Evolution, 43(2), 1989, pp. 362–381

PATTERNS OF SPECIATION IN DROSOPHILA[1]

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Abstract.—To investigate the time course of speciation, we gathered literature data on 119 pairs of closely related *Drosophila* species with known genetic distances, mating discrimination, strength of hybrid sterility and inviability, and geographic ranges. Because genetic distance is correlated with divergence time, these data provide a cross-section of taxa at different stages of speciation.

Mating discrimination and the sterility or inviability of hybrids increase gradually with time. Hybrid sterility and inviability evolve at similar rates. Among allopatric species, mating discrimination and postzygotic isolation evolve at comparable rates, but among sympatric species strong mating discrimination appears well before severe sterility or inviability. This suggests that prezygotic reproductive isolation may be reinforced when allopatric taxa become sympatric.

Analysis of the evolution of postzygotic isolation shows that recently diverged taxa usually produce sterile or inviable male but not female hybrids. Moreover, there is a large temporal gap between the evolution of male-limited and female hybrid sterility or inviability. This gap, which is predicted by recent theories about the genetics of speciation, explains the overwhelming preponderance of hybridizations yielding male-limited hybrid sterility or inviability (Haldane's rule).

Received February 11, 1988. Accepted September 23, 1988

Any theory of evolution must explain genetic changes within species as well as those producing new species. We understand far more about the former than the latter process. This disparity has at least two causes. First, genetic change in populations can be observed and studied during a human lifetime, but the evolution of reproductive isolation generally takes much longer. Second, genetic analysis of species differences is often precluded by their reproductive isolation.

As a result, genetic theories of speciation are often based more on biogeography than on genetical observation or experiment. For example, the founder-flush and transilience theories of speciation were largely inspired by the endemic nature of Hawaiian *Drosophila* species (Carson, 1975; Templeton, 1981). Yet biogeographic patterns may not imply a single evolutionary explanation. Enhanced prezygotic isolation in areas of species overlap, for instance, is often attributed to the reinforcement of mating discrimination by natural selection against maladaptive hybridization (Dobzhansky, 1937). But the observation of stronger premating isolation in sympatry could merely result from a process of differential fusion or extinction: those populations separated by little mating discrimination may simply not persist in sympatry (Templeton, 1981; Butlin, 1987). Species that survive this process will exhibit high levels of mating discrimination, giving the false impression that such discrimination resulted from natural selection to prevent hybridization of sympatric species.

Here we bring together information about reproductive isolation, electrophoretic differentiation, and biogeography in the genus *Drosophila* in a search for patterns to test and motivate theories of speciation. Our goal is to determine the rate at which reproductive isolation evolves; our method is to compare the strength of isolation in species that have diverged for different amounts of time. Using the electrophoretic genetic distance between taxa, which appears to change linearly with time (Nei, 1975, 1987; Kimura, 1983), we can order species pairs by their divergence times. For many of these pairs we also have information about mating discrimination and sterility or inviability of their hybrids. Such data can help answer the following questions.

i) How rapidly does reproductive isolation evolve?—The divergence time of taxa must obviously be correlated with the amount of reproductive isolation between them, because all species begin as populations that

[1] This paper is dedicated to our mentor, Dr. B. S. Grant, without whose help we would now be in lucrative professions.

are not reproductively isolated. Nevertheless, the pattern of this relationship can provide useful information. Reproductive isolation could, for example, increase slowly and at a constant rate up to the limit of complete isolation. Such a "speciation clock" would imply that reproductive isolation results from a gradual process of uniform rate, such as genetic drift or constant selection. On the other hand, reproductive isolation could evolve very quickly once populations are separated, with the rate subsequently slowing. This would imply a faster process, such as rapid adaptation to a new environment or genetic drift during founder events.

ii) Do pre- and postzygotic isolation evolve at the same rate?—We would like to know which type of isolation is most important in reducing gene flow between incipient species, for this factor would be the primary component of speciation. If most species evolve complete mating discrimination well before hybrid sterility or inviability, for example, the study of postmating isolation would be irrelevant to the origin of species.

iii) Do hybrid sterility and inviability evolve at the same rate?—If the evolution of postzygotic isolation is an important cause of speciation, we would like to know which of the above two components evolves first. It is possible, for example, that the evolution of inviability requires far more genetic change than the evolution of sterility.

iv) How does postzygotic isolation increase with time?—There are two common patterns of postzygotic isolation in species hybrids. 1) When only one sex is sterile or inviable, it is almost always the heterogametic sex (Haldane, 1922). This generalization, known as "Haldane's rule," applies in the vast majority of animal hybridizations, regardless of which sex is heterogametic (e.g., Gray, 1954, 1958; Bock, 1984). 2) The sex chromosomes invariably play the largest role in hybrid sterility or inviability (Charlesworth et al., 1987). To explain these two observations, Charlesworth et al. (1987) suggested that sterility and inviability may be pleiotropic effects of recessive or partially recessive alleles that are advantageous in geographically isolated populations. These alleles would be largely hidden from selection if they first arose on autosomes, but would be immediately selected in the heterogametic sex if they were X-linked. If most advantageous mutations with such pleiotropic effects are recessive or partially recessive, it can be further shown that postzygotic isolation will appear first in heterogametic and only later in homogametic hybrids, explaining both Haldane's rule and the large role of the X chromosome (Coyne and Orr, 1989). We can test the prediction that Haldane's rule represents an early stage of speciation by examining genetic distances between species whose crosses yield sterility or inviability in males only, in females only, and in both sexes.

v) Is prezygotic reproductive isolation enhanced by selection when populations become sympatric?—We can test this hypothesis by comparing the degree of mating discrimination between sympatric and allopatric species pairs separated by similar genetic distances. We can also examine whether such a pattern is an artifact of the fusion or extinction of less-reproductively-isolated populations upon secondary contact.

Materials and Methods

We collected information from the literature on electrophoretic genetic distance, degree of mating discrimination in the laboratory, amount of sterility or inviability of interspecific hybrids in reciprocal crosses, and geographic ranges of *Drosophila* species pairs. We included pairs of taxa in our survey only if information was available on genetic distance and at least one form of reproductive isolation. Because we are interested in the initial stages of speciation, we included any pair of recognized taxa showing pre- or postzygotic isolation, however slight (for convenience, we call all pairs of taxa "species pairs").

Genetic Distance.—We used Nei's electrophoretic genetic distance, D (Nei, 1972, 1987), as an index of divergence time. D measures average codon differences between proteins and increases at a constant rate when substitutions occur at a constant rate. This index is 0 when species have identical allozyme frequencies and ∞ when they share no alleles. Among crossable *Drosophila* species, however, D ranges from 0 to 2 (Table 1). At genetic distances greater than

one, the index begins to lose its linearity with time owing to the occurrence of multiple substitutions (the relationship, however, remains monotonic).

The rough constancy of protein substitution over calendar time is, of course, the well-known "molecular clock," for which there is substantial empirical evidence (Nei, 1975, 1987; Wilson et al., 1977; Kimura, 1983). Although much of this evidence was gathered from other organisms, there are at least two reasons to believe that the electrophoretic clock ticks at a fairly constant rate in *Drosophila*. First, *Drosophila* phylogenies constructed from genetic distances are almost completely congruent with those based on chromosome inversions, which probably best approximate true phylogenies (MacIntyre and Collier, 1986). This congruence would not occur if the electrophoretic clock were extremely erratic. Second, independent calibrations of divergence times among taxa are strikingly concordant with those estimated from genetic distances (Nei, 1975; Carson, 1976). While there is a controversy about whether the rough constancy of molecular evolution results from genetic drift or natural selection (Kimura, 1983; Gillespie, 1988), our results depend only on the constancy and not on its cause. Similarly, there is a controversy about whether the clock ticks at the same rate in all organisms, because it may depend upon generation time, particularly at the DNA level (Li and Tanimura, 1987). This issue does not affect our analysis because we use only electrophoretic data and analyze species with similar generation times.

