EVIDENCE FOR EXTENSIVE GENETIC DIFFERENTIATION BETWEEN THE SEX-RATIO AND THE STANDARD ARRANGEMENT OF *DROSOPHILA PSEUDOOBSCURA* AND *D. PERSIMILIS* AND IDENTIFICATION OF HYBRID STERILITY FACTORS

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ABSTRACT

This study deals with sex-ratio genes tightly linked within the Sex-Ratio inversion. By taking advantage of the fact that the Sex-Ratio chromosome of *Drosophila persimilis* [SR(B)] is homosequential to the Standard chromosome of *D. pseudoobscura* [ST(A)], we carried out two reciprocal introgression experiments. Individual segments of SR(B) or ST(A) were introgressed into the genome of *D. pseudoobscura* or *D. persimilis*, respectively. Males possessing a hybrid SR(B)-ST(A) *X* chromosome and a genetic background derived from either of the two species were tested for fertility and sex-ratio expression.——It was found that, in terms of the meiotic drive genes, the Sex-Ratio chromosome differs extensively from the Standard chromosome. Because recombinations of these genes result in a complete loss of sex-ratio expression, this finding lends strong support to the hypothesis of gene coadaptation. Coadaptation, in this context, is the advantage of being transmitted preferentially. In light of this finding, the evolution of the sex-ratio system in these two sibling species is discussed.——Introgression experiments also yielded information about hybrid sterility. With reciprocal introgression, sterility interactions were found to be "asymmetric." The asymmetry is fully expected from the viewpoint of evolution of postmating reproductive isolation.

TO explain the extensive polymorphisms of chromosomal inversions in *Drosophila pseudoobscura*, Dobzhansky (1970 and references cited therein) invoked the concept of a complex of coadapted genes, or supergene, which behaves like a single well-adapted Mendelian gene. The concept of coadaptation includes "both selection of alleles at *different* loci *within* gene arrangements to produce a haploid genome that is physiologically balanced, and selection of alleles of the *same* loci *between* inversions to produce heterosis in heterokary-

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otypes" (PRAKASH and LEWONTIN 1968). It is, however, a formidable task to demonstrate the nature of genes within an inversion; in particular, to identify the number and locations of these genes and the mechanisms by which they together confer high fitness on their carriers. The difficulty is best illustrated by the disagreement over the interpretation of nonrandom associations between arrangements of the third chromosome in *D. pseudoobscura* and the electrophoretic alleles they carry. PRAKASH and LEWONTIN (1968, 1971) suggested that nonrandom associations are evidence that the structural genes coding for the proteins they examined are part of the coadapted gene complex. NEI and LI (1975, 1980) stressed that the observed patterns could be explained without reference to any adaptive difference among electrophoretic alleles.

To support the "supergene" hypothesis, it is sufficient, as well as necessary, to break the tightly linked gene complex and then score for fitness differences. One approach is to bring together the same chromosomal arrangement from different localities (DOBZHANSKY and PAVLOVSKY 1958). If each arrangement represents a different adaptive gene complex, recombination could disrupt the integrity of these supergenes.

To further elucidate the nature of coadapted genes in any inversion, it is, however, necessary to have chromosomal arrangements that are associated with different fitness-related phenotypes. One such system is the Sex-Ratio (SR) inversion polymorphisms present in each of the sibling species, *D. pseudoobscura* and *D. persimilis*.

The right arm of the *X* chromosome (*XR*) of each species has two types of arrangements, referred to as Standard (ST) and Sex-Ratio (SR) arrangement, respectively. Male carriers of the SR chromosome transmit primarily (95–99%) *X*-bearing sperm and produce predominantly daughters. The SR chromosomes, therefore, enjoy a great advantage through meiotic drive. Previous studies showed that the inversions between the SR and ST arrangements in *D. pseudoobscura* almost completely suppress recombination on *XR* (STURTEVANT and DOBZHANSKY 1936).

Since genes that jointly produce the meiotic drive effect double their chance of being transmitted by paternal carriers, it is conceivable that any inversion which completely or partially binds such genes together will increase in the population. The genes within such a new inversion would be coadapted in the sense of meiotic drive, not of viability or fertility. To verify this coadaptation hypothesis, it is necessary to show that, within the SR inversion, there are two or more genes that are indispensible for the complete expression of the sex-ratio trait.

Tests of this hypothesis are possible due mainly to several phenomena. First, the F_1 hybrid females of *D. pseudoobscura* and *D. persimilis* are both viable and fertile, although the F_1 hybrid males are completely sterile (LANCEFIELD 1929; and many later studies). Second, studies of recombination frequencies between the SR chromosome of *D. persimilis* [SR(B)] and the ST chromosome of *D. pseudoobscura* [ST(A)] together with studies of the banding pattern of these two chromosomes suggest that ST(A) and SR(B) are homosequential (STURTEVANT and DOBZHANSKY 1936; DOBZHANSKY 1944; and this study). There are, there-

fore, three *XR* arrangements among the two sibling species. (For an explanation of notation, please refer to the MATERIALS AND METHODS, *Notation*; also, see Figure 1 for graphic representation.) Third, biochemical and visible markers are available on these chromosomes. The fact that ST(A) is homosequential to SR(B) is evidence against the hypothesis that the inversion *per se* (*e.g.*, the position effect) is the cause of meiotic drive. Rather, it supports the hypothesis that the SR inversions only serve to bind the "sex-ratio" (hereafter referred to as *sr* to distinguish them from the chromosomal arrangements) genes tightly together by suppressing any recombination between the SR and ST arrangement.

