THE EVOLUTIONARY HISTORY OF *DROSOPHILA BUZZATII*. XII. THE GENETIC BASIS OF STERILITY IN HYBRIDS BETWEEN *D. BUZZATII* AND ITS SIBLING *D. SERIDO* FROM ARGENTINA

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ABSTRACT

The genetic basis of hybrid sterility has been investigated in backcross segmental hybrids between two sibling species, *Drosophila buzzatii* and *D. serido*. Asynapsis of homologous bands in hybrid polytene chromosomes has been used to identify the *D. serido* chromosome segments introgressed into the *D. buzzatti* genome. All the investigated chromosomes contain male sterility factors. For autosomes, sterility is produced when an introgressed *D. serido* chromosome segment, or combination of segments, reaches a minimum size. On the other hand, any introgressed *X* chromosome segment from *D. serido*, irrespective of its size, produces either male hybrid sterility or inviability.

HYBRID sterility is an important and widespread mechanism of genetic isolation in Drosophila. In general, speciation is made possible by mechanisms of reproductive isolation, which are based on those genetic changes that differentiate incipient species up to the point where gene exchange is prevented. TEMPLETON (1981) postulates three basic architectures for the genetics of reproductive isolation: many segregating units, each one of small effect (type I); one or a few major segregating units, commonly with many epistatic modifiers (type II); and complementary or duplicate loci (type III). The evidence reported so far points to a relative abundance of type I architectures in Drosophila (DOBZHANSKY 1936; PONTECORVO 1943a,b; SPENCER 1944; SPIETH 1949; WEISBROT 1963, KILIAS and ALAHIOTIS 1982; COYNE 1984), but there may be some other cases of type II architectures as well, although the evidence is not conclusive (PRAKASH 1972; VAL 1977). However, the nature, actual number and mode of interaction of those "segregating units" is a matter of high speculation. This is so because genetic dissection of sterility factors requires appropriate markers all over the genomes of closely related species which, additionally, must be able to produce hybrids. COYNE (1984) suggested that the scarcity of these studies was due to the difficulty of finding appropriate pairs of recently diverged species. But, even in those cases where backcross hybrids between two sufficiently related species are subjected to study, the

conclusions are vitiated by a lack of sufficient markers; that usually makes it impossible to distinguish between one and several genetic factors per chromosome arm. Moreover, those studies cannot provide precise information either on the architecture of factors in the same or different chromosomes or on their nature.

D. buzzatii and *D. serido* are two closely related species of the *D. repleta* group for which phylogenetic relationships have been recently established (RUIZ, FONTDEVILA and WASSERMAN 1982). Hybrids are not found in nature, but laboratory crosses between *D. serido* females and *D. buzzatii* males produce sterile hybrid males and fertile hybrid females. Reciprocal crosses never produce offspring. Using a planned scheme of successive backcrosses between hybrid females and *D. buzzatii* males, it is possible to select recombinant strains of *D. buzzatti*, such that each one is introgressed with a different chromosomal segment of *D. serido* in heterozygous condition (segmental hybrids). Identification and selection of segmental hybrids is feasible due to the availability of appropriate marking techniques, mainly interspecific chromosome asynapsis (NAVEIRA, PLA and FONTDEVILA 1986). In Drosophila the degree of asynapsis between homologues can be increased by various environmental and genetic methods (PERJE 1955; BEERMAN 1962; BERENDES 1963), but it is always very much enhanced in interspecific hybrids, even when their banding sequences may be identical (KERKIS 1936; DOBZHANSKY 1957; KAMBYSELLIS 1970; EVGENEV 1971). Recently, RIEDE and RENZ (1983) have shown that pairing of homologous chromosome bundles in hybrids is a property that resides in each specific polytene band, and that the frequency of asynapsis between homologous bands is strongly negatively correlated with the nucleotide sequence homology between them. In hybrids between *D. buzzatii* and *D. serido*, asynapsis is strong and present in all the chromosomes. With very few exceptions, any hybrid region will exhibit asynapsis in more than 60% of the cells, whereas asynapsis never occurs in more than 5% of the regions in the controls of *D. buzzatii* and *D. serido* (NAVEIRA, PLA and FONTDEVILA 1986). This property allows an easy identification of the introgressed chromosome segments, and, consequently, the fine-scale dissection of the genetic architecture of interspecific sterility. Our aim has been to find out what is the minimum number of genetic differences necessary to produce a sterile hybrid male. Briefly, our results show that every piece of the *D. serido X* chromosome tested yields either sterile or inviable males when introgressed into a *D. buzzatii* genetic background. This is not true for autosomes, where small segments from *D. serido* yield fertile males when introgressed, and sterility is only produced when these introgressed segments reach a minimum critical size.

MATERIALS AND METHODS

Biological material: *D. buzzatii* and *D. serido* are two sibling cactophilic species which coexist in many of the arid and semiarid zones of Andean Bolivia and of Northwest Argentina. In South America, *D. serido* has a range of distribution larger than that of *D. buzzatii*, extending into Paraguay and up to the northeastern region of Brazil (Caatinga). *D. buzzatii* is also found in the Old World and Australia.

The two strains used in the present work were derived from collections made in

December 1979 by the senior author (A.F.) and A. RUIZ. The strain of *D. buzzatii* was derived from a single wild inseminated female (isoline) collected at the Sierra de San Luis, Argentina. That of *D. serido* was derived from an inseminated female taken from a population cage founded by the combination of F_1 progenies of 59 wild-inseminated females (isolines) also collected at Sierra de San Luis. Both strains were kept by mass-culturing thereafter. They share the same arrangement in chromosomes *X*, *3* and *4*. Chromosome *2* differs by a series of inversions ($j^9l^9m^9$) in *D. serido* that cover about two-thirds of its length, whereas *D. buzzatii* exhibits the *st* and *j* arrangements. Chromosome *5* differs by a single inversion (*g*) in *D. buzzatii* including about one-third of its length (RUIZ, FONTDEVILA and WASSERMAN 1982).

