitted, as occurred in most attempts to repeat Thoday and Gibson's work, prezygotic isolation did not evolve. The available laboratory evidence therefore provides no support for the reinforcement model of speciation.

Yet some disruptive selection experiments with protocols differing from that of Thoday and Gibson have led to the evolution of high levels of prezygotic isolation, and even complete isolation in some cases. These successful experiments do not simulate the reinforcement model but instead simulate the divergence-with-gene-flow model when isolation evolves via pleiotropy/hitchhiking. To integrate these positive and negative results we first outline the major theoretical objection to the reinforcement model of speciation.

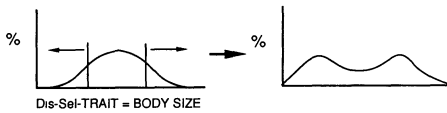
TWO MODELS OF DIVERGENCE-WITH-GENE-FLOW SPECIATION

Two major genetic models exist for the evolution of prezygotic isolation in response to disruptive selection (fig. 1). In the *double-variation*

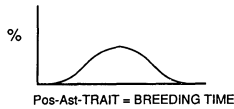
2 Models of Speciation-with-Gene-Flow:

Double-variation Models: ISOLATION VIA LINKAGE DISEQUILIBRIUM

1) DISRUPTIVELY SELECTED TRAIT



2) POSITIVE ASSORTATIVE MATING TRAIT



Single-variation Models: ISOLATION VIA PLEIOTROPY

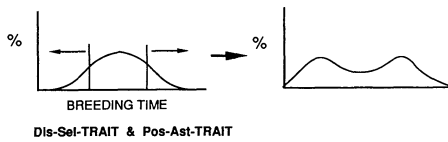


FIG. 1. Two models of speciation when gene flow occurs between populations. See text for description.

model, disruptive selection is applied to genetic variation for a trait (the disruptively selected trait, abbreviated Dis-Sel trait and illustrated by body size in fig. 1), which does not produce positive assortative mating via fortuitous pleiotropy. If disruptive selection is sufficiently strong, a bimodal, or at least hyperdispersed, distribution for body size (Dis-Sel trait) is expected. The evolution of prezygotic isolation requires a second type of genetic variation that produces positive assortative mating (i.e., the positive-assortative-mating trait, abbreviated Pos-Ast trait and illustrated by breeding time in fig. 1).

If there is a random association between the genetic variation for body size and breeding time (Dis-Sel and Pos-Ast traits), then the two modes of the body size distribution mate at the same average time, and no prezygotic isolation is manifest. To make progress toward reproductive isolation a nonrandom association between the genetic variation for the Dis-Sel and Pos-Ast traits must be established. For example, if individuals genetically disposed to be small (large) were also, on the average, genetically disposed to breed earlier (late), then partial reproductive isolation would be manifest between the two modes of the body-size distribution. As the degree of nonrandom association increases, this increases the disparity in breeding time, which in turn results in greater prezygotic isolation. As was first noted

by Felsenstein (1981), the buildup of a nonrandom association (linkage disequilibrium) between genetic variation controlling the Dis-Sel and Pos-Ast traits is the principal genetic constraint on the evolution of reproductive isolation when gene flow occurs between divergently selected populations.

What evolutionary processes affect the development of the requisite linkage disequilibrium? The lowered fitness (intermediate phenotype) of hybrid offspring causes selection to build the appropriate disequilibrium. But acting in opposition to selection is the mixing of genes during meiosis, that is, the homogenizing effect of recombination acts to destroy the nonrandom associations being built up by disruptive selection. When both selection and recombination are modeled simultaneously, computer simulations indicate that the homogenizing effect of recombination overpowers selection and prezygotic isolation is not expected to evolve (in the context of divergence-with-gene-flow or reinforcement) except under the restrictive conditions of oligogenic control of the Dis-Sel and Pos-Ast traits and very tight linkage (Felsenstein 1981). This antagonism between selection and recombination (Sel-Rec antagonism) is the principal theoretical objection to the reinforcement model of speciation.

