Genetic Architecture of Autosome-Mediated Hybrid Male Sterility in Drosophila

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ABSTRACT

Several estimators have been developed for assesing the number of sterility factors in a chromosome based on the sizes of fertile and sterile introgressed fragments. Assuming that two factors are required for producing sterility, simulations show that one of these, twice the inverse of the relative size of the largest fertile fragment, provides good average approximations when as few as five fertile fragments are analyzed. The estimators have been used for deducing the number of factors from previous data on several pairs of species. A particular result contrasts with the authors' interpretations: instead of the high number of sterility factors suggested, only a few per autosome are estimated in both reciprocal crosses involving *Drosophila buzzatii* and *D. koepferae*. It has been possible to map these factors, between three and six per chromosome, in the autosomes *3* and *4* of these species. Out of 203 introgressions of different fragments or combinations of fragments, the outcome of at least 192 is explained by the mapped zones. These results suggest that autosome-mediated sterility in the male hybrids of these species is mediated by a few epistatic factors, similarly to *X*-mediated sterility in the hybrids of other Drosophila species.

THE genetic basis of reproductive isolation is one of the least understood aspects of modern evolutionary theory. The genus Drosophila is especially valuable in studies on the development of interspecific incompatibilites because of the knowledge of its genetics and the large number of interspecific crosses that yield some kind of viable and fertile progeny (BOCK 1984). One of the most common causes of reproductive isolation among Drosophila species is the sterility of the hybrid progeny (reviewed in ZOUROS 1989; COYNE 1992; WU *et al.* 1993; WU and PALOPOLI 1994). Studies analyzing backcross hybrids, where whole chromosomes or fragments of chromosomes of one species are introgressed in a related species, are particularly interesting. Regarding male hybrid sterility, first generation backcross studies show that in well-differentiated pairs of species there are usually "sterility factors", that is genetic elements that induce sterility when introgressed, in every chromosome, although the introgression of the *X* chromosome has a particular strong effect (COYNE 1984, 1985; COYNE and KREITMAN 1986; ORR 1987; ZOUROS *et al.* 1988; ORR and COYNE 1989; HEIKKINEN and LUMME 1991; KHADEM and KRIMBAS 1991). The exceedingly large effect of the *X* chromosome has also been demonstrated in a single study using subspecific taxa. In this case, no apparent effect due to introgression of the autosomes was evident (ORR 1989). By repeatedly backcrossing into one species, the effect of fragments smaller than one chromosome can be assessed (WU and BECKENBACH 1983; COYNE and CHARLESWORTH 1986, 1989; NAVEIRA and FONTDEVILA 1986, 1991a,b; NAVEIRA 1992; JOHNSON *et al.* 1992, 1993; PEREZ *et al.* 1993; WU *et al.* 1993; ZENG and SINGH 1993; CABOT *et al.* 1994; PALOPOLI and WU 1994; see also ORR 1992). Most of these works have focused on the effect of the *X* chromosome. The effects on fertility caused by the introgression *in heterozygosis* of autosomes or fragments of autosomes, as occurs in individuals produced by unidirectional backcrosses, have been less thoroughly studied. This work mainly concerns the genetic basis of this type of sterility, hereafter referred to as autosome-mediated (for a comment on sterility caused by homozygous autosome fragments, see DISCUSSION).

The most complete studies on autosome-mediated sterility analyzed the hybrids between *Drosophila buzzatii* and *D. koepferae* (NAVEIRA *et al.* 1984, 1989; NAVEIRA and FONTDEVILA 1986, 1991a,b) (*D. koepferae* was formerly known as *D. serido* "from Argentina", see FONTDEVILA *et al.* 1988). Using the asynapsis of the polytene chromosomes of the hybrids as a marker of the introgressed fragments (NAVEIRA *et al.* 1986), these authors were able to obtain fertile "segmental" males that carried small autosomal fragments of *D. koepferae* in an otherwise *D. buzzatii* background. Independent fertile males with overlapping introgressed segments were obtained. Put together, some of these fertile overlapping introgressions span the whole third and fourth chromosomes of these species (the other two major autosomes were less extensively studied because they carry inversions). These data demonstrate that there is not a single factor that alone can induce male sterility (NAVEIRA and FONTDEVILA 1986, 1991a,b). However, when large fragments were introgressed, they generally caused sterility. More-

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over, when two individually fertile fragments were introgressed together, they frequently induced sterility (NAVEIRA and FONTDEVILA 1986, 1991a,b). To explain their results, these authors suggested that a critical size (roughly 25–35% of one autosome) exists, above which sterility occurs. Finally, in an experiment where chromosomal fragments of *D. buzzatii* were introgressed in a *D. koepferae* genetic background, a zone of the chromosome *3* was found to produce sterility independently of the size of the carrier fragment (NAVEIRA and FONTDEVILA 1991a). This result shows that the asynapsis method is able to correctly detect major sterility factors and suggests that two different classes of sterility, mono- and polygenic, are acting in these hybrids (NAVEIRA and FONTDEVILA 1991a).