Because the clock is a stochastic one, calculations of D from one or a few loci are unreliable. Moreover, standard errors are large when D is larger than one (Nei, 1987). In virtually all of our species pairs, however, D is calculated from more than ten loci. Furthermore, most of our data analysis is confined to species pairs separated by low genetic distances ($D \ll 1$), where the clock is more accurate.

When D values were not given in an electrophoretic survey, we calculated them from the raw data. If several populations were surveyed for a single species, we used the unweighted mean of allele frequencies among populations. In one case we used a transformation of Rogers' similarity coefficient ($-\ln S$; Rogers, 1972) as the measure of genetic divergence, because the authors presented this value without raw data (values of $-\ln S$ are, however, usually within 10% of D's calculated from the same data [Hedrick, 1975]). When several genetic distances were available for the same species pairs, we chose those values from studies in which other species pairs were also surveyed, so that as many data as possible were obtained with identical electrophoretic methods.

Finally, we note that some of our results depend upon the assumption of an electrophoretic clock. The value of these results will obviously rise or fall with the accuracy of the clock. Other of our results, however, depend only on a rough positive correlation between genetic distance and time and not on a strict linear relation between the two. Still other results are valid regardless of any correlation of genetic distance with time. We note in the Results and Discussion which conclusions are clock-dependent and which are not.

Postzygotic Isolation.—This category includes two components: hybrid inviability and hybrid sterility. The strength of this isolation was calculated in the following way. We first counted the number of sexes in both reciprocal crosses that were either completely sterile or completely inviable. This value, which ranges from 0 (both sexes viable and fertile in both reciprocal crosses) to 4 (both sexes sterile or inviable in both reciprocal crosses), was then divided by 4. This yields an index of postzygotic isolation ranging from 0 (no isolation) to 1 (complete isolation); cases of Haldane's rule are thus classified as 0.5. Zouros (1973) used an identical measure. We classified a cross yielding no offspring as "producing all inviable hybrids" only if there was evidence that females were inseminated, so that we did not confound premating and postmating isolation. (The "insemination reaction," in which sperm are destroyed or immobilized in species crosses, was counted as prezygotic isolation; see below.) A hybrid sex was considered to be viable if any adults of that sex appeared, even rarely. Similarly, it was considered to be fertile if any individuals of that sex were ever fertile. This pro-

cedure is similar to that used by other investigators classifying species hybridizations (e.g., Throckmorton, 1982). Our index is thus a minimum estimate of the true postzygotic isolation between species.

Prezygotic Isolation.—Mating isolation between a pair of species is commonly measured in the laboratory by one of four tests: 1) no choice, in which females of one species are confined with males of another; 2) female choice, in which females of one species are placed with males of their own as well as another species; 3) male choice, the reverse of 2, and 4) multiple choice, in which males and females of both species are tested together. Mating discrimination is measured by either counting the number of females inseminated after a long treatment (methods 1 and 3) or observing the number of copulations during a shorter period (methods 2, 3, and 4).

In some species, insemination is not accompanied by fertilization, because sperm are inactivated or expelled by the female's reproductive tract (Patterson, 1947*b*). In such cases, females may be erroneously classified as unmated, because the insemination is not detected. Sperm inactivation is, however, equivalent to mating isolation because fertilization does not occur. We therefore call this category "prezygotic," and not "premating," isolation.

We constructed an index of prezygotic isolation that can be applied to all four types of mating tests:

$$1 - \frac{\text{frequency of heterospecific matings}}{\text{frequency of homospecific matings}}.$$

This index ranges from $-\infty$ (complete disassortative mating) through 0 (no mating isolation) to 1 (complete isolation), and its computation from choice tests is straightforward. In practice, there is very little disassortative mating among species, so the index almost always ranges from 0 to 1. Five pairs of species showed some disassortative mating in one of the two reciprocal tests, though not in the other. In these instances we rounded the negative mating index to 0 before averaging it with the positive value from the reciprocal test. We used this procedure because it is equivalent to that used to quantify postzygotic isolation and thus allows a fairer comparison between the two (see below): in quantifying sterility or inviability, we assumed that hybrids could not have higher fitness than parental species, which would yield negative postzygotic isolation values. However, none of our results change when we incorporate the negative mating values into our calculations or simply eliminate these species from the analysis.

In no-choice mating tests, we required intraspecific controls as well as measurements of interspecific copulation in both reciprocal tests. The mating-isolation index in this case incorporates the frequency of interspecific insemination (the unweighted average from reciprocal tests) divided by the unweighted average frequency of insemination in the intraspecific controls (the latter is almost always close to 1.0).

When a study presented data from more than a single pair of strains, we averaged the isolation values from the different tests. For "sympatric" species with partially overlapping ranges, we averaged results from both sympatric and allopatric strains (see below).

The index of prezygotic isolation is equivalent to that of postmating isolation, for the latter can be thought of as 1 − (frequency of fertile and viable sexes in heterospecific tests/frequency of fertile and viable sexes in both homospecific controls [always 4]). Thus, values of pre- and postzygotic isolation are comparable measures of how these isolating mechanisms reduce autosomal gene flow between species. When either is 0.5, for example, gene exchange between species is half that occurring within species.

The direct comparison of pre- and postzygotic isolation indexes requires one additional adjustment. While our index of prezygotic isolation is a continuous variable, postzygotic isolation was recorded as a discrete variable that could assume the values of only 0, 0.25, 0.50, 0.75, or 1.0 (corresponding to zero, one, two, three, or four hybrid sexes that were inviable or sterile). Postzygotic isolation is therefore rounded down to a minimum value. We therefore adjust prezygotic isolation in a similar way when comparing the two indices. For such comparisons, we rounded the prezygotic-isolation index down to the next smaller value of 0, 0.25, 0.50, or 0.75. A prezygotic

value of 1.0 could only be attained if no interspecific matings occurred.

For the few species pairs in which mating isolation was studied using different experimental methods, we used those isolation values derived from experiments testing the largest number of related species. Thus as much of the data as possible was gathered by the same protocol.

We are aware of the problems of combining results from different types of mating tests into a single index of isolation and of assuming that laboratory studies indicate the extent of mating discrimination in nature. For example, species that hybridize appreciably in the laboratory may rarely do so when sympatric in nature (this appears to be true of *Drosophila pseudoobscura* and *D. persimilis* [Dobzhansky, 1973]). Such differences may be due to the artificiality of forced confinement in the laboratory, to environmental cues differing between nature and the laboratory, or to microenvironmental differences between the species in the field that lead to encounters less frequently than expected from their co-occurrence at a given site. Finally, the periods of observation differ among the various studies, ranging from one hour in choice tests to a full week in some no-choice tests. We find, however, that the strength of prezygotic isolation (standardized by genetic distance) does not vary significantly with the type of mating test used (Kruskal-Wallis test, $H_2 = 2.194$, $N = 90$, $P > 0.20$). Thus, species pairs of roughly the same age show roughly the same degree of prezygotic isolation, regardless of the type of mating test used.

Sympatry and Allopatry.—We classified species as "sympatric" if their described ranges overlapped in any area or if both appeared in collections from the same site. In 49 of the 53 "sympatric" species pairs, both species were actually collected from the same site. We could not find collection data for the remaining four pairs of species. Species were considered to be "allopatric" if they had no geographic overlap. We did not use geographic data from the cosmopolitan *D. virilis* in North America; even though its New World range overlaps with that of other species in the group, it is largely confined to human habitation and may be microallopatric (Throckmorton, 1982). We did include two other pairs of sympatric cosmopolitan species in our survey: *D. simulans/D. melanogaster* and *D. annanassae/D. pallidosa*. The former pair is broadly sympatric throughout the world, but also in parts of Africa, where the pair is thought to have evolved (Lachaise et al., 1988). The species of the latter pair occur together on the island of Tutuila in Samoa, and the karyotypic, morphological, and reproductive differences among South Pacific populations of *D. annanassae* imply that the species is not a recent introduction (Futch, 1966).

