The Fertility Effects of Pericentric Inversions in *Drosophila melanogaster*

Jerry A. Coyne, Wendy Meyers,[1] Anne P. Crittenden and Paul Sniegowski

Department of Ecology and Evolution, The University of Chicago, Chicago, Illinois 60637

Manuscript received September 15, 1992
Accepted for publication February 17, 1993

ABSTRACT

Heterozygotes for pericentric inversions are expected to be semisterile because recombination in the inverted region produces aneuploid gametes. Newly arising pericentric inversions should therefore be quickly eliminated from populations by natural selection. The occasional polymorphism for such inversions and their fixation among closely related species have supported the idea that genetic drift in very small populations can overcome natural selection in the wild. We studied the effect of 7 second-chromosome and 30 third-chromosome pericentric inversions on the fertility of heterokaryotypic *Drosophila melanogaster* females. Surprisingly, fertility was not significantly reduced in many cases, even when the inversion was quite large. This lack of underdominance is almost certainly due to suppressed recombination in inversion heterozygotes, a phenomenon previously observed in Drosophila. In the large sample of third-chromosome inversions, the degree of underdominance depends far more on the position of breakpoints than on the inversion's length. Analysis of these positions shows that this chromosome has a pair of "sensitive sites" near cytological divisions 68 and 92: these sites appear to reduce recombination in a heterozygous inversion whose breakpoints are nearby. There may also be "sensitive sites" near divisions 31 and 49 on the second chromosome. Such sites may be important in initiating synapsis. Because many pericentric inversions do not reduce the fertility of heterozyotes, we conclude that the observed fixation or polymorphism of such rearrangements in nature does not imply genetic drift in very small populations.

HETEROZYGOTES for some chromosomal rearrangements, such as pericentric inversions and reciprocal translocations, are expected to be semisterile because abnormal segregation or recombination within the inverted region produces aneuploid gametes. Because of this heterozygote inferiority (underdominance), these rearrangements should be quickly eliminated from populations when they first arise. Indeed, polymorphisms for such rearrangements are much rarer than for other rearrangements that do not confer underdominance, such as the well known paracentric inversions in Drosophila (STONE 1955). Nevertheless, some species are polymorphic for rearrangements thought to be underdominant (CARSON and STALKER 1947; WHITE 1973). Moreover, closely related species are sometimes fixed for different rearrangements thought to be underdominant in ancestral heterozygotes (STONE 1955). These observations raise an evolutionary problem because natural selection would oppose the polymorphism as well as the transition from one fixed state to another.

The widely accepted solution to this problem involves genetic drift. In very small populations, drift may overcome natural selection and allow rearrangments that cause underdominance to become more common (LANDE 1979, 1984; HEDRICK 1981). (We refer hereafter to rearrangements that cause underdominance in heterozygotes as "underdominant rearrangements.") To many evolutionists, polymorphism or fixation of such rearrangements has provided strong evidence for genetic drift (*e.g.*, "It is not unreasonable to consider much of the corpus of cytogenetic data *prima facie* evidence that speciation occurs by the geographic isolation of small populations" [FUTUYMA and MAYER (1980), pp. 262–263]). Fixation of such rearrangements has also been adduced as evidence for WRIGHT's (1970, 1982) shifting balance theory of evolution, since the transition between alternate homozygotes requires crossing an "adaptive valley" (a population polymorphic for underdominant rearrangements), and can therefore be seen as a "peak shift" that may require genetic drift. Indeed, cytogenetic data provide some of the only evidence that genetic drift can influence characters of substantial selective importance (COYNE, AULARD and BERRY 1991). The cytogenetic data have also inspired several theories of chromosomal speciation, such as WHITE's (1968, 1978) theory of "stasipatric speciation," which consider chromosome rearrangements to be a major cause of reproductive isolation. These theories again depend critically on genetic drift fixing underdominant rearrangements in small populations.

These speculations assume that chromosome re-

[1] Current address: Center for Population Biology, Storer Hall, The University of California, Davis, California 95616.

arrangements that are theoretically underdominant really reduce the fitness of heterozygotes in nature. There is increasing evidence, however, that this is may not always be true (JOHN 1981; COYNE, AULARD and BERRY 1991; KING 1992). For example, pericentric inversions in Drosophila theoretically reduce the fitness of heterokaryotypic females, as recombination in the inverted region produces eggs with duplications and deficiencies. (The males, which lack recombination, do not have this problem.) However, some pericentric inversions do not markedly reduce egg hatch of their female carriers (ROBERTS 1967), presumably because recombination is somehow suppressed.

COYNE, AULARD and BERRY (1991) recently studied a large pericentric inversion in *D. melanogaster*, *In(2LR)PL*, which covers nearly 40% of the second chromosome. This inversion was polymorphic in two small populations on Indian Ocean islands (Mauritius and Rodriguez), and we initially speculated that the polymorphism resulted from genetic drift. Genetic analysis showed, however, that despite its length *In(2LR)PL* was *not* underdominant: females heterozygous for this inversion and its ancestral *Standard* sequence were perfectly fertile. This normal fertility resulted from a nearly complete absence of recombination in the inverted region, so that these females produced almost no aneuploid gametes. Although the cause of reduced recombination was not clear, it was almost certainly not a gene that inhibited crossing over, because inversion *homozygotes* showed normal levels of recombination. A more likely explanation is some abnormality of synapsis. It is possible that pericentric inversion heterozygotes sometimes fail to form homosynaptic loops during meiosis, but instead pair heterosynaptically (straight), a configuration that prevents recombination. Another possibility is that breakpoints in certain locations somehow inhibit recombination over large areas of the chromosome. This idea is supported by the work of ROBERTS (1970, 1972) and HAWLEY (1980), who showed that the position of breakpoints in *D. melanogaster* strongly affects recombination in heterozygotes for reciprocal translocations.