Cumulative effects on sterility have also been found in other experiments. In first generation backcrosses, the effect of the introgression of several autosomes is sometimes stronger than that of a single autosome (COYNE 1984; COYNE and KREITMAN 1986; HEIKKINEN and LUMME 1991). In the most refined analyses of *X*-linked male hybrid sterility, CABOT *et al.* (1994) and PALOPOLI and WU (1994) (and see also NAVEIRA 1992) have described a situation similar to that found in the *D. buzzatii-D. koepferae* hybrids in three independent introgressions using *D. simulans* and their closer relatives: small fragments that do not induce sterility when introgressed alone produce sterile males when introgressed together. Two different hypotheses have been proposed to account for the cumulative effects on sterility. NAVEIRA and FONTDEVILA (1986, 1991a,b) and NAVEIRA (1992) have suggested that sterility would be determined by the combined effects of a high number of interchangeable sterility factors. The bigger the introgressed fragment, the higher is the probability of including a number of sterility factors that, when above certain threshold, would induce sterility. According to this model, "sterility genes", that is, genes that cause a large qualitative effect, diminishing fertility when introgressed, would be rare or, as an extreme proposal, completely nonexistent. In fact, NAVEIRA and FONTDEVILA (1991a,b) suggested that hybrid male sterility could actually be chromosomal, *i.e.*, due to generalized effects of the introgressed zone, and be independent of the introgressed genes. This situation would be analogous to the sterility caused by the inappropriate inactivation of the *X* chromosome in the germ line of males carrying *X*-autosome translocations in *D. melanogaster* (LINDSLEY and LYFSCHITZ 1972). An alternative hypothesis has been developed by WU and coworkers (PEREZ *et al.* 1993; WU *et al.* 1993; CABOT *et al.* 1994; PALOPOLI and WU 1994). They have suggested that their results on *X*-mediated sterility can be explained by a limited number of closely linked sterility genes acting alone or in small groups (pairs, trios) with strong and probably specific epistatic interactions. The main difference between these two hypotheses, which I will call respectively the "threshold model" and the "epistatic model", concerns the contribution to sterility of independent genes.

It is not easy to determine the number of sterility factors or how they interact. The information provided by introgression experiments is usually very limited, consisting of the approximate localization and sizes of the introgressed fragments together with their effects on fertility. Moreover, only a small number of chromosomal segments are generally tested. I have developed estimators that use this limited information for deducing the number of sterility factors in a chromosome under the simplest multigenic hypothesis, that two factors are required together to produce sterility. By means of simulations, I have tested the general usefulness of these estimators under different conditions (different amounts of sterility factors per chromosome and numbers of fertile introgressed segments examined). The application of these estimators to the available experimental data has suggested that the number of factors per autosome producing sterility in *D. buzzatii-D. koepferae* hybrid males could be much lower than previously supposed. A reexamination of these results shows that it is not necessary to postulate that autosome-mediated sterility in this pair of species is due to a high number of interacting factors. Only a few factors per chromosome acting in pairs can explain practically all of these observations. The biological and evolutionary implications of these results are discussed.

METHODS

Development of the estimators of the number of sterility factors in a chromosome: We start with the supposition that any chromosome contains a large number of genetic factors and that some among them produce sterility in hybrid males when introgressed. The effects of these factors are cumulative, therefore sterility occurs when the number of introgressed factors is above a certain value, x. A particular fragment of this chromosome contains N factors. If p is the probability that any factor is involved in sterility, the average number of sterility factors in this segment is then $\lambda = Np$. Similarly, a larger fragment with a size of $N' = cN (c > 1)$ will contain an average number of factors $\lambda' = cNp = c\lambda$. The increase in the probability of inducing sterility with this increase of size ($\Delta s/\Delta N$) can be estimated according to the difference between two accumulated Poisson distributions:

$$\Delta s/\Delta N = [1 - \sum_{k=0}^{x} \{(c\lambda)^k/(e^{c\lambda}k!)\}] - [1 - \sum_{k=0}^{x} \{\lambda^k/(e^{\lambda}k!)\}] \quad (1)$$

Differentiating equation (1) respect to λ and setting equal to 0, we obtain:

TABLE 1

Conditions that define the estimators of the number of fertility factors in a chromosome

Estimator	Value of c^a	Value of N^a
A	$c \to 1$	$N = F_{max}$
B	$c = 1/F_{av}$	$N = F_{av}$
C	$c = 1/F_{max}$	$N = F_{max}$
D	$c \to 1$	$N = F_{max(s)}$
E	$c = S/F_{av(s)}$	$N = F_{av(s)}$
F	$c = S/F_{max(s)}$	$N = F_{max(s)}$

F_{max}, size of the larger fertile fragment; F_{av}, average size of the fertile fragments; S, size of the sterile fragments inside which one or several fertile fragments are analyzed (see METHODS). (s) refers to the fertile fragments inside this sterile one.