For statistical purposes, we assigned integer values to this category, designating sympatry as "0" and allopatry as "1." We can thus calculate an average degree of sympatry among several related species pairs. In the phylogenetically corrected data set described below, taxa with sympatry-allopatry values greater than 0.5 were considered to be "allopatric," and those with values smaller than 0.5 were considered to be "sympatric." If a phylogenetically corrected taxa pair included an equal number of sympatric and allopatric species pairs (i.e., sympatry-allopatry = 0.5), we excluded these data from analyses involving geography.

Phylogenetic Correction.—Levels of reproductive isolation among all species pairs are not evolutionarily independent because of their phylogenetic relatedness (Felsenstein, 1985*b*). Treating each species pair as an independent datum may hence incorrectly inflate the number of degrees of freedom in testing the correlation between genetic distance and reproductive isolation. To eliminate this problem, we made phylogenetic corrections to produce a set of evolutionarily and statistically independent data points (see Results).

In making these corrections, we relied on published phylogenies derived from electrophoretic data. With the exception of one phylogeny (the *virilis* group [Throckmorton, 1982]), these were taken from the compilation of MacIntyre and Collier (1986), who showed that electrophoretically based phylogenies are highly correlated with those derived from other criteria, such as morphology or chromosome banding. Although we treated these electrophoretic phylogenies as correct, they may actually be statistically indistinguishable from other phylogenies (Felsenstein, 1985*a*). This problem should

not systematically bias our results, because slightly incorrect phylogenies should only obscure whatever true correlations exist among genetic distance, reproductive isolation, and geographic overlap.

Possible Biases in the Data.—It should be noted that the electrophoretic surveys used many different gel types, buffer systems, enzymes, and laboratory protocols, and that several techniques were used to measure mating discrimination (postzygotic isolation is, however, always measured in the same way). Could the use of different methods by different laboratories bias our data, creating artifactual correlations between genetic distance and the strength of reproductive isolation? While such laboratory effects introduce random error into our data, we do not see how these effects could systematically bias our results to yield spurious correlations. Laboratory effects could produce artifactual correlations between D and the strength of isolation only if the same laboratories analyzed both D and reproductive isolation in the same taxonomic group and erred in the same direction when measuring each quantity. This scenario is improbable. First, the same laboratories rarely measured both genetic distance and strength of isolation within a group (for example, reproductive isolation and genetic distances in the *virilis* group were studied 40 years apart by different laboratories). Second, even if D and reproductive isolation were studied by the same workers, there is no obvious reason why laboratories that underestimate D would also underestimate the strength of reproductive isolation (and vice versa). Third, there cannot be much laboratory bias in the measurements of postzygotic isolation, for different laboratories almost always obtain the same results. In over 50 years, for example, there have been no reported exceptions to the rule of sterility in male hybrids of *D. pseudoobscura* and *D. persimilis*. In light of these points, we consider laboratory effects a source of random error but not of systematic bias.

RESULTS

Table 1 summarizes the data on genetic distance, sympatry-allopatry, prezygotic isolation, and postzygotic isolation for each species pair (arranged by species group). To determine whether hybrid sterility and inviability can be treated as a single phenomenon, we compare the rates at which these two mechanisms arise. Figure 1 shows the average genetic distance separating the pairs falling into each of the four severity classes of postzygotic isolation. Crosses yielding hybrid sterility only are displayed separately from those yielding hybrid inviability only or both hybrid inviability and sterility. Any difference between these plots would reflect a difference in the evolutionary rates of sterility and inviability. The plots are almost congruent, showing that sterility and inviability evolve at similar rates. We henceforth treat the evolution of postzygotic isolation as a single process.

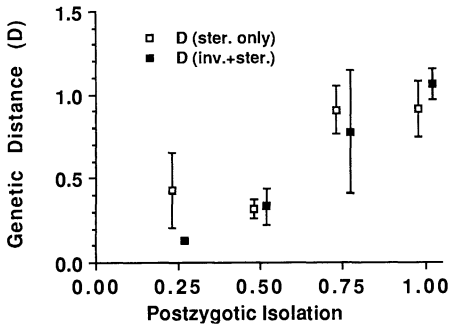


FIG. 1. Average genetic distance (±SE) at which a given amount of postzygotic isolation arises. Hybridizations producing only sterile offspring (open squares) are plotted separately from those producing any inviable offspring (filled squares).

In Figure 2, pre- and postzygotic isolation are plotted versus genetic distance for the total data (prezygotic isolation values are rounded down as noted above). Here we must deal with a statistical difficulty: data points from individual species pairs may not be evolutionarily (and hence statistically) independent because of the phylogenetic relationships among the species (Felsenstein, 1985*b*). Figure 3, for example, shows a phylogenetic tree in which an initial bifurcation, producing species A and B, is followed by a bifurcation of the latter species into C and D. Reproductive isolation between the pairs A-C and A-D does not necessarily represent two independent evolutionary events, but may reflect a single event: the evolution of reproductive isolation between species A and B, the common ancestor of C and D.

We dealt with this problem by employing

TABLE 1. Literature data on genetic distance (D), biogeography, and reproductive isolation (defined in text) of *Drosophila* species pairs. The last 13 entries are *the semispecies of D. paulistorum.* We omit sympatry-allopatry values for all comparisons in which *D. virilis* is a human commensal. Genetic distances for the *virilis* group were provided by L. Throckmorton (pers. comm.).

Species 1	Species 2	Sympatric (0) or allopatric (1)	D	Isolation index		References[a]
				Prezygotic	Postzygotic	
gaucha	*pavani*	0	0.460	0.312	0.75	7, 25, 35
mesophragmatica	*pavani*	1	0.990	—	1.00	24, 35
aldrichi	*mulleri*	0	0.123	0.928	—	36, 45
aldrichi	*mojavensis*	1	0.326	0.817	—	36, 45
aldrichi	*arizonensis*	0	0.289	1.000	—	13, 36, 45
arizonensis	*mojavensis baja*	0	0.212	0.732	0.25	36, 46, 49
arizonensis	*mulleri*	0	0.232	1.000	—	13, 45
mojavensis	*mulleri*	1	0.324	0.972	—	13, 45
americana	*virilis*	—	0.540	0.748	0.00	38, 43
americana	*lummei*	1	0.540	—	0.50	43
americana	*novamexicana*	1	0.430	0.465	0.00	38, 43
americana	*lacicola*	1	1.420	1.000	—	38, 43
americana	*montana*	1	1.480	0.992	1.00	38, 43
americana	*texana*	0	0.010	0.242	0.00	38, 43
borealis	*flavomontana*	0	0.380	—	1.00	38, 43
borealis	*montana*	0	0.210	—	0.50	43
borealis	*littoralis*	1	0.680	1.000	—	37, 43
borealis	*lacicola*	0	0.340	1.000	—	37, 43
borealis	*virilis*	—	1.060	—	1.00	43
flavomontana	*montana*	0	0.290	—	0.50	43
flavomontana	*virilis*	—	1.280	—	0.75	43
flavomontana	*littoralis*	1	0.710	—	0.75	43
flavomontana	*lacicola*	1	0.180	—	0.50	43
lacicola	*novamexicana*	1	1.200	1.000	—	38, 43
lacicola	*texana*	1	1.450	0.992	0.75	38, 43
lacicola	*montana*	0	0.210	0.954	0.00	36, 37, 43
lacicola	*littoralis*	1	0.660	—	0.75	43
lacicola	*virilis*	—	1.170	0.717	0.75	38, 43
littoralis	*montana*	0	0.660	—	0.75	43
littoralis	*virilis*	—	1.060	—	0.00	38, 43
lummei	*virilis*	0	0.350	—	0.00	43
lummei	*texana*	1	0.640	—	0.50	43
montana	*novamexicana*	1	1.220	1.000	0.75	38, 43
montana	*virilis*	—	1.230	0.895	0.50	38, 43
montana	*texana*	1	1.510	0.985	0.50	38, 43
novamexicana	*texana*	1	0.440	0.444	0.00	38, 39, 43
novamexicana	*virilis*	—	0.500	0.493	0.00	38, 43, 46

TABLE 1. Continued.

