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"PATTERNS OF SPECIATION IN *DROSOPHILA*" REVISITED

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In a paper published seven years ago in this journal (Coyne and Orr 1989a), we analyzed the time course of speciation in *Drosophila* by correlating electrophoretic genetic distance between pairs of species (a number roughly proportional to their divergence time) with the strength of reproductive isolation between them. That analysis yielded five conclusions. First, both prezygotic and postzygotic reproductive isolation increase with divergence time between taxa. Second, prezygotic (sexual) isolation evolves more rapidly than postzygotic isolation (sterility and inviability of hybrids). This difference is, however, due entirely to much stronger prezygotic isolation between sympatric than between allopatric pairs of species. We suggested that this difference was due to "reinforcement," or direct selection for sexual isolation that occurs among sympatric taxa that produce unfit hybrids (Dobzhansky 1937). Third, hybrid sterility and inviability evolve at similar rates. This conclusion now appears to be incorrect because average divergence time between taxa is not a sensitive way to measure evolutionary rates of reproductive isolation, and more sensitive analyses show that hybrid sterility may in fact evolve more rapidly than inviability (Wu 1992). Fourth, the usual pathway for the production of postzygotic isolation is the initial appearance of sterility or inviability in hybrid males, followed by its appearance in females. This explains the frequent observation of Haldane's rule: the pattern that if only one gender of hybrids is sterile or inviable in species crosses, it is nearly always the heterogametic (XY or XO) sex (Haldane 1922; Coyne and Orr 1989b). Finally, there is a large increase in genetic distance between those species pairs producing sterile or inviable males only and those producing sterile or inviable hybrids of both sexes. This implies that there is a long time lag between the evolution of postzygotic isolation in males and in females.

While a similar (but much smaller) analysis has since been conducted in salamanders (Tilley et al. 1990), the data from *Drosophila* are unique—and are likely to remain so—because of the large number of crossable species and the ease of estimating sexual and postzygotic isolation in the laboratory. These *Drosophila* data have hence attracted some interest. Because of this, we have continued to accumulate new data as they have appeared. We have also found a few errors in our original data set, and have revised some estimates of reproductive isolation and phylogenetic relatedness when better data became available. We now have data for 171 interspecific hybridizations in *Drosophila*, an increase of 43% over the 119 hybridizations described in our previous paper.

Because DNA sequencing has largely supplanted gel electrophoresis as a way of measuring divergence between species, it is unlikely that this data set will grow much larger; and it will be many years before we possess DNA-based estimates of divergence between many pairs of *Drosophila* species. We therefore thought it timely to check our earlier conclusions using the new and larger data set.

MATERIALS AND METHODS

The reader should consult our previous paper (Coyne and Orr 1989a) for a detailed description of our criteria for including species, determining whether they are sympatric or allopatric, and estimating genetic distances and reproductive isolation. These criteria and all methods of calculation remain unchanged for the data presented here. Briefly, we collected pairs of taxa for which the following information was available: Nei's (1972) genetic distance (D) estimated from gel electrophoresis, the degree of prezygotic isolation as estimated from tests of sexual isolation in the laboratory, and the degree of postzygotic isolation estimated from measurements of fertility and viability of hybrid offspring. Our previous paper also discusses possible biases in the data (none of which appear to invalidate the conclusions) and important caveats about applying our results to reproductive isolation in nature.

Because the same individual species sometimes take part in several different hybridizations within a species group—and because of the phylogenetic relatedness of the taxa studied—our data points may not all be statistically independent. To eliminate this problem, we produced sets of "corrected" data, which include comparisons that are all phylogenetically and hence statistically independent (Coyne and Orr 1989a, pp. 367, 371–372. (The raw data from all species pairs is termed "uncorrected".) We continue to make these phylogenetic corrections in the data presented here, and most of the statistics are calculated using these corrected data. As before (Coyne and Orr 1989a, pp. 365–366), postzygotic isolation is measured as a discrete character that only assumes the values 0.00, 0.25, 0.50, 0.75, and 1.00: 0.00 means that all hybrids were viable and fertile, while 1.00 means that both hybrid sexes in both reciprocal crosses were sterile or inviable. Prezygotic isolation, on the other hand, is measured as a continuous variable ranging between zero and one. Thus,

TABLE 1. Literature data on genetic distance (*D*), biogeography, and reproductive isolation (defined in text) of *Drosophila* species pairs. Data from the species pairs marked with asterisks are revised from Coyne and Orr (1989a), and the remaining data are new.