The Sel-Rec antagonism is bypassed in the *single-variation* model of divergence-with-gene-flow speciation (Slatkin 1982; Rice 1984a, 1987). In this case, disruptive selection is applied to genetic variation that produces positive assortative mating as a fortuitous byproduct of pleiotropy (or genetic hitchhiking). For example, if disruptive selection were applied directly to breeding time in the above example, early breeders would necessarily breed among themselves as do late breeders. In this case, we need not build a nonrandom association (linkage disequilibrium) between two types of genetic variation to achieve prezygotic isolation because pleiotropy causes the Dis-Sel and Pos-Ast traits to be one and the same. Because this linkage disequilibrium need not be built up, the Sel-Rec antagonism is bypassed and the principal theoretical objection to divergence-with-gene-flow speciation is removed.

Linkage disequilibrium does need to be produced, however, among loci affecting the disruptively selected trait unless it is controlled by a single gene. When the Dis-Sel and Pos-Ast traits are one and the same, however, positive assortative mating via pleiotropy is a powerful generator

of this form of linkage disequilibrium and positive assortative mating via pleiotropy (or genetic hitchhiking) greatly reduces the requisite level of divergent selection (Rice 1984a, 1987).

The principal genetic constraint on the single-variation model of divergence-with-gene-flow speciation is the homogenizing effect of gene flow (migration) between subpopulations experiencing divergent selection (the selection-migration antagonism; Sel-Mig antagonism). Experimental and theoretical studies that address the potential for the occurrence of genetic divergence between populations connected by gene flow indicate that nontrivial divergence occurs only when the strength of selection is large relative to the level of gene flow (see below). Prezygotic isolation is therefore expected only between populations experiencing both divergent selection and gene flow when the divergently selected trait(s) lead to positive assortative mating via pleiotropy/hitchhiking, eliminating the Sel-Rec antagonism, *and when* the strength of selection is sufficiently high relative to the level of gene flow, overpowering the Sel-Mig antagonism.

As an aside, we point out that the Sel-Rec antagonism does not preclude the operation of the reinforcement model when postzygotic isolation is virtually complete (and when this is supplemented with strong prezygotic isolation) before secondary contact. The fewer hybrids that survive (or are produced by cross mating), the smaller the homogenizing effect of recombination and the less important the Sel-Rec antagonism. More experiments testing the reinforcement model with very strong but incomplete postzygotic isolation are needed.

DIVERGENCE-WITH-GENE-FLOW SPECIATION

The studies surveyed above in the context of the basic allopatry model provide experimental evidence that reproductive isolation may commonly evolve via pleiotropy/hitchhiking. For reproductive isolation to develop via pleiotropy/hitchhiking when gene flow is present, the only additional constraint on speciation is that populations must become genetically differentiated. Experiments directed at establishing genetic differentiation between populations connected by gene flow were summarized by Mather (1983) who found that substantial genetic differentiation typically occurred only when selection (measured by the percentage of each population removed by artificial selection) was at least as strong

as gene flow (measured as a percentage of the maximum), which is consistent with the prediction from simple oligogenic "island models" of selection-migration antagonism (summarized in Hedrick 1983).

As an example of a study that compared the development of prezygotic isolation as a correlated response with divergent selection with and without gene flow, consider the experiments of Hurd and Eisenberg (1975) who selected divergently (95% of flies removed by selection each generation) for geotaxis in allopatric (0% gene flow) and sympatric (50% gene flow) populations. Over the course of a 16-generation experiment, flies showed a marked response to divergent selection in both the allopatric and sympatric experiments. More importantly with respect to speciation, moderately strong prezygotic isolation (homotypic matings outnumbered heterotypic by more than three to one) evolved between the divergently selected populations irrespective of the presence of gene flow between them. A similar study by Soans et al. (1974) with a different starting stock produced very similar results (part E of table 1).

A shortcoming with the above experiments is that only incomplete reproductive isolation evolved in response to divergent selection with gene flow. One explanation for the limited degree of isolation is that the experimenters applied selection to only a single phenotypic trait, whereas selection in nature is likely to be multifarious, leading to more opportunity for reproductive isolation to evolve as a correlated response. This interpretation is supported by the work of Rice (1985) and Rice and Salt (1988, 1990) who selected on multifarious habitat selection (phototaxis, geotaxis, and chemotaxis as well as developmental time) under conditions of maximal gene flow between divergently selected populations, and tested for the development of reproductive isolation as a correlated response. In these experiments, complete or nearly complete prezygotic isolation evolved as a pleiotropic byproduct of genetic changes that caused the flies to use different spatiotemporal habitats.