Species 1	Species 2	Sympatric (0) or allopatric (1)	*D*	Isolation index		References[a]
				Prezygotic	Postzygotic	
texana	*virilis*	—	0.580	0.749	0.00	38, 43
differens	*silvestris*	1	0.249	0.774	—	23
differens	*heteroneura*	1	0.246	0.434	—	23
differens	*planitibia*	1	0.138	0.196	0.50	11, 12, 23
heteroneura	*planitibia*	1	0.134	0.553	0.50	23
heteroneura	*silvestris*	0	0.026	0.829	0.00	12, 23
planitibia	*silvestris*	1	0.191	0.519	0.50	12, 23
ananassae	*pallidosa*	0	0.091	0.897	0.00	20
bipectinata	*pseudoananassae*	0	0.271	0.843	0.50	6, 48
bipectinata	*parabipectinata*	0	0.145	0.546	0.50	6, 48
bipectinata	*malerkotliana*	0	0.104	0.841	0.50	6, 48
malerkotliana	*pseudoananassae*	0	0.282	0.956	0.50	6, 48
malerkotliana	*parabipectinata*	0	0.226	0.854	0.50	6, 48
parabipectinata	*pseudoananassae*	0	0.366	0.764	0.50	6, 48
mauritiana	*melanogaster*	1	0.500	0.883	1.00	10, 41, 46
mauritiana	*sechellia*	1	0.320	—	0.50	10, 14
mauritiana	*simulans*	1	0.300	0.607	0.50	10, 29, 41, 46
melanogaster	*sechellia*	1	0.620	—	1.00	10, 14
melanogaster	*simulans*	0	0.550	0.914	1.00	10, 42, 46
sechellia	*simulans*	1	0.280	—	0.50	10, 14
athabasca EA	*athabasca* EB	0	0.024	0.169	0.00	22, 33
athabasca WN	*athabasca* EA/EB	0	0.124	0.994	0.00	22, 33
affinis	*tolteca*	1	1.440	0.724	1.00	27, 28, 32
affinis	*athabasca*	0	0.740	0.776	—	27, 28, 32
affinis	*azteca*	0	0.850	1.000	—	27, 28, 32
affinis	*narragansett*	0	0.970	0.985	—	27, 28, 32
affinis	*algonquin*	0	0.740	1.000	—	27, 28, 31
algonquin	*athabasca*	0	0.650	0.988	—	27, 28, 32
algonquin	*tolteca*	1	1.250	0.932	1.00	27, 28, 32
algonquin	*azteca*	0	1.250	1.000	—	27, 28, 32
athabasca	*tolteca*	1	1.250	0.615	1.00	27, 28, 32
athabasca	*narragansett*	0	1.100	0.960	1.00	27, 28, 32
athabasca	*azteca*	0	1.250	0.507	1.00	17, 27, 28, 32
azteca	*narragansett*	1	0.970	0.991	1.00	27, 28, 32
azteca	*tolteca*	0	1.100	0.563	0.25	27, 28, 32
narragansett	*tolteca*	1	0.970	0.913	1.00	27, 28, 32
ambigua	*persimilis*	1	1.660	—	1.00	9, 27, 28
ambigua	*pseudoobscura*	1	1.660	—	1.00	27, 28
bifasciata	*miranda*	1	1.810	0.980	—	16, 27, 28

TABLE 1. Continued.

Species 1	Species 2	Sympatric (0) or allopatric (1)	*D*	Isolation index		References[a]
				Prezygotic	Postzygotic	
bifasciata	*pseudoobscura*	1	1.810	0.942	—	16, 27, 28
bifasciata	*imaii*	0	0.560	0.969	0.50	16, 28, 34
bifasciata	*subobscura*	1	1.180	0.936	—	6, 16, 48
bifasciata	*persimilis*	1	1.810	0.980	—	16, 27, 28
imaii	*persimilis*	1	1.950	0.980	—	16, 27, 28
imaii	*subobscura*	1	1.100	0.980	—	16, 27, 28
imaii	*miranda*	1	1.950	0.969	—	16, 27, 28
imaii	*pseudoobscura*	1	1.950	0.726	—	16, 27, 28
loweii	*pseudoobscura*	0	1.050	—	1.00	21, 27, 28
miranda	*pseudoobscura*	0	0.560	0.990	1.00	16, 27, 28
miranda	*persimilis*	0	0.560	1.000	1.00	16, 27, 28
miranda	*subobscura*	1	1.950	0.947	—	16, 27, 28
persimilis	*subobscura*	1	1.660	0.990	—	16, 27, 28
persimilis	*pseudoobscura*	0	0.410	0.953	0.50	16, 27, 28
pseudoobscura	*subobscura*	1	1.660	0.958	—	16, 27, 28
pseudoobscura Bogota	*pseudoobscura* USA	1	0.194	0.222	0.25	1, 40
kikkawai	*leontia*	0	0.419	—	0.50	5, 44
equinoxialis	*paulistorum*	0	0.621	0.938	—	4, 8
equinoxialis	*insularis*	1	1.090	0.924	—	4, 15
equinoxialis	*willistoni*	0	0.656	0.997	—	4, 8
equinoxialis	*tropicalis*	0	0.665	1.000	—	4, 8
insularis	*tropicalis*	1	0.883	0.930	1.00	4
insularis	*willistoni*	0	1.070	0.915	—	4, 15
insularis	*paulistorum*	1	1.270	0.968	—	4, 15
paulistorum	*pavlovskiana*	0	0.232	—	0.50	4
paulistorum	*willistoni*	0	0.524	1.000	—	4, 8
paulistorum	*tropicalis*	0	0.609	0.474	—	4, 8
tropicalis	*willistoni*	0	0.413	0.979	—	4, 8
equinoxialis caribbensis	*equioxialis equinoxialis*	1	0.246	0.205	0.50	3
willistoni quechua	*willistoni willistoni*	1	0.214	0.299	0.25	2
paulistorum Amazonian	*paulistorum* Andean-Brazilian	0	0.170	0.860	0.75	4, 18, 19
p. Amazonian	*p.* Centroamerican	0	0.152	0.820	0.50	4, 18, 19
p. Amazonian	*p.* Orinocan	0	0.189	0.807	—	4, 18, 19
p. Andean-Brazilian	*p.* Orinocan	0	0.165	0.800	0.50	4, 18, 19, 30
p. Andean-Brazilian	*p.* Transitional	0	0.067	0.707	0.00	4, 19, 30
p. Andean-Brazilian	*p.* Interior	0	0.127	—	0.25	4, 19
p. Andean-Brazilian	*p.* Centroamerican	1	0.071	0.646	0.50	4, 19, 30
p. Centroamerican	*p.* Transitional	1	0.043	—	0.00	4, 19
p. Centroamerican	*p.* Interior	1	0.127	—	0.50	4, 19

TABLE 1. Continued.

Species 1	Species 2	Sympatric (0) or allopatric (1)	*D*	Isolation index		References[a]
				Prezygotic	Postzygotic	
p. Centroamerican	*p.* Orinocan	0	0.209	0.881	0.50	4, 18
p. Interior	*p.* Orinocan	1	0.074	—	0.50	4, 19
p. Interior	*p.* Transitional	1	0.149	—	0.50	4, 19
p. Orinocan	*p.* Transitional	0	0.198	—	0.50	4

[a] Key to references: 1) Ayala and Dobzhansky, 1974; 2) Ayala and Tracey, 1973; 3) Ayala et al., 1974*a*; 4) Ayala et al., 1974*b*; 5) Baimi et al., 1980; 6) Bock, 1978; 7) Brncic and Koref Santibañez, 1957; 8) Burla et al., 1949; 9) Buzzati-Taverso and Scossiroli, 1955; 10) Cariou, 1988; 11) Craddock, 1974; 12) Craddock and Johnson, 1979; 13) Crow, 1942; 14) David et al., 1974; 15) Dobzhansky et al., 1957; 16) Dobzhansky et al., 1968; 17) Dobzhansky and Koller, 1938; 18) Ehrman, 1965; 19) Ehrman and Powell, 1982; 20) Futch, 1973; 21) Heed et al., 1969; 22) Johnson, 1985; 23) Kaneshiro, 1976; 24) Koref-Santibañez, 1963; 25) Koref-Santibañez and Del Solar, 1961; 26) Lachaise et al., 1988; 27) Lakovaara and Saura, 1982; 28) Lakovaara et al., 1976; 29) Lemeunier and Ashburner, 1976; 30) Malogolowkin-Cohen, 1965; 31) Miller, 1950; 32) Miller and Kleager, 1971; 33) Miller and Westphal, 1967; 34) Moriwaki et al., 1967; 35) Nair et al., 1971; 36) Patterson, 1947*a*; 37) Patterson, 1952; 38) Patterson et al., 1947; 39) Patterson and Stone, 1949; 40) Prakash, 1972; 41) Robertson, 1983; 42) Sturtevant, 1929; 43) Throckmorton, 1982; 44) Tsakas and Tsakas, 1984; 45) Wasserman, 1982; 46) Watanabe and Kawanishi, 1979; 47) Wood and Ringo, 1980; 48) Yang et al., 1972; 49) Zouros, 1973.

