Lack of Underdominance in a Naturally Occurring Pericentric Inversion in *Drosophila melanogaster* and Its Implications for Chromosome Evolution

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ABSTRACT

In(2LR)PL is a large pericentric inversion polymorphic in populations of *Drosophila melanogaster* on two Indian Ocean islands. This polymorphism is puzzling: because crossing over in female heterokaryotypes produces inviable zygotes, such inversions are thought to be underdominant and should be quickly eliminated from populations. The observed fixation for such inversions among related species has led to the idea that genetic drift can cause chromosome evolution in opposition to natural selection. We found, however, that *In(2LR)PL* is not underdominant for fertility, as heterokaryotypic females produce perfectly viable eggs. Genetic analysis shows that the lack of underdominance results from the nearly complete absence of crossing over in the inverted region. This phenomenon is probably caused by mechanical and not genetic factors, because crossing over is not suppressed in *In(2LR)PL* homokaryotypes. Our observations do not support the idea that the fixation of pericentric inversions among closely related species implies the action of genetic drift overcoming strong natural selection in very small populations. If chromosome arrangements vary in their underdominance, it is those with the least disadvantage as heterozygotes, like *In(2LR)PL* , that will be polymorphic or fixed in natural populations.

THE strongest evidence that genetic drift can overcome natural selection is the existence of fixed differences among related species for chromosome rearrangements that are underdominant (*i.e.*, deleterious as heterozygotes). Heterokaryotypes for many such rearrangements, including pericentric inversions, centric fissions, translocations and centric or tandem fusions, may be semisterile because aberrant meiotic segregation or recombination within the arrangements produce aneuploid gametes (WHITE 1973) . Because such arrangements always originate as heterokaryotypes, they should be eliminated immediately by natural selection in randomly mating populations. Indeed, these arrangements are rarely found as polymorphisms in nature. In Drosophila, for example, there are thousands of polymorphisms for paracentric inversions, which are not underdominant, but almost no polymorphisms for pericentric inversions (PATTERSON and STONE 1952).

Although polymorphisms for underdominant chromosomes are rare, closely related species may sometimes be fixed for such differences (STONE 1955; WHITE 1973). This fixation implies that populations or species have undergone an evolutionary transition from one arrangement to another, a transition that would be opposed by natural selection.

The most widely accepted explanation for such transitions is genetic drift. In populations that are sufficiently small, drift can increase the frequency of a new underdominant arrangement above the unstable equilibrium frequency, after which it is fixed by selection. [This corresponds to a shift between adaptive peaks, an important part of WRIGHT's (1970) "shifting balance" theory of evolution.] When the underdominance is severe, this process requires extremely small effective population sizes (on the order of 10; LANDE 1979, 1984; HEDRICK 1981; WALSH 1982). The fixation of chromosome arrangements thus implies small effective species sizes: "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). WHITE's (1968, 1978) theory of stasipatric speciation, which proposes that chromosome arrangements are an important cause of reproductive isolation, also requires genetic drift in very small populations.

There are two other explanations for the fixation of chromosomes that are underdominant for fertility: natural selection and meiotic drive. If a new chromosome rearrangement contains favorable alleles, the net fitness of the arrangement may be positive despite underdominance for fertility. This could cause either polymorphism or fixation (DOBZHANSKY 1951; CHARLESWORTH 1985; BICKHAM and BAKER 1979).

Alternatively, if a new arrangement was linked to

an allele that segregated preferentially in meiosis, it could be fixed even if opposed by natural selection (WHITE 1978; HEDRICK 1981). While possible, meiotic drive seems less plausible than drift. Genetic analysis has shown no evidence for fixed meiotic-drive alleles among closely related species, and the parapatric geographic distribution of some chromosome arrangements could not occur if they were meiotically driven (COYNE 1986, 1989).

Here we describe the genetic properties of a chromosome arrangement whose polymorphism in nature was surprising because of its supposed underdominance. In 1985, a pericentric inversion was found in populations of *Drosophila melanogaster* on two Indian Ocean islands: Mauritius, about 1000 km east of Madagascar, and Rodriguez, about 600 km east of Mauritius (AULARD 1990). This inversion is very large, so recombination should severely reduce the fertility of heterokaryotypic females. Yet the inversion's occurrence on two islands in moderate frequencies (3 and 7%, respectively) suggested that it must have been present for at least several generations. With the exception of the very small inversion associated with the *Segregation-distorter* allele (HARTL and HIRAIZUMI 1976), this is the only pericentric inversion reported from more than one population of *D. melanogaster*.

Because *D. melanogaster* is rare on these islands (DAVID *et al.* 1989), we originally suspected that this inversion might be strongly underdominant yet maintained by genetic drift. As we show below, genetic analysis disproved these expectations, for the inversion was not underdominant for fertility. This lack of underdominance leads us to question the idea that chromosome evolution provides strong evidence for the ability of genetic drift to overcome natural selection in small populations.

MATERIALS AND METHODS

The inversion: In August, 1985, MICHEL SOLIGNAC collected *D. melanogaster* on Mauritius and Rodriguez, sampling both wild and domestic habitats (DAVID *et al.* 1989). *D. melanogaster* was relatively rare on both islands, occurring only in warehouses in the harbors. Aulard (1990) karyotyped single chromosomes from 14 isofemale lines from Rodriguez and 30 from Mauritius. One line from each population contained a large pericentric inversion with breakpoints at cytological positions 31F and 51C on the second chromosome (Figure 1A). We name this inversion *In(2LR) Port Louis*, abbreviated *In(2LR)PL*, after the town in Mauritius from which it came. In the rest of this paper, however, we refer to it simply as *2LR*.

2LR is therefore large, covering nearly half the cytological length of the chromosome, and roughly symmetrical around the centromere. It also includes the well known loci *black*, *purple*, *cinnabar*, *alcohol dehydrogenase* and *vestigial*, which have been mapped to small regions of the polytene chromosomes (LINDSLEY and ZIMM 1985, 1990). We estimate the recombinational length of the inversion to be about 30 centimorgans, because *daughterless* (map position 41.3) has been cytologically located between 31C and 32A, and *curved* (map position 75.5) is just outside the inversion at 52D

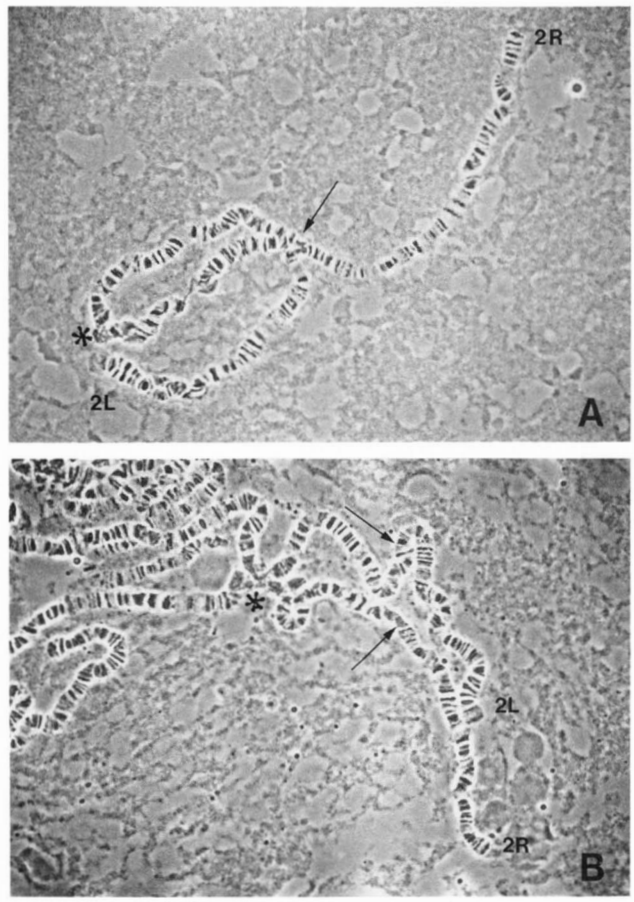

FIGURE 1.—Polytene second chromosomes in larval salivary glands. Asterisk marks the chromocenter, and arrows show the position of the breakpoints. A, *2LR/ST* heterokaryotype. B, $2LR^{Mau}/2LR^{Rod}$ homokaryotype, showing cytological identity of the inversions from the two islands.