By introgressing SR(B) into *D. pseudoobscura* by repeated backcrossing or, reciprocally, by introgressing ST(A) into *D. persimilis*, the gene complexes bound together by the inversions can be broken up. The chromosomal segments of SR(B) can then be studied either individually or in various combinations for the expression of the sr trait.

Reciprocal introgression procedures are necessary because they are complementary for these reasons: It was hypothesized (and confirmed in this study) that several hybrid sterility factors are distributed on the *X* chromosome. If the sr trait is controlled by a large number of genes dispersed on SR(B), a substantial portion of it has to be introgressed to *D. pseudoobscura* before *sr* genes can be mapped. This will tend to give rise to sterile rather than *sr* males. It would be more fruitful in this case to introgress a small segment (or small segments) of ST(A) into *D. persimilis*. On the other hand, introgression of SR(B) into *D. pseudoobscura* would be quite feasible for mapping *sr* genes if *sr* expression is controlled by only one or two loci. This procedure, in complement with the reciprocal introgression, also provides information on modification of *sr* expression by genes of the sibling species.

In addition to information on the genetics of the *sr* trait, the introgression of segments of foreign *X* chromosome would also yield information about genetic interactions underlying sterility in hybrid males. Most studies on the relative effects of *whole* chromosomes of Drosophila on various properties of

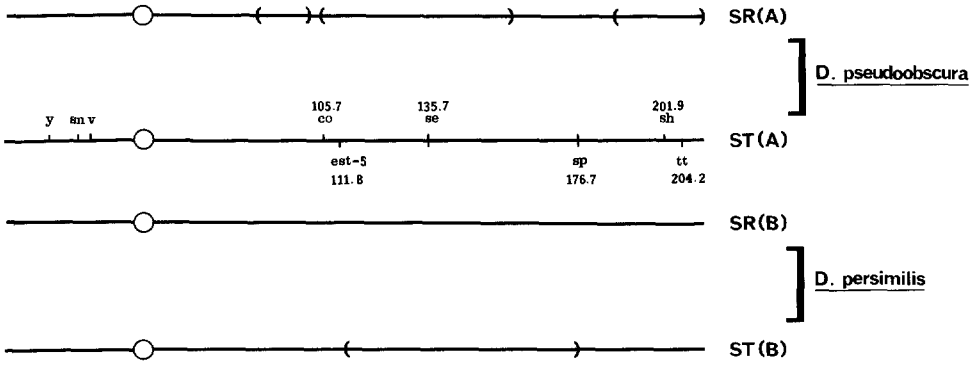

FIGURE 1.—Graphic representation of inversions of *XR* of the sibling pair. The segments within parentheses are inversions relative to the ST(A) [=SR(B)] arrangement. The mutant genes shown are not drawn in correspondence to either the break points of inversions or the centromeres.

interspecific hybrids suggest the overwhelming importance of the *X* chromosome [*e.g.*, DOBZHANSKY (1936) on sterility; PONTECORVO (1943) on viability; TAN (1946) and ZOUROS (1981) on ethological isolation]. Studies on the genetic divergence of *segments* of the *X* chromosome resulting in hybrid sterility will undoubtedly throw some light on the evolution of reproductive isolation between the two sibling species.

MATERIALS AND METHODS

Notation: The most commonly used notation, SR for Sex-Ratio chromosome and ST for Standard, presents a serious difficulty when attempting to localize sex-ratio genes within the inversions and when comparing *D. pseudoobscura* and *D. persimilis*. In this paper, SR and ST refer specifically to the *chromosomal arrangements* carrying sex-ratio and standard genes, respectively, regardless of the banding patterns. The lower case terms, sr and st, refer to the phenotypes, whereas *sr* and *st* refer to the genes responsible for the phenotypes. We now have four arrangements (two of them homosequential): SR(A) for the SR arrangement in *D. pseudoobscura*; ST(A) = SR(B) for the ST arrangement in *D. pseudoobscura*; and SR arrangement in *D. persimilis*, respectively; and ST(B) for the ST arrangement in *D. persimilis*. Within each species (that is, before gene introgression), SR and ST are associated with sr and st phenotypes, respectively. The letter A is for *D. pseudoobscura* and the letter B for *D. persimilis*. Both follow the pre-1944 "race" designations for these two species.

Strains: Both ST(B) and SR(B) were recently collected and isolated from Vancouver Island populations. The SR(A) strains was collected in San Diego, California, and was made available by D. HAYMER. The ST(A) strains were from the National Drosophila Stock Center at the University of Texas, Austin. These two strains carry *y sn v co se sh* and *se ll sp tt*, respectively. (Figure 1; for descriptions, see ANDERSON and NORMAN 1977). The mutant *ll* was disregarded.

Inversions between SR(A) and ST(A), ST(A) and SR(B), and SR(B) and ST(B) mentioned previously were identified cytologically in the strains used in this study. Of most interest is the identical banding pattern of ST(A) and SR(B). This was inferred from data on recombination frequencies and from cytological comparisons of banding patterns. In this study it was found that ST(A) and SR(B) chromatids in the salivary gland cells of hybrid females are apparently capable of complete synapsis, although the affinity between ST(A) and SR(B) seems to be weaker than that between conspecific chromatids.