Species 1	Species 2	Sympatric (0) or allopatric (1)	*D*	Isolation index			References[a]
				Prezygotic	Postzygotic	Total	
Hawaiian species							
silvestris W	*silvestris* E	1	0.049	0.136	0.000	0.136	1, 24
immigrans group							
nasuta	*albomicans*	1	0.530	0.000	0.000	0	5, 22, 26
melanogaster group							
auraria	*triauraria*	0	0.222	0.881	0.500	0.941	13, 16, 19
biauraria	*auraria*	0	0.229	0.902	0.000	0.902	13, 16, 19
biauraria	*triauraria*	0	0.221	0.931	0.000	0.931	13, 16, 19
kikkawai	*bocki*	1	0.336	1.000	—	1.000	3, 16, 19
kikkawaii	*pennae*	0	0.344	—	0.000	—	3, 16, 19
leontia	*bocki*	0	0.190	1.000	—	1.000	3, 16, 19
leontia	*pennae*	—	0.366	—	0.250	—	3, 16, 19
simulans	*sechellia*	1	0.280	0.376	0.500	0.688	6
quadraria	*auraria*	1	0.278	0.587	—	—	13, 16, 19
quadraria	*biauraria*	1	0.284	0.760	0.000	0.760	13, 16, 19
quadraria	*triauraria*	1	0.093	0.045	0.250	0.284	13, 16, 19
obscura group							
**athabasca* EB	*athabasca* EA	0	0.024	0.992	0.000	0.992	18, 27
**imaii*	*bifasciata* Japan	0	0.480	0.969	0.500	0.985	7, 14
**leontia*	*kikkawai*	0	0.244	—	0.500	—	3, 19
**miranda*	*bifasciata* Japan	1	1.950	0.980	—	0.980	7, 14
**persimilis*	*bifasciata* Japan	1	1.950	0.980	—	0.980	7, 14
**pseudoobscura*	*bifasciata* Japan	1	1.950	1.000	—	1.000	7, 14
**pseudoobscura*	*imaii*	1	1.950	1.000	—	1.000	7, 14
**subobscura*	*bifasciata* Japan	1	1.100	0.936	—	0.936	7, 14
athabasca WN	*athabasca* EA	0	0.120	0.925	0.000	0.925	18, 27
athabasca WN	*athabasca* EB	1	0.140	0.622	0.000	0.622	18, 22
azteca	*affinis*	0	0.850	1.000	1.000	1.000	11, 14
bifasciata Europe	*bifasciata* Japan	1	0.480	—	0.000	—	14, 15
subobscura	*madeirensis*	0	0.405	—	0.500	—	4, 12
repleta group							
**aldrichi*	*mulleri*	0	1.051	0.928	1.000	1.000	10
borborema	*martensis*	1	1.489	—	—	1.000	17, 23
borborema	*richardsoni*	1	1.318	—	—	1.000	17, 23
borborema	*stalkeri*	1	1.603	—	—	1.000	17, 23
borborema	*starmeri*	1	1.795	—	—	1.000	17, 23
borborema	*uniseta*	1	1.609	—	—	1.000	17, 23
borborema	*venezolana*	1	1.719	—	—	1.000	17, 23
buzzatii	*borborema*	0	0.744	—	—	1.000	17, 23
buzzatii	*richarsoni*	1	1.610	—	0.750	—	17, 23
buzzatii	*stalkeri*	1	1.214	—	—	1.000	17, 23
buzzatii	*starmeri*	1	1.680	—	—	1.000	17, 23
buzzatii	*uniseta*	1	1.424	—	—	1.000	17, 23
huaylasi	*mulleri*	1	0.362	—	0.750	—	10
huaylasi	*aldrichi*	1	1.080	—	1.000	1.000	10
koepferae Bolivia	*koepferae* Argentina	1	0.131	—	0.000	—	9
martensis	*richardsoni*	1	1.120	—	—	1.000	17, 23
martensis	*stalkeri*	1	1.010	—	—	1.000	17, 23
martensis	*starmeri*	0	0.901	—	—	1.000	17, 23
martensis	*uniseta*	0	0.637	—	—	1.000	17, 23
martensis	*venezolana*	0	1.038	—	—	1.000	17, 23
nigrodumosa	*aldrichi*	0	1.171	—	1.000	1.000	10
nigrodumosa	*huaylasi*	1	0.321	—	0.750	—	10
nigrodumosa	*mulleri*	1	0.538	—	0.750	—	10
serido	*borborema*	1	0.656	—	—	1.000	17, 23
serido	*buzzatii*	0	0.673	—	—	1.000	17, 23
serido	*koepferae*	1	0.814	0.802	0.500	0.901	9
serido	*martensis*	1	1.601	—	—	1.000	17, 23
serido	*uniseta*	1	1.584	—	—	1.000	17, 23
serido	*venezolana*	1	1.755	—	—	1.000	17, 23
stalkeri	*richardsoni*	1	0.770	—	—	1.000	17, 13
starmeri	*stalkeri*	1	0.724	—	—	1.000	17, 13
starmeri	*uniseta*	0	0.702	—	—	1.000	17, 13

TABLE 1. Continued.

Species 1	Species 2	Sympatric (0) or allopatric (1)	D	Isolation index			References[a]
				Prezygotic	Postzygotic	Total	
starmeri	*venezolana*	0	0.471	—	0.750	—	17, 13
uniseta	*richardsoni*	1	1.403	—	—	1.000	17, 13
uniseta	*stalkeri*	1	1.132	—	—	1.000	17, 13
uniseta	*venezolana*	0	0.824	—	—	1.000	17, 13
venezolana	*richardsoni*	1	1.090	—	—	1.000	17, 13
venezolana	*stalkeri*	1	0.793	—	—	1.000	17, 13
virilis group							
americana	*littoralis*	1	1.210	—	—	1.000	21, 25
montana	*ezoana*	0	0.610	1.000	—	1.000	20, 25
novamexicana	*littoralis*	1	0.970	—	1.000	1.000	21, 25
texana	*littoralis*	1	1.220	—	—	1.000	21, 25
willistoni group							
**paulistorum* Am	*pulistorum* An-Br	0	0.170	0.860	0.500	0.930	2, 8