[a] Values of c and N that are used for estimating n according to equations 4 and 5.

$$\sum_{k=0}^{x} [k e^{\lambda(c-1)}\lambda^{k-1} - e^{\lambda(c-1)}\lambda^{k} - k c^{k}\lambda^{k-1} + c^{k+1}\lambda^{k}] = 0 \quad (2)$$

The value of λ from equation (2) can be algebraically solved for $x = 0$ (a single factor causes sterility) and, more interestingly, $x = 1$ (two factors are required together). It may be noted that although the general development of the model postulated cumulative effects, when $x = 1$, this is equivalent to assuming epistatic interactions between pairs of factors, so they are required together for sterility to occur. The solutions for these cases are:

$$\text{if } x = 0 \quad \lambda_0 = (\ln c)/c - 1 \quad (3)$$

$$\text{if } x = 1 \quad \lambda_1 = (\ln c^2)/c - 1 \quad (4)$$

The values of λ when $x > 1$, have to be solved numerically. The average number of sterility factors, n, in a chromosome that contains a total number of factors equal to T for a certain value of x will be:

$$n = \lambda_x T/N \quad (5)$$

From these formulae, it is concluded that we could estimate the number of factors n by determining the value of N (and therefore of λ) that maximizes the function $\Delta s/\Delta N$ for a certain value of c. However, the information provided by the introgression experiments is generally insufficient for determining this function. Usually only the sizes of one or a few fragments that allow fertility when introgressed are known. Different estimators can be suggested when the information is so limited. Six different estimators, defined according to the assumptions about c and N used to calculate n, are listed in Table 1. These estimators have been selected according to different considerations. The sets of conditions A, B and C consider the whole chromosome, that is supposed to cause sterility when introgressed. They can be used either when the information is minimal (only one segment has been studied, the carrier hybrids being fertile) or when a large number of fragments are considered. The set A uses a constant value of c, and thus of λ (when $c \to 1$, $\lambda = 2$). The sets B and C use values of c that depend, respectively, on the average and maximum sizes of the fertile fragments examined. B and C have the same value when only one fragment per chromosome has been obtained. The sets of conditions D, E and F are equivalent to A, B and C, respectively, except that they are based on "local" comparisons among fertile fragments and a sterile fragment that contains them, but is smaller than the whole chromosome. An attractive feature of these estimators is that several zones of the same chromosome can be examined independently and the estimates compared to see whether large discrepancies exist. They can also been used for estimating more precisely the number of factors in a determined zone. The obvious disadvantages are that any estimate for the whole chromosome will be an extrapolation and, because the number of fragments per zone considered is generally low, they will produce larger associated errors.

Simulations for evaluating the estimators: Using the values of c and N that define each estimator (Table 1), it has been possible to test their usefulness and to apply them to estimate the number of sterility factors from the data already available. Simulations have been used to establish the usefulness of the estimators defined in the previous section assuming the simplest polygenic model, that is $x = 1$. The reason for focusing the analysis on this particular case is that the current results suggest that, in general, male sterility is not mediated by single genes (reviewed in WU and PALOPOLI 1994). Moreover, demonstrating that two factors acting together are sufficient to explain experimental data would argue against the "threshold model", that postulates higher order interactions, therefore favouring the "epistatic model".

In these simulations, variable numbers of sterility factors have been randomly situated in "chromosomes" consisting of 1000 possible positions (because the calculations use relative sizes, the estimates are independent of this number. The same results are found with "chromosomes" with 10,000 positions). For the sets of conditions A, B and C, fragments of random size were sampled from each of these "chromosomes", the number of sterility factors included in these fragments was counted and the fragments were consequently classified as fertile or sterile. This procedure was repeated until a certain number of fertile fragment was obtained. The maximum or average size of the fertile fragments was then used to calculate the values of n, according to Table 1 and equations (4) and (5). For the sets of conditions D, E and F, the procedure consisted of two parts. First, fragments of random size were generated until one of them contained two or more sterility factors (*i.e.*, "caused sterility"). Then, fragments inside this sterilizing fragment were obtained until a certain number could be classified as fertile. Again, the maximum

or mean values of the fertile fragment(s) were used to estimate n. To calculate an average value of n for each combination of the two variables (number of sterility factors per chromosome and number of fertile fragments sampled), a total number of 1000 replicates (1000 different "chromosomes") were generated.

Estimation of the number of sterility factors in previously reported introgressions: The defined estimators have been used to calculate the number of sterility factors in the *X* chromosome introgressions analyzed by PEREZ *et al.* (1993), CABOT *et al.* (1994) and PALOPOLI and WU (1994), as well as the best analyzed cases (chromosomes *3* and *4*) in NAVEIRA and FONTDEVILA (1986, 1991a,b). Again, it has been supposed that $x = 1$, that is, that two factors are required to produce sterility. For NAVEIRA and FONTDEVILA's results, the sizes of the fragments were determined by the authors. Sometimes, they expressed the chromosomal sizes relative to that of the chromosome *2* of these species. For doing so, they divided the size of the fragments coming from the chromosome *4* by 1.23. Those values that were so normalized have been multiplied again by this number, so that they now reflect the size relative to chromosome *4*. Because the chromosomes *2* and *3* have very similar sizes, the data for this latter chromosome have not been corrected. In the cases studied by WU and coworkers, the determination of the sizes is more complicated because they used markers that delimit only a maximum and minimum size for their introgressions. I have made two assumptions for estimating these sizes. First, because all the species that they used are homosequential with *D. melanogaster*, I have assumed that the distances between markers are the same. The positions of the markers where then approximated using data on the distribution of the DNA content per band in the polytene chromosomes of *D. melanogaster* (HEINO *et al.* 1994). Second, I have assumed that fragments that have one extreme between two markers end in the middle between them.