(LINDSLEY and ZIMM 1985; ASHBURNER 1991).

The isofemale lines from Mauritius were maintained in the laboratory until 1989, when we began this analysis. All but one of the isofemale lines from Rodriguez were combined in 1986 and maintained as a single stock.

Drosophila stocks used in the analysis:

CyO: Second-chromosome balancer *In(2LR)O,* dp^{lvl} *Cy pr* $cn^2/In(2LR)bw^{V1}$.

FM7a: X chromosome balancer *In(1)*sc^8 + *In(1) dl-49,* y^{31d} sc^8 w^a v^{Of} *B.*

Compound-2 entire: C(2)EN bw sp, a stock having both second chromosomes fused into a single entity. Produced by NOVITSKI (1976), this chromosome was used to measure nondisjunction.

vg(ST): A stock homozygous for the second chromosome-mutation *vestigial* (2-67.0) and made homokaryotypic for the Standard arrangement of the second chromosome.

b cn(ST): A stock homozygous for the second chromosome-mutations *black* (2-48.5) and *cinnabar* (2-57.5) and made homokaryotypic for the Standard arrangement of the second chromosome.

b cn vg(ST): This stock, also homokaryotypic for the Standard arrangement, was used to measure double recombination in the heterokaryotype.

w m: This stock, containing the *X*-linked mutations *white* (1-1.5) and *miniature* (1-36.1), as well as the *ST* sequence of the second chromosome, was used to measure recombination on the *X* chromosome.

all(ST): This stock, containing the mutations *al* (2-0.01),

dp (2-13.0), *b* (2-48.5), *pr* (2-54.5), *c* (2-75.5) *px* (2-100.5) and *sp* (2-107.0), as well as the ST arrangement of the second chromosome, was used to measure recombination on the second chromosome.

ru h th: This stock, containing the mutations *roughoid* (3-0.0), *hairy* (3-26.5), and *thread* (3-43.2), as well as the *ST* sequence of the second chromosome, was used to measure recombination on the third chromosome.

nod^{DTW}: A dominant allele of the *X*-linked *nod* locus (1-36), *nod^{DTW}* causes nondisjunction of nonexchange chromosomes, with a similar but much weaker effect on chiasmatic bivalents (WRIGHT 1974; ZHANG and HAWLEY 1990; ZHANG *et al.* 1990; RASOOLY *et al.* 1991). Drosophila females have two meiotic pairing systems: exchange pairing, which ensures normal disjunction of recombinant chromosomes, and distributive pairing, which ensures normal disjunction of nonrecombinant chromosomes such as the fourth (see GRELL 1976). Like other alleles at this locus, *nod^{DTW}* disturbs distributive pairing, causing nondisjunction of nonexchange chromosomes. The amount of *nod^{DTW}*-induced nondisjunction is hence a good measure of the frequency of nonexchange chromosomes.

Originally called *TW-6^{cs}* by WRIGHT (1974), *nod^{DTW}* was kept balanced in males in the stock *C(1)Dx y f/B^{S}Y* × *nod^{DTW} l(1)f^{96A}/B^{S}Y*, and was used obtain indirect measurements of recombination in genotypes lacking morphological markers.

cn bw Rsp^{S}: A stock homozygous for the second chromosome mutations *Responder-sensitive* (2-56.6), *cinnabar* (2-57.5) and *brown* (2-104.5) (BRITTNACHER and GANETZKY 1983). When *SD* alleles are present in *Rsp^{s}/Rsp^{i}* heterozygotes, meiotic drive largely eliminates the chromosome carrying *Rsp^{s}*.

Iv: A stock homokaryotypic for the *Standard* arrangements on all major chromosomes. It was founded 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 a new stock by combining 21 of these lines that were that were homokaryotypic for the Standard arrangement on all chromosomes.

Rearing of flies: All crosses were made on standard agar-yeast-cornmeal medium, and reared in 8-dram vials at 24° on a 12-hr light/dark cycle.

Chromosome analysis: Salivary glands from third-instar larvae were dissected in Ringer's solution, squashed, and orcein-stained according to standard protocol (ASHBURNER 1989).

Egg hatchability: Virgin females used to lay the eggs for scoring were placed in vials for 2 days with an equal number of males. Flies were then transferred to egg-laying chambers containing a small portion of colored medium. After 17–20 hr, unhatched eggs were picked from the medium, washed in 70% ethanol, and placed in groups of 40 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 24 hr. Preliminary tests showed that all viable eggs hatched during this period.

Measuring segregation, recombination, and nondisjunction: Two or three females (depending on the stock) were crossed to an equal number of males in 8-dram food-containing vials. Adults were removed after five days, and offspring scored periodically until 8 days after the first eclosion.

Measuring fecundity: One male and one newly hatched virgin female were placed in a food vial to mate, and transferred 2 days later to an 8-dram vial containing colored food. The pairs were transferred daily and their eggs counted over the next 7 days. Males who died during this period were replaced, and if the female died the data were discarded.

***Adh* RFLP study of inverted and standard chromosomes:** Using the *CyO* balancer, we extracted a single second chromosome from each of ten isofemale lines from the island populations: three lines from Rodriguez (one *2LR* and two *ST*) and eight from Mauritius (one *2LR* and seven *ST*). Those lines carrying *2LR* could not be made isochromosomal because they carried recessive lethals, so these were balanced over a chromosome, A178, which carries a deletion that includes the *Adh* structural locus. DNA was extracted according to the procedure of KREITMAN and AGUADÉ (1986). Approximately 3 μg of DNA was digested overnight in sealed microtiter plates with 0.5 μg of RNAase I and 5 units of the following enzymes: *Alu*I, *Dde*I/*Bam*HI, *Hae*III, *Hha*I, *Hin*fI, *Msp*I, *Sau*3AI and *Taq*I. DNA was precipitated with NaOAc and isopropanol, washed in 70% ethanol, dried and resuspended in 3 μl of formamide loading buffer (KREITMAN and AGUADÉ 1986). Heat-denatured samples were electrophoresed on a 30 cm × 40 cm × 0.4 mm 5% polyacrylamide/7 M urea sequencing gel with a NaOAc electrolyte gradient (SHEEN and SEED 1988) and electrophoretically transferred to nylon membranes, UV cross-linked, hybridized to a 2.7-kb fragment incorporating the *Adh* structural locus, washed and autogradiographed according to KREITMAN and AGUADÉ (1986).

From the published *Adh* sequence (KREITMAN 1983), we know the expected fragment lengths and can therefore identify exactly the positions of restriction site gain/losses. The positions and sizes of insertions and deletions, however, must be estimated. Overall, these nine enzymes survey about 19% of all possible substitutions within the probed region [*i.e.*, 19 × 2700 = 513 base pairs of sequence (KREITMAN and AGUADÉ, 1986)] and all insertions and deletions.

RESULTS

Extraction of the inversion: Karyotypic analysis showed that the isofemale line from Mauritius and the mixture of isofemale lines from Rodriguez still contained a high frequency of *2LR* chromosomes after five years of laboratory culture. Second chromosomes from each strain were extracted using the *CyO* balancer. Fourteen of the chromosomes from the Mauritius isofemale line produced homozygous-viable offspring, and six lines produced no homozygous offspring. The lethal chromosomes were kept balanced against *CyO*. Karyotypic analysis showed that all of the homozygous-viable second chromosomes carried the *Standard* (*ST*) arrangement, while the homozygous-lethal chromosomes were all *2LR*. Crossing tests showed that all of these lethals from Mauritius were allelic.

Eight chromosomes were extracted from the Rodriguez line; six of these were homozygous viable and two were homozygous lethal. All of the viable chromosomes carried the *ST* arrangement, while the two lethal chromosomes again carried *2LR*, and the lethals were allelic.