[a] Key to references: (1) Ahearn and Templeton 1989; (2) Ayala et al. 1974; (3) Baimi et al. 1980; (4) Cabrera et al. 1983; (5) Chang and Ayala 1989; (6) J. Coyne, unpubl. data; (7) Dobzhansky et al. 1968; (8) Ehrman and Powell 1982; (9) Fontdevila et al. 1988; (10) Fontdevila et al. 1990; (11) Goddard et al. 1990; (12) Khadem and Krimbas 1991; (13) Kurokawa et al. 1982; (14) Lakovaara et al. 1976; (15) Lakovaara and Saura 1982; (16) Lemeunier et al. 1986; (17) Marin et al. 1993; (18) Miller and Westphal 1967; (19) Ohnishi et al. 1983b; (20) Ohnishi et al. 1983a; (21) Patterson 1952; (22) Rao and Ranganath 1990; (23) Sanchez 1986; (24) Sene and Carson 1987; (25) L. Throckmorton pers. comm.; (26) Wilson et al. 1969; (27) Yoon and Aquadro 1994.

when comparing prezygotic with postzygotic isolation, we adjusted the former figure downward (to the nearest 0.00, 0.25, 0.50, 0.75, or 1.00) to make the two indices comparable estimates of the reduction in gene flow.

RESULTS

Table 1 shows all of our new or revised data. The hybridizations marked with an asterisk are corrections of data presented in Table 1 of our 1989 paper, which included a few errors of transcription, erroneous descriptions of biogeography, or phylogenies that were not consistent with more recent estimates. The remaining 59 pairs represent new data. A few of these are pairs from our previous paper for which estimates of either prezygotic or postzygotic isolation have recently become available, and the remaining data are completely new. In addition, three hybridizations used in our earlier paper were removed because we found large and conflicting differences in the genetic distances between published studies: *D. mojavensis/D. aldrichi*, *D. aldrichi/D. arizonae* (formerly *D. arizonensis*), and *D. mulleri/D. arizonae*. The data presented here, when combined with those from table 1 of our previous paper, yield a total of 171 hybridizations.

In addition to the column for "prezygotic" and "postzygotic" isolation in Table 1, we present a column for "total" reproductive isolation, a combined figure calculated by assuming that the prezygotic and postzygotic isolation act sequentially and multiplicatively to reduce gene flow (see below). In some cases, we give a total reproductive isolation of 1.00 (no gene flow) when there are no separate estimates for prezygotic and postzygotic isolation. These figures come from studies in which males and females of different species were forcibly confined for extended periods but failed to produce offspring. Although reproductive isolation between such pairs is complete, we could not determine the relative involvement of sexual isolation versus hybrid inviability because the authors presented no data on mating frequencies or percentage of females inseminated. Nevertheless, the total reproductive isolation in such cases is 1.00.

When we correct our data set of 171 species pairs for phylogenetic dependence, we obtain 50 independent pairs of taxa for prezygotic isolation, and 56 pairs for postzygotic isolation (in our previous paper we had 42 such pairs for each form of isolation). The list of these corrected taxon pairs is not given here but is available on request.

Using the larger data set does not alter the conclusions of our previous study. Indeed, virtually all of our conclusions—particularly the remarkable difference between sympatric and allopatric species in their degree of sexual isolation—are strengthened. We present our analysis of the new data in the same order as that given in our 1989 paper.

Prezygotic and Postzygotic Isolation Increase with Time

Figures 1a,b show the relationship in the uncorrected data between genetic distance and prezygotic or postzygotic isolation. As in our previous work (Coyne and Orr 1989a, pp. 365–366), we have rounded down the estimates of prezygotic isolation to make them comparable to postzygotic isolation, which was measured as a discrete variable. Both forms of reproductive isolation increase with genetic distance. Using the uncorrected data, Kendall's rank correlation between prezygotic isolation and Nei's D yields a γ of 0.230 ($n = 101$, $P = 0.0007$); for postzygotic isolation, Kendall's $\gamma = 0.507$ ($n = 107$, $P < 0.0001$). Figures 2a,b show the correlation in the phylogenetically corrected data. Again, both forms of reproductive isolation are correlated with genetic distance (prezygotic isolation: Kendall's $\gamma = 0.251$, $n = 50$, $P = 0.0102$; postzygotic isolation: Kendall's $\gamma = 0.529$, $n = 56$, $P < 0.0001$).

Prezygotic Isolation Is Stronger than Postzygotic Isolation

In our previous paper, we found that prezygotic isolation was stronger than postzygotic isolation but that this differ-