Figure 1 shows the arrangements of these chromosomal arms relative to one another. In *D. pseudoobscura*, recombination between the *co-sh* region of ST(A) and the corresponding region of SR(A) is so rare that we consider *sr* genes in *D. pseudoobscura* to be completely linked in this region (STURTEVANT and DOBZHANSKY 1936).

Every *XR* used in this study carries a distinct *Est-5* allele and, hence, is electrophoretically distinguishable from every other one. The electrophoresis procedure is identical with that of BECKENBACH (1983).

Introgression by repeated backcrossing

Experiment I: In this experiment, SR(B) was introgressed into the genome of *D. pseudoobscura*. Figure 2 illustrates the procedure. Note that the integrity of SR(B) was maintained in stage I since recombination between SR(A) and SR(B) was completely suppressed. Only in stage II was recombination on *XR* allowed.

Recombinant males with part of *XR* from SR(B) and the rest of the genome from standard males of *D. pseudoobscura* were then obtained and tested for fertility and *sr* expression. Examination of the phenotypes of these recombinant males provided information on the locations of introgressed SR(B) segments.

Experiment II: This experiment involved introgression of ST(A) of *D. pseudoobscura* into the genome of *D. persimilis*. Figure 3 illustrates the introgression protocol. Note that, unlike SR(A)/SR(B) in experiment I, recombination between ST(A) and ST(B) is not completely suppressed. There was a rather small percentage of crossing over between *se* and *sh*, but double crossing over

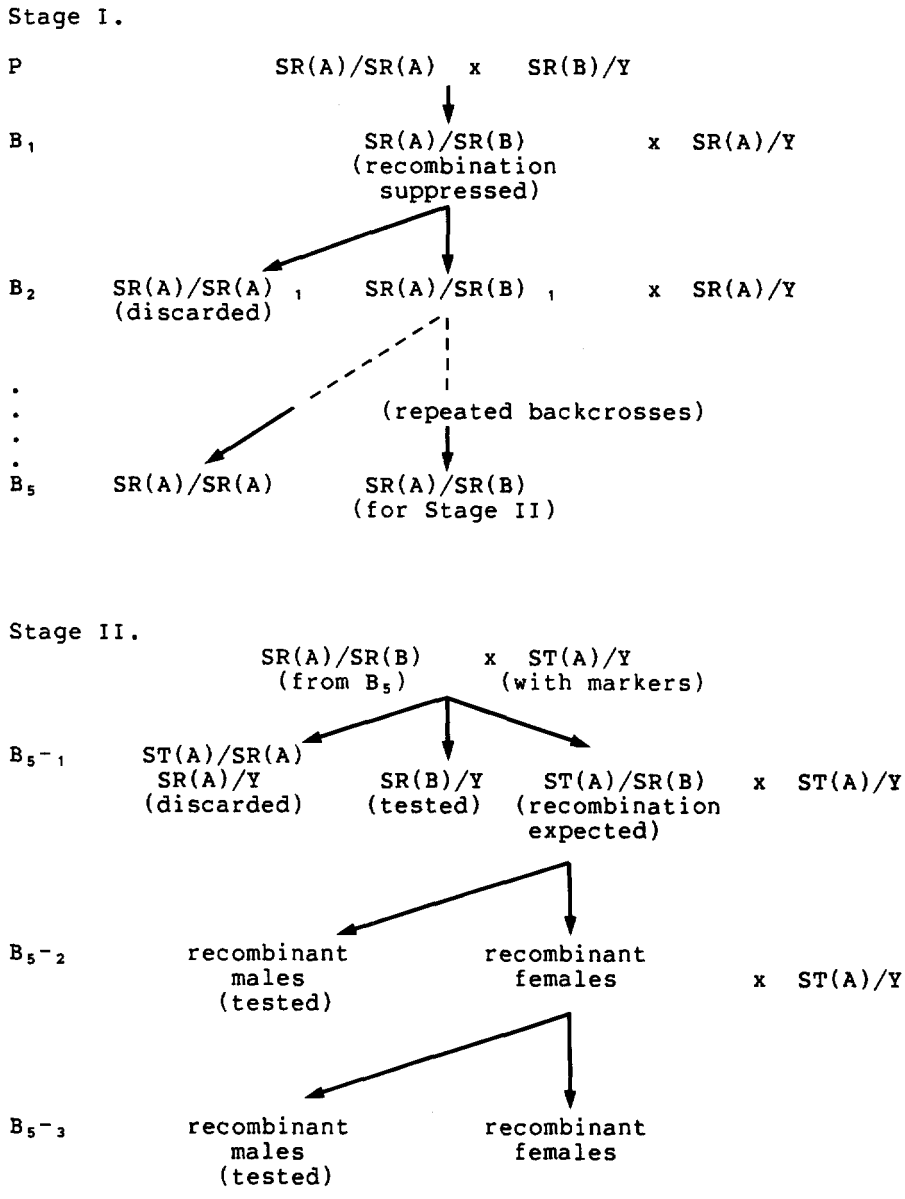

FIGURE 2.—Introgression protocol for experiment I. SR(B) was introgressed into the genome of *D. pseudoobscura* in stage I. Recombinant males with a hybrid *XR* were obtained in stage II. 1Females were allowed to lay eggs and then were assayed for *Est-5* alleles. Homozygotes were discarded.

is quite unlikely. Among $B_{2\text{-}1}$ and $B_{3\text{-}1}$ (Figure 3) females, only those whose sons possess all three markers on ST(A) were used ensuring that the whole ST(A) arm was introgressed.