The pericentric inversions from the different islands did not, however, carry allelic lethals, because numerous wild-type offspring were produced upon intercrossing the *CyO/2LR* balanced strains from the two islands. The viability of homozygous *2LR^{Mauritius}/2LR^{Rodriguez}* offspring allowed us to determine that the

TABLE 1

Egg hatchability of heterokaryotypic females in two experiments, one using a *2LR* from Maritius, and the other a *2LR* from Rodriguez

	Experimental *ST/2LR* × *Iv*		Control *Iv* × *ST/2LR*			
Inversion	Eggs	Fraction hatched	Eggs	Fraction hatched	χ^2	95% C.I. Expt.-Control
A. $2LR^{Maur}$	2360	0.961	2560	0.956	0.84	0.005 ± 0.011
B. $2LR^{Rod}$	3180	0.977	2960	0.974	0.74	0.003 ± 0.008

Females are given first in the cross description.

pericentric inversions in both populations were cytologically identical, showing perfect pairing in salivary gland preparations (Figure 1B).

To determine if the nonallelic lethals could be removed by recombination in homokaryotypes, we began a stock with the wild-type $2LR^{Mauritius}/2LR^{Rodriguez}$ progeny and reextracted second chromosomes after fifteen generations. Eight of the eighteen homozygous chromosomes were viable and fertile, indicating that the lethals had been removed. Five of these lines were tested cytologically and confirmed to be *2LR/2LR*.

These results imply that *2LR* originally arose on a lethal-free chromosome The subsequent accumulation of two lethals also implies that *2LR* was probably segregating in nature for tens to hundreds of generations before it was discovered, a suggestion supported by its occurrence on two distant islands. Finally, the removal of the lethals through recombination shows that they are not associated with the inversion breakpoints.

Is *2LR* underdominant for fertility in heterokaryotypes? To determine the effect of the inversion on the production of aneuploid eggs, we produced heterokaryotypic females by crossing males from *CyO/2LR* balancer stocks to *ST/ST* females from the homokaryotypic *Iv* strain. The non-Curly F_1 females (*ST/2LR*), were backcrossed to *Iv* males, and scored for egg hatch. The control experiment consisted of *ST/2LR* males (from the same F_1 cross) backcrossed to *ST/ST Iv* females. The offspring of these two crosses are genotypically nearly identical, differing only in the source of their *X* chromosome. Only the heterokaryotypic females, however, are expected to produce aneuploid eggs, as males have no recombination. This experiment was performed twice, once using the pericentric inversion from Mauritius and once the inversion from Rodriguez.

The hatchability of eggs laid by heterokaryotypic females in both experiments was over 95% (Table 1), and did not differ significantly from that of control eggs. The 95% confidence intervals show that the maximum possible reduction of egg hatchability of heterokaryotypic females compared to controls is only 0.6%. This figure corresponds to the maximum frequency of single crossing over in the inverted region that could have gone undetected in our study. As the inverted region is about 30 centimorgans long, heterokaryotypic females have at most only 2% as much crossing over in the region as *ST/ST* females. We confirmed the suggestion of reduced recombination in the inverted region with the following experiments.

Is there crossing over in heterokaryotypes? Although single crossovers in the inverted region of heterokaryotypes lead to aneuploid gametes, two-strand double-recombinants yield normal gametes. If the high hatchability of eggs produced by *2LR/ST* females results from restricted crossing over in the inverted region, this region should also show a substantial reduction of *double* crossing over. To test this possibility, we crossed female $CyO/2LR^{Mauritius}$ to *b cn vg(ST)* males. The non-Curly F_1 females were heterokaryotypic and heterozygous for the three markers. These were backcrossed to homozygous *b cn vg* females and double-recombination scored in the progeny. As a control, we used *ST/ST* instead of *2LR/ST* females in the original cross.

Only two double-recombinants were recovered among 16,919 offspring of the *2LR/ST*, a frequency of 1.18×10^{-4}. (As expected, there were no single recombinants). The control cross produced 70 recombinants among 15,171 offspring, a frequency of 4.6×10^{-3}. (The expected frequency of double-recombination in *ST/ST* homokarytypes, based on map distances and assuming no interference, is 8.6×10^{-3}). The difference between the estimates from *ST/ST* and *ST/2LR* is highly significant ($P < 10^{-15}$ using Fisher's exact test). There is a 40-fold difference in the amount of double-recombination between the two classes of females, substantiating the predictions from egg-hatch experiments. Further measurements of recombination are described below.

This large reduction in recombination was confirmed in a separate experiment using the nod^{DTW} allele, which causes nondisjunction of nonrecombinant chromosomes and a small percentage of exchange chromosomes. *C(1)Dx y f/B*^{s}*Y* × nod^{DTW} *l(1)* $f^{36A}/B^{s}Y$ males, which have the *Standard* arrangement of the second chromosome, were crossed to $CyO/2LR^{Mauritius}$ females. The wild-type female offspring are *ST/2LR* and heterozygous for the dominant nod^{DTW}. These virgin females were crossed to *C(2)EN bw sp* males at a density of three males and three females per vial. Offspring will appear only when the heterokaryotypic mother has no crossing over on the

TABLE 2

Measurement of nondisjunction when homokaryotypic or heterkaryotypic females (both heterozygous for the dominant allele nod^{DTW}) are crossed to C(2)EN bw sp males

Female	Total offspring (wild-type)	Mean female fecundity (SE)
ST/2LR	802 (750)	106.1 (2.45)
ST/ST	100 (94)	105.2 (4.95)

Twenty-five vials, each containing three males and three females, were scored for each karyotype. Mean female fecundity is given for the total number of eggs laid over the 7-day test period (11 females tested per genotype).

second chromosome, leading to nondisjunction and complementation of the nullo-2 or diplo-2 sperm produced by the compound-2 fathers.

To determine the baseline level of nondisjunction in *ST/ST* homokaryotyes lacking nod^{DTW}, we made the same cross using mothers from an *ST/ST* isochromosomal line from Mauritius. To determine whether any difference in offspring number between control and experimental females was due only to a difference in female fecundity and not the rate of nondisjunction, we measured egg production of both type of females when mated to males from the *Iv* strain.

Table 2 shows that the heterokaryotypic mothers produce eight times more offspring than *ST/ST* mothers, a highly significant difference when compared to the null expectation of equal nondisjunction ($\chi^2_{[1]} = 546.3$, $P < 10^{-6}$). [For both genotypes, many more wild-type than *C(2) bw sp* progeny were found, which is expected because compound-2 males produce more nullo-2 than compound-2 sperm (S. HAWLEY, personal communication).] The difference in progeny number is not due to inherent differences in fecundity, as the two genotypes of females do not differ (Table 2; an unpaired comparison of the fecundity of females from the two groups gives $t_{[22]} = 0.151$, $P = 0.88$).

The eightfold difference in nondisjunction implies an eightfold reduction in recombination in *ST/2LR* females compared to *ST/ST* females. In *ST/2LR* females, the reduction in recombination is measurable only outside the inverted region, because single-recombinants within the region are not recovered. This reduction of recombination in heterokaryotypes is, however, far more than one expects simply from the reduced length of the chromosome in which recombination can be observed. Additional tests described below demonstrate that heterokaryotypic females show reduced recombination over the entire second chromosome.

Do heterokaryotypes have increased nondisjunction? It is possible that although heterokaryotypic females do not produce a high frequency of aneuploid gametes by recombination, they may still do so by having increased nondisjunction. We tested this possibility by crossing the *ST/2LR* heterokaryotypes of both sexes to the *C(2)EN c bw* tester stock and counting

TABLE 3

Measurement of nondisjunction when homokaryotypic or heterokaryotypic females and males are crossed to C(2)EN bw sp individuals of the opposite sex

Karyotype and sex tested	Total offspring (wild type)	Female fecundity (SE)
ST/2LR male	23 (15)	
ST/2LR female	21 (19)	90.27 ± 2.32
Total *ST/2LR*	44 (41)	
ST/ST male	39 (25)	
ST/ST female	20 (16)	64.73 ± 3.84
Total *ST/ST*	59 (41)	

Seventy-five vials, each containing three males and three females, were made for each determination. Average female fecundity is estimated as the total number of eggs laid over a 7-day period (11 females were measured for each genotype). Number of wild-type offspring (as opposed to *bw sp*) are given for each total.

offspring. Seventy-five replicate vials, each containing three males and three females, set up for each of the two reciprocal crosses. The number of offspring produced by homo- and heterokaryotypic females were nearly equal (Table 3), but offspring from heterokaryotypic males had significantly *fewer* progeny than homokaryotypic males ($\chi^2_{[1]} = 4.12$, $P < 0.05$ under expectation of equal progeny number). Summing both sexes to yield a total frequency of nondisjunction, we find no significant difference between karyotypes under the assumption of equal fertility ($\chi^2_{[1]} = 2.18$). However, homokaryotypic females produced only two-thirds as many offspring as heterokaryotypes, a significant difference (comparison of the two groups of 11 females for weeklong fecundity gives $t_{[20]} = 5.67$, $P < 10^{-4}$). This reduction in fecundity, also described below, could be due to the *ST/ST* females' isogenicity for the entire second chromosome.