Here we report studies of additional pericentric inversions in *D. melanogaster*. We have obtained many such inversions and have measured their effect on the fertility of female heterokaryotypes. Our purpose is twofold: to determine whether heterozygous pericentric inversions may often be innocuous rather than underdominant (this will give us an idea of how often such inversions may arise), and to identify factors determining how deleterious they are. As noted above, the relative frequency of underdominant rearrangements has important implications for the shifting balance theory of evolution and for theories of chromosomal speciation. In addition, we were interested in which properties of an inversion–especially length and location of the breakpoints–most strongly affect its underdominance.

MATERIALS AND METHODS

Strains: We obtained 30 third-chromosome inversions and 7 second-chromosome inversions (see Table 1) from a variety of sources, the most important being the Mid-America Drosophila Stock Center (Bowling Green, Ohio) and the Indiana University Drosophila Stock Center. All but two of these inversions were generated by radiation, and many of them carry recessive lethal alleles. These inversions were maintained as heterozygotes against balancer chromosomes. The other two inversions, *In(2LR)Rev*^{B} and *In(2LR)PL*, were spontaneous; the latter was polymorphic in two natural populations and was extensively studied by COYNE, AULARD and BERRY (1991).

The standard wild-type tester strain was *Ives*, originally created in 1975 by combining the progeny of 200 isofemale lines collected by P. IVES in Amherst, Massachusetts. In 1977, B. CHARLESWORTH extracted isofemale lines from this stock and founded our strain by combining 21 lines homokaryotypic for the *Standard* banding sequence on all chromosomes.

Other recessive-marker stocks (*ebony* and *cinnabar*) were obtained from the National Drosophila Stock Center at Bowling Green, Ohio. Each of these mutants was put onto a background of the *Ives* genome by two cycles of outcrossing and reextraction.

Flies were reared on standard agar-yeast-cornmeal medium in 8-dram vials at 24° on a 12-hr light/dark cycle.

Cytology: All stocks were checked to ensure that they still carried a pericentric inversion and that the described breakpoints were correct. Males from inversion-carrying strains were crossed to *Ives* females, which are homokaryotypic for the *Standard* (*ST*) sequence on all chromosomes. Salivary glands from third-instar F_1 larvae were dissected in 40% acetic acid, stained with aceto-orecin and squashed according to standard protocols (ASHBURNER 1989).

Crossing scheme: The crossing scheme [similar to that used by ROBERTS (1967) and COYNE, AULARD and BERRY (1991)] permitted us to compare the hatchability of eggs produced by heterokaryotypic females with that of eggs fertilized by heterokaryotypic males when both of those genotypes were mated to the *Ives* tester strain. The two types of eggs have similar genetic endowments but differ in whether the heterokaryotypic parent has undergone recombination. Some of the eggs from heterokaryotypic females should be inviable due to deficiencies and duplications produced by recombination in the inverted region. One therefore expects eggs from the "experimental" cross (heterokaryotypic mothers) to show lower hatchability than those from the "control" cross (heterokaryotypic fathers).

The crossing scheme depended on whether the pericentric inversion was homozygous viable or whether it was homozygous lethal and balanced against a marker chromosome. In the latter case, if the balancer contained a clearly scorable dominant allele, males from the inversion/balancer stock were crossed to *Ives* females. F_1 flies not showing the dominant allele (and therefore carrying the pericentric inversion) were then backcrossed to *Ives* flies to produce the eggs scored for hatchability.

If the balancer chromosome did not contain an easily scorable dominant allele but did have an easily scorable recessive (*e.g.*, TM6B, which contains the poorly penetrant *Tubby* allele but also the recessive allele *ebony*), we crossed

inversion/balancer females to males from a strain homozygous for the recessive allele. F_1 flies not showing the recessive phenotype are heterozygous for the pericentric inversion, and these were crossed to the *Ives* strain.

For stocks containing a homozygous-viable inversion, we crossed males from this strain to *Ives* females, and backcrossed the F_1 males and females to the *Ives* stock.

This procedure produces two groups of flies (experimental and control) containing identical combinations of autosomes and with cytoplasm ultimately deriving from the *Ives* strain. Offspring of the two crosses differ somewhat in their sex chromosomes, with offspring of the control cross having only *Ives X* chromosomes, and those from the experimental cross having a mixture of sex chromosomes from both the *Ives* and inversion-bearing strains. These genetic differences could account for some of the slight viability differences between control and experimental crosses, especially those in which the hatchability of eggs from experimental crosses exceeded that of the controls (see below). The experimental design also precluded hybrid dysgenesis from affecting the results: temperatures were too low for the appearance of P-M hybrid dysgenesis, and the use of the naturally occurring Ives strain as a female parent precludes the occurrence of I-R dysgenesis (BREGLIANO and KIDWELL 1983; KIDWELL 1983).