Stage II of this experiment allowing recombination between SR(B) and ST(A) is crucial. As BECKENBACH and CURTSINGER (BECKENBACH, CURTSINGER and POLICANSKY 1982) independently showed, all males from stage I carrying ST(A) were sterile, although some of them were expected to have the rest of the genome completely from *D. persimilis*.

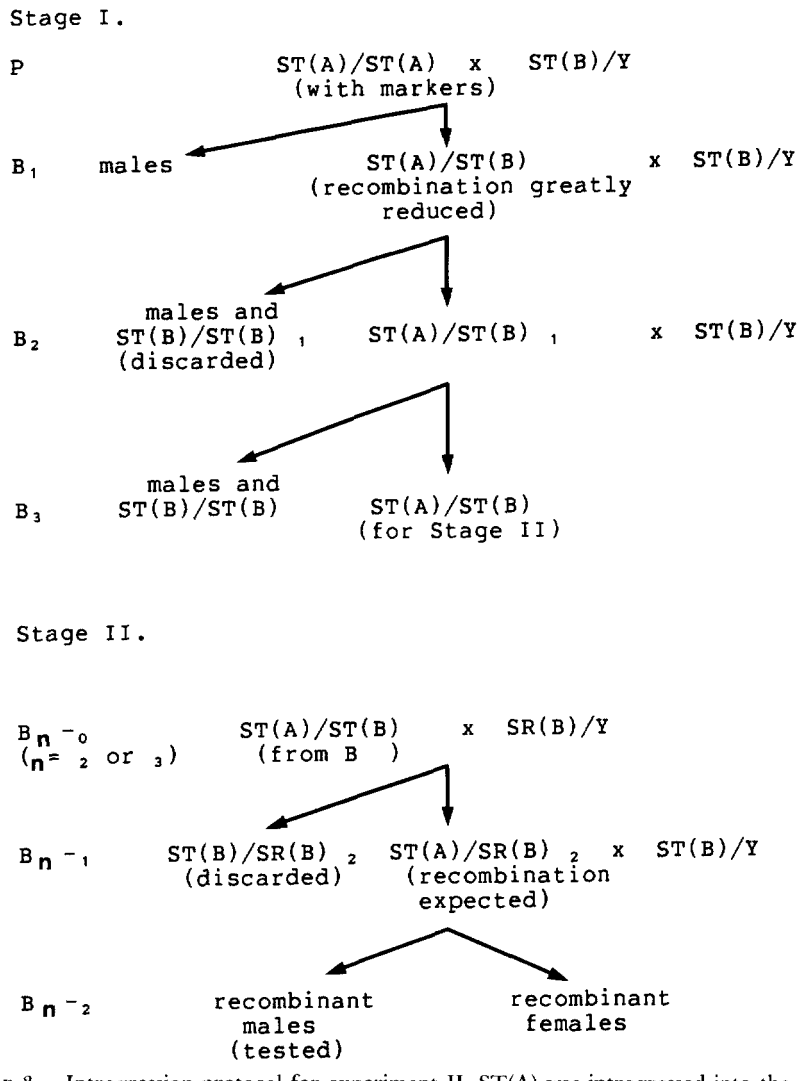

FIGURE 3.—Introgression protocol for experiment II. ST(A) was introgressed into the genome of *D. persimilis* in stage I. Recombinant males with a hybrid *XR* were obtained in stage II. $_1$Females were allowed to lay eggs and then were assayed for *Est-5* alleles. Homozygotes were discarded. $_2$Females were discarded, if none of their sons carried proper mutants.

The recombinant males thus obtained carried segments of ST(A), identifiable by visible genetic markers, in a genome derived mostly from SR(B) males. These males were tested for fertility and for their *sr* expression.

Other procedures: Virgin females used to test for fertility and for *sr* expression of recombinant males were from the species matching the genetic background of the tested males. *D. pseudoobscura* females were used in experiment I, and *D. persimilis* females were used in experiment II. Most of females used in the experiments were F_1's from ST/ST × SR/Y crosses (few males emerged in these cultures) and were aged for 4–6 days before mating. The vials containing the virgin females were subsequently examined for any unexpected insemination. None was detected.

For each recombinant male tested, three to four virgin females were used to minimize failure of insemination caused by behavioral complications. To minimize viability differences in the F_1 caused by crowding, fertile parents were discarded or transferred when a sufficient number of

eggs were laid. In most fertile matings, more than 30 F_1 flies were recovered from each male parent. Progeny having fewer than 5% males were classified as sr. Among these matings, only a few male parents, all with the same genotype, could not be unambiguously classified as either sr or st. These males, producing 10–20% of sons in their progeny, were classified as partial sr. Among those males producing fewer than 30 offspring, only one could not be classified as either sr or st. It was also classified as partial sr.

As a final check of the genotypes of recombinant males, some of them were mated to virgin females homozygous for all recessive mutants in question. Examination of F_1 females, and sometimes of F_2 males, obtained from these matings suggests that the penetrance of the marker genes, *y sn v co se* and *sh*, is nearly complete in males.