The higher fecundity of the heterokaryotype implies that this genotype has slightly less nondisjunction than homokaryotypes. The lack of increased nondisjunction of *2LR/ST* individuals also gives evidence that the distributive pairing system operates in nature.

Does *2LR* carry a gene that reduces crossing over? The lack of recombination in heterokaryotypic females could be due to either mechanical factors, such as a lack of proper meiotic synapsis, or to genetic factors, such as a linked allele that reduces crossing over. Because recombination eliminated the lethals in $2LR^{Maur}/2LR^{Rod}$ homokaryotypes, inversion homokaryotypes must undergo at least some crossing over. We examined the effect of *2LR* on recombination outside the inversion as well as on other chromosomes.

In the former experiment, we made *2LR/ST* heterokaryotypic females in which the ST chromosome was the multiply marked *all* genotype. *Black* and *purple* lie within the inverted region (LINDSLEY and ZIMM 1985, 1990), while *aristaless, dumpy, plexus, curved* and *speck* are outside the inversion (Ashburner, 1991). A control cross estimated recombination rates in *all* heterozygotes that were homokaryotypic for the

TABLE 4

Recombination in females heterozygous for the *all* chromosome when homokaryotypic or heterokaryotypic

Region	Map distance in *ST/2LR* females	Map distance in *ST/ST* females	Proportional reduction in heterokaryotypes (column 2/column 3)
1 (*al-dp*)	0.020	0.108	0.19
2 (*dp-b*)	0.031	0.288	0.11
3 (*b-pr*)	0.0003[a]	0.057	0.005
4 (*pr-c*)	0.0003[a]	0.178	0.002
5 (*c-px*)	0.040	0.217	0.18
6 (*px-sp*)	0.030	0.055	0.55
Sample size	3384	1675	
Frequency of doubles in inverted region (3 and 4)	0.0003	0.012	0.025

[a] A single double-recombinant (*al dp b c px sp*).

TABLE 5

Effect of the second-chromosome inversion on recombination on other chromosomes

Cross and region	Recombination in *ST/2LR* females	Recombination in *ST/ST* females	$G_{[1]}$[a]
w-m	0.412	0.375	7.14*
ru h th			
Region 1 (*ru-h*)	0.273	0.217	5.47**
Region 2 (*h-th*)	0.254	0.206	5.08**

The sample size in the *w m* cross (*X* chromosome) was 1806 for *ST/2LR* females and 1661 for *ST/ST* females. The sample size in the *ru h th* cross (third chromosome) was 1045 for *ST/2LR* females and 719 for *ST/ST* females.

[a] Significance levels: *$P = 0.02$; **$P = 0.008$.

Standard sequence. Table 4 shows the results of both crosses.

Heterokaryotypes again produced no single recombinants in the inverted region, and double-recombinants were found only 2% as frequently as in homokaryotypes. As is commonly observed (ALEXANDER 1952; ROBERTS 1967), the reduction of recombination in heterokaryotypes is more severe inside than outside the inversion. From the egg hatchability tests, we know that *ST/2LR* females have at least a 50-fold reduction of recombination in the inverted region compared to *ST/ST* females. Outside the region, however, recombination is reduced only 2–10-fold, with the highest levels between markers farthest from the breakpoints.

The great reduction of recombination inside as compared to outside the inversion supports a mechanical and not a genetic cause for the reduced crossing over: if a gene were involved, its effects would have to diminish sharply outside the inversion breakpoints.

We also compared *ST/ST vs. ST/2LR* karyotypes for their effects on recombination on the *X* and third chromosome (Table 5). In both cases, the presence of the heterokaryotype slightly but significantly *increases* recombination on the other chromosomes. This is the well known "interchromosomal effect" observed for many arrangements (LUCCHESI 1976), and again supports a mechanical *vs.* a genic explanation for the reduced crossing over.

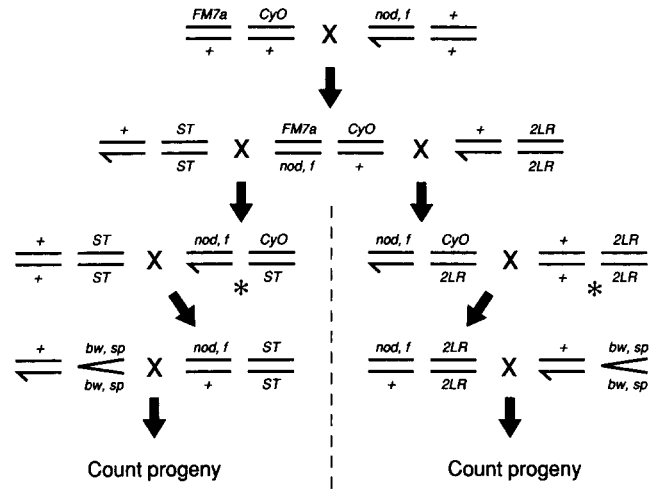

FIGURE 2.—Crossing scheme to construct all three chromosomal genotypes carring the nod^{DTW} allele. Only the *X*, *Y* and second chromosome are shown. The two genotypes marked with an asterisk were intercrossed to produce nod^{DTW}/+; *ST/2LR* females. All three genotypes were then crossed to *C(2)EN bw sp* males, as shown, to provide a measure of nondisjunction and hence crossing over. See text for further details.

Finally, a more indirect test of recombination was made using the nod^{DTW} allele in heterokaryotypes and the two homokaryotypes according to the crossing scheme shown in Figure 2. Recombination in the nod^{DTW}-containing female genotypes was estimated by crossing them to *C(2)EN bw sp* males and counting the offspring, almost all of which are produced when there is no crossing over on the second chromosome. The females were also crossed to *Iv* males and scored for fecundity during 1 week.

In this analysis, the heterokaryotype produces the most offspring, again indicating reduced crossing over (Table 6). The *2LR/2LR* homokaryotype, however, produces only one-third as many offspring as *ST/ST* (an unpaired *t* test on the number of offspring produced in individual vials gives $t_{[88]} = 8.57$, $P < 0.001$). This implies that crossing over is even higher in *2LR/*

TABLE 6

Number of offspring produced by three karyotypes containing nod^{DTW} when each is crossed to C(2)EN bw sp males

Genotype	Total offspring (wild-type)	Fecundity, eggs/week (SE)
nod^{DTW}; *ST/ST*	372 (342)	78.8 (7.4)
nod^{DTW}; *ST/2LR*	1660 (1515)	101.3 (9.2)
nod^{DTW}; *2LR/2LR*	116 (105)	94.2 (8.8)

Forty-five vials (each containing three mated pairs) were scored for each genotype. Egg production is given as average fecundity of each female over a week of measurement (six females were scored per genotype). The proportion of *bw sp* offspring produced by the three genotypes (data not shown) is homogeneous ($G_{[2]} = 0.28$, $P = 0.87$).

2LR than in *ST/ST* females. The difference in progeny production is not due to a lower fecundity of *2LR/2LR* females, as they are significantly *more* fecund than *ST/ST* females over 1 week ($t_{10} = 3.42$, $P = 0.007$). We therefore find no evidence for reduced recombination in *2LR/2LR* as compared to *ST/ST* homokaryotypes.

The above experiment gives an indirect estimate of recombination in homozygotes for *2LR* chromosomes extracted from nature. A more direct test of recombination can be made by placing mutant markers on a *2LR*-containing second chromosome, although in this case the *2LR* chromosome will be a mosaic of wild and laboratory genomes.

We constructed females heterozygous for*2LR* and the multiply marked *all* chromosome containing the *ST* sequence. These females were backcrossed to *all* males. From approximately 20,000 backcross offspring, we collected one *al dp 2LR px sp/al dp b pr c px sp ST* male, having two markers on either side of the inversion. The *CyO* marker chromosome, which contains *pr*, was used to extract *2LR* and produce a stock homozygous for *al dp 2LR px sp*. A similar cross was used to manufacture a stock homozygous for the *al dp ST px sp* chromosome.