As a first approximation, I have considered as fertile any introgressed male that produced at least some offspring, including the quasi-sterile lines obtained by CABOT *et al.* (1994) in their introgressions of *D. mauritiana* in *D. simulans* or the semisterile males observed by NAVEIRA and FONTDEVILA (1991b). Moreover, to estimate the total number of factors in the *X* chromosome in the introgressions by PEREZ *et al.* (1993), CABOT *et al.* (1994) and PALOPOLI and WU (1994), extrapolations were performed considering only those factors deduced by the authors that are both necessary and sufficient to explain complete sterility. This assumption eliminates the factor named "fixB" in the introgression of *D. mauritiana* in *D. simulans* in CABOT *et al.* (1994) (required to explain a quasisterile phenotype) as well as the factor "3" in PALOPOLI and WU (1994) (required for explaining the transition to a more severe sterile phenotype).

RESULTS

Usefulness of the estimators: Table 2 shows the average number of n obtained in the simulations. As a general result, these estimators provide acceptable average values for the number of sterility factors when n is small and tend to underestimate their number when it is higher. Among those estimators that consider the whole chromosome, *A* is generally better than *B* or *C*, except when only one or a few fertile fragments have been analyzed. Estimators *B* and *C* produce underestimates with five or more factors per chromosome. Estimators, *D*, *E* and *F*, do not generally provide better results than *A*. Therefore, I favor *A*, twice the inverse of the relative size of the largest fertile fragment, when five or more fertile fragments have been characterized. It is important to note, however, that the value of *A* when an infinite number of fertile fragments is determined for a certain chromosome can range from two to three when $n = 2$ and from two to $2n - 2$ when $n > 2$. This dependence on the distribution of the factors means that there is no way of knowing, even when a high number of fragments is examined, whether the value provided by *A* is an underestimate or an overestimate of the true number of sterility factors. The distribution of *A* depends on the number of sterility factors and fertile fragments. To show the variability associated to the estimator, Table 2 also lists the values of n that are bigger than, respectively, 5% and 95% of the cases observed in the simulations using estimator *A*.

Estimations of the number of sterility factors in previously reported introgressions: Table 3 summarizes the results obtained when these estimators are applied to the results obtained by NAVEIRA and FONTDEVILA (1986, 1991a,b), PEREZ *et al.* (1993), CABOT *et al.* (1994) and PALOPOLI and WU (1994). This table also includes the number of factors that the chromosome would carry according to extrapolations based on the authors' analysis (see METHODS). Concerning the different introgressions of the *X* chromosome by WU and coworkers, the estimated number is in general agreement with the extrapolations except in the case of the introgression of the proximal end of *D. mauritiana* in *D. simulans* (PEREZ *et al.* 1993). This is easily explained: when a small zone is studied that contains a single gene or cluster of genes that produce sterility, the size of the fertile fragments tend to be small because none of them can cross the position where this gene (or cluster) is located. The extrapolation to the whole chromosome is then strongly biased. The most interesting result is that concerning autosome-mediated sterility. Although the authors of these studies never stated precisely the number of sterility factors in these chromosomes, they considered that this number had to be very high, probably in the hundreds. However, as it is shown in Table 3, supposing that $x = 1$, the number of factors estimated