RESULTS

Experiment I

The results of this experiment are presented in Tables 1 and 2. The wild-type alleles of the marker genes were introgressed from SR(B), whereas the mutant alleles were from the ST(A) chromosome. The rest of the genome was from *D. pseudoobscura*, with, on the average, about 1% still remaining hybrid. No chromosomal segment containing only one of the three marker gene loci was capable of expressing the sr trait by itself in the genetic milieu of *D. pseudoobscura* (Tables 1 and 2). Nor could any combination of these segments do so. Failure of a particular chromosomal segment to express the sr trait does not mean that it is dispensable for *sr* expression. However, it is clear that no gene or segment is sufficient by itself. There two possible explanations for this result. (1) Some genes, either autosomal or *Y* linked, from *D. pseudoobscura* may be capable of suppressing the *sr* expression of SR(B). (2) An element essential for *sr* expression may be closely linked to hybrid sterility factors. This second explanation is elaborated next.

Hybrid sterility: From the second row of Table 1, it is reasonable to suggest that the region of SR(B) between the co^+ and the se^+ locus does not interact with genes from *D. pseudoobscura* in the background to produce sterile males. Data of Table 1 were, therefore, arranged without regard to the genotypes at the *co* locus.

Since only about 38% of the males with introgressed sh^+ and about 30% of those with introgressed se^+ were fertile (Table 1), the middle third of the 66.2 map units separating these two loci must contain two or more factors capable of producing male sterility. Thus, the failure of any males to express sr phenotype may be explained if the putative *sr* gene is located within a segment of approximately 20 map units in the region between *se* and *sh*. Evidence for the existence of this *sr* gene is presented in experiment II. We do not believe that the male sterility observed in this study was the consequence of any behavioral aberration. Some of the males were observed to complete courtship and copulation, but no viable offspring were produced. Finally, Tables 1 and 2, containing data of backcrosses to different mutant strains, are consistent with each other both in terms of *sr* expression and of sterility factors.

To determine which of the two explanations, modifiers or close linkage to sterility factors, was responsible for elimination of sr phenotype, the reciprocal introgression was carried out.

TABLE 1

Tests of fertility and sr expression of recombinant males obtained in experiment I
(st = co se sh)

Genotypes	Proportion of fertile males		Proportion of sr among fertile males
co se sh	10/10	25/26 (96.2)	0
+ *se sh*	15/16		
co se +	6/16	8/21 (38.1)	0
+ *se* +	2/5		
co + *sh*	4/16	14/47 (29.8)	0
+ + *sh*	10/31		
co + +	0/17	0/44 (0)	
+ + +	0/27		

Numbers in parentheses are percentages.

TABLE 2

Tests of fertility and sr expression of recombinant males obtained in experiment I
(st = se sp tt)

Genotypes	Proportion of fertile males		Proportion of sr among fertile males
se sp tt	8/9	14/16 (81.3)	0
se sp +	6/7		
se + +	2/11	3/14 (21.4)	0
se + *tt*	1/3		
+ *sp tt*	9/34	9/35 (25.7)	0
+ *sp* +	0/1		
+ + +	0/22	0/40 (0)	
+ + *tt*	0/18		

Numbers in parentheses are percentages.

Experiment II

Mutant alleles for the marker genes were introgressed from ST(A), whereas the wild-type alleles were those from SR(B). The rest of the genome came from *D. persimilis*, with an average of one-eighth ($B_{2\text{-}2}$) or one-sixteenth ($B_{3\text{-}2}$) remaining in the hybrid state. Since the bulk of the genetic background and

sr genes were from the same species (*D. persimilis*), no suppressor modification of sr phenotype has to be considered. Also, males carrying any essential *sr* gene closely linked to hybrid sterility factors would be fertile, since such factors would not produce sterility in the genetic milieu of their own species.

The results of this experiment are presented in Tables 3 and 4. To interpret these results, it is important to note that the absence of sr trait suggests that an essential *sr* gene was replaced by an introgressed *st* allele from the ST(A). Thus, the loss of sr phenotype indicates the presence of an *sr* gene on the SR(B).

In both Tables 3 and 4, a majority of males retaining the wild-type allele from SR(B) at every marker locus (designated +++) are sr males. This indicates that the introgression procedure does not interfere with the expression of *sr* genes. Those +++ males that have lost *sr* expression can be readily explained by hidden recombination. The +++ males with the st phenotype in Table 3 must be the result of double crossing over since *co-se-sh* covers nearly the whole SR linkage complex. The proportion of double crossing over in the *se-sh* region among those having no crossing over or having double crossing over in that region is 26 of 143 = 0.182. (From Table 4, 19 males are of the genotype + *sp* + and seven are *se* + *tt*, among 143 males with the genotypes se^{+}-tt^{+} or *se-tt*.) We observed in Table 3 that 12 of 71 (ca. 17%) +++ males lost the *sr* expression. Since, as we will see later, every chromosomal segment containing any mutant gene locus used in this study is indispensible for *sr* expression, the agreement between the rate of double crossing over and loss of *sr* expression is good. Similarly, those st +++ males in Table 4 can be explained by single crossing over between *co* and *se*. (Data of Table 4 were obtained using an ST(A) strain with markers only to the right of *se*.)

The most striking observation in Tables 3 and 4 is that replacement of any segment of SR(B), except the ones very close to the tip of the chromosome (in the *sh-tt* region), always results in the loss of the sr trait. This suggests that there are *sr* genes very closely linked to co^{+}, se^{+}, sp^{+} and sh^{+} which are about 30–40 map units away from their neighboring markers. Based on the number of males found to have lost the *sr* expression, the upper limit of the distance between each *sr* factor and the nearest marker (except sh^{+}) is estimated to be approximately 2 map units. (Since mapping of these *sr* genes involves double crossing over, the actual distances could be larger if interference is severe.)