Virgin females in the control and experimental crosses were placed for 3 days in vials containing an equal number of males. Sixty males and 60 females were then transferred to egg-laying chambers containing a small portion of colored medium. After 17–20 hr, eggs were picked from the medium, washed in 70% ethanol and placed in groups of 50 on small squares of black paper. Each square was placed in an 8-dram vial containing food and incubated at 24°. Egg hatch was scored after 31 h of incubation; preliminary tests showed that all viable eggs hatched during this period. Eggs for each pair of crosses were picked over a period of 4 days, with equal numbers scored daily for the experimental and control crosses.

RESULTS

Fertility effects of inversions: Table 1 gives the breakpoints of the inversions and the inviability of eggs produced by their carriers. For each inversion, the difference in egg viability between the experimental and control cross is expressed as a "corrected inviability" and inversions are listed in order of increasing values of this statistic. "Corrected inviability" is calculated by assuming that eggs from the experimental cross experience two independent sources of mortality: one source from environmental effects and normal genetic constitution that also operate in the control cross, and an additional source due to any recombination in the inverted region. The viability of eggs in the experimental cross is assumed to equal the product of the viabilities remaining after each source of mortality operates. The inviability of eggs in the experimental cross due to recombination alone is thus calculated as $1 - (V_E/V_C)$, where V_E is the observed viability of eggs in the experimental cross and V_C the observed viability of eggs in the control cross. Because control viabilities nearly always exceeded 90%, these corrected inviabilities are close to the simple arithmetic difference in inviability between control and experimental eggs.

As expected, most of the corrected inviabilities are positive (this is true for 6 of the 7 second-chromosome and 19 of the 30 third-chromosome inversions; a sign test on the combined data gives $\chi^2 = 4.56$, 1 d.f., $P < 0.05$), indicating that recombination in heterokaryotypic females generally reduces egg viability. However, we also observed instances in which inviability of control eggs exceeded that of experimental eggs. This is probably due to either uncontrolled environmental differences between crosses or, more likely, slight genetic differences between control and experimental eggs (see MATERIALS AND METHODS). The absolute values of the negative estimates are, however, significantly smaller than the absolute values of the positive estimates according to the nonparametric Mann-Whitney U test ($U_{[11,19]} = 186$, $P < 0.002$), indicating the expected effects of recombination on egg mortality.

Table 1 does not show significance levels for control *vs.* experimental inviabilities because, using Fisher's exact test, only four inversions show no significant difference [*In(2LR)PL*, *In(2LR)434.93*, *In(3LR)C190* and *In(3LR)234*]. Under two-tailed tests, all other comparisons are significant–most of them highly so.

A one-tailed Fisher's exact test is used when investigating mortality due to recombination, for this source of mortality would always produce higher inviabilities in the experimental than in the control cross. Under this test, no negative values of corrected inviability are considered statistically significant. Two of seven second chromosomes (*In(2LR)PL* and *In(2LR)434.93*) and 11 of 30 third chromosomes (all those with negative corrected inviabilities) show no significant excess inviability in experimental crosses. Thus, a substantial number of inversions do not show significant underdominance, and are similar to *In(2LR)PL* studied previously (COYNE, AULARD and BERRY 1991). Some of the non-underdominant inversions are quite long. *In(2LR)PL*, *In(3LR)234* and *In(3LR)C190*, for example, all show normal egg viability even though they encompass 20 cytological divisions, the size of an entire chromosome arm.

The fertility effects of two of our inversions have been studied previously with comparable results. ROBERTS (1967) found, as did we, that *In(3LR)C190* showed normal hatchability of eggs in experimental crosses. He found, however, that *In(3LR)269* reduced the egg viability of heterokaryotypic females by 46% compared to controls. In our hands, this reduction is only 20%. This disparity between our results and those of ROBERTS could be due to environmental differences, genetic changes occurring over the last quarter century, or differences between tester strains in the rate of recombination.

TABLE 1

Inviability of eggs in control and experimental crosses for second- and third-chromosome inversions

Inversion	Breakpoints	Sample Size (each group)	Fraction inviable (control)	Fraction inviable (exptl.)	Corrected inviability (exptl.)
Second chromosomes					
PL[a]	31F-51C	5540	0.0344	0.0298	−0.0046
434.93	27D-41A/B	2600	0.0565	0.0573	0.0008
Rev^{B}	40-52C-E	2850	0.0621	0.0470	0.0161
bw[V32g]	40F-59E	2700	0.0596	0.1344	0.0795
432.3	23C-41A	2700	0.0563	0.1722	0.1228
lt(G10)	40-60A/B	2650	0.0404	0.1630	0.1278
lt(G16)	40-57D/E	2750	0.0506	0.2302	0.1892
Third chromosomes					
273	68A-88A	2700	0.1089	0.0463	−0.0702
238	80D/F-89B	2500	0.0812	0.0336	−0.0518
281	67B/C-87E/F	2850	0.0670	0.0323	−0.0372
LD31	67C-81F	2850	0.0547	0.0228	−0.0338
265	68E/F-85B/C	2800	0.0582	0.0282	−0.0319
224	69E-83B/C	2700	0.0467	0.0182	−0.0300
275	68F/69A-88F/89A	2850	0.0540	0.0291	−0.0263
277	65/66-83C/D	2700	0.0641	0.0374	−0.0258
280	68E/F-82F/83A	2750	0.0546	0.0302	−0.0258
C190	69F-89D	3000	0.0667	0.0613	−0.0057
234	67D-88A/B	2250	0.0431	0.0382	−0.0051
260	64C/D-83A/B	3000	0.0847	0.1073	0.0248
LD12	64F-81F/82A	2950	0.0529	0.0766	0.0251
252	64C-88C/D	3000	0.0470	0.0917	0.0469
270	64C-85E	3000	0.0623	0.1103	0.0512
Sep	65E-85E	3100	0.0258	0.0913	0.0672
259	63F-86A/B	2100	0.0329	0.1062	0.0758
267	66A/B-81F	2795	0.1488	0.2250	0.0895
111	64A/B-97A/B	3000	0.0453	0.1417	0.1009
278	61/62-85	2900	0.0831	0.1814	0.1072
268	64A-84A	2800	0.0836	0.1832	0.1087
250	62E/89D	2900	0.0817	0.1835	0.1108
279	61A/B-89B/C	2900	0.1169	0.2397	0.1390
272	62B-86D	2600	0.0681	0.2192	0.1622
282	65F/66A-81	2700	0.1152	0.2633	0.1674
257	79-95F/96A	2350	0.0570	0.2332	0.1868
C269	78C-98F	2600	0.0923	0.2735	0.1996
LD3	61F-82A	1390	0.0547	0.2432	0.1994
208	61E-86C	2700	0.0589	0.2552	0.2086
271	61-82E/F	2800	0.1504	0.3850	0.2762