FIG. 2. Strength of isolation plotted versus the genetic distance (*D*) between taxa (uncorrected data). A) Prezygotic isolation (rounded down); B) postzygotic isolation.

a procedure recommended by Felsenstein (1985*a*). This method permits only one comparison between species on either side of a phylogenetic bifurcation. We averaged together all comparisons between pairs of species spanning a node to produce a single estimate of genetic distance, pre- and postzygotic isolation, and degree of sympatry. For example, in Figure 3 there are three possible comparisons between species pairs: A versus C, A versus D, and C versus D. The first two comparisons, however, may not be independent. We therefore obtain one data point by calculating average genetic distances, pre- and postzygotic isolation indices, and sympatry-allopatry values of the A-C and A-D comparisons. The comparison between C and D is, however, independent of the others, because it reflects isolation evolving after the divergence of A and B. We dealt with unresolved trichotomies by averaging values from all three species.

Data on pre- and postzygotic isolation

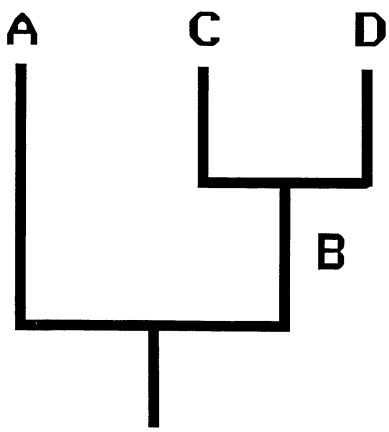
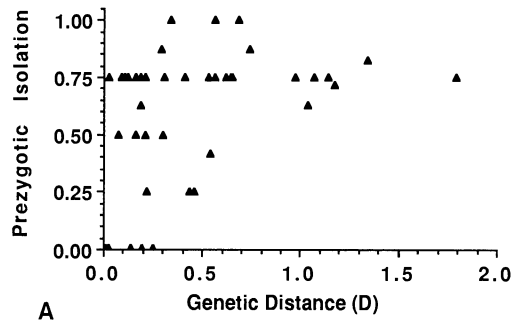


FIG. 3. Sample phylogeny used to show method of phylogenetic correction. See text for explanation.



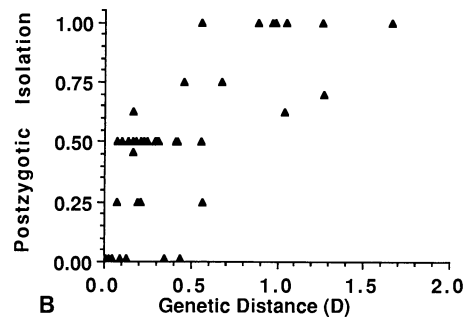


FIG. 4. Strength of isolation versus genetic distance (D) between taxa, plotted using corrected data. A) Prezygotic isolation (rounded down); B) postzygotic isolation.

were pooled separately, because information about both forms of isolation was sometimes unavailable for a given species pair. These pooled data are termed "corrected," and they represent the fewest possible evolutionarily independent data points. Phylogenetic pooling reduced our sample from 119 to 42 comparisons in both the corrected prezygotic and postzygotic data sets (these data sets do, however, include some different species pairs). We present statistical results from only the corrected data. Phylogenetic correction did not qualitatively alter our results: the statistical significance of every test (P greater or less than 0.05), though not the exact level of significance, was consistent for the corrected and uncorrected data.

Prezygotic isolation and postzygotic isolation are plotted separately versus genetic distance from the corrected data in Figure 4. Both forms of isolation are significantly correlated with Nei's D (prezygotic: Kendall's $\tau = 0.272$, $N = 42$, $P < 0.02$; postzygotic: $\tau = 0.569$, $N = 42$, $P < 0.001$).

The patterns of pre- and postzygotic isolation differ: in both the corrected and uncorrected data sets, prezygotic isolation increases more quickly at relatively low genetic distances. Both forms of isolation level off at higher genetic distances. Because we are most interested in the beginning of speciation and because reproductive isolation must reach an asymptote at higher genetic distances, we limit our comparison of pre- and postzygotic isolation to those species pairs separated by low genetic distances ($D \leq 0.5$; this value was chosen before data were analyzed, but lower threshholds produce qualitatively similar results). This comparison shows significantly greater prezygotic isolation than postzygotic isolation (Mann-Whitney U test: $Z = 2.541$, $N_1 = 26$, $N_2 = 29$, $P < 0.01$; all probabilities two-tailed). This result is not an artifact of different average genetic distances among the species pairs in the pre- and postzygotic data sets, for the average D's are nearly identical ($D_{\text{prezygotic}} = 0.209 \pm 0.025$, $N = 26$; $D_{\text{postzygotic}} = 0.201 \pm 0.024$, $N = 29$). Similarly, if one compares the strength of pre- and postzygotic isolation for each species pair at $D \leq 0.5$, more pairs have greater pre- than postzygotic isolation than is expected by chance (uncorrected data, Wilcoxon's sign rank test: $Z = 2.189$, $N = 32$, $P < 0.05$; this test cannot be performed on the corrected data, because the pre- and postzygotic data sets include some different

species pairs). We note that this result does not depend upon the accuracy (or even the existence) of the molecular clock: within any pair of species, there has been exactly as much time for the evolution of pre- and postzygotic isolation.

The rates at which pre- and postzygotic isolation evolve can provide information about whether these forms of isolation result from different genetic mechanisms. Charlesworth et al. (1987) have proposed that the initial stages of postzygotic isolation result from the rapid accumulation of recessive or partially recessive alleles (h [the dominance coefficient] < 0.5) at sex-linked loci causing sterility or inviability of the heterogametic sex in reciprocal hybridizations. Under this hypothesis, it can be shown that genes causing sterility or inviability in females will accumulate more slowly than those causing sterility or inviability in males (Coyne and Orr, 1989). These theories predict that: a) classes 0.25 and 0.50 of postzygotic isolation consist largely of species pairs obeying Haldane's rule; b) classes 0.25 and 0.50 evolve fairly rapidly; c) assuming an appreciable frequency of recessive or partially recessive mutations, the transition from class 0.50 to class 0.75 (the latter must include female effects) is slower than the transition from class 0 to class 0.25. There is no analogous genetic model predicting the course of prezygotic isolation with time.

Prediction a is fulfulled: classes 0.25 and 0.50 consist almost entirely of hybridizations obeying Haldane's rule (in the corrected data, 19 of 21 pairs in these classes have sterility and inviability limited to male hybrids; in the uncorrected data, the corresponding numbers are 37 of 43 pairs). This shows that male hybrids in both reciprocal crosses are almost always affected before female hybrids in either cross. This result cannot be inferred from the simple observation of Haldane's rule, which could result from males and females from one reciprocal cross becoming sterile or inviable before males and females from the other (with male effects preceding female effects in each cross).