All stocks and experimental cultures were kept at 25°.

Genetic markers: Detection of segmental hybrids has been possible using two kinds of independent chromosomal markers: first, diagnostic electromorphs for each species, marking specific chromosome regions; and second, the presence of asynapsis of homologous polytene chromosomes in the backcross hybrids (Figures 1 and 2). This kind of asynapsis is frequently characterized by the presence of chromatin fibers connecting both homologues (incomplete pairing), a feature never observed in intraspecific spontaneous asynapsis (Figure 1, arrows). Asynapsis appears in every hybrid cell, although the frequency of pairing varies from band to band (EVGENEV and POLIANSKA 1976). Each band of the polytene chromosomes in the *D. serido* × *D. buzzatii* hybrids has a certain probability (usually very low) of being paired (NAVEIRA, PLA and FONTDEVILA 1986). Six to eight karyotypes were examined per larva. The absence of asynapsis in all the cells was taken to mean that the studied segment was homozygous. Using this criterion, the probability of a hybrid segment not being detected was always less than 0.01.

Cytological methods: *D. buzzatii* and *D. serido* have the standard *D. repleta* group polytene karyotype, consisting of five rod-like chromosomes and a tiny dot-like chromosome. Number *1* corresponds to the *X*, numbers *2–5* to the long acrocentric chromosomes, and number *6* to the dot. Cytological maps of both species were constructed by RUIZ, FONTDEVILA and WASSERMAN (1982) from the *D. repleta* maps of WHARTON (1942). Each chromosome is subdivided into cytological intervals, identified by capital letters and numbers. Each interval contains a series of bands, which are identified by lowercase letters, in alphabetical order from telomere to centromere.

Polytene chromosomes from the salivary glands of third instar larvae (Figure 1) and from the Malpighian tubes of adult males (Figure 2) were examined according to the following procedures:

Salivary glands: Third instar larvae were dissected in acetic alcohol (3:1), and their salivary glands were extracted and placed on a slide in a small drop of lactic-acetic orcein (1:1) for half an hour; the preparation was then covered with a coverslip and squashed. Slides were kept at 4°.

Malphighian tubes: Well-fed, adult males were etherized and dissected in 50% acetic acid, and their Malpighian tubes were extracted and placed in acetic alcohol (3:1) for 3 min, then transferred to acetic orcein for 2 min and, finally, to lactic-acetic orcein (1:1) for 1 min. Afterwards, the preparation was covered with a coverslip, squashed and kept at 4°.

Malpighian chromosomes of adults are harder to identify than salivary chromosomes of larvae (Figures 1 and 2), and it is difficult to determine the precise extremes of the introgressed segments by observing asynapsis in Malpighian chromosomes. However, it is always possible to identify the asynapsed chromosomes and, more important, to locate the introgressed segment in them with a precision of one or two cytological intervals (10–20 bands) at both ends. This accuracy is enough to corroborate the indirect evidence that sterile males are always hybrids for an introgressed segment longer than a critical minimum size.

Biochemical methods: Starch gel electrophoresis of larva and adult homogenates was performed at several stages of the experimental procedure. Three different allozyme