Inversions are listed in order of increasing effect on inviability. Under a two-tailed test, four inversions show no difference in viability between control and experimental: *In(2LR)PL*, *In(2LR)434.93*, *In(3LR)C190* and *In(3LR)234*. All other inversions show a significant difference between control and experimental egg viabilities, with all probabilities (except for *In(2LR)Rev*^{B}) less than 0.001.

[a] Data from COYNE, AULARD and BERRY (1991).

Cytological correlates of fertility: We further analyzed the large sample of third-chromosome inversions for factors that might influence egg viability. Figure 1 shows the cytological breakpoints of these inversions and the corrected inviability of eggs laid by heterokaryotypes. It is clear that we do not have a sample of breakpoints distributed across the entire third chromosome: most breakpoints on 3L are between cytological divisions 60 and 70, while most on 3R are between divisions 80 and 90. Several other pericentric inversions have been described in *D. melanogaster* (see LINDSLEY and ZIMM 1992), but most of these are no longer available.

The cytological length of each inversion was estimated as the difference between numbered cytological positions of the left and right breakpoints. BRIDGES (1935) divided the *D. melanogaster* salivary gland chromosomes into 102 divisions; the second chromosome covering divisions 21 to 60 and the third chromosome divisions 61 to 100. BRIDGES also divided each numbered division into six subdivisions designated by letters A–F; we converted these letters into equally spaced fractions (*e.g.*, 62A = 62.0, 62B = 62.17, 62F = 62.83, etc.). If no letter was associated with an inversion breakpoint, we designated it as falling at the beginning of the numbered section (*e.g.*, a breakpoint listed as 69 would be given the value 69.0).

The correlation between the length of an inversion

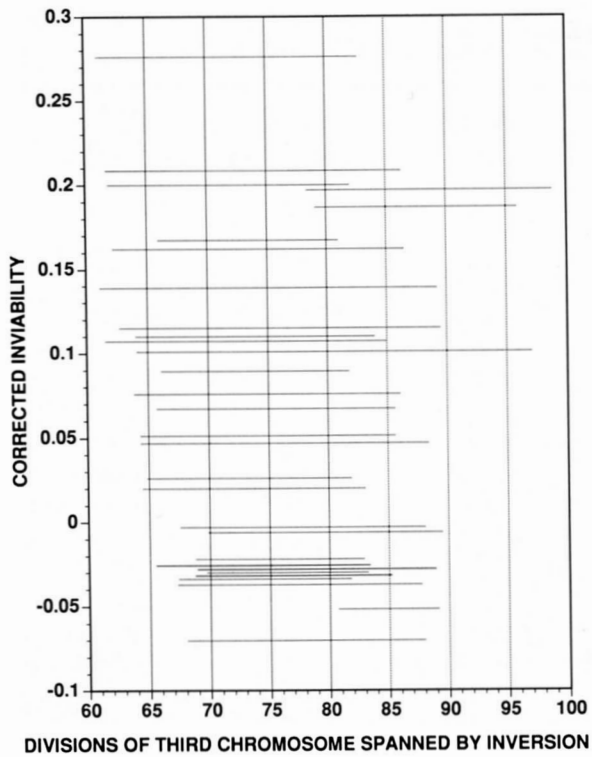

FIGURE 1.—Cytological extent of third-chromosome pericentric inversions and the corrected inviability of eggs laid by heterokaryotypic females. The extent of the inversion is shown by its span over the *x*-axis [using BRIDGES' (1935) numbered divisions of salivary-band chromosomes], and the height of the inversion on the *y*-axis indicates the corrected inviability of eggs relative to control. The centromere is located at 81A.