In addition, there are very few species pairs separated by low genetic distances that fall into classes 0.75 or 1.00, which must include female effects. Of the 47 species pairs with $D \leq 0.5$, only four belong to these two

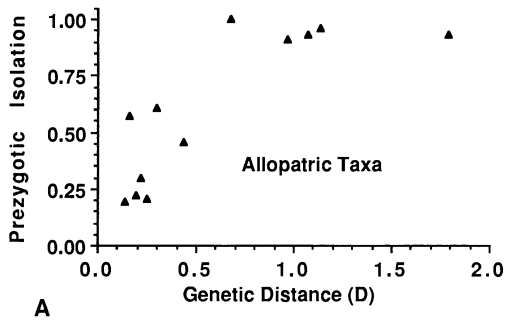
TABLE 2. Mean and standard error of genetic distance at which a given level of post- and prezygotic isolation occurs in *Drosophila,* using corrected data (isolation indices are rounded down to nearest 0.00, 0.25, 0.50, 0.75, or 1.00). Groups spanned by vertical lines are homogeneous by Scheffe's F test.

Isolation index	Mean genetic distance ± SE (N)	
	Postzygotic	Prezygotic
0.00	0.138 ± 0.058 (8)	0.122 ± 0.046 (5)
0.25	0.251 ± 0.083 (5)	0.370 ± 0.078 (3)
0.50	0.249 ± 0.032 (16)	0.257 ± 0.080 (5)
0.75	0.722 ± 0.198 (5)	0.578 ± 0.098 (24)
1.00	0.991 ± 0.127 (8)	0.523 ± 0.089 (5)

postzygotic isolation classes. Thus, Haldane's rule does not result from a combination of two evolutionary processes, one of which produces sterility and inviability in males only (followed by females) with the other producing sterility and inviability simultaneously in both sexes in both reciprocal crosses. Rather, male-limited sterility/inviability in both reciprocal crosses appears to be the ubiquitous first step in the evolution of postzygotic isolation.

To test predictions b and c above, we calculate the average genetic distance at which a given level of pre- or postzygotic isolation appears (Table 2). Note that for postzygotic isolation, classes 0.25 and 0.50 arise at low genetic distances, but the transition from class 0.50 to class 0.75 requires substantially more genetic distance. Post hoc analysis shows that the only significant jump in genetic distance between adjacent classes occurs between classes 0.50 and 0.75, as predicted (in fact, all comparisons spanning the 0.50–0.75 boundary are significant except the class 0.25 versus 0.75 comparison, in which both samples are very small). In sum, hybridizations obeying Haldane's rule are on average much younger than those showing female sterility or inviability, confirming the predictions of Charlesworth et al. (1987). We emphasize that this interpretation does not depend on a linear relationship between genetic distance and time but requires only a positive correlation between genetic distance and time.

There is no analogous stalling of prezygotic isolation at class 0.50 (see Table 2). In fact, Scheffe's F test (Sokal and Rohlf, 1981 p. 253) reveals no significant jump in ge-



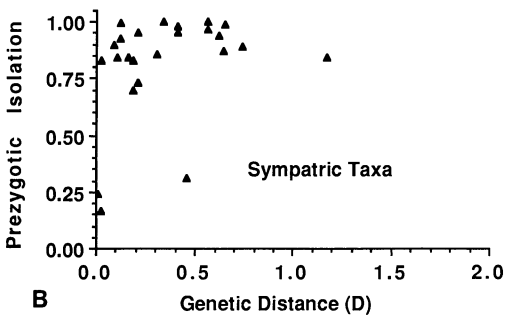


FIG. 5. A) Prezygotic isolation plotted versus genetic distance (D) among allopatric taxa using corrected data; B) prezygotic isolation plotted versus D among sympatric taxa using corrected data.

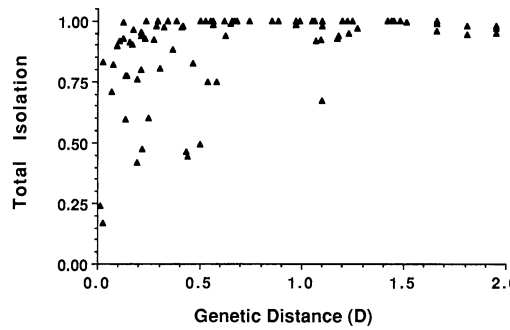


FIG. 6. "Total" reproductive isolation (T) versus genetic distance.

netic distance between any prezygotic isolation classes, adjacent or not.

We can next compare the evolutionary rates of reproductive isolation in allopatric versus sympatric species pairs from the corrected data (Fig. 5; exact instead of rounded prezygotic isolation indices are shown, because we do not compare them with the discrete postzygotic isolation values). All cases of strong prezygotic isolation at low genetic distances occur in species pairs that are sympatric. At $D \leq 0.5$, there are no cases of prezygotic isolation greater than 0.75 among the corrected allopatric species pairs, but there are 12 such cases among the 17 sympatric species pairs (in the uncorrected data, there are only four such cases among 15 allopatric species pairs, but 20 cases among the 26 sympatric pairs). The mean degree of premating isolation for sympatric pairs at $D \leq 0.5$ (0.768 ± 0.065, $N = 17$) is more than twice as large as that for allopatric pairs (0.365 ± 0.067, $N = 7$).

The greater prezygotic isolation in sympatry than in allopatry can be demonstrated in several other ways. a) There is a significant difference between the strength of prezygotic isolation in sympatric and allopatric species pairs at low genetic distances ($D \leq 0.5$) (Mann-Whitney U test: $Z = 2.890$, $N_1 = 17$, $N_2 = 7$, $P < 0.01$). This result is not an artifact of differences in genetic distances between allopatric and sympatric species pairs, as they do not differ ($D_{\text{allopatric}} = 0.241 \pm 0.038$, $N = 7$; $D_{\text{sympatric}} = 0.199 \pm 0.034$, $N = 17$). b) Significantly more sympatric than allopatric species pairs have greater prethan postzygotic isolation ($G_2 = 10.64$, $P < 0.01$): in allopatry, two species pairs have greater pre- than postzygotic isolation, and four have greater post- than prezygotic isolation (five species pairs are tied). However, in sympatry, 15 species pairs have greater pre- than postzygotic isolation while only one has greater post- than prezygotic (four are tied). c) Similarly, the previously noted result that prezygotic isolation is greater than postzygotic isolation within species pairs at $D \leq 0.5$ is attributable entirely to sympatric taxa: there is no significant difference between the strength of pre- and postzygotic isolation within allopatric species pairs (Wilcoxon's signed rank test $Z = 1.19$, $N = 11$, $P > 0.2$). In sympatry, however, many more species pairs have greater pre- than postzygotic isolation than is expected by chance (Wilcoxon's $Z = 3.011$, $N = 20$, $P < 0.01$). We note that tests b and c do not depend upon the accuracy or even the existence of the molecular clock, because, as noted above, these are tests within pairs of species.

It appears that premating isolation evolved faster among sympatric than among allopatric pairs of species. Similar analysis shows that geographic overlap has no effect on the strength of postzygotic isolation (Mann-Whitney U test on corrected data: $Z = 0.052$, $N_1 = 18$, $N_2 = 10$, $P > 0.90$). We consider processes that could produce these patterns in the Discussion.

Finally, we may combine indices of pre- and postzygotic isolation to measure the evolutionary rate of "total" reproductive isolation. Assuming that the two forms of isolation act sequentially (and hence multiplicatively) within a species pair, the appropriate index is

$$T = \text{Pre} + (1 - \text{Pre}) \times \text{Post}$$

where T is total isolation, Pre is prezygotic isolation, and Post is postzygotic isolation.

Total isolation is plotted versus genetic distance in Figure 6 (to improve accuracy, we used exact values of prezygotic isolation). This plot includes several data points from species pairs having prezygotic isolation greater than 0.90 but no information about postzygotic isolation. In such cases we set "total" isolation equal to the level of prezygotic isolation, because the maximum possible error in these cases is only 10%. Total isolation is strongly correlated with genetic distance ($\tau = 0.350$, $N = 91$; $P < 0.001$). High values of total isolation are frequent at low genetic distances; this is attributable largely to strong mating discrimination between closely related sympatric species. At higher genetic distances, almost all species pairs show strong reproductive isolation.