TABLE 2

Results of the simulations

No. factors[a]	No. fertile fragments[b]	Average number of factors ± SEM according to the estimators					
		A	*B*	*C*	*D*	*E*	*F*
2	1	19.19 ± 3.06 (2.61, 47.62)[c]	3.79 ± 0.05	3.77 ± 0.05	22.72 ± 2.36	4.97 ± 0.10	4.98 ± 0.10
2	2	6.13 ± 0.21 (2.37, 14.93)	3.50 ± 0.03	3.07 ± 0.03	7.24 ± 0.44	4.43 ± 0.06	3.87 ± 0.06
2	3	4.57 ± 0.11 (2.31, 9.48)	3.38 ± 0.02	2.79 ± 0.02	5.53 ± 0.16	4.49 ± 0.09	3.72 ± 0.07
2	5	3.62 ± 0.05 (2.27, 6.31)	3.33 ± 0.02	2.59 ± 0.01	4.35 ± 0.09	4.39 ± 0.08	3.42 ± 0.06
2	10	3.13 ± 0.03 (2.19, 4.67)	3.28 ± 0.02	2.44 ± 0.01	3.69 ± 0.05	4.26 ± 0.06	3.15 ± 0.04
2	20	2.87 ± 0.02 (2.12, 4.26)	3.28 ± 0.01	2.36 ± 0.01	3.47 ± 0.06	4.32 ± 0.07	3.11 ± 0.05
2	50	2.64 ± 0.02 (2.09, 3.66)	3.26 ± 0.01	2.28 ± 0.01	3.24 ± 0.04	4.24 ± 0.06	2.98 ± 0.04
3	1	24.67 ± 3.27 (3.00, 68.97)	4.21 ± 0.05	4.19 ± 0.06	30.51 ± 3.45	6.05 ± 0.13	6.12 ± 0.13
3	2	7.73 ± 0.40 (2.85, 18.02)	3.79 ± 0.03	3.34 ± 0.03	9.00 ± 0.30	5.52 ± 0.09	4.83 ± 0.08
3	3	5.51 ± 0.10 (2.67, 11.63)	3.71 ± 0.02	3.08 ± 0.02	7.07 ± 0.18	5.47 ± 0.10	4.49 ± 0.08
3	5	4.49 ± 0.06 (2.51, 7.75)	3.66 ± 0.02	2.86 ± 0.01	5.63 ± 0.13	5.39 ± 0.14	4.17 ± 0.11
3	10	3.84 ± 0.04 (2.42, 5.93)	3.60 ± 0.01	2.67 ± 0.01	4.70 ± 0.08	5.22 ± 0.09	3.84 ± 0.07
3	20	3.42 ± 0.03 (2.38, 4.82)	3.60 ± 0.01	2.56 ± 0.01	4.40 ± 0.07	5.34 ± 0.09	3.81 ± 0.06
3	50	3.20 ± 0.02 (2.27, 4.39)	3.59 ± 0.01	2.48 ± 0.01	4.16 ± 0.10	5.33 ± 0.13	3.70 ± 0.09
4	1	39.82 ± 4.99 (3.52, 125.00)	4.50 ± 0.06	4.57 ± 0.06	35.85 ± 3.29	6.88 ± 0.14	7.21 ± 0.17
4	5	5.44 ± 0.07 (3.03, 9.95)	3.95 ± 0.02	3.11 ± 0.02	6.49 ± 0.12	5.86 ± 0.10	4.54 ± 0.08
4	10	4.52 ± 0.05 (2.79, 7.30)	3.89 ± 0.02	2.88 ± 0.01	5.64 ± 0.10	5.97 ± 0.13	4.39 ± 0.09
4	20	3.96 ± 0.03 (2.70, 5.65)	3.84 ± 0.01	2.73 ± 0.01	5.05 ± 0.09	5.85 ± 0.11	4.15 ± 0.08
4	50	3.70 ± 0.03 (2.56, 5.09)	3.86 ± 0.01	2.66 ± 0.01	4.72 ± 0.08	5.88 ± 0.11	4.06 ± 0.08
5	1	44.78 ± 5.56 (4.15, 133.33)	4.76 ± 0.06	4.70 ± 0.06	45.51 ± 4.44	7.76 ± 0.21	7.51 ± 0.16
5	5	6.40 ± 0.10 (3.28, 12.20)	4.17 ± 0.02	3.28 ± 0.02	7.64 ± 0.15	6.60 ± 0.14	5.12 ± 0.11
5	10	4.99 ± 0.05 (2.96, 7.84)	4.08 ± 0.02	3.03 ± 0.01	6.29 ± 0.12	6.42 ± 0.14	4.70 ± 0.11
5	20	4.56 ± 0.04 (2.97, 6.45)	4.08 ± 0.02	2.89 ± 0.01	5.64 ± 0.08	6.44 ± 0.10	4.53 ± 0.07
5	50	4.19 ± 0.03 (2.87, 5.71)	4.07 ± 0.01	2.80 ± 0.01	5.37 ± 0.09	6.53 ± 0.13	4.48 ± 0.09
7	1	44.14 ± 3.87 (5.20, 166.67)	5.10 ± 0.06	5.09 ± 0.06	54.81 ± 5.19	8.49 ± 0.18	8.99 ± 0.23
7	5	7.87 ± 0.12 (4.07, 14.49)	4.53 ± 0.02	3.57 ± 0.02	8.99 ± 0.15	7.19 ± 0.12	5.57 ± 0.09
7	10	6.23 ± 0.07 (3.70, 10.05)	4.45 ± 0.02	3.31 ± 0.02	7.83 ± 0.18	7.71 ± 0.20	5.63 ± 0.15
7	20	5.61 ± 0.05 (3.56, 8.33)	4.43 ± 0.01	3.17 ± 0.01	7.08 ± 0.13	7.65 ± 0.17	5.40 ± 0.12
7	50	5.08 ± 0.04 (3.44, 7.17)	4.41 ± 0.01	3.02 ± 0.01	6.43 ± 0.10	7.37 ± 0.14	5.04 ± 0.10
10	1	56.36 ± 4.58 (6.90, 222.22)	5.62 ± 0.06	5.56 ± 0.06	66.27 ± 6.13	9.81 ± 0.24	9.60 ± 0.22
10	5	10.28 ± 0.17 (5.04, 18.69)	4.97 ± 0.02	3.95 ± 0.02	12.30 ± 0.32	9.22 ± 0.35	7.16 ± 0.27
10	10	8.13 ± 0.09 (4.69, 12.66)	4.92 ± 0.02	3.67 ± 0.01	9.51 ± 0.17	8.71 ± 0.20	6.31 ± 0.15
10	20	7.11 ± 0.06 (4.48, 10.52)	4.87 ± 0.01	3.48 ± 0.01	9.01 ± 0.30	9.11 ± 0.39	6.40 ± 0.28
10	50	6.27 ± 0.05 (4.05, 8.81)	4.86 ± 0.01	3.35 ± 0.01	7.87 ± 0.12	8.53 ± 0.17	5.79 ± 0.12
20	1	117.57 ± 8.84 (11.49, 400.0)	6.65 ± 0.06	6.71 ± 0.07	105.86 ± 7.61	13.28 ± 0.37	13.30 ± 0.36
20	5	17.98 ± 0.26 (8.55, 33.33)	5.96 ± 0.02	4.82 ± 0.02	20.00 ± 0.46	11.68 ± 0.40	9.08 ± 0.21
20	10	14.18 ± 0.16 (7.87, 23.26)	5.91 ± 0.02	4.50 ± 0.02	16.60 ± 0.29	12.48 ± 0.37	9.13 ± 0.27
20	20	11.93 ± 0.10 (7.43, 17.54)	5.88 ± 0.01	4.27 ± 0.01	13.92 ± 0.26	11.18 ± 0.32	7.83 ± 0.24
20	50	10.60 ± 0.08 (6.87, 15.15)	5.86 ± 0.01	4.07 ± 0.01	12.70 ± 0.37	11.70 ± 0.46	7.94 ± 0.35
50	1	219.12 ± 11.63 (25.0, 1000)	8.16 ± 0.06	8.23 ± 0.06	214.44 ± 11.62	19.61 ± 0.64	19.75 ± 0.65
50	5	43.80 ± 0.78 (17.24, 86.96)	7.49 ± 0.03	6.20 ± 0.03	45.14 ± 0.74	17.71 ± 0.48	14.02 ± 0.36
50	10	32.27 ± 0.41 (14.71, 57.14)	7.43 ± 0.02	5.81 ± 0.02	35.04 ± 0.47	17.84 ± 0.50	13.20 ± 0.36
50	20	26.40 ± 0.27 (13.79, 41.67)	7.38 ± 0.02	5.54 ± 0.02	29.28 ± 0.41	16.49 ± 0.49	11.70 ± 0.36
50	50	22.33 ± 0.21 (11.05, 33.33)	7.33 ± 0.02	5.24 ± 0.02	25.91 ± 0.34	17.25 ± 0.49	11.66 ± 0.33