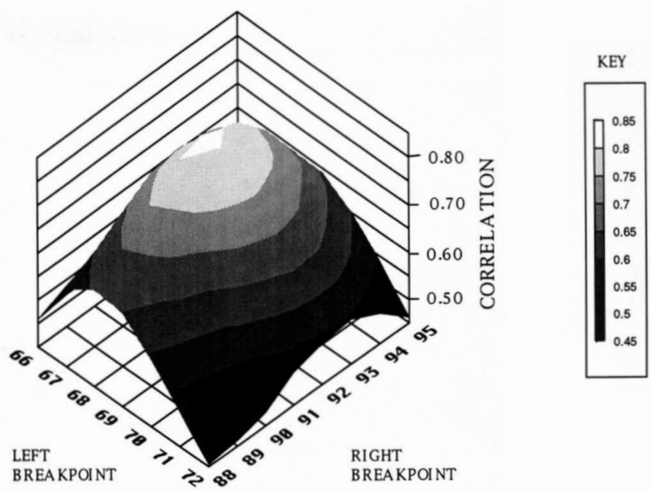

FIGURE 2.—Graph showing the position of the "recombination-sensitive sites" on the third chromosome. The horizontal axes give the pairs of chromosome positions picked on the left and right arms, respectively, and the vertical axis shows, for each pair of positions, the correlation obtained between corrected inviability of the, 30 inversions and the distance of their breakpoints to the nearest of the positions. The peak with the highest correlation (0.801) occurs with sensitive sites at divisions 68 and 92. Legend to the right shows which correlations correspond to the shades of the contour surface. See text for further details.

and its corrected inviability is significant for third-chromosome inversions ($r = 0.43$, 28 d.f., $0.01 < P < 0.05$), but not for the small sample of second-chromosome inversions ($r = 0.524$, 5 d.f.). On at least one chromosome, then, longer inversions have a greater effect on viability; this is expected because longer inversions should allow more recombination in heterokaryotypes. However, Figure 1 also suggests that the position of an inversion may also be important: heterozygotes with breakpoints near the middle of each arm (roughly at divisions 70 and 90) seem to have less inviability of eggs. This agrees with the finding of ROBERTS (1970, 1972) and HAWLEY (1980) that translocation heterozygotes with breakpoints in the middle of the arm show reduced recombination over large sections of the chromosome.

To locate more precisely any sites that might affect inviability (and by inference the amount of recombination), we calculated correlations between breakpoint position and inviability of third-chromosome inversions. We assumed that each of the two arms harbored one "sensitive site," and that the closer a breakpoint was to the site, the greater the suppression of recombination and hence the lower the inviability of eggs produced by heterozygous females. Our goal was to find the "sensitive sites" that best explained the inviability of inversions. Because of ROBERTS' and HAWLEY's observation that a single breakpoint near a sensitive site would impede recombination over large areas of the chromosome, we determined for each inversion the distance of its breakpoints to the *nearest* putative sensitive site. For example, if we chose the pair of sensitive sites to be at divisions 70 and 90 (see below), and the inversion was *In(3LR)272*, with breakpoints of 62B and 86D, the distance of breakpoints from the two sensitive sites would be would be 7.83 and 3.50, respectively. The distance to the nearest sensitive site would therefore be taken as 3.50 divisions.

By determining the correlation of inviability with many pairs of sensitive sites chosen near the middle of each arm, we found that the highest correlation was obtained for sensitive sites around division 70 on 3L and division 90 on 3R. We then narrowed down the location by picking 56 pairs of sensitive sites representing all pairwise combinations of integrally numbered divisions between 66 and 72 on 3L and 88 to 95 on 3R. For each pair of sites, we calculated the correlation between the corrected inviability of the 30 third-chromosome inversions and the distance from their breakpoints to the nearest of the two sensitive sites. Figure 2 is a three-dimensional graph showing the correlation associated with each chosen pair of "sensitive sites" on the third chromosome. The highest correlation [$r = 0.801$, 28 d.f., $P < 0.005$ according to the sequential Bonferroni correction for multiple comparisons (RICE 1989)] was obtained using "sensitive sites" 68 and 92; it is worth noting that these

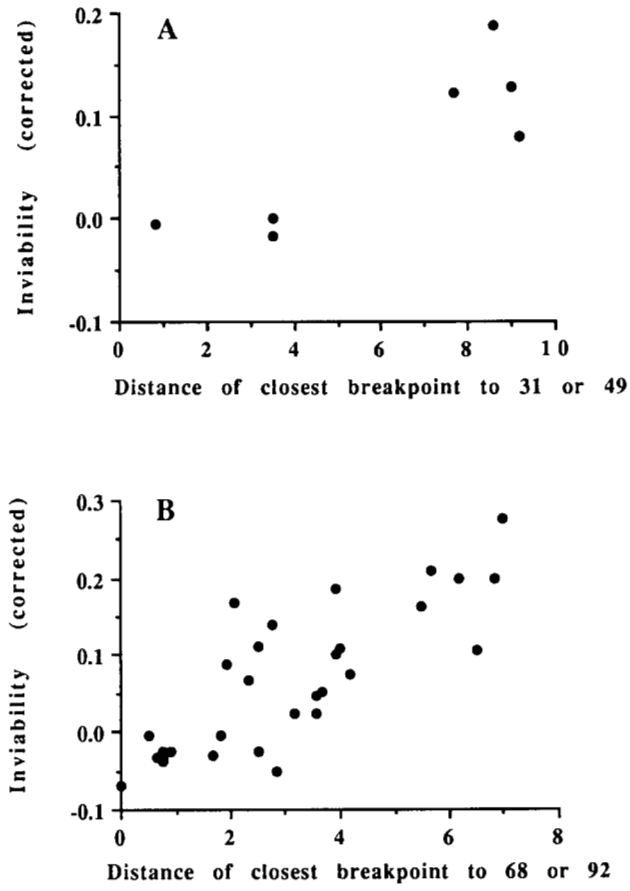

FIGURE 3.—The correlation between corrected inviability of eggs laid by heterokaryotypes and the distance from their breakpoints to the nearest "sensitive site." (A) Second-chromosome inversions and (B) third-chromosome inversions. Graphs are given only for the pair of sensitive sites showing the highest correlation with inviability (cytological divisions 31 and 49 for second-chromosome inversions and 68 and 92 for third-chromosome inversions). See text for further details.

sites are roughly equidistant from the tips of the two chromosome arms. Figure 3 displays the strong correlation between the inviability of 3LRs and the distance of their closest breakpoint to these sites.