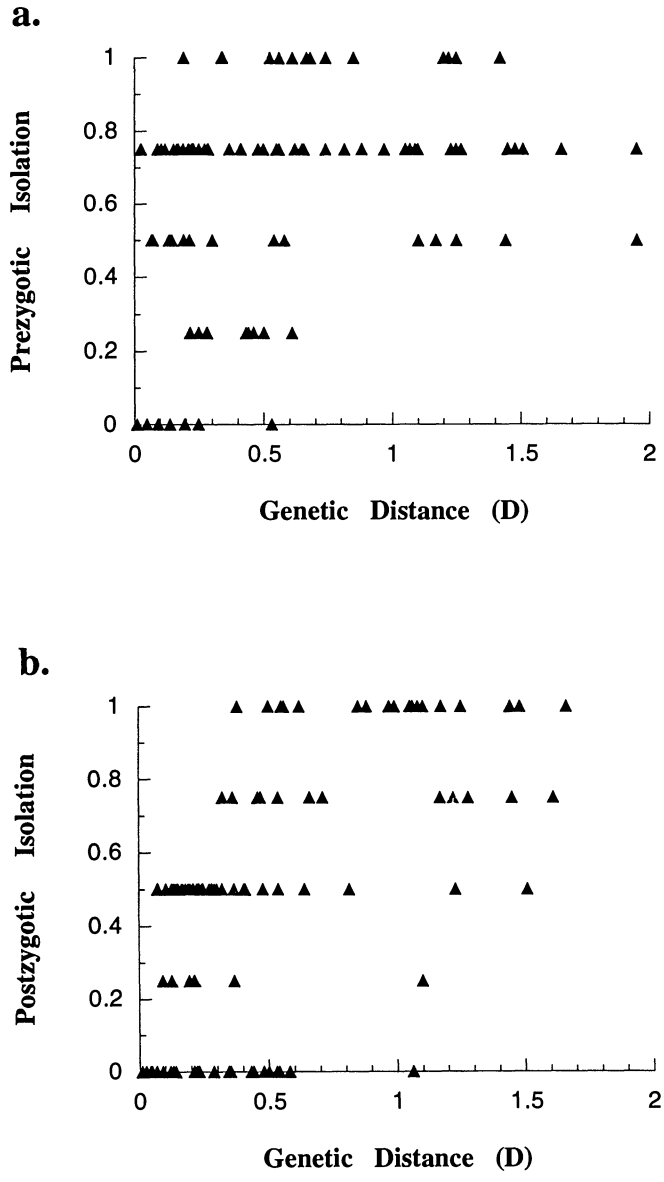

FIG. 1 Strength of isolation plotted against genetic distance (D) between taxa (uncorrected data). (a) Prezygotic isolation (rounded down); (b) postzygotic isolation.

ence was due entirely to elevated levels of prezygotic isolation in sympatric taxa. As before, we limit our reanalysis to those species pairs separated by low genetic distances ($D \leq 0.5$), as we are most interested in what happens in the early stages of speciation. Using the corrected data, we again find significantly greater prezygotic than postzygotic isolation in this group of young species (mean prezygotic isolation = 0.563, $n = 32$; mean postzygotic isolation = 0.319, $n = 40$). These values differ significantly (Mann-Whitney U-test: $Z = 3.47$, $P = 0.0005$). This difference is not an artifact of different average genetic distances among species pairs in these two data sets, as these values are nearly identical (mean $D_{prezygotic} = 0.217 \pm 0.023$ [SE], $n = 32$; mean $D_{postzygotic} = 0.225 \pm 0.022$, $n = 40$). The difference between prezygotic and postzygotic isolation is also highly significant in the uncorrected data (analysis not shown).

Using the uncorrected data, we may also compare the level of prezygotic versus postzygotic isolation *within* individual pairs of species. We have 41 hybridizations with $D \leq 0.5$ for which there is information on both prezygotic and postzygotic isolation. Applying Wilcoxon's signed-rank test to these paired values, we find that prezygotic isolation significantly exceeds postzygotic isolation ($Z = 3.12$, $P = 0.0018$). As we show below, however, *this difference is due entirely to high levels of prezygotic isolation in sympatric taxa*; among allopatric taxa the levels of prezygotic and postzygotic isolation do not differ.

Large Time Lag between the Appearance of Postzygotic Isolation in Male versus Female Hybrids

For each pair of species, there are two reciprocal hybridizations, each producing males and females, so there are four "gender classes" that can be either completely sterile or inviable (we counted genders with partial sterility or inviability as if they were fully fertile or viable). Each hybridization can be placed in either one of these four classes or the "zero" class (no genders completely sterile or inviable), allowing us to determine the average genetic distance between pairs of species falling into each class. In our previous paper, we found that species pairs in which one or two gender classes were sterile or inviable were much younger (i.e., had lower Ds) than those in which three or four of these classes were sterile or inviable. In nearly all cases, pairs with two or fewer afflicted classes showed postzygotic isolation in male hybrids alone (as expected under Haldane's rule), while those with three or more such classes necessarily included females. There was thus a large gap in time between the appearance of male versus female postzygotic isolation.

The left half of Table 2 shows our reanalysis of this problem using the corrected data. For each class, we present the average genetic distances (and standard error) at which a given level of postzygotic isolation arises. (The "isolation index" is the proportion of the four gender classes whose members were completely sterile or inviable.) Of the 24 pairs of taxa falling into the 0.25 and 0.50 categories, 22 have the sterility and inviability limited to male hybrids. Post hoc analysis (Scheffe's F-test; see Table 2) shows that there is a large and significant jump in genetic distance between categories 0.5 and 0.75. Biologically, this means that there is a long stall after the evolution of hybrid male sterility and inviability and *before* the appearance of hybrid female sterility and inviability. There is no analogous "stalling" of *prezygotic* isolation: as the right side of Table 2 shows, there is no significant jump in genetic distance between any of the prezygotic isolation "classes." Identical results are obtained with the total uncorrected data (not shown).

Prezygotic Isolation Is Enhanced in Sympatric Species

The present reanalysis strengthens one of the most striking findings of our previous paper: for species pairs of a given age, the amount of sexual isolation is much greater when they are sympatric than when they are allopatric. Figures 3a,b show the relationship between genetic distance and prezy-