Eight of 83 ++*sh* males (Table 3) tested retained some *sr* expression and three showed complete meiotic drive. The recovery of *sr* males in this phenotypic class indicated that the immediate vicinity of *sh* is not crucial for *sr* expression. However, because the majority of ++*sh* males (75 of 83) lost the sr trait completely, it is reasonable to infer the existence of one more essential *sr* genes near and to the left of sh^{+}. Finally, since five of the eight males showing some expression of sr trait retained only partial expression, there may be an additional minor *sr* gene near sh^{+}. Replacement of such a minor *sr* gene may result in only partial loss of expression, and may account for the three of 71 sr males observed in the +++ phenotypic class (Table 3).

Hybrid sterility: As in experiment I, it is evident that certain regions of the *X* chromosome, when introgressed, result in male sterility. Introgression of the

TABLE 3

Tests of fertility and sr expression of the recombinant males obtained in experiment II
(st = co se sh)

Genotypes	Phenotypes	Source of recombinant males		
		$B_{2\text{-}2}$	$B_{3\text{-}2}$	Total
+ + +	sr	36	20	
	sr′	1	2	
	st	8	4	
	Fertile	45/59 (76.3)	26/30 (86.7)	71/89
+ + *sh*	sr	0	3	3
	sr′	4	1	5
	st	48	27	75
	Fertile	52/72 (72.2)	31/35 (88.6)	83/107
Total (+ + −)	Fertile	97/131 (74.0)	57/65 (87.7)	(P = 0.014)
	(No sr males among the following genotypes)			
+ *se* +	Fertile	14/22	3/7	31/58 (53.4)
+ *se sh*	Fertile	12/18	2/11	
co + +	Fertile	2/10	12/34	20/60 (33.3)
co + *sh*	Fertile	5/7	1/9	
co se +	Fertile	1/41	0/25	2/126 (1.4)
co se sh	Fertile	1/25	0/35	

Numbers in parentheses are percentages. sr′ = partial sr.

sh allele appears to have no effect on the proportion of fertile males (Table 3). If *sh* is ignored, more than half the males with introgressed *se* [but co^+] and a third of those with introgressed *co* [but se^+] were fertile. Since only 1% of males carrying both introgressed markers were fertile, it appears that at least one factor is present between them that is capable of producing male sterility when introgressed from *D. pseudoobscura* to *D. persimilis*.

This experiment provides evidence of dominant autosomal sterility factors as well. Among those males with +++ or ++*sh* in Table 3, the $B_{3\text{-}2}$ generation had a greater proportion of fertile males than the previous backcross generation, $B_{2\text{-}2}$, even though both carried the *XR* and *Y* chromosomes from the same source. Among the residual genes from *D. pseudoobscura*, if there are n independently assorting dominant sterility factors, the proportion of fertile males would be $(7/8)^n$ among $B_{2\text{-}2}$ and $(15/16)^n$ among the $B_{3\text{-}2}$ generation. For n = 2, the expected proportions are 0.77 for $B_{2\text{-}2}$ and 0.88 for $B_{3\text{-}2}$. We observed

TABLE 4

Tests of fertility and sr expression of recombinant males obtained in experiment II
(st = se sp tt)

Genotypes	Phenotypes	Source of recombinant males	
		$B_{2\text{-}2}$	$B_{3\text{-}2}$
+ + +	sr	6	9
	sr′		3
	st	2	9
	Fertile	8/21 (38.1)	21/32 (65.6)
+ + *tt*	sr		
	sr′	1	
	st	5	
	Fertile	6/11	0/1
	(No sr males among the following genotypes)		
+ *sp* +	Fertile	1/4 (25.0)	13/15 (86.7)
+ *sp tt*	Fertile	10/26 (38.5)	10/12 (83.3)
se + +	Fertile	1/21 (4.8)	5/19 (26.3)
se + *tt*	Fertile	1/7	
se sp +	Fertile	0/3 (0)	2/9 (22.2)
se sp tt	Fertile	2/50 (4.0)	2/14 (14.3)

Numbers in parentheses are percentages. sr′ = partial sr.

0.76 and 0.72 for the former and 0.87 and 0.89 for latter. The agreement is very good. Thus, the results can be explained if there are two independently segregating autosomal loci, or chromosome segments, capable of producing hybrid male sterility when introgressed in this direction.

Some of the recombinant males obtained in experiment II possessed genes introgressed from the *y-sn-v* region of *XL* of *D. pseudoobscura* with the rest of the genome (including *XR*) derived from *D. persimilis*. Those males carrying only the *v* gene were invariably very fertile. On the other hand, none of 12 hybrid males carrying all three mutant genes was fertile. Sterility factors on *XL* must be on the left of *sn*.

DISCUSSION

The sr trait: The classical theory of chromosomal inversions as coadapted gene complexes seems to be strongly supported by the evidence presented in this paper. The new inversion in *D. pseudoobscura*, SR(A), has emerged because it carries *every* essential gene for *sr* expression which gives the inversion a great advantage in transmission. The new inversion in *D. persimilis* is presumably ST(B) [based on a comparison of *X* chromosomes of these two species and *D. miranda*, see also DOBZHANSKY and TAN (1936)] which, just like SR(A), suppresses recombination between the SR and ST gene complexes within a species.

(It has been emphasized that the inversions *per se* are not responsible for *sr* expression.)