Taken collectively, these studies strongly support the conclusion that reproductive isolation can easily evolve between populations connected by gene flow whenever divergent selection is strong relative to gene flow. They also demonstrate that speciation without allopatry is genetically feasible whenever isolation evolves the same way that it must evolve under allopatric

conditions, that is, via fortuitous pleiotropy/hitchhiking of genes adapting the populations to differing environmental conditions.

BOTTLENECK-INDUCED SPECIATION

There have been four major studies of the population-bottleneck model of speciation. The first concerns experiments done with lines made to be completely homozygous in a single generation (Templeton 1979) and will not be discussed here because, as pointed out by Charlesworth et al. (1982) and acknowledged later by Carson and Templeton (1984, p. 22), there appears to be little relevance of these experiments to the natural speciation process.

The basic design of the first two of the remaining three studies was to mix many geographically separated populations to form a highly genetically diverse starting population. From this composite population, a set of singly mated females is drawn haphazardly and each mated female is used to found a separate isofemale line. Over the next few generations, descendants from the singly inseminated female form a population numbering many thousands. At this point, a new, singly mated female is drawn from each line and her descendants, over a few generations, are used exclusively to continue the line until it again numbers many thousands of individuals. Repetition of this bottleneck/exponential-growth cycle is continued and then the populations are assayed for pre- and postzygotic reproductive isolation.

Powell (1978) and Dodd and Powell (1985) used *Drosophila pseudoobscura* as a model system. There were eight bottleneck/exponential growth and two control lines. After four bottleneck/exponential-growth cycles, there was no evidence for postzygotic isolation. Low, but persistent and statistically significant, prezygotic isolation was observed in two of the experimental lines but neither of the control lines. This difference (two of eight lines versus zero of two lines) in isolation between experimental and control lines was not statistically significant (2×2 contingency test, $P = 0.6$). Mean isolation indices were virtually identical for the experimental and control lines.

Ringo et al. (1985) used a *D. simulans* model system. There were eight bottleneck/exponential-growth lines and eight "control" lines (mass-mated, changing-selection lines, which were used for other purposes but also served, to a limited degree, as a type of control). After six bottleneck/

exponential-growth cycles, there was low, but statistically significant postzygotic isolation in both the experimental and changing-selection lines, as described earlier. Prezygotic isolation was manifest in one of the eight experimental lines and none of the changing-selection lines, but this difference (one of eight lines versus zero of eight lines) was not statistically significant ($P = 0.5$, 2×2 contingency test). A weak but statistically significant trend toward increasing prezygotic isolation with increasing numbers of bottlenecks was observed, but by the end of the experiment, mean isolation indices were virtually identical for the experimental and control lines.

The remaining study by Meffert and Bryant (1991) used a similar protocol with a *Musca domestica* model system with the exceptions that (a) they started with flies collected from a single geographical locality; (b) each of six independent bottleneck/exponential-growth lines began with 1 singly mated female, but the subsequent five bottlenecks were 1, 4, and 16 singly mated females (two replicated lines of each size); and (c) exponential growth continued to a size of 1000 pairs of flies. In 16 tests for prezygotic isolation examining a subset of all possible pairwise combinations of lines, two tests were individually statistically significant (one with positive and one with negative assortative mating), but no tests were significant after adjustment for multiple tests using the sequential Bonferroni method. Clear evidence appeared, however, for changes in some components of mating behavior among lines, but none leading to levels of prezygotic isolation beyond what could be reasonably expected by chance alone in a collection of 16 tests.

These studies collectively demonstrate that the bottleneck/exponential-growth cycles can lead to measurable pre- and postzygotic isolation. It is not clear, however, that a "genetic revolution" is the mechanism responsible for the isolation that has been reported. Barton and Charlesworth (1984) review other alternative interpretations of the bottleneck data.