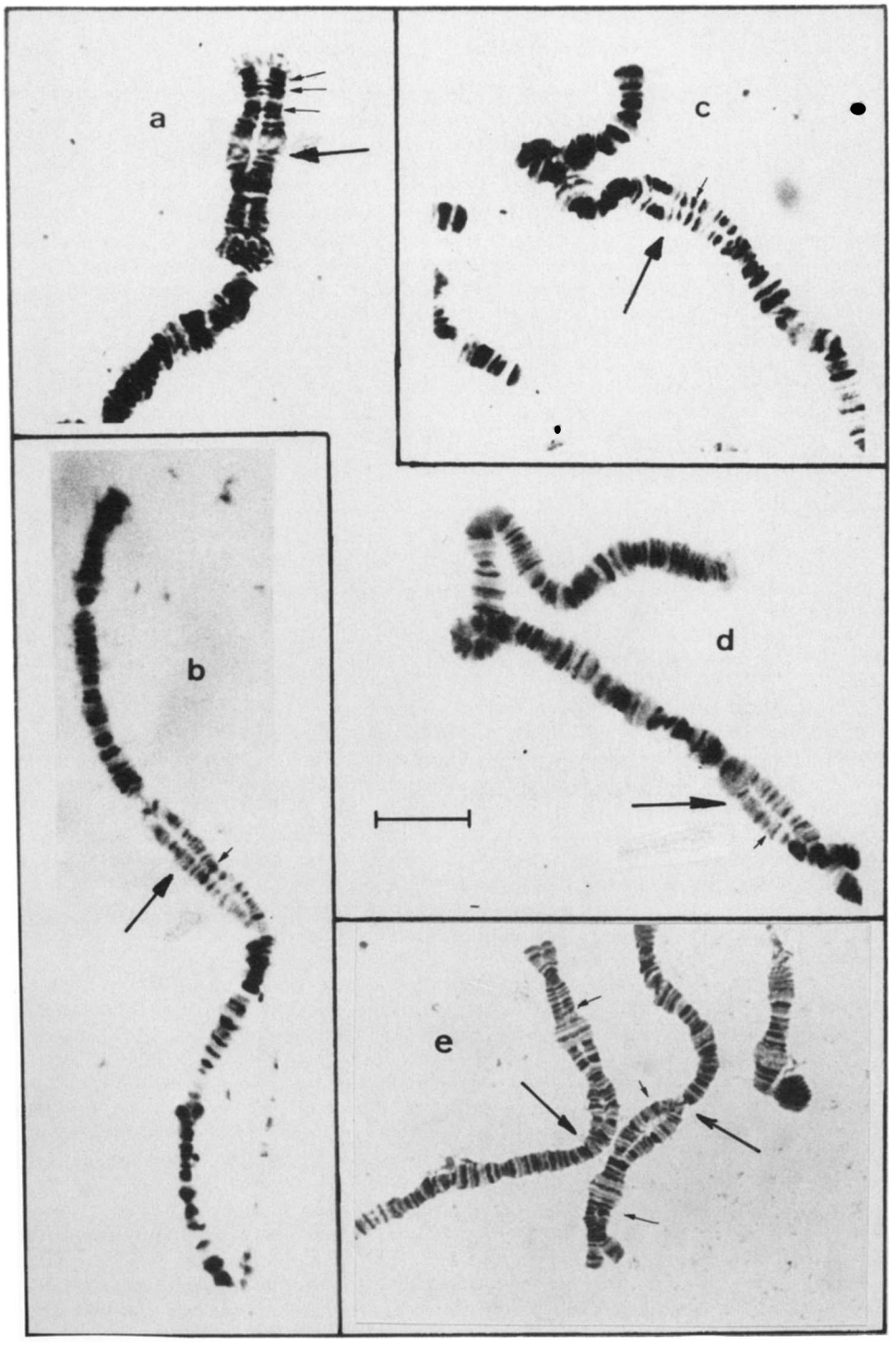

FIGURE 1.—Polytene chromosomes of salivary glands from segmental hybrid larvae. Long arrows signal the asynapsed segments, and short arrows point at some of the bands connected by chromatin fibers (incomplete pairing). a, Segmental hybrid female for the interval *X*A—C4b from *D. serido*. b, Segmental hybrid for *4*C3b—E3a. c, Segmental hybrid for *3*C4c—E2f. d, Segmental hybrid for *2*F4b—H. e, Segmental hybrid for *5*A—C1d. Bar represents 10 μm.

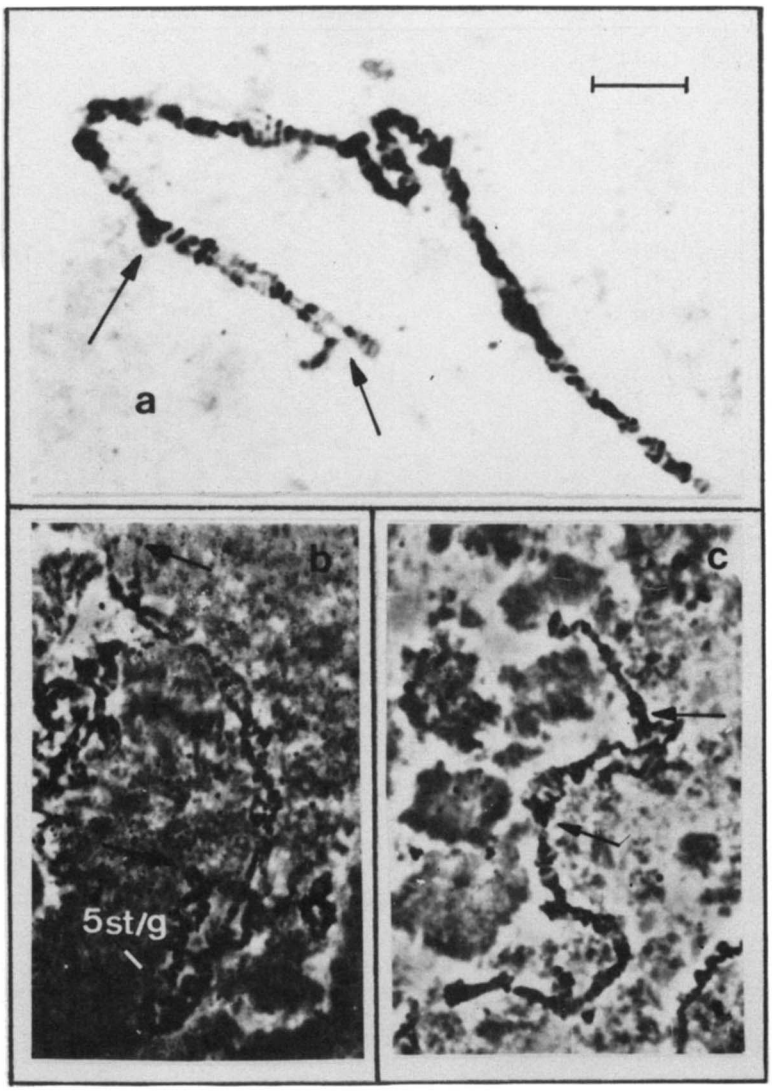

FIGURE 2.—Polytene chromosomes of Malpighian tubes from hybrid sterile adult males. Arrows point at both ends of asynapsed segments. a, Segmental hybrid for the interval *3*A—A5e. b, Hybrid for the whole chromosome *5*, showing the inversion loop *5*st/g. c, Segmental hybrid for *3*A4f—E3d. Bar represents 10 μm.

loci were analyzed: Octanol dehydrogenase (*Odh*), Alcohol dehydrogenase (*Adh*) and Phosphoglucomutase (*Pgm*), with slight modifications of the method of POULIK (1957). *Odh* is on chromosome *2*; *Adh* on chromosome *3*, in the cytological interval *3*F4c—F4f; and *Pgm*, on chromosome *4*, in the interval *4*A1a—A1g (NAVEIRA, PLA and FONTDEVILA 1986).

Introgression method: The arrow diagram of Figure 3 shows the detailed procedure to introgress one marked chromosome segment from *D. serido* (M^s) into the nuclear background of *D. buzzatii*. The homologous segment in *D. buzzatii* is designated by M^b. This entire series of crosses is called an introgression line.