Although the coadaptation hypothesis would have predicted genetic differentiation between the SR and ST gene complex, the observations that SR differs from ST at or near every marker gene used and that replacement of any of these chromosomal segments by its homologue on ST results in a complete loss of *sr* expression were quite unexpected. These observations immediately argue against the intutive notion that all of the genes on SR (denoted *sr*) were derived from their alleles on ST (denoted *st*) for the following reason: Carriers of SR are known to be less fit than carriers of ST (*cf.* BECKENBACH 1983), but the deleterious SR (and, hence, *sr* genes) are "meiotically driven" to a stable equilibrium. Since an individual *sr* gene will not enjoy the advantage of meiotic drive in the absence of any of the other *sr* genes, it will never increase in frequency in the first place.

Therefore, we propose that many or most (but certainly not all) of the *sr* genes on SR(B) reported in Tables 1–4 were the ancestral genes, whereas the *st* alleles were derived later. The model is shown in Figure 4 in which all but one of the *st* genes are derived from their *sr* alleles. This is essentially a model for the evolution of modifier genes (*i.e.*, *st* genes) that suppress meiotic drive, and we denote *st* as *m* and *sr* as *M*. Interestingly, the modifier *m*, if equivalent to its allele *M* in fitness, will not increase in frequency because *M* enjoys the advantage of transmission distortion in its favor when associated with *sr* genes.

To find the conditions under which *m* will increase requires a multilocus model of sex-linked genes with meiotic drive. An analysis of a two-locus model (C.-I. WU, unpublished results) suggests that the most critical factor for *m* to increase and to reach a stable polymorphism with *M* is sufficiently tight linkage between the *sr-st* locus and the *M* locus. This is not unlike the condition required for a stable polymorphism in an autosomal two-locus model (KARLIN and FELDMAN 1969; CHARLESWORTH and HARTL 1978). With tight linkage (10% or less recombination), even a very slight advantage of *m* is sufficient for maintaining the *M-m* polymorphism (stage 2 of Figure 4). A new inversion binding *sr* and *M* (or binding *st* and *m*) can be shown, in theory, to increase in frequency (stage 3). The new inversion reduces recombination and allows the emergence of more modifiers in the region of the ST chromosome where crossing over is sufficiently reduced (stage 4). This newly established polymorphism will again facilitate the spread of any new inversion which further reduces recombination (stage 5). The final stage of Figure 4 represents the structure of the two gene complexes of the present day *D. pseudoobscura*. Since any inversion that reduced recombination between the *sr-st* and the *M* loci would have increased in frequency, it could have occurred on the ST gene complex as well. This was indeed what happened in *D. persimilis*. This model, therefore, easily explains the once very puzzling question that the same chromosomal arrangement in the two species [ST(A) = SR(B)] manifests different phenotypes, whereas the same phenotype in each species is associated with different inversions [SR(A) $\neq$ SR(B), ST(A) $\neq$ ST(B)]. The model would also expect very extensive differentiation between the two gene complexes. It is,

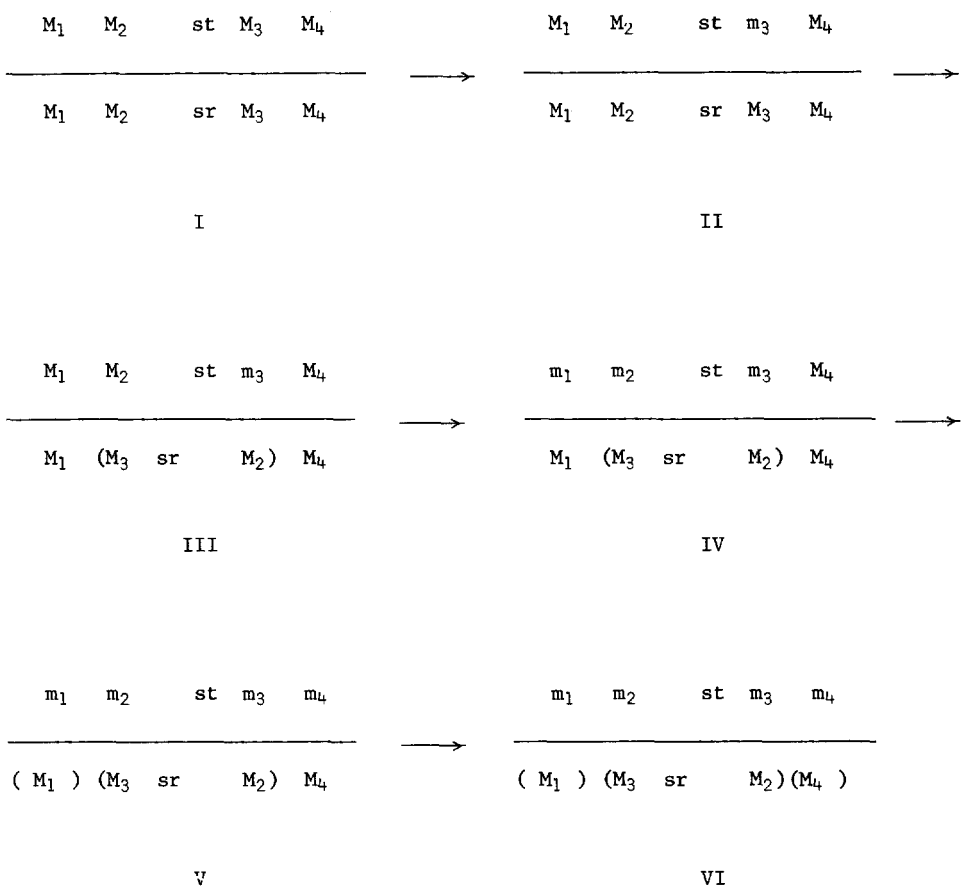

FIGURE 4.—A model for the extensive differentiation between the SR (the lower one) and ST (the upper one) chromosomes of *D. pseudoobscura*. Males with the *sr* allele and all of the other nonmodifier *M* alleles express the sr trait and are otherwise of an st phenotype. Note the substitution of *m* for *M*.

after all, quite reasonable that ST(A) would have been saturated with suppressor modifiers.