Discussion

Our survey of the literature yields five major observations. First, both pre- and postzygotic reproductive isolation increase with divergence time between taxa. Second, prezygotic isolation evolves faster than postzygotic isolation. This difference, however, is completely attributable to higher prezygotic isolation between sympatric species. Third, hybrid sterility and inviability evolve at similar average rates. Fourth, the usual pathway for the evolution of postzygotic isolation is the early appearance of sterility or inviability in male hybrids from both reciprocal crosses, followed by the appearance of these anomalies in female hybrids. Fifth, there is a large jump in genetic distance between hybridizations producing sterile or inviable males only (cases of Haldane's rule) and hybridizations yielding female effects as well. We discuss these conclusions in turn.

i) Prezygotic isolation and postzygotic isolation increase with time.—This is the conventional wisdom about speciation, predicted by any theory that reproductive isolation results from gradual genetic change within populations. Indeed, the only theories refuted by this observation are those of special creation and instantaneous speciation (e.g., by infectious agents). We also note that "total" reproductive isolation increases with time, so that at high genetic distances all taxa are strongly isolated.

Two previous studies used similar methods to delineate the course of speciation. Zouros (1973) correlated genetic distance with hybrid inviability or sterility in 12 pairs of *Drosophila* species. He found that Nei's D was significantly correlated with inviability but not with sterility. Assuming that electrophoretic loci represent a random sample of genes altered by natural selection, Zouros concluded that inviability requires far more genetic change than sterility. His 12 data points, however, are not phylogenetically independent, and Zouros' correlations become nonsignificant when we apply Felsenstein's (1985*a*) correction.

In a study of the *Drosophila willistoni* group, Ayala (1975) showed that genetic distance was correlated with taxonomic rank (i.e., populations of a species are separated by lower D values than subspecies within a species, which in turn are less differentiated than sibling species, etc.). Like Zouros (1973), Ayala apparently assumed that electrophoretic divergence between taxa reflects genetic change caused by natural selection. Under this assumption, Nei's genetic distance is an index of the amount of genomic change required to achieve a given taxonomic status.

Our work differs from that of Zouros and Ayala in several respects. First, we examine factors not considered by these workers, including geographic distribution, pre- versus postzygotic isolation, and sex differences in

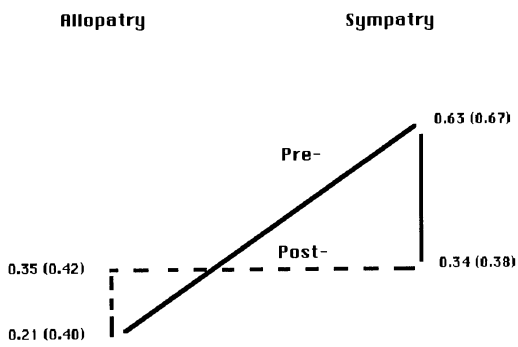


FIG. 7. Average strength of pre- and postzygotic isolation in allopatric versus sympatric species pairs. Only species pairs with Nei's $D \leq 0.5$ are included. Prezygotic values are rounded down as described in text. The first mean is from the corrected data; the mean in parentheses is from uncorrected data. Values connected by solid lines are significantly different by the Mann-Whitney U test ($P < 0.05$), and those connected by broken lines are not significantly different (the statistical significance does not differ using corrected or uncorrected data).

hybrid sterility and inviability. Second, we consider electrophoretic differentiation between taxa as a measure of their divergence time and not of the effects of selection. (Ayala [1975], for instance, observed that in the *willistoni* group, the average genetic distance between subspecies was similar to the genetic distance between semispecies. Assuming that semispecies are older than subspecies [because the former show premating isolation while the latter do not], he concluded that the evolution of premating isolation does not require much additional genetic change. We conclude, on the other hand, that these subspecies and semispecies are equally old, and we propose that the higher prezygotic isolation among semispecies results more from reinforcement in sympatry than from age.) While electrophoretic genetic distance is surely correlated both with time of divergence and with genetic change caused by natural selection, Kimura (1983) argues convincingly that the former correlation exceeds the latter.

ii) Prezygotic isolation evolves more rapidly than postzygotic isolation in sympatric species pairs, but not in allopatric pairs.— At low genetic distances we find strong prezygotic isolation far more often than strong postzygotic isolation. It is remarkable that in our corrected data, *every* case of strong prezygotic isolation at low genetic distances occurs in a pair of species that is sympatric. In allopatric populations, however, there is no significant difference between the evolutionary rates of pre- and postzygotic isolation (Fig. 7). In geographically isolated populations, then, both forms of isolation are significant components of speciation.

There are three possible explanations of heightened reproductive isolation among sympatric species pairs. The first is that introgression of enzyme alleles among a sympatric pair may artificially lower the genetic distance between them. Reproductive isolation may thus appear to be "enhanced" in sympatry merely because sympatric taxa are older than allopatric taxa of the same genetic distance. This hypothesis, however, fails to explain why prezygotic but not postzygotic isolation is greater among sympatric than among allopatric taxa. Because both forms of isolation increase with genetic distance between allopatric taxa, both forms should appear to be enhanced in sympatry under the introgression hypothesis. They are not.

Second, as we noted in the Introduction, a pattern of enhanced prezygotic isolation could result from a process of fusion or extinction in sympatry. If allopatric species pairs differ from each other in the strength of prezygotic isolation, less-isolated species are more likely to fuse or drive each other extinct if they become sympatric. As Templeton (1981) notes, this mechanism could produce—without any reinforcement—a pattern of higher average prezygotic isolation among sympatric species.

However, several features of our data do not support the fusion/extinction hypothesis. Again, we find that only prezygotic isolation increases in sympatry although pre- and postzygotic isolation are equally strong in allopatric species (Fig. 7). This pattern is predicted by the reinforcement hypothesis. The fusion/extinction hypothesis, however, predicts that both pre- and postzygotic isolation will be stronger in sympatry, because *any* factor that reduces gene flow should inhibit fusion or extinction. Second, the fusion/extinction hypothesis predicts that degrees of prezygotic isolation observed among sympatric species form a subset of degrees seen among allopatric species pairs (sym-

patric species pairs were once strongly isolated allopatric pairs). We do not observe this pattern. Instead, sympatric species show a high degree of isolation not seen among allopatric pairs.

The pattern of elevated prezygotic (but not postzygotic) isolation between sympatric species is predicted only by the hypothesis that natural selection has reinforced mating discrimination among some sympatric species. Selection against the disadvantageous results of hybridization can select for increased mating discrimination in sympatry (Dobzhansky, 1937), but such selection is very unlikely to affect the amount of postzygotic isolation in *Drosophila* (Coyne, 1974). Two previous studies suggest the possibility of reinforcement for prezygotic isolation: in *Drosophila* species with partially overlapping ranges, mating isolation is greater between individuals taken from sympatric than from allopatric populations (Wasserman and Koepfer, 1977; Ehrman and Powell, 1982).

There are several ways that such reinforcement can evolve.

a) *Reinforcement of prezygotic isolation by natural selection.* Increased prezygotic isolation may result from natural selection against the production of sterile or inviable hybrids (Dobzhansky, 1937). This mechanism requires that sympatric species having strong prezygotic isolation also show some postzygotic isolation. However, only five of the 12 closely related, sympatric pairs of taxa with strong prezygotic isolation (corrected data; Pre > 0.75, $D \leq 0.5$) show any postzygotic isolation in the laboratory (four show none, and there is no information for the other three). This casts some doubt on reinforcement by natural selection, but for three reasons we cannot rule it out. First, our criteria for postzygotic isolation may be too strict: hybrids could well be partially sterile or inviable, although no sex is completely sterile or inviable. We classify such species pairs as showing no postmating isolation. Second, it is possible that the benign laboratory environment masks postzygotic isolation that exists in the wild. Third, hybrids may be disadvantaged not because they are sterile or inviable, but because they fall between the niches of two ecologically isolated taxa or cannot mate properly with either parental species.

b) *Reinforcement of prezygotic isolation by sexual selection, given postzygotic isolation.* Natural selection against the production of unfit hybrids could favor assortative mating. This, in turn, could initiate sexual selection and rapidly increase isolation (Fisher [1958 Ch. 6], Maynard Smith [1978 p. 172], and Lande [1982] suggest this possibility but provide no mathematical models). Like explanation a, this model requires some initial postzygotic isolation and cannot explain the reinforcement of prezygotic isolation in hybridizations lacking sterility or inviability.

c) *"Reinforcement" of prezygotic isolation by sexual selection, given no postzygotic isolation.* In the absence of any postzygotic isolation, reinforcement may nevertheless occur if the courtship behavior of hybrids makes it difficult for them to secure mates. Assortative mating would again be favored, and sexual selection would then occur. This process differs from traditional mechanisms of reinforcement and has not been modeled mathematically.