The one thing that is not open to alternative interpretations from the bottleneck experiments, however, is that bottlenecks, even multiple episodes, have led to only weak levels of pre- and postzygotic isolation. This is in sharp contrast to studies on the pleiotropy/hitchhiking models where strong to complete isolation has been experimentally demonstrated. A major strength of the various bottleneck hypotheses is that they

are based on microevolutionary phenomena (bottleneck → “genetic revolution” → strong reproductive isolation), which should be readily observed in laboratory experiments. No bottleneck experiment has produced levels of isolation even approaching those needed for speciation. This fact leads to the following conclusion: available laboratory data indicate that bottlenecks may facilitate (see e.g., Bryant and Meffert 1988) but not cause the speciation process.

Some might argue that genetic revolutions are so rare that thousands of experiments, perhaps more, must be done with consistent negative results to effectively falsify the bottleneck model. Such a philosophy makes the model virtually impossible to reject experimentally. We think that the burden of proof is on the proponents of the bottleneck model, thus until experiments demonstrate levels of isolation commensurate with speciation we suggest a “wait and see” attitude.

DISCUSSION AND CONCLUSIONS

Overview.—The data from experiments attempting to duplicate all or part of the speciation process collectively support the following conclusions: we have substantial experimental evidence for the evolution of strong reproductive isolation via pleiotropy/hitchhiking, with or without allopatry, but not via any other mechanism. Although no individual study is definitive, the experiments that have accumulated over the last 40 yr provide no evidence supporting the reinforcement and bottleneck models, strong support for the basic allopatry model, and strong support for the divergence-with-gene-flow model when reproductive isolation evolves as it is presumed to evolve in the allopatry model, that is, via incidental pleiotropy/hitchhiking.

Role of Geography in Speciation.—There appears to be widespread agreement among evolutionary biologists that the geographical model of speciation is a feasible and important speciation mechanism. The experimental studies are fully consistent with the operation of this speciation model. But what aspect(s) of geographical separation is crucial to the speciation process? Mayr (1963, and elsewhere) has strongly advocated the view that geographical separation produces allopatry (zero gene flow) and that this is the critical role of geographical separation promoting speciation. Laboratory experiments do not support this extreme view when reproductive isolation evolves via pleiotropy/hitchhiking.

The available experiments indicate that the more important role of geographical separation is to *restrict* gene flow and produce *sharp discontinuities* in *strong, multifarious* divergent selection. Geographical separation of regions of suitable habitat is an obvious and simple way to reduce, but not necessarily eliminate, gene flow between them. It also can produce abrupt changes in selection, thereby eliminating the development of “bridging populations” that are adapted to intermediate habitats along an environmental cline, and which maintain gene flow between divergently selected populations (e.g., see Endler 1973). Substantial genetic divergence also can occur with weak selection along a continuous cline or via sampling drift because of isolation by distance. Although theoretical work summarized by Endler (1977, on natural selection) and extended by Lande (1982, on runaway sexual selection) suggest that speciation may be possible in the context of weak clinal selection, the critical experiments testing these ideas are conspicuously absent in the literature.

Geographical separation, with or without allopatry, also facilitates the simultaneous operation of many different selective factors (e.g., differences in climate, soil, community composition, etc.) thereby promoting strong, multifarious divergent selection. Laboratory experiments collectively indicate that *multifarious, strong, discontinuous, divergent* selection can readily lead to complete reproductive isolation via pleiotropy (e.g., Rice and Salt 1990), but that *single-factor, strong, discontinuous, divergent* selection will typically lead to only incomplete reproductive isolation (e.g., Soans et al. 1974; Hurd and Eisenberg 1975).