[a] Number of factors randomly situated in the simulated chromosomes.

[b] Number of fertile fragments examined (in the whole chromosome for *A, B,* and *C;* inside a sterile fragment in *D, E, F;* see METHODS).

[c] Inside the parentheses: values above, respectively, 5% and 95% of the cases observed in the simulations using estimator *A*.

is small, five to seven according to estimator *A*. Taking into account the results of the simulations (Table 2), that show that *A* gives very good estimations when the number of factors is low, and considering that the number of fertile fragments analyzed in these works is six to 23 (see Tables 4 and 5 for a summary), this estimator should provide a reasonable aproximation of the real number of sterility factors per chromosome. As a matter of fact, simulations using six to 23 fertile fragments have shown that having as low as 20 factors per chromosome is very unlikely in the *D. buzzatii-D. koepferae* introgressions: at most 2% of the simulated chromosomes produce estimates of n smaller than those shown in Table 3.

Mapping sterility factors in the introgressions of *D. koepferae* in *D. buzzatii:* Obviously, more complicated

TABLE 3

Estimations of the number of factors per chromosome according to previously reported experiments

Species pairs[a]	Introgressed chromosome	Values of *n* according to the estimators						Extrapolations	References
		A	*B*	*C*	*D*	*E*	*F*		
(*D. k.*) × *D. b.*	*3*	5.4	3.6	3.2	6.3 ± 0.2	5.4 ± 0.4	4.8 ± 0.4	High number	1
(*D. k.*) × *D. b.*	*4*	7.0	3.8	3.5	8.4 ± 0.7	6.8 ± 0.1	6.4 ± 0.2	High number	1
(*D. k.*) × *D. b.*	*3*	5.2	3.9	3.1	9.6 ± 1.3	7.5 ± 0.7	7.1 ± 0.9	High number	2, 3
(*D. k.*) × *D. b.*	*4*	6.6	3.9	3.4	6.9 ± 0.1	5.7 ± 0.2	5.1 ± 0.2	High number	3
(*D. b.*) × *D. k.*	*3*	4.9	3.6	3.0	5.6 ± 0.4	5.8 ± 0.3	5.1 ± 0.3	High number	2, 3
(*D. b.*) × *D. k.*	*4*	5.0	3.6	3.1	5.5 ± 0.4	5.0 ± 0.4	4.3 ± 0.3	High number	3
(*D. m.*) × *D. sim.*	*X* (prox.)	17.6	5.4	4.9	23.7 ± 3.5	19.7 ± 1.7	18.2 ± 2.0	2.9	4
(*D. sech.*) × *D. sim.*	*X* (prox.)	9.5	4.2	3.9	—	—	—	—	4
(*D. m.*) × *D. sim.*	*X* (distal)	10.1	5.0	4.0	10.1	11.6	8.7	7.6	5
(*D. sech*) × *D. sim.*	*X* (distal)	8.6	4.7	3.8	8.6	10.7	8.1	7.6	5
(*D. sim.*) × *D. m.*	*X* (prox.)	11.8	4.9	4.3	9.7 ± 2.1	14.9 ± 2.3	12.9 ± 2.3	14.2	6

—, Not determinable with the available results. *D. k., Drosophila koepferae; D. b., D. buzzatii; D. m., D. mauritiana; D. sim., D. simulans; D. sech., D. sechellia.* References: 1, NAVEIRA and FONTDEVILA (1986); 2, NAVEIRA and FONTDEVILA (1991a); 3, NAVEIRA and FONTDEVILA (1991b); 4, PEREZ *et al.* (1993); 5, CABOT *et al.* (1994); 6, PALOPOLI and WU (1994).