Figure 2 shows that these correlations decrease progressively as either of the "sensitive sites" deviates from positions 68 or 92, so those locations appear to have important effects on recombination. We should note, however, that we lack inversions having breakpoints between 69 and 78 on 3L and 90 and 95 on 3R, so a larger sample of inversions might modify slightly the location of these "sensitive sites."

The correlations obtained by using only the nearest breakpoint are not increased when one incorporates information from both breakpoints. For example, the correlation between corrected inviability of an inversion and the *average* distance of its two breakpoints from divisions 68 and 92 is 0.621, lower than the value of 0.801 obtained using only the nearest breakpoint. The proximity of a breakpoint to the closest sensitive site therefore seems to be the major determinant of an inversion's underdominance.

Because both length and breakpoint position affect inviability produced by third-chromosome inversions, we determined which factor explained the greater variance in viability. Length and breakpoint position are, in fact, correlated with each other: for example, the correlation between the length of a third-chromosome inversion and the distance of its nearest breakpoint to either positions 68 or 92 is 0.373 (28 d.f., $P < 0.05$). A multiple-regression analysis using these two factors as independent variables and corrected inviability as the dependent variable shows that the distance from sensitive sites 68 or 92 is a highly significant determinant of inviability ($t_{28} = 6.40$, $P < 10^{-4}$), but that length has no significant effect ($t_{28} = 1.25$). The underdominance of an inversion therefore depends far more on the position of its breakpoints than on its length.

Because we had only seven second-chromosome inversions, we calculated correlations between inviability and only those sites that were equidistant from the tip of each arm. The highest correlation, shown in Figure 3B, was obtained using "sensitive sites" 31 and 49 ($r = 0.854$, 5 d.f., $P < 0.05$). One should not make too much of this because the data from this chromosome are limited. Nevertheless, it is clear that pericentric inversions on both chromosomes often fail to show the substantial underdominance expected when there is recombination in female heterokaryotypes.

DISCUSSION

Although we do not have a large sample of pericentric inversions with breakpoints scattered throughout the autosomes, several conclusions are clear. First, a large proportion of sampled inversions (2 of 7 on the second chromosome and 11 of 30 on the third chromosome) do not significantly reduce the viability of eggs laid by heterokaryotypic females. Hence, nonunderdominant inversions, such as *In(3LR)C190* (ROBERTS 1970) and *In(2LR)PL* (COYNE, AULARD and BERRY 1991), may be more common than is generally assumed. Based on the genetic studies of *In(2LR)PL* and *In(3LR)C190*, the lack of underdominance of such pericentric inversions is almost certainly due to suppressed recombination in heterokaryotypic females.

Because the control and experimental groups were not genetically identical, we must consider to what extent our results could be due only to the different frequencies of *X* chromosome between these groups and not to the inversions themselves. We cannot invoke *X* effects to explain the many cases in which heterokaryotypic females produce offspring with near-normal viability. Whatever the genetic makeup of an *X* chromosome, it cannot rescue an aneuploid egg produced by recombination within an inversion.

Therefore, our observation that many pericentric inversions do not appreciably reduce fertility must be real, regardless of any effects of sex chromosomes.

The remaining question is whether the reduced fertility caused by the remaining inversions on fertility may really be due to their possession of fitness-reducing *X* chromosomes and not to recombination. There are two reasons why we do not consider this a major cause of sterility. First, we found a very strong correlation between the position of an inversion's breakpoints and its effect on fertility. This would be very difficult to explain if the inviability was due to the *X* chromosome and not to the inversion. Second, as discussed above, the inversions that have the smallest effect on fertility are those with breakpoints near the middle of the chromosome arms. As noted above, this parallels observations of heterozygotes for reciprocal translocations, which have reduced recombination when the breakpoints are near the middle of the arms. Moreover, we previously found that heterozygotes for *In(2LR)PL* (COYNE, AULARD and BERRY 1991), an inversion with breakpoints near the middle of both arms, showed strongly reduced recombination. The results of these three studies are almost certainly not coincidental. Therefore, while some of the infertility of heterokaryotypes may be caused by their *X* chromosomes, it is much more likely that this is due to aneuploidy caused by recombination within the inversion.

For inversions on the third chromosome, and possibly for those on the second as well, the reduction of recombination depends far more on the location of the breakpoints than on the length of the inversion. Inversions with at least one breakpoint near the middle of an arm (in particular, near divisions 31 and 49 on the second chromosome and 68 and 92 on the third chromosome) are less likely to be underdominant. It is important to note that these locations are near the regions found by ROBERTS (1970, 1972) to decrease recombination in translocation heterozygotes: divisions 29–30 and 53 on the second chromosome, and 67–69 and 93–94 on the third.