We should add that there are formidable difficulties with all models of reinforcement (see Spencer et al., 1986), and other explanations may be possible.

iii) Hybrid sterility and inviability evolve at similar rates.—This similarity suggests that sterility and inviability are by-products of similar genetic processes, a conclusion strengthened by the fact that both obey Haldane's rule.

iv) The usual pathway for the evolution of postzygotic isolation is the initial appearance of sterility/inviability in hybrid males, followed by its appearance in females.—There are almost no cases of female sterility or inviability early in speciation. Haldane's rule thus characterizes the first step in the evolution of postzygotic isolation, and the evolutionary explanation of this rule becomes an important goal of speciation theory.

v) There is a significant increase in genetic

distance between those species pairs producing sterile or inviable males only and those producing sterile or inviable hybrids of both sexes.—As noted above, almost all species pairs belonging to our postzygotic classes 0.25 and 0.50 show effects limited to hybrid males. Classes 0.75 and 1.00, on the other hand, include female effects. The large jump in genetic distance between class 0.50 and 0.75 is hence associated with the appearance of postzygotic isolation in females and represents a "stalling" between male-only and female effects. Prezygotic isolation, on the other hand, shows no significant jump between any two adjacent classes (or, in fact, between any classes). The "stalling" between the appreance of male and female effects confirms several predictions of the Charlesworth et al. (1987) and Coyne and Orr (1989) theories, which we discuss in turn.

The observation that Haldane's rule is an early stage of speciation resolves a potential difficulty in interpreting genetic analyses of reproductive isolation. Such analyses almost always reveal a disproportionately large effect of the X chromosome on hybrid sterility and inviability (Wu and Beckenbach, 1983; Charlesworth et al., 1987). As Charlesworth et al. (1987) note, it is possible that this large effect of the X chromosome is observed merely because autosomal alleles causing hybrid sterility/inviability are more likely to affect both sexes, yielding species pairs that cannot be genetically analyzed (some viable and fertile hybrids are required for genetic studies). There could thus be an empirical bias favoring detection of X-chromosome effects, and analyzable cases of postzygotic isolation (usually those obeying Haldane's rule) would not necessarily represent taxa undergoing the first steps of speciation.

We find, however, that hybridizations showing sterility or inviability of both sexes are much older than those obeying Haldane's rule. The ubiquity of Haldane's rule in recently diverged pairs of species suggests that the large effect of the X chromosome does not result from a biased sample of crossable species. It also implies that there are not two distinct evolutionary pathways in speciation, one causing effects in hybrid males only and the other in both sexes simultaneously. Rather, there appears to be a single evolutionary pathway that culminates in the sterility and/or inviability of all hybrids.

This pathway, however, is characterized by two phases: an initial accumulation of alleles causing postzygotic isolation in male hybrids, followed by accumulation of alleles causing isolation in female hybrids. Such a pathway is predicted by the theories of Charlesworth et al. (1987) and Coyne and Orr (1989). However, these theories require the assumption that the genetic basis of postzygotic isolation differs fundamentally from that of other observable differences between species (the latter are usually caused by additive substitutions spread throughout the genome). There is no independent evidence for this assumption.

Our postzygotic-isolation data also explain why cases of Haldane's rule are so numerous. Bock (1984), for example, shows that over half of all successful *Drosophila* hybridizations are cases of Haldane's rule (the other crosses yield hybrid effects involving both males and females). Haldane (1922) noted that cases of hybrid unisexual inviability or sterility almost invariably involve males, but he did not explain why such a large proportion of hybridizations show this pattern instead of sterility or inviability of both sexes. The obvious explanation is that the delayed evolution of female anomalies stalls many species pairs at the stage of Haldane's rule.

We note parenthetically that we observe no cases of sterility/inviability of both males and females in only one direction of hybridization at low genetic distances [$D \leq 0.5$]. One might expect such patterns if cytoplasmic factors were frequently responsible for postzygotic isolation.

vi) The systematic status of allopatric taxa.—Our data provide us with a protocol for deciding when allopatric taxa are distinct species. The strength of isolation between sympatric species is obviously sufficient for them to remain distinct. A reasonable conclusion is that allopatric taxa with reproductive isolation as strong as that between sympatric species should also remain distinct upon secondary contact. The mean level of total isolation among sympatric species pairs is 0.907 ± 0.026 (uncorrected data, $N = 44$). The lower bound of the 95% confidence interval about this

mean is 0.854. Thus, total isolation of 0.85 or greater is probably enough to prevent the fusion of allopatric taxa upon secondary contact.

This calculation can be applied to the difficult problem of how to use the biological species concept (Mayr, 1963) with allopatric taxa. We suggest that any two allopatric taxa with values of total isolation below this cutoff be regarded as conspecific (there are several of these in our data). If, however, reproductive isolation is increased by selection upon secondary contact, our criterion is too conservative, and taxa below the cutoff may nevertheless remain distinct once sympatric. Conversely, any allopatric taxa with isolation above this level can be regarded as distinct species that would maintain their integrity upon secondary contact.

Finally, we estimate the genetic distance required to attain species status, i.e., to reach a "total isolation" value of 0.85. We calculated second-order least-squares regressions of "total isolation" on genetic distance, forcing these regressions through the origin. This assumes that two species begin as populations that differ by very low D's (as is commonly observed). Regressions were performed on the uncorrected data, using data from all species pairs, allopatric pairs alone, and sympatric pairs alone. These equations were then solved for the value of genetic distance predicting a "total isolation" of 0.85. These D's were 0.53 for the total data, 0.66 for allopatric species, and 0.31 for sympatric species. Species status is therefore reached twice as quickly in sympatric as in allopatric taxa. It is difficult to estimate the absolute time corresponding to these values, because the molecular clock is not well calibrated for *Drosophila*. Nei (1987), however, suggests that in many organisms, a D value of 1 corresponds roughly to 5,000,000 years of divergence. This calibration would place the time required for speciation between 1,500,000 and 3,500,000 years. This is, of course, a rule of thumb, and there are taxa that attain species status at D's well below the mean values given here. (In fact, over 15% of all sympatric taxa with total isolation exceeding 0.85 are separated by genetic distances smaller than 0.20, implying a divergence time of less than 1,000,000 years.)

In sum, we find that in allopatric taxa both pre- and postzygotic isolation contribute significantly to reproductive isolation, so that no single isolating mechanism is the "stuff of speciation." In sympatric taxa, however, prezygotic isolation predominates, perhaps implying direct selection for mate discrimination. We note, however, that the evolution of enhanced prezygotic isolation may be triggered by postzygotic isolation. Even in sympatry, then, both types of isolation may play important roles in speciation.

The recognition of patterns in nature is the source of most evolutionary theory. Theories of speciation, however, require historical information about the evolution of reproductive isolation, and are therefore slow to bear fruit. We hope that further synthesis of genetic analysis with estimates of divergence time will reveal patterns clarifying Darwin's "mystery of mysteries," the origin of species.

Acknowledgments

We thank M. L. Cariou, D. Lachaise, and L. Throckmorton for permission to cite their unpublished data, B. Hedges for calculating some genetic distances, and S. Arnold, B. Charlesworth, D. Futuyma, B. Grant, R. Lande, S. Orzack, D. Schemske, M. Turelli, P. Verrell, M. Wade, and P. Ward for criticism of the manuscript. This work was supported by grants from the National Institutes of Health (GM 32221 and 38462), National Science Foundation (BSR 18558), and the Louis Block Fund of The University of Chicago.

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