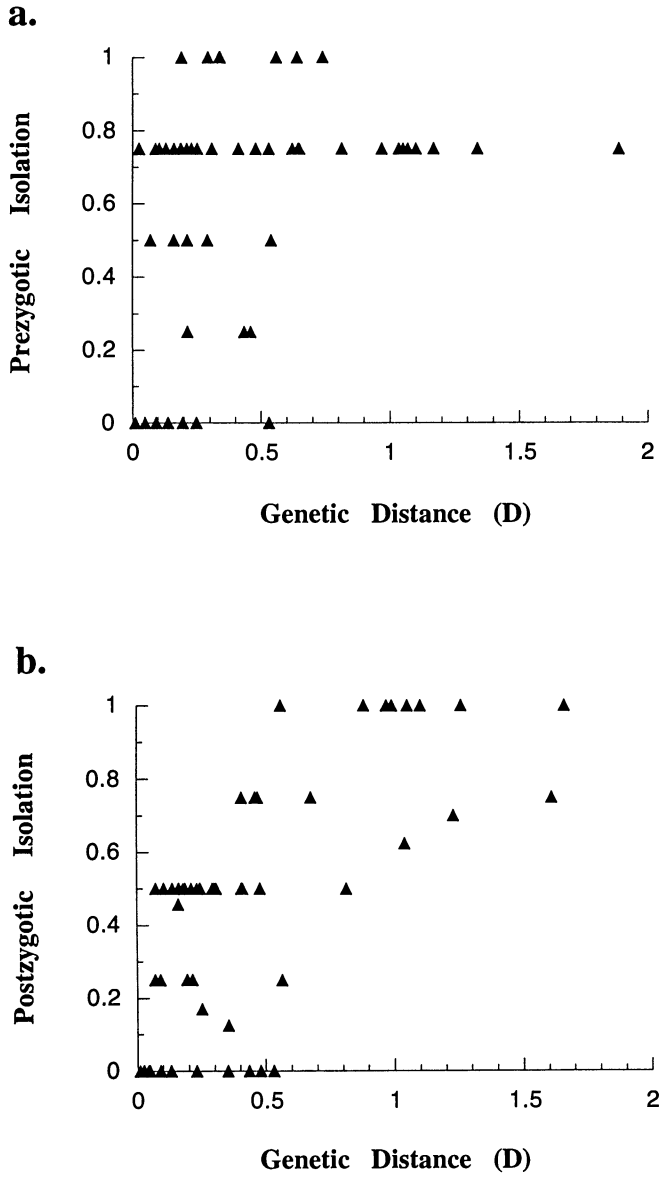

FIG. 2. Strength of isolation plotted against genetic distance (D) between taxa (corrected data). (a) Prezygotic isolation (rounded down); (b) postzygotic isoloation.

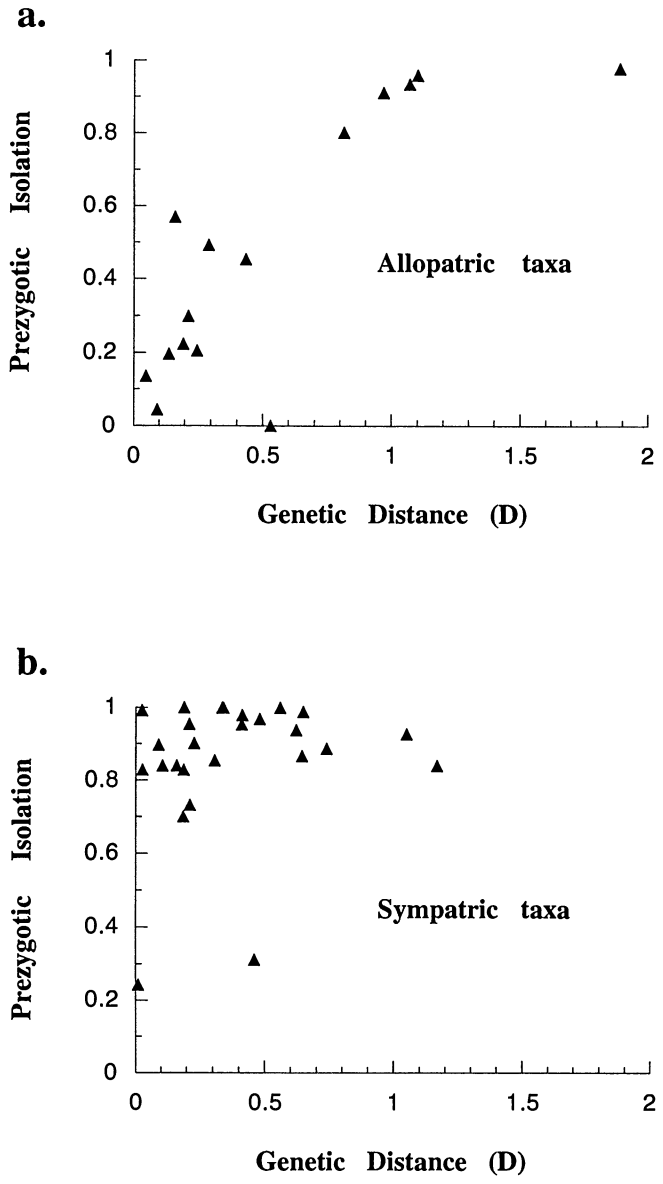

FIG. 3. (a) Prezygotic isolation plotted against genetic distance (D) among allopatric taxa (corrected data); (b) prezygotic isolation plotted against genetic distance (D) among sympatric taxa (corrected data).