There are two reasons why recombination might be suppressed in some inversion heterozygotes (COYNE, AULARD and BERRY 1991). First, inversion loops may not be formed, and heterozygotes may then either fail to pair in the inverted region or instead pair heterosynaptically (straight), which inhibits recombination. (Although all of our inversions show loops when heterzygous in salivary gland preparations, this does not mean that they do so in the female germ cells.) Heterosynaptic pairing of pericentric inversions in meiosis has been described in several species (MCCLINTOCK 1933; MARTIN 1967; NUR 1968; ASHLEY, MOSES and SOLARI 1981; GREENBAUM and REED 1984; HALE 1986; GABRIEL-ROBEZ *et al.* 1988; BOJKO 1990).

Second, synapsis could proceed normally, forming inversion loops in heterozygotes, but recombination might be inhibited by a mechanical or genetical effect of heterozygosity at the breakpoints. As far as we know, there have been no reports of this phenomenon. Although these alternatives could be resolved by cytological observations of female meiosis, it is presently impossible to obtain such preparations in Drosophila.

Both of these explanations must deal with the problem of why some locations on the chromosome are more important than others in inhibiting recombination. There are again two possibilities: some sites may be important in the mechanical alignment of chromosomes, or, as suggested by ROBERTS (1972, 1976) some sites may be important in initiating or maintaining synapsis or recombination. A mechanical explanation seems less likely, because both para- and pericentric inversion heterokaryotypes often show reduced recombination for considerable distances *outside* the inverted region (NOVITSKI and BRAVER 1954; COYNE, AULARD and BERRY 1991). Since inversion loops are not formed in these regions, there is no obvious reason why they should be mechanically prevented from homologous pairing.

If there are indeed "recombination-sensitive" sites, further investigation must determine, as our experiments suggest, whether there is only one such site per chromosome arm, as well as why the inhibition of recombination is not an all-or-none phenomenon but increases gradually as the breakpoints approach the sensitive sites. Also, it is not clear why disruption of recombination requires *heterozygosity* at such sites. COYNE, AULARD and BERRY (1991) found that although recombination was strongly inhibited in *In(2LR)PL/ST* heterozygotes, it was normal in *In(2LR)PL* homozygotes. ROBERTS (1970) also found normal recombination in homozygotes for reciprocal translocations.

COYNE, AULARD and BERRY (1991) discuss the evolutionary implications of their observation that *In(2LR)PL* was not underdominant. These conclusions are strengthened with our finding that a sizeable fraction of radiation-generated pericentric inversions also fail to show underdominance when heterozygous with the *Standard* arrangement. The most important conclusion is that one cannot automatically assume that fixed or polymorphic pericentric inversions give evidence for strong genetic drift overcoming natural selection in very small populations. One is also not entitled to assume that such fixations represent Wrightian "peak shifts" across adaptive valleys. If an inversion is not underdominant, then the adaptive valley does not exist, and such inversions can be fixed by either weak genetic drift or positive selection. If some members of a class of rearrangments are not

underdominant, one cannot then assume that the fixation of such rearrangements causes speciation, for such speciation requires that the heterozygote be sterile [see FUTUYMA and MAYER (1980) and BARTON and CHARLESWORTH (1984) for further arguments against chromosomal speciation].

It is nevertheless clear that most pericentric inversions arising in nature, like those in our sample, are underdominant. In Drosophila, such inversions are polymorphic or fixed far less often than paracentric inversions, which are not underdominant. STONE (1955), for example, estimated that between 6,100 and 36,500 paracentric inversions, but only 32 pericentric inversions, were polymorphic or fixed among 650 Drosophila species. If, however, a *subset* of such inversions are not underdominant, it is just those inversions that are most likely to attain high frequencies in nature. We are not, in fact, aware of a single case of a fixed or polymorphic pericentric inversion in which underdominance has been demonstrated (COYNE, AULARD and BERRY 1991).

Our results, coupled with STONE's (1955) demonstration that fixed or polymorphic pericentric inversions are extremely rare, raise an obvious question. If, as our data suggest, roughly 40% of pericentric inversions are not underdominant, why are not they more common in nature? There are four possible answers. First, our inversions may not be random sample of those arising in nature. We do not have a sample of inversions with breakpoints distributed throughout the autosomes. Moreover, most of our inversions were not found in nature but were generated by radiation, and many were detected by observing a reduction in single crossovers with a tester strain (see, for example, ROBERTS 1967, 1970). We do not consider this last point a major source of bias, because virtually all pericentric inversions, underdominant or not, would reduce the frequency of detected crossovers in these tests. (In underdominant inversions, single crossovers produce inviable eggs, and in non-underdominant inversions, the suppression of recombination would reduce crossing over between markers.) In our opinion, nonrandom sampling cannot explain the disparity between the high frequency of viable inversions in our experiment and the rarity of pericentric inversions in nature.

Second, the rate of spontaneous mutation to pericentric inversions may be lower than to paracentric inversions. We know of no reason why this should be so.

Third, chromosomes in nature may break only infrequently near the "sensitive sites," so that non-underdominant inversions are not often produced. This explanation is probably wrong because there are plenty of naturally occurring *paracentric* inversions with breakpoints in these regions (LEMEUNIER *et al.* 1986).