We used these two stocks to estimate recombination among the four markers in all three possible karyotypes. To measure recombination in *2LR* homozygotes, we crossed *al dp 2LR px sp* males to the stock homozygous for the unmarked, extracted *2LR* chromosome. The *al dp 2LR px sp/al*$^+$ *dp*$^+$ *2LR px*$^+$ *sp*$^+$ females were backcrossed to *all* males and the 16 possible genotypes scored in the offspring. A similar estimate was made for *ST/ST* homozygotes using *al dp ST px sp/al*$^+$ *dp*$^+$ *ST px*$^+$ *sp*$^+$ females. Finally, we measured recombination in heterokaryotypes using *al dp 2LR px sp/al*$^+$ *dp*$^+$ *ST px*$^+$ *sp*$^+$ females.

As expected, the amount of recombination in *ST/2LR* heterokaryotypes was much less than in homokaryotypes (Table 7). The *2LR/2LR* and *ST/ST* homokaryotypes, however, showed nearly equal frequencies of recombination and showed no difference in the

TABLE 7

Recombination across the inverted region in the two homokaryotypes and the heterokaryotype (see text for details)

Genotype	Region 1 *al-dp*	Region 2 *dp-px*	Region 3 *px-sp*	*N*
2LR al dp px sp/2LR	0.110	0.486	0.048	1013
ST al dp px sp/ST	0.113	0.443	0.045	1276
2LR al dp px sp/ST	0.019	0.030	0.016	880

Recombination fractions for each region are calculated from the sixteen genotypes segregating in each cross (data not shown). Actual map lengths of each region from (LINDSLEY and ZIMM 1985, 1990) are: region 1, 12.6 cM; region 2, 86.5 cM; region 3, 6.5 cM.

proportion of individuals in the 16 genotypic classes (data not shown; heterogeneity, $\chi^2_{[15]} = 23.23$, $P = 0.08$). Again, there is no evidence that *2LR* contains a gene that reduces crossing over. In this cross, as opposed to the cross using the nod^{DTW} allele, there is no sign of higher recombination in *2LR* than in *ST* homokaryotypes. This difference may be due to either different genetic backgrounds in the two tests or the mosaic nature of the *al dp 2LR px sp* chromosome.

Because heterokaryotypes do not decrease recombination on other chromosomes, because crossing over in heterokaryotypes is reduced far more inside than outside the inverted region, and because *2LR/2LR* females do not show less recombination than *ST/ST* females, we conclude that crossing over in heterokaryotypes is reduced by mechanical and not genetic factors.

Does *2LR* show segregation distortion? Although *2LR* is not obviously underdominant, it does carry a lethal and hence should eventually be eliminated from laboratory cultures (this should also be true in nature if all *2LR*s carry lethals). Three obvious explanations for its persistence are genetic drift, *overdominance* of the inversion (*i.e.*, *2LR/ST* genotypes are more fit than the *ST/ST* and lethal *2LR/2LR* karyotypes), and segregation distortion, such that heterokaryotypes preferentially produce gametes carrying the inversion. Several tests were done to check this last hypothesis.

Because the *Segregation-distorter* locus is in the inverted region (LINDSLEY and ZIMM 1990), one obvious possibility is that *2LR* contains the *SD* allele, which segregates at very high frequencies when heterozygous with a second chromosome carrying the sensitive allele at the linked *Responder* locus (*Rsp*^{s}; HARTL and HIRAIZUMI 1976). To test this possibility, we produced a genotype heterozygous for the *2LR* chromosome and a *Standard*-sequence chromosome carrying *Responder-sensitive* and two recessive markers (*2LR/ST Rsp*^{S} *cn bw*). If *2LR* males carry the *SD* allele, nearly all of their sperm should contain *2LR* and they should produce nearly all wild-type offspring when crossed to *cn bw* females.

These males, however, produced progeny in Mendelian proportions: the proportion of *cn bw* offspring

TABLE 8

Segregation ratios of *2LR* versus *ST* chromosome from heterokaryotypes

	Female parent	Male parent	Total	Proportion mutant	G_1
A.	*b cn/b cn*	*b cn (ST)/b⁺ cn⁺ (ST^{Mau})*	6316	0.461	1.15 (n.s.)
	b cn/b cn	*b cn (ST)/b⁺ cn⁺ ($2LR^{Mau}$)*	2802	0.472	
B.	*vg(ST)/vg⁺ (ST^{Mau})*	*vg/vg*	2332	0.484	4.32 ($p < 0.05$)
	vg(ST)/vg⁺ ($2LR^{Mau}$)	*vg/vg*	2784	0.513	
C.	*vg(ST)/vg⁺ (ST^{Rod})*	*vg/vg*	1649	0.445	17.98 ($p < 10^{-4}$)
	vg(ST)/vg⁺ ($2LR^{Rod}$)	*vg/vg*	1292	0.523	

was 0.470 ($N = 967$), not significantly different from the frequency of 0.447 ($N = 664$) obtained in the control reciprocal cross ($G_{[1]} = 0.86$, $P = 0.36$). We also determined in a separate cross that *2LR* from both islands carry the Rsp^S allele (data not shown).

Three other tests of segregation ratios from heterokaryotypic males or females were conducted using heterozygotes for *2LR* chromosomes and *Standard* chromosomes containing recessive mutations. These heterozygotes were backcrossed to stocks homozygous for the mutations; the ratios of mutant *vs.* nonmutant progeny give the segregation ratios of *ST vs. 2LR* chromosomes. Control tests for viability examined the segregation ratios in *ST/ST* individuals.

One of the three tests showed no deviation from Mendelian ratios (Table 8), while the other two showed slight but significant *underrepresentation* of *2LR* in the progeny. These deviations may be due to differences in fitnesses of the *vestigial* homozygote on different genetic backgrounds. At any rate, these differences do not indicate a meiotically driven *2LR* chromosome because the inversion is underrepresented in the offspring of *ST/2LR* females.

It is possible that although the inversion does not show meiotic drive against the marked tester chromosomes, it might do so against *ST* chromosomes taken from the same natural population. It is difficult to test this possibility, as it requires measuring segregation ratios of unmarked chromosomes. To do so, we constructed *2LR/ST* genotypes by crossing *CyO/2LR* females to males isochromosomal for *ST* chromosomes extracted from the same population as the *2LR*. (Inversions from the two islands were studied separately.) The *2LR/ST* males were crossed to homozygous *b cn vg(ST)* females. We tested only heterokaryotypic males because meiotic drive is nearly always limited to that sex (ZIMMERING, SANDLER and NICOLETTI 1970). These males produce two types of offspring: *b cn vg(ST)/b⁺ cn⁺ vg⁺(ST)*, and *b cn vg(ST)/b⁺ cn⁺ vg⁺(2LR)*. Females of the former genotype will produce recombinants when backcrossed crossed to homozygous *b cn vg* males; females of the latter genotype produce no recombinants. The ratio of females producing no recombinants to those producing recombinants thus gives the ratio of *ST* to *2LR* sperm produced by heterokaryotypic males.

Every female hatching from three vials of each cross was collected as a virgin and backcrossed to two *b cn vg/b cn vg* males. With the exception of the few females who escaped or died, all of the females produced more than the 55 offspring needed to determine with 99% confidence whether the chromosome was *ST* or *2LR*. (This frequency was determined in separate estimates of recombination in *ST b cn vg/ST b⁺ cn⁺ vg⁺* heterozygotes.)

Heterokaryotypes from both populations showed Mendelian segregation. In the cross using Mauritius females, the proportion of *2LR* gametes was 0.495 ($N = 275$), not significantly different from the expected 0.5 ($\chi^2_{[1]} = 0.03$, $P > 0.75$). In Rodriguez females, the proportion of *2LR* gametes was 0.514 ($N = 253$), again not differing from Mendelian expectation ($\chi^2_{[1]} = 0.19$, $P > 0.5$).