TABLE 2. Mean and standard error of genetic distance at which a given level of postzygotic and prezygotic isolation occurs in *Drosophila,* using corrected data. The "isolation index" is the proportion of the four gender classes that are completely sterile or inviable; the isolation index is averaged among taxa and then rounded down to the 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	0.209 ± 0.047 (15)	0.180 ± 0.066 (7)
0.25	0.224 ± 0.073 (6)	0.370 ± 0.078 (3)
0.50	0.275 ± 0.041 (18)	0.255 ± 0.080 (5)
0.75	0.757 ± 0.173 (8)	0.574 ± 0.090 (28)
1.00	1.003 ± 0.113 (9)	0.443 ± 0.077 (7)

gotic isolation for sympatric and allopatric taxa considered separately (corrected data). The difference is impressive. We can confirm this effect statistically in several ways. First, among the nine pairs of allopatric taxa separated by low genetic distances ($D \leq 0.5$), none shows prezygotic isolation greater than 0.75. In contrast, among the 19 pairs of sympatric species with $D \leq 0.5$, fifteen have prezygotic isolation greater than 0.75. The mean degree of prezygotic isolation among the allopatric species below this D-value is 0.291 ± 0.059 (SE), while for sympatric species of similar age it is 0.833 ± 0.049. Prezygotic isolation is thus nearly three times stronger among sympatric than allopatric species of similar age. This difference is highly significant (Mann-Whitney U-test: $Z = 3.864$, $n_1 = 19$, $n_2 = 9$, $P < 0.001$; this is test "a"

discussed by Coyne and Orr 1989a, p. 374). This effect cannot be an artifact of a difference in mean genetic distance between sympatric and allopatric pairs, as none is seen ($D_{allopatric}$ = 0.202 ± 0.039; $D_{sympatric}$ = 0.230 ± 0.034). Analysis of the uncorrected data yields nearly identical results: sexual isolation is significantly stronger in young sympatric than in young allopatric taxa (analysis not shown).

The effect of sympatry on sexual isolation can be demonstrated in several other ways. One of these involves comparing prezygotic and postzygotic isolation *within* pairs of species for which we have information about both forms of reproductive isolation (test "b" in Coyne and Orr 1989a, p. 374). This comparison requires us to use the uncorrected data. Among allopatric species pairs with $D \leq 0.5$, prezygotic isolation exceeds postzygotic isolation in five cases, is less than postzygotic isolation in six cases, and equals postzygotic isolation in six cases (again, prezygotic isolation is rounded down to allow a fair comparison with postzygotic isolation). In contrast, the corresponding numbers among sympatric taxa are 21, one, and two cases, respectively. These distributions differ significantly (G_2 = 15.44, P = 0.0005). Thus, within species pairs, prezygotic isolation exceeds postzygotic significantly more often when taxa are sympatric than allopatric.

Finally, we can compare the magnitudes of prezygotic and postzygotic isolation within species pairs when they are sympatric versus when they are allopatric (test "c" in Coyne and Orr 1989a, p. 374). We find no difference in the strength of prezygotic and postzygotic isolation among young ($D \leq 0.5$) allopatric taxa (corrected data, Wilcoxon signed rank test: Z = 0.262, n = 10, P = 0.794). For sympatric taxa, however, prezygotic isolation significantly exceeds postzygotic isolation (Z = 3.741, n = 22, P = 0.0002).

As emphasized in our previous paper, these last two tests ("b" and "c") do not depend on the accuracy or even the existence of a molecular clock since, *within* each species pair, there has been exactly as much time for the evolution of prezygotic and postzygotic isolation.

In contrast to sexual isolation, the degree of *postzygotic* isolation among taxa is not affected by whether they are sympatric or allopatric: among young taxa ($D \leq 0.5$), geographic overlap has no effect on postzygotic isolation (corrected data, Mann-Whitney U-test, Z = 0.565, n_1 = 22, n_2 = 15, P = 0.572). The results are the same when one uses the uncorrected data (P = 0.943).

Using our more complete data, then, the enhancement of prezygotic isolation among sympatric species pairs is even more striking than seen in our previous paper—every statistical test yields a more significant result.

"Total" Reproductive Isolation Increases with Time

As in our previous paper, we can calculate "total" reproductive isolation by assuming that prezygotic and postzygotic isolation operate sequentially and multiplicatively to reduce gene flow. If T represents total reproductive isolation, *Pre* represents prezygotic isolation and *Post* represents postzygotic isolation, then $1 - T = (1 - Pre)(1 - Post)$. When this equation is rearranged, $T = Pre + (1 - Pre) \times Post$. (This "total" reproductive isolation, of course, neglects some sources of reproductive isolation that may operate in the wild, such as ecological or temporal divergence.) As in our previous paper, for species in which prezygotic isolation exceeded 0.9 but for which we had no information about postzygotic isolation, we set the "total" isolation equal to level of prezygotic isolation; in such cases the maximum possible error is only 10%.

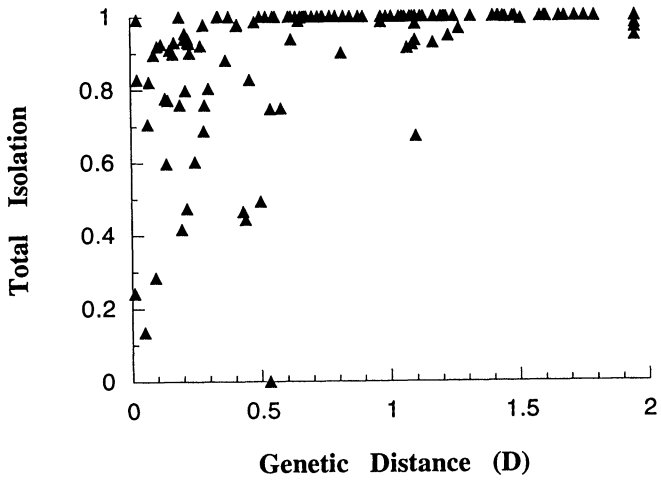

FIG. 4. Total reproductive isolation (T) plotted against genetic distance (D).

Figure 4 shows the relationship between total isolation and genetic distance. T increases quickly with time (i.e., with larger values of D), and virtually all species pairs separated by a D of 0.6 or more are completely isolated. The rank correlation between T and D from Kendall's test is γ = 0.432 (n = 134, $P < 0.0001$).