The laboratory experiments demonstrating that divergent selection can lead to genetic divergence between populations experiencing gene flow are irrefutable (reviewed in Endler 1977), as are those demonstrating reproductive isolation via pleiotropy/hitchhiking (reviewed here). Experiments combining both of these factors clearly demonstrate the evolution of strong, even complete, reproductive isolation via pleiotropy/hitchhiking when gene flow is present. It therefore seems inescapable to conclude that any environmental context generating restricted gene flow and producing strong, discontinuous, and multifarious divergent selection should also frequently lead to speciation via pleiotropy/hitchhiking. We will refer to this set of requisite conditions, which extant experiments indicate induce speciation, as

SPECIATION VIA DIVERGENCE-WITH-GENE-FLOW

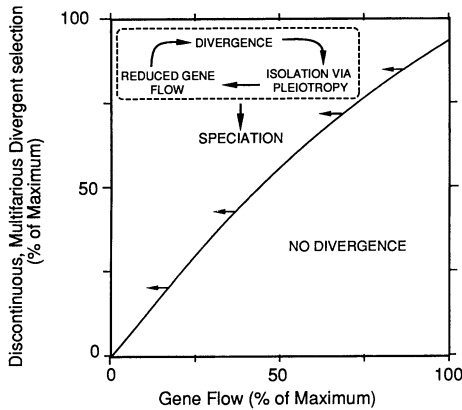


FIG. 2. A graphical model for speciation via pleiotropy (or genetic hitchhiking). The curve is based on experimental results tabulated by Mather (1983). Arrows point toward the region of the plot where speciation is possible. See text for further details.

the Restricted-Strong-Discontinuous-Multifarious-conditions, that is, Restricted gene flow with Strong, Discontinuous, and Multifarious divergent selection.

An aspect of divergence-with-gene-flow speciation via pleiotropy/hitchhiking that seems to be unappreciated is its positive feedback or runaway nature. Consider two populations inhabiting large blocks of habitat that are geographically separated but connected by moderate gene flow. Experiments surveyed by Mather (1983) suggest that, early on, divergence is expected only for those characters on which there is at least moderately strong divergent selection. The strength of divergent selection may be too weak for most characters to diverge initially. But if some characters respond and if at least some of these produce partial reproductive isolation via pleiotropy/hitchhiking, then the initial level of gene flow will be reduced. As gene flow abates, new genetic variation, which could not initially diverge because selection was overpowered by migration, can be recruited into the divergence process, and this will produce further isolation via pleiotropy/hitchhiking. Each new cycle of this process will recruit new genetic variation into the divergence process and progressively expand the genetic base for divergence. Reproductive isolation via pleiotropy/hitchhiking is therefore expected to evolve at an accelerated rate and ultimately develop to a level far exceeding that predicted from the initial conditions of divergent selection and gene flow.

Interestingly, in a natural setting the process of reinforcement might be mimicked by nonreproductive character divergence at the zone of overlap between divergently selected populations. This would occur when ecological competition between partially isolated populations accentuates divergent selection (and hence phenotypic divergence via the positive feedback system described above) and thereby leads to increased prezygotic isolation via pleiotropy/hitchhiking. Only detailed genetic and historical data can resolve the *cause*, reinforcement versus pleiotropy/genetic hitchhiking, of the increased level of prezygotic isolation frequently observed at areas of overlap between genetically differentiated populations. This dual interpretation for the same empirical pattern illustrates why observational data alone rarely provide resolution among alternative speciation mechanisms.

Figure 2 summarizes the domains for speciation via pleiotropy/hitchhiking by plotting the strength of discontinuous, multifarious divergent selection versus the initial level of gene flow between populations. The Y-axis alone (ordinate) represents the domain of the allopatry model and the origin (intersection of the coordinate axes) of the allopatric speciation via sampling drift model. The remainder of the coordinate system above the curve defines the domain of speciation via the divergence-with-gene-flow model. When selection is sufficiently strong relative to gene flow, genetic differentiation is expected, and experiments surveyed here suggest that partial reproductive isolation will frequently accrue as a correlated character. This partial isolation will reduce gene flow further and initiate the positive feedback system described earlier. Local adaptation acting to reduce the competitive ability of migrants may play an important role in the gradual reduction in gene flow.