We therefore find no evidence for segregation distortion of *2LR* over *ST* chromosomes from the same population. Of course, our power to detect small amounts of distortion is limited by the restricted sample of offspring. The upper 95% confidence limit for the proportion of *2LR* sperm from heterokaryotypes is 0.575 for the Rodriguez cross and 0.553 for the Mauritius cross.

Do the inversions from the two islands have a single origin? A limited RFLP study of the *Adh* region examined the likelihood of a single origin of the inversions from the two islands. We analyzed 11 lines: seven *ST* second chromosomes from Mauritius, one *2LR* chromosome from Maritius, two *ST* chromosomes from Rodriguez and one *2LR* chromosome from Rodriguez. The *ST* chromosomes came from different isofemale lines.

There was striking uniformity among these chromosomes, all having identical RFLP patterns except for two *ST*s from Mauritius [one with a loss of *Alu*I 423 and the other a loss of *Alu*I 423 and a loss of *Msp*I 503 (numbering after KREITMAN 1983)] and the two *ST* lines from Rodriguez (both having loss of *Alu*I 423, gain of *Hae*III 817, and a 35-bp insertion in fragment 498–573). Homozygosity is higher than has been seen in other tested populations of *D. melano-*

gaster: using the same techniques in a survey of populations on the east coast of the United States, A. BERRY and M. KREITMAN (personal communication) found a homozygosity of *Adh* 4-cutter haplotypes of only 0.08 (113 haplotypes were seen in 1533 lines from 25 populations). This contrasts with the homozygosity of 0.383 for the combined lines from the two islands. The limited RFLP variation suggests that the flies have restricted population sizes on the islands, which is further supported by their rarity.

While the RFLP results do not rule out the possibility that *2LR* has originated more than once, the similar RFLP patterns for the two inversions despite the differences among *ST* chromosomes supports the idea of a unique origin. The cytological identity of the breakpoints of the *2LR*s from the two islands (Figure 1) would also be a remarkable coincidence if the inversion arose more than once.

DISCUSSION

Despite the widespread idea that heterozygotes for pericentric inversions are semisterile, we find no such underdominance for fertility in *2LR*. The lack of such underdominance almost certainly comes from a drastic reduction of crossing over in the inverted region, so that heterokaryotypic females produce almost no aneuploid eggs. Heterokaryotypes also show no increase in nondisjunction over homokaryotypes, an observation made on other inversions (STONE and THOMAS 1935; STURTEVANT and BEADLE 1936; MORAN 1981).

The reduced crossing over is not caused by a gene linked to the inversion, because *2LR* homokaryotypes show at least as much recombination as *ST* homokaryotypes, and the presence of *2LR* does not reduce recombination on other chromosomes. Instead crossing over is apparently reduced by mechanical factors; we suspect heterosynaptic meiotic pairing or a disruption of pairing sites (see below).

The inversion's lack of underdominance for fertility undoubtedly contributes to its persistence in nature and in laboratory cultures. With our present data, however, we cannot discriminate between the possibilities that *2LR* is neutral with respect to *ST*, that it is deleterious as a homozygote but maintained in the population by drift, or that it is overdominant because it contains advantageous alleles but cannot be fixed because of the included lethal. We can conclude, however, that stong drift in very small populations is not required to explain the polymorphism.

There is one previous report of a pericentric inversion in which reduced crossing over in heterokaryotypic females leads to nearly normal fertility. ROBERTS (1967) measured fertility and crossing over in heterokaryotypes for three X-ray-induced pericentric inversions on the third chromosome of *D. melanogaster*. Two inversions showed substantial underdominance (with egg hatch reduced by 25 and 43%, respectively), as well as frequent double-recombination within the inverted region. Heterokaryotypes for *In(3LR)190*, however, showed no reduction in fertility and virtually no double recombination. Roberts speculated that this inversion was not underdominant because it spanned a region of the chromosome having low recombination.

Other anecdotal evidence that such inversions may not be underdominant comes from a failure to find semisterility in mice heterokaryotypic for both X-linked and autosomal pericentrics (NACHMAN and MYERS 1989), although sample sizes were very small. In addition, polymorphism for pericentric inversions has been reported in *Drosophila robusta* by CARSON and STALKER (1947), who speculated that the polymorphism may have been permitted by reduced crossing over in heterokaryotypic females. Populations of other animal species are sometimes polymorphic for pericentric inversions, and in many of these cases chiasmata are not observed in the inverted region (see below).

The most important implication of our results relates to the widespread idea that fixed differences among taxa for "underdominant" inversions necessarily imply extreme population bottlenecks and shifts between adaptive peaks. We emphasize first that most newly arising pericentric inversions in Drosophila must certainly be underdominant. In contrast to paracentric inversions, they are almost never polymorphic in natural populations, and are also fixed much less frequently. STONE (1955) estimated that in the 650 *Drosophila* species known at that time, only 32 pericentric inversions were either polymorphic or had been fixed, compared to between 6,100 and 36,500 paracentric inversions. Unless the two types of inversions occur at drastically different rates, which seems unlikely, this difference must reflect an innate difference in fitness.

If, however, pericentric inversions vary in their rate of recombination (and hence their fertility) as heterokaryotypes, it is those with the least underdominance that will become fixed or polymorphic. Because this study and others have shown such variation, the observation of species fixed for different inversions need not imply a peak shift or strong episode of genetic drift. Such a conclusion requires that the supposed underdominance be tested by direct studies of meiosis and fertility in hybrids. Moreover, these studies are complicated by the fact that genic as well as chromosomal differences can cause meiotic problems and hybrid sterility (DOBZHANSKY 1951).

If such inversions are not underdominant, we need not invoke special mechanisms such as meiotic drive to explain their fixation. Indeed, the segregation studies show not the slightest evidence for meiotic drive of *2LR*. Normal Mendelian segregation was also found

in species hybrids heterokaryotypic for a pericentric inversion fixed between two phylads of the *Drosophila virilis* group (COYNE 1989).

Our failure to find genes linked to *2LR* that reduce crossing over in heterokaryotypes implies the involvement of mechanical factors. It also militates against the idea that pericentric inversions persist because they are linked to genes reducing recombination, either fortuitously or as a result of selection (CARSON and STALKER 1947).

The mechanical causes of reduced crossing over are unclear. One possibility is heterosynapsis: during meiosis in female heterokaryotypes, the homologues may pair without forming inversion loops, leading to synapsis of nonhomologous DNA. Chiasmata are apparently not formed in heterosynaptic regions because molecular recombination requires homology between paired segments of DNA (WATSON *et al.* 1976).

Heterosynaptic pairing of paracentric and pericentric inversions is often seen in other species, including corn (MCCLINTOCK 1933), fungi (BOJKO 1990), mice (ASHLEY, MOSES and SOLARI 1981; MOSES *et al.* 1982; GREENBAUM and REED 1984; GREENBAUM, HALE and FUXA 1986; HALE 1986), chironomids (MARTIN 1967), orthopterans (COLEMAN 1948; NUR 1968; MORAN 1981), and humans (GABRIEL-ROBEZ *et al.* 1988). Some inversions initially pair as a loop and then "adjust" to straight heterosynaptic pairing during pachytene (MOSES *et al.* 1982; BOJKO 1990), others pair heterosynaptically throughout meiosis (HALE 1986), while still others vary in their pairing behavior among cells (MCCLINTOCK 1933; NUR 1968; GREENBAUM and REED 1984). Cell-to-cell variation in pairing could account for the very low frequency of double-recombination seen in our studies. Unfortunately, it is difficult to determine whether heterosynaptic pairing occurs in Drosophila because it is impossible to get good chromosome preparations of female meiosis.

Another explanation for reduced crossing over is that the breakpoints of *2LR* may lie in regions important for synapsis and exchange. Using X-rays to produce a number of 2-4 and 3-4 reciprocal translocations, ROBERTS (1970) found that the position of the breakpoints had a large effect on recombination in heterozygotes, with some translocations lowering recombination throughout the entire chromosome arm. Breakpoints with the largest effect occurred roughly in the middle of each autosomal arm (ROBERTS 1976, Figure 7). It is notable that both breakpoints of *In(2LR)PL* fall within these regions, as do those of *In(3LR)190*, ROBERTS' (1967) inversion discussed above. HAWLEY (1980) found similar area effects on recombination in heterozygotes for *X*-4 translocations, although in this case the reduced recombination occurred over smaller regions of the chromosome.