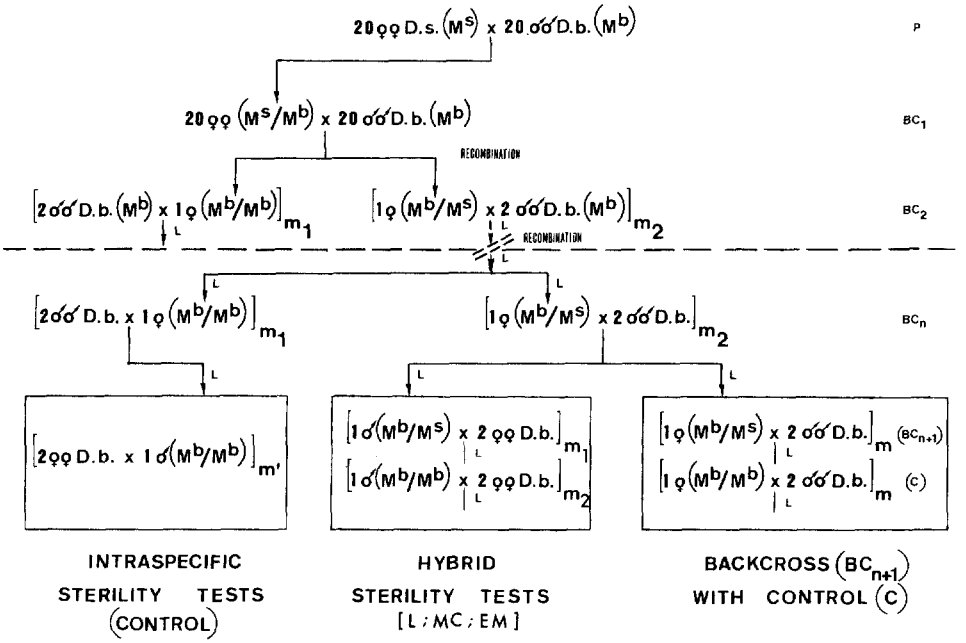

FIGURE 3.—Arrow diagram showing the experimental method to obtain segmental hybrids and to test them for male sterility. Tests are performed in the unspecified backcross generation (BC_n) in which each particular *D. serido* segment (M^s) becomes introgressed. M^b stands for the homologous *D. buzzatii* interval. Selection of an introgression line in hybrid females is performed analyzing polytene chromosomes in larvae (L) from each backcross (BC_n) trio offspring (1♀ × 2♂♂). The number of analyzed females ($m_1 + m_2$) in trios ranges from 20 to 30 each generation. Hybrid sterility tests are performed analyzing the offsprings of 30 males in trios ($m_1 + m_2 = 30$) for polytene chromosomes in fertile male larva offspring and for Malpighian chromosomes (MC) plus electrophoretic markers (EM) in sterile males. Offspring analysis of sister hybrid females (BC_{n+1}) is used as a test for introgressed segments not found in fertile males.

Several groups (usually five) of 20 *D. serido* virgin females and 20 *D. buzzatii* males, each 2–4 days old, were placed in food vials and transferred to fresh food every week. Hybrid females (F_1 hybrids) were backcrossed to *D. buzzatii* males in the same way (20 × 20). From each first backcross, 20–30 females (BC_1 offspring) were randomly chosen and individually backcrossed to two *D. buzzatii* males (1♀ × 2♂♂). The offspring of each of these backcrossed females (BC_2) is called an introgression subline. The polytene chromosomes of eight larvae from each subline were analyzed in order to find out the chromosomal constitution of the backcrossed parent female. The probability is lower than 0.01 that a maternal *D. serido* chromosome element would not be detected. Thus, backcrossed females were divided into two groups: those that were homozygous *D. buzzatii* for all the chromosomes (M^b/M^b) and those that were heterozygous (hybrid) for chromosomes or chromosome segments (M^s/M^b). Sublines from homozygous females were always discarded. Sublines from hybrid females whose segmental karyotypes seemed interesting were selected. Twenty to 30 females were then chosen in each selected subline (BC_2 hybrids) and were individually backcrossed once more to two *D. buzzatii* males (1♀ × 2♂♂). This procedure was repeated several times, in successive generations. In each generation, crossing over in hybrid females produces recombinant chromosomes, which can be identified by the change in the typical pattern of asynapsis of the hybrid. This method allowed us to reduce progressively the length of the introgressed segments. The end result was the production of different introgression sublines, each one bearing one or, at most, two different segments from *D. serido* in heterozygous

state in an otherwise unaltered *D. buzzatii* genome (segmental hybrids). Thus, the length of the introgressed segments in these sublines ranges from one or a few polytene bands to a whole chromosome, and the sterility effects of a wide array of individual segments can be checked.

Observations performed in the introgression sublines: The sex-ratio (number of sons *vs.* daughters) and the frequency of sterile males were estimated in the offspring of each selected hybrid female, using the first 50 progeny to emerge and the first 30 males, respectively. The karyotypes of various fertile and sterile individuals were then determined.

Sterility tests: Thirty offspring males, 6–7 days old, from each hybrid female were placed individually with two *D. buzzatii* females in small food vials (Figure 3). The cultures were examined 8 days later. Five to ten of the males from cultures without larvae were dissected, and their seminal vesicles were checked for the presence of sperm in order to verify their sterility. In addition, those males from sublines introgressed with autosomal segments were used either to analyze the polytene chromosomes of their Malpighian tubes or to detect the biochemical markers by electrophoresis. Males from sublines introgressed with *X* segments could not be detected cytologically because they were hemizygous for the *X* chromosome, for which we have no allozyme marker available.

Females of these same five to ten cultures with no larvae on the eighth day, together with all the other trios without progeny on that same date, were transferred to fresh food vials and kept until their death. Each one of the 30 males was finally identified as fertile or sterile, according to the presence or absence of progeny in the vials. Whenever a male produced progeny, the polytene chromosomes of eight larvae were analyzed, and the male karyotype was inferred from these data.

Controls of these sterility tests were performed by analyzing the progeny of nonhybrid sisters of the selected hybrid females (Figure 3). At least two replicates were run of each subline, thus making a total of 60 males examined for the effect of a given chromosome segment.

Karyotypic analysis: Karyotypes of fertile individuals were inferred by direct chromosomal analysis of third instar larvae in their progeny. On the other hand, karyotypes of sterile individuals could be inferred indirectly, by comparing the karyotype of their mother with those of their fertile sibs. The karyotype of the mother shows the maximum size of segment that would have been transferred to her sons in the absence of crossing over. Since the karyotypes of the fertile sons show a set of recombinant segments that do not prevent fertility, comparing the two would reveal whether there exists any maternal segment that is never present in the fertile sons. This segment would be a good candidate for causing hybrid male sterility. This indirect method was checked by analyzing either the electrophoretic pattern of the sterile males or the polytene chromosomes of their Malpighian tubes.