[a] The name in parentheses refers to the species from which the introgressed fragments are obtained.

hypotheses, involving higher order interactions among a large number of factors, could also be compatible with the observed sizes of fertile and sterile fragments. However, these results suggest that, if indeed the combined effects of pairs of factors are the cause of sterility, it should be possible to map a few specific zones in each chromosome that would cause sterility when introgressed together. I have used an exclusion procedure, similar to that used by CABOT *et al.* (1994) and PALOPOLI and WU (1994), for mapping factors in the *D. buzzatii-D. koepferae* hybrids. Basically, whenever there is a fertile fragment contained within a sterile one, sterility factors are assigned to the nonoverlapping regions. Using this procedure on the data describing the introgressions of *D. koepferae* in a *D. buzzatii* background (NAVEIRA and FONTDEVILA 1986), four zones can be mapped in each of the chromosomes *3* and *4* of *D. koepferae* that produce sterility when at least two of them are introgressed together. The intervals that define these zones are: 3 A5e-B5h, 3 C3b-D1b, 3 E1g-F1e, 3 G1f-G1h; 4 A-A4b, 4 B4c-B4e, 4 D5b-E1a, 4 F3a-F4d [this nomenclature refers to the maps of *D. buzzatii* (RUÍZ *et al.* 1982)]. A perfectly congruent mapping for the chromosome *3* is found in introgression experiments that used other stocks (NAVEIRA and FONTDEVILA 1991a): 3 B5d-C2f, 3 D1a-D1e, 3 E1a-E3c and 3 F1h-G2a. Assuming that the genetic basis of the sterility found in both introgressions is the same, these results narrow down the positions in this chromosome to 3 B5d-B5h, 3 D1a-D1b, 3 E1g-E3c and 3 G1f-G1h. Out of the 143 fragments or combinations of fragments in the chromosomes *3* and *4* obtained by these authors, these positions can explain 135 (94%) (Table 4).

Results of the reciprocal introgressions: When the less extensively analyzed introgressions of *D. buzzatii* in *D. koepferae* (NAVEIRA and FONTDEVILA 1991a,b) are considered, the results are similar. Five to six factors in chromosome *3* (located in 3 A4f, 3 C4c-D1a, 3 E1c, two factors in 3 E1e-E1h, and probably another one in 3 F2g-H, the uncertainty due to lack of data in the proximal end) and at least three factors in chromosome *4* (4 B1a-B3c, 4E1e-E3e, 4G1b-G1c) explain 57 out of 60 results (95%) (Table 5). These factors do not map in the positions obtained in the reciprocal experiments, a result also found in other species (PEREZ *et al.* 1993; PALOPOLI and WU 1994). Introgression of the region 3 E1e-E2f always produced sterility. I have considered that this zone carries two closely linked factors. However, as suggested by NAVEIRA and FONTDEVILA (1991a), hypothesizing a single factor of major effect would be simpler.

Semisterility is partially explained by the effect of individual factors: Until now I have considered as equally fertile all those individuals able to produce even a limited number of offspring. However, some segmental males are actually semisterile. When the semisterile cases analyzed by NAVEIRA and FONTDEVILA (1991b) are considered, a clear relationship between the zones deduced to carry sterility factors and semisterility is observed. No matter how small they are, all the introgressions of chromosomes *3* and *4* that produce semisterility (45 cases) include one of these factors. However, approximately half of the fragments examined that apparently do not produce any effect on fertility also carry one of them. This result suggests that the sterility factors mapped are also involved in the semisterile cases and that, as in the production of complete sterility, their individual effects are necessary but not sufficient.

DISCUSSION

A number of studies have focused on *X*-mediated hybrid male sterility in Drosophila species. Two im-

TABLE 4

Fragments of *Drosophila koepferae* introgressed in *D. buzzatii*, observed results and expectations according to the hypothesis of pairs of epistatic factors