Parapatry (two adjacent regions of different habitat) is a special case of geographical separation in which a nonhabitable region does not intervene between regions of suitable habitat. If the ecotonal zone at the interface of the regions is narrow relative to the organism's dispersal capabilities, then theory and experiments (reviewed in Endler 1977) indicate that migration will overpower local selection and prevent an ecotone-adapted "bridging population" from developing in the region of contact. Reproductive isolation is also selected for indirectly on a population-wide basis, that is, not just at the interface of the habitats, when it develops via plei-

otropy/hitchhiking as a correlated response to habitat-specific divergent selection. In this case, the Restricted-Strong-Discontinuous-Multifarious conditions can be achieved just as they would with complete geographical separation. We think that it is important to point out that all of the theoretical objections to the parapatry model (e.g., Paterson 1978) are based on "double-variation" models (e.g., reinforcement) and do not apply to isolation via pleiotropy/hitchhiking.

When the ecotonal region between adjacent suitable habitats is extensive, a clinal gradation of genotypes may develop in the ecotonal region and the discontinuous feature of the Restricted-Strong-Discontinuous-Multifarious conditions will not be achieved. Theoretical work (e.g., see Endler 1977; Lande 1982) suggests that speciation may also occur in this context, but we have found no laboratory experiments, pro or con, addressing this context of speciation with gene flow.

Habitat mosaics, in which many of two (or more) discrete habitat types are broadly interspersed over a large geographical area, is another context in which the Restricted-Strong-Discontinuous-Multifarious conditions can sometimes be met. Restricted gene flow can be achieved despite migration among habitat patches when the organisms mate on or near their selected or natal habitats. That is, positive assortative mating is a byproduct of habitat preference in combination with local mating. When the habitat types are discrete and sufficiently different, then strong, discontinuous and multifarious divergent selection is expected, ecotonal "bridging populations" are not expected to develop, and divergence-with-gene-flow speciation is genetically feasible as shown experimentally by Rice (1984a) and Rice and Salt (1990).

Complete sympatry, where geography does not enforce some degree of positive assortative mating, makes it far more difficult to achieve the Restricted-Strong-Discontinuous-Multifarious conditions. When there is substantial positive assortative mating via pleiotropy/hitchhiking, experiments surveyed by Mather (1983) in combination with those of Soans et al. (1974) and Hurd and Eisenberg (1975) support the possibility of this speciation mechanism but only in ecological contexts where extremely strong, multifarious divergent selection is manifest.

In populations that are allopatric but where strong, multifarious divergent selection is absent, two mechanisms can lead to speciation via plei-

otropy/hitchhiking. The first and most obvious is for allopatric populations to become genetically differentiated via sampling drift. Reproductive isolation accrues because of drift-induced genetic differentiation. Experiments demonstrating isolation between inbred lines support the potential operation of this model, but the fact that none of these microevolutionary experiments has generated strong isolation suggests that pleiotropy/hitchhiking-based speciation via drift may be a very slow process.

An alternative to sampling drift for the genetic differentiation of allopatric populations experiencing very similar environments is perpetual coevolution between the sexes caused by sexual and sexually antagonistic selection. Lande (1981, 1982) has modeled the case of runaway sexual selection via female choice leading to speciation without allopatry, but other forms of more subtle coevolution between the sexes may be more generally applicable. Several lines of evidence (e.g., Coyne 1983; Eberhard 1985; Lee and Vacquier 1992; Thomas and Singh 1992; Schiff et al. 1992; Agaude et al. 1992) suggest that the sexes may be continuously evolving in response to sex-specific selection as well as changes in each other's phenotype. Laboratory experiments suggest that both sexual selection (e.g., Prout 1971) and sexually antagonistic selection (Rice 1992) can be very strong and therefore potentially lead to rapid and substantial genetic differentiation among allopatric populations experiencing similar environments. The large effect of the X sex chromosome in coding for infertility-based postzygotic isolation (e.g., Coyne and Orr 1989; Wu and Davis 1993) and perhaps also prezygotic isolation (e.g., Wood and Ringo 1980; Kawanishi and Watanabe 1981) is consistent with the prediction of several models of sexual and sexually antagonistic selection (Rice 1984b; Charlesworth et al. 1987; Coyne and Orr 1989; Wu and Davis 1993).