RESULTS

A summary of the results is presented here, and a more detailed account of the data is available upon request. Table 1 gives a set of overlapping *D. serido* chromosomal segments found in fertile hybrid males from offspring sublines. Segments included in a larger segment already found in another fertile male are not shown in this table. Recombination between homologous segments is very frequent in F_1's and first backcrosses, but it is rather infrequent in the following backcrosses when the introgressed segments become very small. These small introgressed segments are very rarely broken up by crossing over and segregate as single units.

The most extensive information is provided by chromosomes *3* and *4*. These chromosomes are homosequential in both species, and recombination may oc-

TABLE 1

Chromosomal structure of fertile segmental hybrid males

Cytological intervals of single introgressed segments	Percentage size relative to chromosome *2*	Cytological intervals of two simultaneous introgressed segments	Percentage size relative to chromosome *2*
*2*F4b—H	18.2	Same chromosome	
*2*B3e—B1d (21^9)	17.4	*3*A—A4f; F1g—H	37.2
		*3*A—A5d; D1c—E3d	31.0
*3*A—C3a	34.5	*3*A—A4f; D5c—F1a	28.3
*3*D4c—G1e	30.6	*3*A—A4f; C4c—E1f	26.3
*3*F1f—H	27.5		
*3*D5a—F1h	20.5	*4*E1b—F1a; G2e—H	20.8
*3*C1b—D4e	19.0	*4*E1b—F1e; G1a—G3d	20.3
*3*C39—D3c	14.7		
		Different chromosomes	
*4*F1b—H	23.2	*3*A—C1a; *4*F4e—C4a	37.2
*4*D3d—F1e	22.9	*4*C3b—E3a; *5*A—A5d	31.4
*4*C1a—E1g	21.3	*3*A5—C1c; *4*G3a—H	24.8
*4*D2c—F1b	21.3	*4*C3b—E3a; *6* (whole)	24.0
*4*A4c—D1d	20.5	*4*C3b—E3a; *6* (whole)	24.0
*4*B3a—D3c	18.6	*4*B1a—D1a; *6* (whole)	20.9
*4*B1a—D1a	17.0	*3*C4c—E2b; *6* (whole)	16.7
*4*A—B3d	16.7	*3*C4c—D4b; *6* (whole)	15.9
*4*E3a—F4d	16.7	*5*A—A5d; *6* (whole)	15.1
*4*A5a—C3b	16.3	*4*C2a—D3a; *6* (whole)	13.9
*4*C2a—D3a	10.1	*4*G3b—H; *5*A—A3c	11.6
*5*A—C2a	27.9		
*5*A2e—C3c	27.5		
*5*A4d—C4f	26.3		
*5*G1a—H	13.2		
6 (whole)	3.9		

cur anywhere along the chromosome. A set of overlapping segments covering the whole of chromosomes *3* and *4* has been found in fertile hybrid males. The chromosomal intervals covered by these segments are given in Table 1 and are depicted graphically in Figure 4. Therefore, major sterility factors cannot be mapped in these chromosomes using the overlapping method described in NAVEIRA, PLA and FONTDEVILA (1986), and we may conclude that, in chromosomes *3* and *4*, no single gene exists that is able to produce interspecific sterility by itself. The same seems to be true for chromosome *6*, the dot, since male hybrids for this chromosome are always fertile.

Chromosomes *2* and *5* contain species-specific inversions that prevent recombination in large central chromosomal regions (see Figure 4). Consequently, their genetic architectures are not amenable to fine-scale analysis of sterility. However, recombinant segments outside the inverted regions can be tested. Table 1 shows that, in freely recombining regions, it is also impossible to map any major sterility factor, since a series of introgressed segments covering the totality of those regions are found in fertile males.