DISCUSSION

Our analysis of the updated data set yields essentially the same conclusions as in our previous paper. All of the patterns that were previously significant are now even more so, including two of the most intriguing observations: the difference in sexual isolation between allopatric and sympatric species pairs, and the temporal gap between the evolution of postzygotic isolation in males and females. We briefly discuss the five results outlined above.

First, both prezygotic and postzygotic isolation increase with divergence time between taxa. This is not surprising, of course, as the increase of reproductive isolation with time is predicted by any reasonable theory of speciation. We can, however, combine our prezygotic and postzygotic estimates into an estimate of "total" reproductive isolation (see below) and thereby get a rough idea of how much time is required to attain a given level of reproductive isolation.

Second, prezygotic isolation evolves faster than postzygotic isolation. As Figure 5 shows, however, this disparity is due entirely to higher prezygotic isolation between sympatric than between allopatric pairs of species. In our previous paper (Coyne and Orr 1989a, pp. 376–377), we considered various explanations for this pattern and concluded that the most likely is reinforcement: the enhancement of sexual isolation by natural selection when two taxa that produce unfit hybrids become sympatric (Dobzhansky 1940). Reinforce-

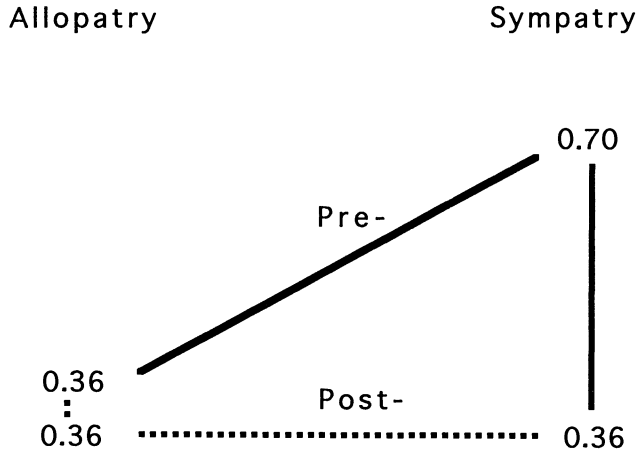

FIG. 5. Average strength of prezygotic and postzygotic isolation in allopatric versus sympatric species pairs. Only species pairs with Nei's $D \leq 0.5$ are included. Prezygotic values are rounded down and means are from uncorrected data (corrected data sets yield similar values). Values connected by solid lines are significantly different (by Mann-Whitney U-test), while those connected by dashed lines are not.

ment has, of course, been controversial. Once considered ubiquitous, this process has recently been questioned because of a lack of supporting data and the inability of theoretical models to demonstrate reinforcement with any frequency (Spencer et al. 1986; Butlin 1987). More recently, however, evidence has begun to accumulate in its favor. This includes not only the comparative data shown here, but also direct observations of increased sexual isolation among sympatric versus allopatric populations (Butlin 1995; Noor 1995). In addition, newer theoretical models show that reinforcement may be both a plausible and a likely outcome of secondary contact between allopatric taxa (Liou and Price 1994; Kelly and Noor 1996). Although we are not ardent champions of reinforcement, and certainly do not share Dobzhansky's (1940) view that it is a universal phase of speciation, we think that these recent results resurrect reinforcement as a serious possibility.

Finally, we find a large jump in genetic distance between hybridizations producing sterile or inviable males only, and hybridizations producing postzygotic isolation in females as well. Moreover, in almost all younger hybridizations, hybrid sterility or inviability is limited to males. Taken together, these observations show that speciation does *not* proceed along two paths, one path in which hybrid males become sterile or inviable first (followed by females) and the other path in which hybrid male and females become simultaneously sterile or inviable. (Note that the simultaneous existence of these two paths would still give rise to Haldane's rule.) Instead, there seems to be a single, and almost requisite, path along which postzygotic isolation evolves: hybrid males succumb early in the process, and hybrid females much later.

The observed "stall" before the evolution of hybrid female sterility and inviability can be explained in two ways. First, such a lag is expected if hybrid sterility and inviability result from alleles that act as partial recessives in hybrids: males quickly succumb to such recessives because the alleles are fully expressed on the (hemizygous) X-chromosome. It will take much longer, however, before females are affected by the accumulation of partially recessive "speciation genes," as hybrid females are heterozygotes at all such loci (Turelli and Orr 1995; Orr and Turelli 1996). Second, alleles causing sterility only in hybrid males appear to accumulate faster than those causing sterility only in females, at least in *Drosophila* (Wu and Davis 1993; True et al. 1996). This would obviously cause a lag between the evolution of hybrid male and female sterility.

One could assess the relative roles of these two forces in *Drosophila* by looking at the duration of the lag when considering hybrid inviability alone. Because hybrid-inviability genes appear to affect both sexes equally (Orr 1993), any stalling before the evolution of hybrid female inviability must reflect recessivity alone. Unfortunately, we do not have enough inviability data to allow a meaningful estimate of this lag time. However, consideration of other taxa suggests that the second scenario is not a general explanation of Haldane's rule. Birds and butterflies, which have heterogametic females, also conform to Haldane's rule, that is, hybrid females are preferentially sterile or inviable (Haldane 1922; Coyne and Orr 1989b). This pattern cannot be explained by a more rapid evolution of male-specific sterility alleles.