Either heterosynapsis or chromosome breakage at pairing sites could account for the well known but unexplained phenomenon of reduced crossing over in females heterokaryotypic for *paracentric* inversions (NOVITSKI and BRAVER 1954; ISHII and CHARLESWORTH 1977). In such genotypes, double exchange is often reduced dramatically inside the inversion and single-exchange reduced outside the inversion; the latter effect often reaches far beyond the breakpoints. We suggest that such chromosomes do not synapse properly.

There may, of course, be other explanations for reduced recombination in heterokaryotypes for pericentric inversions. The inverted sections, for example, may simply fail to pair because loop formation is difficult in germ cells. Such explanations will be testable when it becomes possible to observe meiotic pairing in female Drosophila.

The lack of underdominance of polymorphic pericentric inversions may also apply to other arrangements such as Robertsonian fusions, tandem fusions, and translocations. While it is clear that heterokaryotypes for Robertsonian fusions or reciprocal translocations can be semisterile because of aberrant segregation, the degree of sterility differs among arrangements, with some showing normal segregation and no underdominance (STONE 1949; LEWIS and JOHN 1957; BRUÈRE and ELLIS 1979; ELDER and PATHAK 1980; PORTER and SITES 1985; MORITZ 1986). Summarizing the data on segregation of heterozygous arrangements in hybrid zones, SHAW (1981) found little evidence that naturally occurring arrangements disrupted meiosis.

As with pericentric inversions, each putative case of underdominance must be demonstrated by direct investigation of meiosis and hybrid fertility. Such studies must also ensure that the meiotic problems result from the chromosomal differences and not from genic divergence, and also that heterokaryotypes have lowered fertility (in some cases, abnormal gametes may be eliminated before fertilization) (BRUÈRE and ELLIS 1979). Other requirements for demonstrating underdominance for fertility are reviewed by SITES and MORITZ (1987) and BAKER and BICKHAM (1986).

If such arrangements are not underdominant, then of course they cannot cause reproductive isolation and speciation as proposed by WHITE (1968, 1978). The most vigorous advocate of chromosomal speciation, WHITE based much of his theory on fixed and polymorphic pericentric inversions in orthopterans. But as JOHN (1981, p. 43) noted, "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."

If chromosome evolution does not constitute strong support for peak shifts in nature, are we left with any evidence that genetic drift can overcome natural selection? The one convincing case is that of coiling in snails. In some species, right- and left-hand coiling are controlled by single Mendelian alleles showing maternal inheritance (BOYCOTT *et al.* 1930; MURRAY and CLARKE 1966). In a population fixed for one coiling allele, an individual of opposite coiling is at a disadvantage because it has great difficulties achieving copulation (LIPTON and MURRAY 1979; GITTENBERGER 1988). Indeed, polymorphisms for coiling are quite rare in nature. Nevertheless, populations of some species are fixed for different coiling morphs, implying that evolution has produced transitions between adaptive peaks. Such switches in coiling may be initiated by genetic drift, which occasionally increases the frequency of a rare allele above the unstable equilibrium frequency, after which it becomes fixed by selection (ORR 1991). This scenario is facilitated by the limited mobility of snails, their small populations, and the maternal inheritance of the alleles, which allows them to increase in frequency without producing many individuals having unfit phenotypes (JOHNSON 1982; GITTENBURGER 1988).

A lone example, however, cannot buttress strong claims about evolutionary change. Much more evidence is required if models of evolution involving genetic drift, such as the shifting balance theory of WRIGHT (1970), are to be taken seriously.

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LITERATURE CITED

ALEXANDER, M. L., 1952 The effect of two pericentric inversions upon crossingover in *Drosophila melanogaster*. Univ. Texas Pub. **5204:** 219–226.

ASHBURNER, M., 1989 *Drosophila, a Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

ASHBURNER, M., 1991 *Drosophila* Genetic Maps. Drosophila Inform. Serv. **69.**

ASHLEY, T., M. J. MOSES and A. J. SOLARI, 1981 Fine structure and behaviour of a pericentric inversion in the sand rat, *Psammomys obesus*. J. Cell Sci. **50:** 105–119.

AULARD, S., 1990 Polymorphisme chromosomique de *Drosophila melanogaster*, en Afrique et dans les îles de l'Océan Indien. Thesis, Université Pierre et Marie Curie, Paris.

BAKER, R. J., and J. W. BICKHAM, 1986 Speciation by monobrachial centric fusions. Proc. Natl. Acad. Sci. USA **83:** 8245–8248.

BICKHAM, J. W., and R. J. BAKER, 1979 Canalization models of chromosome evolution. Bull. Carnegie Mus. Nat. Hist. **13:** 70–84

BOJKO, M., 1990 Synaptic adjustment of inversion loops in *Neurospora crassa*. Genetics **124:** 593–598.

BOYCOTT, A. E., C. DIVER, S. GARSTANG and F. M. TURNER, 1930 The inheritance of sinistrality in *Limnaea peregra* (Mollusca, Pulmonata). Phil. Trans. R. Soc. Lond. B **219:** 51–131.

BRITTNACHER, J. G., and B. GANETZKY, 1983 On the components of segregation distortion in *Drosophila melanogaster*. II. Deletion mapping and dosage analysis of the *SD* locus. Genetics **103:** 659–673.

BRUÈRE, A. N., and P. M. ELLIS, 1979 Cytogenetics and reproduction of sheep with multiple centric fusions (Robertsonian translocations). J. Reprod. Fertil. **57:** 363–375.

CARSON, H. L., and H. D. STALKER, 1947 Gene arrangements in natural populations of *Drosophila robusta* Sturtevant. Evolution **1:** 113–133.

CHARLESWORTH, B., 1985 Recombination, genome size, and chromosome number, pp. 489–513 in *Evolution of Genome Size*, edited by T. CAVALIER-SMITH. John Wiley & Sons, Chichester.

COLEMAN, L. C., 1948 The cytology of some western species of *Trimerotropis* (Acrididae). Genetics **33:** 519–528.

COYNE, J. A., 1986 Meiotic segregation and male recombination in interspecific hybrids of Drosophila. Genetics **114:** 485–494.

COYNE, J. A., 1989 A test of the role of meiotic drive in chromosome evolution. Genetics **123:** 241–243.

DAVID, J. R., S. F. MCEVEY, M. SOLIGNAC and L. TSACAS, 1989 *Drosophila* communities on Mauritius and the ecological niche of *D. mauritiana* (Diptera, Drosophilidae). Rev. Zool. Afr. **103:** 107–116.

DOBZHANSKY, T. 1951 *Genetics and the Origin of Species*, Ed. 3. Columbia University Press, New York.

ELDER, F. F. B., and S. PATHAK, 1980 Light microscopic observations on the behavior of silver-stained trivalents in pachytene cells of *Sigmodon fulviventer* (Rodentia, Muridae) heterozygous for centric fusion. Cytogenet. Cell Genet. **27:** 31–38.

FUTUYMA, D. J., and G. C. MAYER, 1980 Non-allopatric speciation in animals. Syst. Zool. **29:** 254–271.

GABRIEL-ROBEZ, O., C. RATOMPONIRINA, M. CROQUETTE, J. COUTURIER and Y. RUMPLER, 1988 Synaptonemal complexes in a subfertile man with a pericentric inversion in chromosome 21. Cytogenet. Cell Genet. **48:** 84–87.

GITTENBERGER, E., 1988 Sympatric speciation in snails; a largely neglected model. Evolution **42:** 826–828.

GREENBAUM, I. F., D. W. HALE and K. P. FUXA, 1986 Synaptic adaptation in deer mice: a cellular mechanism for karyotypic orthoselection. Evolution **40:** 208–213.

GREENBAUM, I. F., and M. J. REED, 1984 Evidence for heterosynaptic pairing of the inverted segment in pericentric inversion heterozygotes of the deer mouse (*Peromyscus maniculatus*). Cytogenet. Cell Genet. **38:** 106–111.

GRELL, R. F., 1976 Distributive pairing, pp. 435–486 in *The Genetics and Biology of Drosophila*, Vol. 1a, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

HALE, D. W., 1986 Heterosynapsis and suppression of chiasmata within heterozygous pericentric inversions of the Sitka deer mouse. Chromosoma **94:** 425–432.