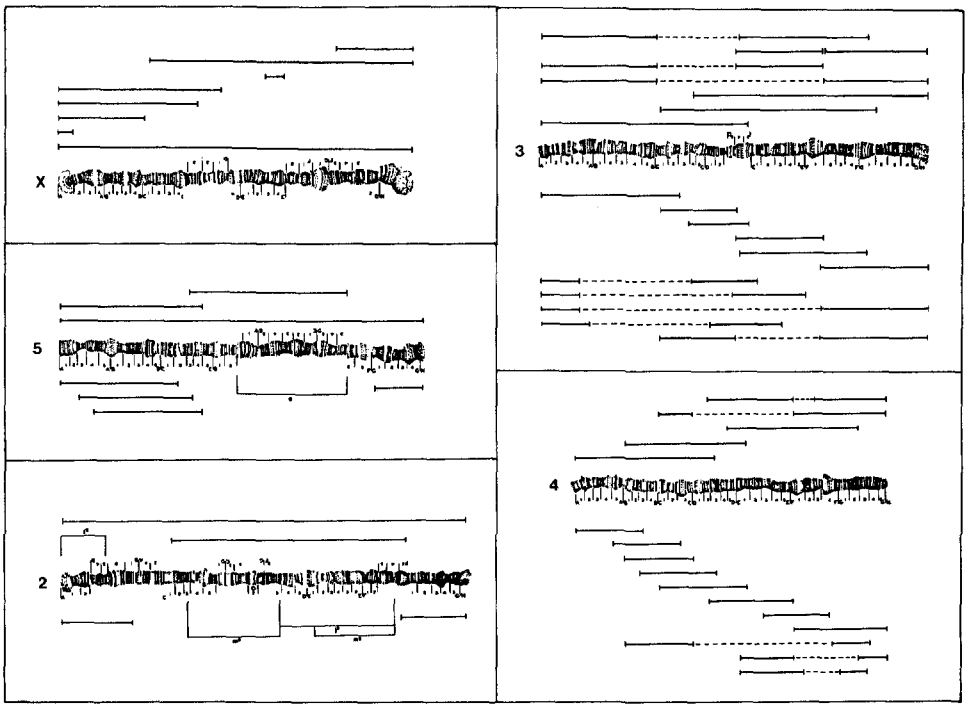

FIGURE 4.—Graphical distribution of some *D. serido* chromosomal segments introgressed into *D. buzzatii* genomic background. Bars (|——|) represent length and position of introgressed segment relative to each *D. buzzatii* chromosome. Segments producing male hybrid sterility are drawn above each *D. buzzatii* chromosome, while those not producing sterility are drawn below. Each row with a single segment corresponds to an individually introgressed segment in a subline. Two segments united by dots in a row means that they are introgressed simultaneously in the same subline. *D. buzzatii* chromosomes are drawn following RUIZ, FONTDEVILA and WASSERMAN (1982), and inverted parts due to cytological evolution are shown upside down. In chromosomes 2 and 5, the extent of inverted segments due to species-specific inversions for *D. serido* ($2j^9$, $2m^9$, $2l^9$) and for *D. buzzatii* (5*g*) is shown.

In sharp contrast, no recombinant *D. serido* segments of chromosome *X* have ever been found in fertile hybrid males, suggesting that sterility factors of major effect may be traced to many different parts of the sex chromosome. The actual distribution of introgressed *X* segments in sublines (Figure 4) may suggest erroneously a minimum of four or five gene loci for sterility (or inviability), using the logic of deletion maps. However, our method has much more power than a deletion map, and it is the absence of *X* chromosome segments among the offspring fertile males that suggests a much greater number of sterility factors in the *X* chromosome. This is so because an introgressed segment undergoes recombination in the hybrid female and produces an array of new, smaller segments that must show up in her offspring. Their absence in fertile hybrid sons must mean that even a few polytene bands of the *X* chromosome are able to severely disturb spermatogenesis when introgressed into a foreign genetic background. Table 2 gives a list of those introgressed segments never found in fertile males. Among them, autosomal segments have

TABLE 2

Frequency of sterile males (f_s) and sex-ratio (SR) in offspring sublines from hybrid females introgressed with chromosomal segments that were never recorded in their fertile sons

Hybrid females		Offspring	
Cytological interval	Percentage size relative to chromosome *2*	f_s	SR
X (whole)	86.8	0.73***	0.58*
*X*C1e—H	64.3	0.40	0.78
*X*A—D1d	39.0	0.05***	0.27***
*X*A—D3c	34.1	0.10***	0.36***
*X*A—C1a	20.9	0.00***	0.45***
*X*F5c—H	18.2	0.23***	0.74
*X*E3b—E4h	5.0	0.45	0.41***
*X*A—A2a	3.9	0.56	0.93
2 (whole)	100.0	0.52	1.08
*2*C1g—F3d	56.6	0.49	1.12
*3*A—G1e	79.1	0.50	0.87
*3*B2f—H	76.0	0.58	0.85
*3*C4c—H	56.6	0.50	1.15
*3*C1b—G2a	52.7	0.44	1.17
*3*A—D3c	46.1	0.52	0.82
*3*D5a—G2a	34.1	0.60	0.85
*4*A—D3c	34.1	0.40	1.00
*4*D4e—G3a	32.1	0.57	0.92
*4*B1a—E1g	29.8	0.52	1.06
5 (whole)	88.7	0.52	
*5*C3b—F2h	39.5	0.44	1.27
*5*A—C4f	34.1	0.57	0.89

χ^2 probability values (P) are as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The remaining figures for f_s and SR are statistically nonsignificantly different from the expected values of 0.50 and 1.00, respectively.

been found in approximately one-half of the females of each offspring subline, as expected if the parental female is a segmental hybrid. A similar segregation in males should give one-half of the males with the segment. None of these males has been found among the fertiles, and consequently, they must be in the class of steriles. This indirect evidence of a relationship between these large autosomal segments and male hybrid sterility is also reinforced by several independent observations. First, frequencies of sterile males are not significantly different from 0.50 (Table 2), as expected in the backcross offspring of a hybrid female for a dominant male-sterility factor. Second, the analysis of Malpighian polytene chromosomes (5–10 per subline) revealed that, within the limitations of this technique (see MATERIALS AND METHODS), the expected segment has been observed in the vast majority (>97%) of sterile males. Third, using species-specific electrophoretic markers we have been able to demonstrate that practically all of the sterile males which we studied are heterozy-

TABLE 3

Critical sizes (%) relative to chromosome *2*, showing the differential effect of the chromosomes in the determination of hybrid male sterility

	Size		
	Chromosome *3*	Chromosome *4*	Chromosome *5*
Maximum for fertility	34.5	23.2	27.9
Minimum for sterility	34.1	29.8	34.1

gotes. As an example, in each of the sublines, *2*C1g—F3d, *3*D5a—G2a; *3*C1b—G2a; *3*C4c—H and *4*A—D3c, more than 95% of the sterile males have always been found heterozygous for the electrophoretic markers (see MATERIALS AND METHODS). These evidences, when combined, are sufficient to substantiate the hypothesis that segments in Table 2 produce sterility when introgressed.

One feature that distinguishes autosomal segments producing sterile males from those found in fertiles is size. Tables 1 and 2 show also the sizes of the introgressed segments relative to the total length of chromosome *2* for comparison among autosomes. Generally, *D. serido* segments producing male sterility are longer than those carried by fertile males. In Table 3 we have shown the longest segment that maintains fertility and the shortest segment that produces sterility in chromosomes *3*, *4* and *5*. The size effect is chromosome-dependent and may suggest a nonuniform distribution of sterility factors among chromosomes. Chromosome *4* is the most effective in producing sterility because it shows the lowest minimum size (29.8) for sterility. This minimum is 34.1 for chromosomes *3* and *5*. In chromosome *3* there is an overlap between the maximum size for fertility (34.5) and the minimum size for sterility (34.1). This may simply reflect that sterility factors are not evenly distributed in this chromosome. Thus, the longest tested segment (*3*A–C3a) producing fertility is situated in a different chromosomal region from the shortest tested segment (*3*D5a—G2a) producing sterility (Figure 4).