The essential feature of this model is that the extensive differentiation between SR and ST results from the accumulation on ST of suppressor modifiers, their nonmodifier (*sr*) alleles being the ancestral state. Two supplementary observations support this hypothesis. First, ST(B) can be inferred to be still carrying the *sr* alleles near the marker *sh*, whereas ST(A) is known to possess the modifiers in that region. This is because, if ST(B) also carries the modifiers near *sh*, one would have expected to find *D. persimilis* males that are of an SR karyotype but otherwise have a standard phenotype (for details, see WU 1982). Second, autosomal suppressor modifiers of *sr* expression can be and have become very common in some species of Drosophila (STALKER 1961) as long as linkage relationship and selection are conducive for their emergence (WU 1983). The same should apply to *X*-linked modifiers.

Finally, an alternative to the model of Figure 4 for explaining the extensive differentiation between SR and ST can be found in the work by WU (1982). It invoked the action of either autosomal or *Y*-linked modifiers and requires more assumptions than the present model does.

Hybrid sterility: DOBZHANSKY'S (1936) comprehensive study on sterility of hybrid males between *D. pseudoobscura* and *D. persimilis* underscored the overwhelming effect of chromosomal interactions. Backcross males in his study with all chromosomes from either of the two species were fertile irrespective of the source of their cytoplasm. Therefore, between these two species, only interactions of chromosomal genes need to be considered. POWELL'S (1983) recent study also supports this view.

From this study, it is immediately clear that sterility interactions identified are invariably "asymmetric." For example, the *se-sh* region of ST(A) is compatible with the autosome and *XL* of *D. persimilis*, whereas the reciprocal introgression results in male sterility. Asymmetry, more specifically, means that, if *M*(A)-*M*(B) and *N*(A)-*N*(B) are corresponding alleles in species A and B at the *M* and *N* loci and if the *M*(A)-*N*(B) interaction results in hybrid inviability or sterility, the reciprocal combination, *M*(B)-*N*(A), is normal in viability and fertility. Sterility interactions involving the *co-se* region are also asymmetric.

This phenomenon is very important to understanding the evolution of postmating reproductive isolation. If sterility interaction is symmetric and the ancestral condition is, for example, *M*(A) and *N*(A), neither *M*(B) nor *N*(B) would have any chance to increase its frequency in the populations. A new *M*(B) allele could only be associated with *N*(A) and give rise to sterile individuals. It is also true for *N*(B). The fact that interspecific sterility *per se* has no advantage was emphasized by Darwin in his debate with Wallace on the role of natural selection in "the infertility of crosses" (see WALLACE 1889; MAYR 1959).

It is, therefore, reasonable to expect asymmetric sterility interactions between closely related species, such as sibling species. If the *M*(A)-*N*(B) combination is observed to cause hybrid sterility, then the reciprocal combination, *M*(B)-*N*(A), may have been the ancestral condition that subsequently became *M*(A)-*N*(A) and *M*(B)-*N*(B) in species A and species B, respectively. Alternatively, species B may have diverged from species A via the intermediate stage *M*(B)-*N*(A). This latter scheme is quite feasible if species B originated from a small isolated population in which genetic drift may have accelerated the fixation of alleles. A haploid stochastic model of reproductive isolation by NEI, MARUYAMA and WU (1983) gives strong support for such a view.

Conceivably, symmetric sterility interactions may result after species had been separated long enough for further independent allelic substitutions. Nevertheless, asymmetry seems to be a very common pattern of sterility interactions between races or closely related species when *both* reciprocal combination of alleles were tested for fertility (*cf.* PRAKASH 1972; OKA 1974, 1978; SCHAEFER 1978).

In the previous analysis, sterility interactions between the introgressed elements on *XR* from one species and the rest of *XR* from the other were not considered. If the within-*XR* interaction of this kind is of any importance, we

would not expect a steady decrease in the proportion of fertile males when the size of the introgressed chromosomal segment gradually increases. Instead, introgression of *part* of *XR* is expected to exhibit the maximal level of sterility interaction. Results in Tables 1 and 3 showed a gradual decrease in the proportion of fertile males when more foreign marker genes were introgressed. Finally, there is also evidence for higher order sterility interactions between the two species that involve at least three gene loci (*Wu* 1982).

The peculiar inversion polymorphisms and the associated sr trait in *D. pseudoobscura* and *D. persimilis* offer an excellent opportunity to study the genetics of reproductive isolation. Previous studies on genetic divergence of Drosophila species rested their analysis on the effects of the whole chromosomes (*e.g.*, DOBZHANSKY 1936; EHRMAN 1961; ZOUROS 1981). With the SR system, it is possible to study the effects of individual segments of *X* chromosomes. Since most studies confirm the overwhelming importance of the *X* chromosome in hybrid viability and fertility, the sex-ratio system promises to be a rewarding target for further detailed investigations.

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