Speculation on the Importance of Different "Engines of Speciation".—If we conclude that strong divergent selection, sampling drift, and sexual/sexually antagonistic selection are the major "engines" leading to speciation via pleiotropy/hitchhiking, then the relative importance of each needs to be addressed. Paleontological evidence suggests that there are periodic episodes of mass extinction followed by periods of extensive speciation (see, e.g., Raup 1992), although Vermeij (1987, chapters 13, 14) provides evidence for a delay between a major extinction

event and the following pulse of speciation. Other extrinsic events, such as changes in sea level, biotic exchange between previously isolated communities, and so forth may also stimulate major episodes of speciation (Vermeij 1987). The relative importance of drift, divergent selection, and sexual/sexually antagonistic selection may differ between periods such as the present and those characterized by bursts of speciation.

After an episode of mass extinction, many previously filled ecological niches would simultaneously become available. The reduced levels of interspecific competition could, in many cases, generate very strong divergent selection on the remaining species and facilitate the process of allopatric and nonallopatric speciation via pleiotropy/hitchhiking.

A microcosm of this process has apparently led to "species flocks" of fish in many prehistoric and extant large lakes (reviewed in Echelle and Kornfield 1984). Here a large lake providing many ecological niches is formed in a region where dispersal into the lake by preadapted, extant species is highly improbable. Systematic and paleontological evidence indicates that once a single founding fish species invades, it can rapidly speciate into tens to hundreds of new species, adapting to the available, empty niches. It is not at all clear that allopatry has played any important role in these explosive episodes of speciation, but it is clear that reduced interspecific competition leads to a rapid pulse of speciation when preadapted species are precluded by dispersal barriers.

Because the Restricted-Strong-Discontinuous-Multifarious conditions are most likely to be met after a period of mass extinction, speciation via pleiotropy/hitchhiking because of divergent selection, with or without allopatry, may be most common at these times. During more typical times, interspecific competition will be higher, the Restricted-Strong-Discontinuous-Multifarious conditions are less likely to be met, and sampling drift and sexual/sexually antagonistic selection may be the predominant "engines" of speciation.

Importance of Divergence-with-Gene-Flow Speciation.—If we conclude that there is strong theoretical and experimental support for the genetic feasibility of divergence-with-gene-flow speciation, then why have most biologists previously concluded that this form of speciation rarely if ever occurs? We think this stems from a lack of appreciation of the distinction between

"double-variation models" of speciation, where isolation evolves via linkage disequilibrium, and "single variation models" of speciation, where isolation evolves via pleiotropy/hitchhiking. Researchers have focused on the untenable double-variation models, and virtually all of the theoretical and experimental evidence marshaled against divergence-with-gene-flow speciation implicitly assumes a double-variation model. We have concluded that the single-variation model of divergence-with-gene-flow speciation has been largely overlooked (but see Slatkin 1982; Rice 1984a, 1987) because it represents a "theoretician's nightmare," that is, it can be completely deduced from experimental results, it is simple, and it is obvious. There just does not seem to be much that is theoretically interesting about divergence-with-gene-flow speciation via pleiotropy/hitchhiking, despite its potential (we would argue likely) importance in nature.

Because we will probably never have "time machines," the relative importance of various speciation mechanisms will never be known with certainty. We can, however, experimentally determine the genetic feasibility of alternative speciation mechanisms and the requisite ecological conditions for those deemed genetically plausible. Only the process of speciation via pleiotropy/hitchhiking is strongly supported by extant experimental evidence. This may be driven by divergent selection, sampling drift, and sexual/sexually antagonistic selection. It is now clear from direct experimentation that divergence-with-gene-flow speciation is genetically feasible. Paleontological evidence suggests that the requisite Restricted-Strong-Discontinuous-Multifarious conditions may have been common in the past. The common assertion that only the allopatry model is important in animal speciation is no longer tenable.

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NOTE ADDED IN PROOF

After the completion of this manuscript, an extensive experimental study of bottleneck-induced speciation was published (Galiana, A., A. Moya, and F. J. Ayala. 1993. Founder-flush speciation in *Drosophila pseudoobscura*: a large-scale experiment. *Evolution* 47:432–444). These published results as well as a large number of yet unpublished experiments (F. J. Ayala pers. comm. 1994) are fully consistent with the conclusion reported here, that is, repeated bottlenecks do not produce levels of prezygotic reproductive isolation even approaching those required for speciation.