In spite of these size overlaps, the existence of a size threshold that elicits male sterility seems to stand out from our results. This threshold can range between 25 and 35% relative to the size of chromosome *2*. Our criterion in classifying a segment as a producer of sterility must be brought up at this point. We consider that a segment must *never* be present in fertile males in order to be considered as a sterility producer. Since we test two replicates of 30 male offspring per subline, the presence of at least one hybrid fertile male in this sample of 60 would place the segment among the fertiles. Using this strict criterion, it is clear that some segments found in fertile males may produce some sterile males as well. As an example, sublines for the long segments *3*A—C3a and *4*F1b—H produce only 68 and 70% of fertile hybrid males, the remaining hybrid males being sterile. These segments should be classified, in the strictest sense, as producers of "semisterility." Nonetheless, these segments are very close to the size threshold, and the transition from semisterility (68–

TABLE 4

Crosses between sublines introgressed with segments that do not produce sterility in hybrid males

Cross			Frequency of sterile sons	Frequency of fertile sons				Added size of the two segments	No. of (1/2) karyotypes over examined sterile males
(1)	×	(2)		(1)	(2)	(1/2)	(0)		
*3*D4c—G1e	×	*2*B3e—B1d	0.23	0.20	0.30	0.00	0.27	0.480	5/5
*3*A—C1a	×	*3*F2a—H	0.30	0.20	0.30	0.00	0.27	0.539	5/5
*3*D4c—G1e	×	*5*A—C1d	0.23	0.30	0.20	0.00	0.27	0.566	5/5
*3*D4c—G1e	×	*3*A—C1a	0.23	0.23	0.27	0.00	0.27	0.593	5/5
*3*D4c—G1e	×	*4*F1b—H	0.37	0.30	0.16	0.00	0.17	0.538	4/5
*3*A—C1a	×	*3*D5a—F1h	0.27	0.33	0.20	0.00	0.20	0.492	5/5
*3*D5a—F1h	×	*2*B3e—B1d	0.23	0.37	0.17	0.00	0.23	0.379	5/5
*4*B1a—D1a	×	*4*F1b—H	0.23	0.33	0.20	0.00	0.24	0.402	5/5
*3*D5a—F1h	×	*4*F1b—H	0.20	0.27	0.20	0.00	0.33	0.437	5/5
*3*D5a—F1h	×	*3*F2a—H	0.23	0.13	0.40	0.00	0.24	0.457	5/5
*4*F1b—H	×	*2*B3e—B1d	0.30	0.30	0.20	0.00	0.20	0.406	5/5
*4*D2c—F1b	×	*4*F3a—H	0.23	0.30	0.20	0.00	0.27	0.395	5/5
*5*A—C1d	×	*2*B3e—B1d	0.07	0.20	0.30	0.20	0.23	0.434	2/2
*5*A—A5d	×	*2*B3e—B1d	0.00	0.20	0.33	0.20	0.27	0.286	
*5*A—A5d	×	*4*F1b—H	0.03	0.27	0.23	0.23	0.24	0.344	1/1
*5*A—A5d	×	*3*F2a—H	0.00	0.20	0.30	0.30	0.20	0.364	
*5*A—A5d	×	*3*D4c—G1e	0.00	0.27	0.23	0.20	0.30	0.418	
*4*F4e—G4a	×	*4*B1a—D1a	0.03	0.23	0.27	0.13	0.34	0.255	1/1
*3*C1b—D4e	×	*4*B1a—D1a	0.00	0.17	0.30	0.17	0.36	0.360	
*3*C1b—D4e	×	*3*F2a—H	0.07	0.23	0.23	0.20	0.34	0.442	2/2
*3*F2a—H	×	*2*B3e—B1d	0.07	0.27	0.23	0.17	0.33	0.426	2/2
*3*A—C1a	×	*4*F4e—G4a	0.03	0.30	0.17	0.23	0.27	0.372	0/1
*3*A5c—C1c	×	*4*C3a—H	0.00	0.17	0.37	0.20	0.26	0.248	
*4*C3b—E3a	×	*5*A—A5d	0.00	0.30	0.20	0.27	0.23	0.314	

(1) Segment contributed by the male; (2) segment contributed by the female; (1/2) both segments simultaneously; (0) *D. buzzatti*. Sizes are relative to chromosome *2*

70% of fertile males) to sterility (0%) is very sharp with a minimum increase in segment length.

One way to test the dependency of sterility upon the introgressed size is by crossing two fertile sublines introgressed with segments of different size and then analyzing their offspring. Table 4 shows the crosses we performed. They include a large array of segments that do not produce sterility independently. They are derived from fertile hybrid males and are kept in the form of 1♂ hybrid × 2♀♀ *D. buzzatii*. Crosses between sublines involved several individual pairs of flies, and the sterility analysis of each offspring cross was performed with a sample of 30 males. Frequencies of sterile and fertile sons per karyotypic class are also given in Table 4. The added size of the two combined segments is, as a general rule, larger in crosses producing hybrid sterile males than in those giving hybrid fertile males. Some exceptions occur due to the differential effect on sterility among chromosomes. In particular, crosses involving segments of chromosome *4* tend to produce hybrid sterility with smaller intro-

gressed sizes than do crosses with segments of other autosomes. Some combined segments (*i.e.*, *5*A—C1d × *2*B3e—B1d; *3*C1b—D4e × *3*F2a—H; *3*F2a—H × *2*B3e—B1d) may be classified as semisteriles they produce some sterile hybrid males (0.07). However, the transition from fertility to sterility is not gradual, and it shows in general a large discontinuity in the frequency of fertile sons, ranging from the expected numbers (0.25) to zero.