Fourth, natural selection may be extremely effective in eliminating pericentric inversions whose underdominance is too small to be detected in our studies. Some of our inversions that were not underdominant (*i.e.*, "experimental" higher than "control" hatchability) may actually be slightly underdominant on a uniform genetic background, but this underdominance may have been masked in our studies by the viability effects of slight genetic differences between control and experimental progeny. In addition, our sample sizes allowed us to detect only those inversions affecting fitness by more than one or two percent. If natural populations are sufficiently large, newly arisen pericentric inversions could be eliminated even if they were only slightly deleterious. This seems to us the best explanation for the rarity of pericentric inversions in nature.

No matter which explanation is true, however, our conclusion still holds: a surprisingly large number of pericentric inversions are not *strongly* underdominant. Strong underdominance has long been assumed to characterize pericentric inversions and is the reason why their fixation in nature is assumed to require genetic drift in very small populations.

Another obvious question is whether pericentric inversions that are polymorphic or fixed in the *D. melanogaster* group are those having breakpoints near the sensitive regions. Unfortunately, this question cannot be answered. Eighteen naturally occurring pericentric inversions have been described in *D. melanogaster*, 10 on the second chromosome and 8 on the third (AULARD 1990). However, with the exception of *In(2LR)PL* (which is not underdominant), and the very small pericentric inversion containing the *Segregation-Distorter* locus, all of these rearrangements were seen only once and hence were not polymorphic. Moreover, only one pericentric inversion has been fixed in the *melanogaster* group: *In(2LR)a*, which is a diagnostic difference between the *D. melanogaster* subgroup on the one hand and *Drosophila erecta*, *Drosophila teissieri* and *Drosophila yakuba* on the other (LEMEUNIER and ASHBURNER 1976). The best estimates of its breakpoints, 36C and 46D, are not near the "recombination-sensitive" regions we observed on the second chromosome. However, this fixed difference is accompanied by a large non-overlapping paracentric inversion on 2L. Such an inversion could help fix a normally deleterious pericentric inversion by inhibiting recombination over large sections of the chromosome.

Polymorphic or fixed pericentric inversions have been described in other species, but in no case does there appear to be substantial recombination in heterozygotes. M. J. D. WHITE, for example, based much

of his theory of stasipatric speciation on the observed fixation of pericentric inversions in closely related populations and species of grasshoppers (WHITE 1968, 1973, 1978). As JOHN (1981, p. 43) points out, however, "In all these cases there is straight, nonhomologous pairing of the relatively inverted segments at male meiosis and no reverse looping. Because such straight pairing precludes the production of unbalanced gametes in the heterozygotes, it also precludes them from generating hybrid sterility. Indeed, there is no case that I am aware of where fixed differences involving genuine pericentric inversions do lead to reproductive isolation."

Similar conclusions hold for other chromosome rearrangements that are thought to be underdominant, such as reciprocal translocations, Robertsonian fusions, and tandem fusions. As noted by SITES and MORITZ (1987) and COYNE, AULARD and BERRY (1991), each type of rearrangement may vary dramatically in its effect on fertility, with some examples showing normal segregation and no underdominance in heterozygotes. Reviewing the data on fitness effects of chromosome rearrangements segregating in hybrid zones, SHAW (1981) found little evidence for underdominance in nature.

Finally, our data relate to one current hypothesis for the origin of sex. BERNSTEIN, HOPF and MICHOD (1988) proposed that recombination evolved and is maintained primarily because of its adaptive role in repairing damage to DNA. Recombination initiated by double-stranded breaks in damaged DNA (ORR-WEAVER and SZOSTAK 1985) can cause the damaged region to be replaced with DNA synthesized using the homolog as a template. Failure to repair such double-stranded breaks via recombination would produce dominant lethals, strongly reducing the viability of offspring from individuals lacking recombination.

Such damage to DNA must be substantial if it is to explain why its repair could overcome the evolutionary cost of meiosis (MAYNARD SMITH 1978). Critics of the recombination-repair theory have pointed out that in some groups, like Drosophila, males lack recombination but suffer no obvious loss of fitness despite their inability to repair DNA through recombination (MAYNARD SMITH 1988). In response, BERNSTEIN, HOPF and MICHOD (1988) suggested that recombinational repair may not be important in male germ lines because metabolic activity leading to production of DNA-damaging oxidative compounds is lower in spermatogenesis than in oogenesis. However, COYNE, AULARD and BERRY (1991) found that eggs of *D. melanogaster* females heterozygous for a large pericentric inversion (which effectively suppressed crossing over) hatch at normal rates, and in this paper we show similar results for several large inversions. We therefore fail to see the substantial loss of female fitness expected under the recombination-repair hypothesis for the evolution of sex.

It can no longer be assumed without proof that fixed or polymorphic chromosome rearrangements are underdominant in nature. Future studies should concentrate on the actual fitness effects of naturally occurring inversions, translocations, and fusions. We predict that, with the exception of monobrachial centric fusions, whose fixation does not require drift (BAKER and BICKHAM 1986), many of these rearrangements will not reduce the fitness of heterozygotes.

This work was supported by grant GM 38462 to J.A.C. from the National Institutes of Health. We thank KATHY MATTHEWS and B. CHARLESWORTH for fly stocks, MICHELE PRUYN for technical assistance and B. CHARLESWORTH, K. HUGHES, M. NOOR and H. A. ORR for advice and comments.

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Communicating editor: A. G. CLARK