HARTL, D. L., and Y. HIRAIZUMI, 1976 Segregation distortion, pp. 616–666 in *The Genetics and Biology of Drosophila*, Vol. 1b, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

HAWLEY, R. S., 1980 Chromosomal sites necessary for normal levels of meiotic recombination in *Drosophila melanogaster*. I. Evidence for and mapping of the sites. Genetics **94:** 625–646.

HEDRICK, P. W., 1981 The establishment of chromosome variants. Evolution **35:** 322–332.

ISHII, K., and B. CHARLESWORTH, 1977 Associations between allozyme loci and gene arrangements due to hitch-hiking effects of new inversions. Genet. Res. **30:** 93–106.

JOHN, B., 1981 Chromosome change and evolutionary change: a critique, pp. 23–51 in *Evolution and Speciation. Essays in Honor of M. J. D. White*, edited by W. R. ATCHLEY and D. S. WOODRUFF. Cambridge University Press, Cambridge.

JOHNSON, M. S., 1982 Polymorphism for direction of coil in *Partula suturalis*: behavioural isolation and positive frequency dependent selection. Heredity **49:** 145–151.

KREITMAN, M., 1983 Nucleotide polymorphism at the Alcohol Dehydrogenase locus of *Drosophila melanogaster*. Nature **304:** 412–417.

KREITMAN, M., and M. AGUADÉ, 1986 Genetic uniformity in two populations of *Drosophila melanogaster* as revealed by filter hybridization of four-nucleotide-recognizing restriction enzyme digests. Proc. Natl. Acad. Sci USA **83:** 3562–3566.

LANDE, R., 1979 Effective deme sizes during long-term evolution estimated from rates of chromosomal rearrangement. Evolution **33:** 234–251.

LANDE, R., 1984 The expected fixation rate of chromosomal inversions. Evolution **38:** 743–752.

LEWIS, K. R., and B. JOHN, 1957 Studies on *Periplaneta americana*. II. Interchange heterozygosity in isolated populations. Heredity **11:** 11–22.

LINDSLEY, D., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*. Part 1: Genes A-K. Drosophila Inform. Serv. **62.**

LINDSLEY, D., and G. ZIMM, 1990 The genome of *Drosophila melanogaster*. Part 2: Genes L-Z, balancers, and transposable elements. Drosophila Inform Serv. **68.**

LIPTON, C. S., and J. MURRAY, 1979 Courtship of land snails of the genus *Partula*. Malacologia **19:** 129–146.

LUCCHESI, J. C., 1976 Interchromosomal effects, pp. 315–329 in *The Genetics and Biology of Drosophila*, Vol. 1b, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

MARTIN, J., 1967 Meiosis in inversion heterozygotes in Chironomidae. Can. J. Genet. Cytol. **9:** 255–268.

MCCLINTOCK, B., 1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellforsch. Mikrosk. Anat. **19:** 191–237.

MORAN, C., 1981 Spermatogenesis in natural and experimental hybrids between two chromosomally differentiated taxa of *Caldeia captiva*. Chromosoma **81:** 579–591.

MORITZ, C., 1986 The population biology of Gehyra (Gekkonidae): chromosomal change and speciation. Syst. Zool. **35:** 46–67.

MOSES, M. J., P. A. POORMAN, T. H. RODERICK and M. T. DAVISSON, 1982 Synaptonemal complex analysis of mouse chromosomal rearrangements. IV. Synapsis and synaptic adjustment in two paracentric inversions. Chromosoma **84:** 457–474.

MURRAY, J., and B. CLARKE, 1966 The inheritance of polymorphic shell characters in *Partula* (Gastropoda). Genetics **54:** 1261–1277.

NACHMAN, M. W., and P. MYERS, 1989 Exceptional chromosomal mutations in a rodent population are not strongly underdominant. Proc. Natl. Acad. Sci. USA **86:** 6666–6670.

NOVITSKI, E., 1976 The construction of an entire compound two chromosome, pp. 562–568 in *The Genetics and Biology of Drosophila*, Vol. 1b, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

NOVITSKI, E., and G. BRAVER, 1954 An analysis of crossing over within a heterozygous inversion in *Drosophila melanogaster*. Genetics **39:** 197–209.

NUR, U., 1968 Synapsis and crossing over within a paracentric inversion in the grasshopper, *Camnula pellucida*. Chromosoma **25:** 198–214.

ORR, H. A., 1991 Is single-gene speciation possible? Evolution **45:** 764–769..

PATTERSON, J. T., and W. S. STONE, 1952 *Evolution in the Genus Drosophila*. Macmillan, New York.

PORTER, C. A., and J. W. SITES, JR., 1985 Normal disjunction in Robertsonian heterozygotes from a highly polymorphic lizard population. Cytogenet. Cell Genet. **39:** 250–257.

RASOOLY, R. S., C. M. NEW, P. ZHANG, R. S. HAWLEY and B. S. BAKER, 1991 The genetic analysis of microtubule mtors in *Drosophila melanogaster*. II. A dominant mutation at the nod locus is associated with a single base change in the putative ATP-binding domain. Genetics (in press).

ROBERTS, P. A., 1967 A positive correlation between crossing over within heterozygous pericentric inversions and reduced egg hatch of Drosophila females. Genetics **56:** 179–187.

ROBERTS, P. A., 1970 Screening for X-ray-induced crossover suppressors in *Drosophila melanogaster*: prevalence and effectiveness of translocations. Genetics **65:** 429–448.

ROBERTS, P. A., 1976 The genetics of chromosome aberrations, pp. 67–184 in *The Genetics and Biology of Drosophila*, Vol. 1a, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

SHAW, D. D., 1981 Chromosomal hybrid zones in orthopteroid insects, pp. 146–170 in *Evolution and Speciation. Essays in Honor of M. J. D. White*, edited by W. R. ATCHLEY and D. S. WOODRUFF. Cambridge University Press, Cambridge.

SHEEN, J., and B. SEED, 1988 Electrolyte gradient gels for DNA sequencing. Biofeedback **6:** 942–944.

SITES, J. W. JR., and C. MORITZ, 1987 Chromosomal evolution and speciation revisited. Syst. Zool. **36:** 153–174.

STONE, W. S., 1949 The survival of chromosomal variation in evolution. Univ. Texas Pub. **4920:** 18–21

STONE, W. S., 1955 Genetic and chromosomal variability in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **20:** 256–270.

STONE, W., and I. THOMAS, 1935 Crossover and disjunctional properties of X chromosome inversions in *Drosophila melanogaster*. Genetics **17:** 170–184.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversion in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

WALSH, J. B., 1982 Rate of accumulation of reproductive isolation by chromosome rearrangements. Am. Nat. **120:** 510–532.

WATSON, J. D., N. H. HOPKINS, J. W. ROBERTS, J. A. STEITZ and A. M. WEINER, 1976 *Molecular Biology of the Gene*, Ed. 4. Benjamin Cummings Publishing Co., Menlo Park, Calif.

WHITE, M. J. D., 1968 Models of speciation. Science **159:** 1065–1070.

WHITE, M. J. D., 1973 *Animal Cytology and Evolution.* Cambridge University Press, Cambridge.

WHITE, M. J. D., 1978 *Modes of Speciation.* Freeman Company, San Francisco.

WRIGHT, S., 1970 Random drift and the shifting balance theory of evolution. pp. 1–31 in *Mathematical Topics in Population Genetics*, edited by K. KOJIMA. Springer-Verlag, Berlin.

WRIGHT, T. R. F., 1974 A cold-sensitive zygotic lethal causing high frequencies of nondisjunction during meiosis I in *Drosophila melanogaster* females. Genetics **76:** 511–536.

ZHANG, P., and R. S. HAWLEY 1990 The genetic analysis of distributive segregation in *Drosophila melanogaster*. II. Further genetic analysis of the *nod* locus. Genetics **125:** 115–127.

ZHANG, P., B. A. KNOWLES, L. S. B. GOLDSTEIN and R. S. HAWLEY, 1990 A kinesin-like protein required for distributive chromosome segregation in Drosophila. Cell **62:** 1053–1062.

ZIMMERING, S., L. SANDLER and B. NICOLETTI, 1970 Mechanisms of meiotic drive. Annu. Rev. Genetics **4:** 409–436.

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