These results not only confirm the size-dependent threshold effect of the *D. serido* introgressed segments but also prove that two segments can cooperate in an additive way, no matter whether they are introgressed in the same (*cis*-action) or in different autosomes (*trans*-action), to produce sterility once their cumulative size exceeds a critical value.

Sex chromosome effects deserve an independent and close examination. Table 2 and Figure 4 show that any introgressed segment produced sterility, irrespective of its size and localization in the chromosome. Besides, introgression of *X* chromosome segments is frequently associated with small sex ratios. This suggests that *X* segments may also be involved in determining the viability of males. Sublines *X*A—D1d, *X*A—D3c and *X*A—C1a are specially illuminating in this respect (Table 2). Their sex ratios are significantly smaller than one, which probably indicates a high mortality in males. Interestingly, most or all of the viable males are fertile (fs = 0.05, 0.10 and 0.00, respectively), but none of them carries the introgressed *X* segment or any of its recombinant products. This segment is still segregating, as witnessed by chromosomal analysis of fertile hybrid females. The most plausible explanation for these data is that hybrid males for any of these *X* chromosome segments are inviable. In other cases, *X* chromosome segments are responsible for male sterility and male viability, as well, but the relationship between size and these male effects is not straightforward.

DISCUSSION

Asynapsis is not only a characteristic of mitotic chromosomes but also of meiotic pairing (EVGENEV and POLIANSKA 1976), which apparently reduces the probability of two nonconspecific homologous chromosome segments being involved in crossover events. This property is beneficial for our purposes. Many different chromosome segments from *D. serido* in the *D. buzzatii* background were initially obtained, due to the high recombination frequency in F_1 and first backcross hybrid females. Later, they were isolated and their effect on fertility was checked, taking advantage of the reduction of recombination frequency in segmental hybrids.

The major novelty of the present data is that we can go further toward unveiling the genetic basis of hybrid sterility than has been possible before. Whereas all tested *X*-chromosome segments from *D. serido* in the *D. buzzatii* background produce hybrid male sterility, in autosomes the sterility is size-dependent, a chromosome segment with a minimum length being necessary. One way or another, the number of different combinations of chromosome sections that might bring about hybrid sterility is enormous. None of the analyses of the genetic determinants of hybrid sterility performed so far by

other authors has provided comparable information, or, at least, not so explicitly. Nearly all of them have been performed at the chromosomal level, like ours, but their results are in terms of a few marked chromosome segments. Their power of resolution depends on the number of genetic markers available, always very small. In our case, we are only limited by the frequency of recombination that allows us to obtain a large array of chromosomal segments marked by their somatic asynapsis. However, we are still unable to explain some important differences with other authors' results.

Our results might be an example of the type I architecture described by TEMPLETON (1981). However, their relationship to earlier evidence provided by other authors on sterility in Drosophila interspecific hybrids (for a review, see EHRMAN 1962), is not straightforward. Several different kinds of interspecific F_1 sterility have been found so far. The F_1 may consist of (1) both sterile males and sterile females, as in the case of the hybrids between *D. melanogaster* and *D. simulans*, where sterility is produced by a small number of "polygenic sets" (MULLER and PONTECORVO 1940, 1941; PONTECORVO 1943b); (2) sterile males and fertile females, as in our study; (3) both fertile males and fertile females, but where sterile males appear among the F_2 and/or backcrosses due to some special combinations of chromosomes of both species. There is still another kind of hybrid sterility (4) exemplified by *D. paulistorum*, where the sterility of backcross hybrid males between races, or incipient species, is due to the presence of microorganisms (EHRMAN 1960; SOMERSON *et al.* 1984).

The second (2) and third (3) classes have been traditionally considered as equivalent, insofar as they deal with male-specific sterility factors. But they result most probably from different genetic determinants, or, at least, differentially organized. Thus, amongst the third class one frequently finds *Y*-autosomal interactions as the main genetic determinants of hybrid sterility (STONE 1947; ALEXANDER, LEA and STONE 1952; HENNIG 1977; SCHAEFER 1978; ZOUROS 1981), although in other cases the results are much more confusing and difficult to interpret (DOBZHANSKY 1974, 1975), perhaps corresponding to a more advanced stage of evolutionary divergence. In contrast, sterility of the class (2) seems to be brought about by a relatively high number of genes carried by all the chromosomes. This is so, for example, in the hybrids between *D. pseudoobscura* and *D. persimilis*, where sterility is due to a *minimum* of eight loci (DOBZHANSKY 1936), and the effectiveness of a chromosome in producing sterility is generally proportional to its length (WU and BECKENBACH 1983), a result that agrees quite well with our findings (NAVEIRA, HAUSCHTECK-JUNGEN and FONTDEVILA 1984). The same is true for hybrids between *D. mauritiana* and *D. simulans*, where sterility is produced by *at least* five loci (one on each chromosome), although probably there are many more (COYNE 1984).

We have gone one step further in the understanding of the genetic basis of hybrid sterility. Our results show that there are at least two types of hybrid sterility determinants, and analysis of more stocks may unveil new ones. First, there are many *X*-linked specific factors distributed all over the chromosome, any one of which produces sterility by itself when introgressed. Second, there are nonspecific factors spread all over the autosomes, and these produce *dom-*

inant sterility only when accumulated in critical amounts; that is, when the introgressed segments exceed roughly a size equivalent to 25–35% of the chromosome 2 length. Differences between chromosomes, or within a given chromosome, in this respect, may or may not reflect underlying differences in the distribution of these nonspecific factors. For example, it cannot be concluded that the number of these factors is greatest in chromosome 4 because the length of a chromosome is not closely related to the length of its DNA, and packing ratios of chromatin (euchromatin *vs.* heterochromatin) may also vary within the chromosome.

The results reported in this paper are concerned with a single type of introgression into *D. buzzatii.* However, reciprocal introgression experiments into *D. serido* using different stocks, and introgression experiments designed to test the role of specific *D. serido* inversions in the determination of hybrid sterility, seem to produce essentially similar results (H. NAVEIRA and A. FONTDEVILA, unpublished results).

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