Finally, from the rate of increase in "total" reproductive isolation, we can make rough calculations about the time required for taxa to attain status as full species (see Coyne and Orr 1989a, p. 379). As before, we consider that the appropriate criterion for species status is the ability of two taxa to remain distinct in sympatry. We thus calculate the level of reproductive isolation between successfully coexisting sympatric species.

The mean level of total reproductive isolation among sympatric species pairs from the uncorrected data is 0.936 (n = 55), and the lower bound of the 95% confidence interval about this mean is 0.903. Thus, total reproductive isolation of magnitude 0.903 or greater is probably sufficient to prevent the fusion of sympatric taxa. We then estimate the genetic distance associated with this degree of reproductive isolation from the total data and from sympatric and allopatric pairs of taxa considered separately. For each of these three data sets, we fit an asymptotic exponential regression between genetic distance (independent variable) and total reproductive isolation (dependent variable), forcing the regression through the origin. This procedure assumes that species begin as genetically undifferentiated populations with no reproductive isolation and eventually proceed to complete reproductive isolation ($T = 1$). These regressions are of the form $T = 1 - e^{-kD}$, where T is total reproductive isolation, D is genetic distance, and k is a constant estimated from the regression line. The constant k is 12.87 for all species considered together, 4.35 for allopatric species, and 59.25 for sympatric species.

After calculating these regressions for our three data sets, we computed the D-value corresponding to $T = 0.903$, the degree of isolation allowing sympatric coexistence. These values were $D = 0.18$ for all species, $D = 0.54$ for allopatric species pairs, and $D = 0.04$ for sympatric species pairs. In our previous paper, these values were calculated as 0.53, 0.66, and 0.31, respectively. The disparities between the two sets of values are due to the greater effect of sympatry on sexual

isolation found in our newer data, and to our use of an exponential regression (which reaches an asymptote) instead of our earlier second-order regression (which does not). If the relationship between D and divergence time is roughly linear (Nei 1987), taxa that became secondarily sympatric must speciate in less than a tenth of the time required for allopatric taxa. If one further assumes that $D = 1$ roughly corresponds to 5 million yr of divergence (Nei 1987), speciation requires approximately 200,000 yr among taxa that become sympatric and approximately 2.7 million yr among taxa that remain allopatric.

These are, of course, very crude estimates that should not be taken too seriously. The time calibration of the electrophoretic clock, for example, may be considerably off (indeed, Nei's calibration was based not on *Drosophila* but on mammals), and a few species pairs deviate considerably from these regression lines. Nevertheless, these calculations provide rough estimates of the average time required for the completion of speciation and show once again the profound effect on sympatry on the rate of speciation. This effect must, in fact, be even more dramatic than the above analysis suggests. Unless one believes that sympatric speciation is common, our "sympatric" taxa must represent cases of secondary contact between taxa that had first been geographically separated. Some of these taxa, however, must have become sympatric more recently than others, even when all are separated by similar genetic distances. The striking increase in prezygotic isolation seen in virtually *all* sympatric taxa thus suggests that the effect of sympatry is not only profound, but is also rapid.

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MATING BEHAVIOR IN *DROSOPHILA MELANOGASTER* SELECTED FOR ALTERED LONGEVITY

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In experimental systems, the interaction between behavior and the expression of life-history traits has seldom been investigated. We present here the results of a study concerning both male and female reproductive behaviors in relation to selection for increased longevity. It is found that the selection process had opposite effects on male and female mating behavior early in life.

Studies of life-history traits using *Drosophila melanogaster* differentially selected for early-versus late-life fitness characteristics (producing short- and long-lived stocks) are extensive (Luckinbill et al. 1984; Rose 1984; Rose et al. 1984; Service et al. 1988; Partridge and Fowler 1992). Much of this work is devoted to identifying correlated responses to selection and inferring genetic relationships between longevity and a variety of other traits. Almost exclusively, the characters examined in this way are physiological or developmental, and the complexity of such relationships are revealed by the myriad of confusing and contradictory results among different laboratories (see Chippindale et al. 1994; Curtsinger et al. 1995).

Considering the range of traits that has been investigated in flies selected for late- versus early-life fitness, relatively little work has been devoted to understanding how behavioral traits have been affected by the selection process, and conversely, how the selection process may have been affected by the plasticity of behavioral traits (but see Service 1993). Behavioral strategies regarding the propensity to mate during certain age periods can have direct effects on current and future survival, and therefore may be involved in a response to selection for increased longevity (Reznick 1992; Tatar et al. 1993). In *D. melanogaster*, males experience a temporary increase in mortality when exposed to the opposite sex (Partridge and Farquhar 1981; Partridge and Andrews 1985; Service 1989), while female costs may include both a temporary and permanent increase in mortality (Partridge et al. 1986; Partridge et al. 1987a; Chapman et al. 1995). These results establish a link between early reproductive behavior and extended longevity.

The arguments above suggest that, for both male and female fruit flies, one way to increase the probability of surviving late in life is to be aversive to early mating. Mating speeds have been shown to have a genetic basis characteristic of a standard polygenic trait (Manning 1961; Parsons 1964; Kessler 1969) and therefore may show a response to direct or indirect selection. The extent to which males and females of long- versus short-lived stocks differ in their propensity to mate at early ages has not been extensively studied. Service (1993) investigated age-specific mating characteristics of long- and short-lived males exposed to common females from a foreign stock, but he did not examine female behavior in the selected lines or the specific dynamics occurring within each selection regime (e.g., males exposed to females of their own stock).

We report here the results of a large experiment designed to investigate the early life mating behavior of both male and female *D. melanogaster* selected for either early- or late-life