NAVEIRA and FONTDEVILA (1986) (Stocks: KSL/BSL)			NAVEIRA and FONTDEVILA (1991a,b) (Stocks: K0/B0)			NAVEIRA and FONTDEVILA (1991b) (Stocks: K0/BIII)		
Introgressed fragments	Phenotypes		Introgressed fragments	Phenotypes		Introgressed fragments	Phenotypes	
	Obs.	Exp.		Obs.	Exp.		Obs.	Exp.
3 A-G1e	S	S	3 A1a-H	S	S	3 A1a-H	S	S
3 B2f-H	S	S	3 C1e-H	S	S	3 C3-H	S	S
3 C4c-H	S	S	3 D2d-H	S	S	3 A1-C5/D1	S	(S)[a]
3 C1b-G2a	S	S	3 A-D3e	S	S	3 E1/E2-G3	F(ss)	(F)
3 A-D3c	S	S	3 B1a-D2e	S	S	3 C5-F1/F2	S	S
3 D5a-G2a	S	S	3 B3g-E1d	S	S	3 A1-C1	F(ss)	F
3 A-C3a	F	F	3 B3g-D4a	S	S	3 C1-E2	S	(S)
3 D4c-G1e	F	F	3 B5c-D4a	S	S	3 D1-E4	F(ss)	(F)
3 F1f-H	F	F	3 D5f-G2b	S	S	3 A1-E2	S	S
3 D5a-F1h	F	F	3 A-C3b	F(ss)	F	4 A1-H	S	S
3 C1b-D4e	F	F	3 B5c-C5e	F	F	4 B2-F4	S	S
3 C3?-D3c	F	F	3 C3a-D3e	F	F	4 D3-H	S	S
			3 D2a-E2f	F	F	4 F1-H	F(ss)	F
4 A-D3c	S	S	3 E1b-F1g	F	F	4 E3/E4-G1/G2	F(ss)	F
4 D4e-G3a	S	S	3 E3d-H	F(ss)	F	4 F4a-H	F(ss)	F
4 B1a-E1g	S	S	3 E5a-H	F(ss)	F			
4 F1b-H	F	F	3 B3g-D3e	S	S	3 B3-H	S	S
4 D3d-F1e	F	F	3 F1a-H	F	F	3 A-D4	S	S
4 C1a-E1g	F	F	3 A-C1d	F(ss)	F	3 E1/E2-H	S	(S)
4 D2c-F1b	F	F	3 A-B5h	F	F	3 E4-H	F(ss)	F
4 A4c-D1d	F	F	3 F2a-H	F	F	3 C1-E4	S	S
4 B3a-D3c	F	F	3 B4a-D3a	S	S	3 B3/B4-D3	S	S
4 B1a-D1a	F	F	3 F3c-H	F	F	3 D5-H	S	S
4 A-B3d	F	F	3 E3d-G1f	F	F	3 C3-F4	S	S
4 E3a-F4d	F	F	3 A5a-C1d	F	F	3 A-C5/D2	S	S
4 A5a-C3b	F	F	3A1b-B3c	F	F	3 A-C3	F(ss)	F
4 C2a-D3a	F	F	3 E5e-G1g	F	F	3 C1-D4	F(ss)	F
			3 C1a-D2e	F(ss)	F			
3 A-C1a+			3 A-B1e	F	F	4 D1-H	S	S
3 F2a-H	S	S	3 A-B1d	F	F	4 E1-H	S	(S)
3 D4c-G1e+			3 E5c-F1d	F	F	4 A-D3	S	S
3 A-C1a	S	S				4 C2-F1/F2	S	F(*)
3 D4c-G1e+			4 A1a-H	S	S	4 E4-H	F(ss)	F
4 F1b-H	S	S	4 A-F1c	S	S	4 F2-H	F(ss)	F
3 A-C1a+			4 D1f-H	S	S	4 B2-E2/E3	S	S
3 D5a-F1h	S	S	4 C1d-F2b	S	F(*)	4 E1-F4	F(ss)	(F)
4 B1a-D1a+			4 D1b-G3a	S	S			
4 F1b-H	S	S	4 D5b-H	S	S			
3 D5a-F1h+			4 E2b-H	S	F(*)			
4 F1b-H	S	S	4 A-D2e	S	S			
3 D5a-F1h+			4 C2b-F1f	S	F(*)			
3 F2a-H	S	S	4 F1b-H	F(ss)	F			
4 D2c-F1b+			4 C2b-E3a	F(ss)	F			
4 F3a-H	S	S	4 A-C1d	F(ss)	S(*)			
4 F4e-G4a+			4 E4d-G3a	F(ss)	F			
4 B1a-D1a	F	F	4 F1e-H	F(ss)	F			
3 C1b-D4e+			4 D4c-F1f	F(ss)	F			
4 B1a-D1a	F	S(*)	4 B1a-D1e	F(ss)	F			
3 C1b-D4e+			4 E5c-G3a	F(ss)	F			
3 F2a-H	S/F[b]	S	4 A-B4b	F	F			
3 A-C1a+			4 B1a-D1b	F(ss)	F			
4 F4e-G4a	F	F	4 F3d-H	F	F			
3 A5c-C1c+			4 E1a-F2b	F(ss)	F			
4 G3a-H	F	F	4 D3d-E5a	F	F			

TABLE 4

Continued

Naveira and Fontdevila (1986) (Stocks: KSL/BSL)			Naveira and Fontdevila (1991a,b) (Stocks: K0/B0)			Naveira and Fontdevila (1991b) (Stocks: K0/BIII)		
Introgressed fragments	Phenotypes		Introgressed fragments	Phenotypes		Introgressed fragments	Phenotypes	
	Obs.	Exp.		Obs.	Exp.		Obs.	Exp.
			4 B4c-D3d	F	F			
			4 D5d-F1b	F	F			
			4 D1a-E2b	F	F			
			4 B1a-C2b	F	F			
			4 G1c-H	F	F			
			4 A-A5b	F	F			
			3A-D3e+					
			3 F1A-H	S	S			
			3 E3d-H+					
			3 A-B1e	S	F(*)			
			3 E5e-G1g+					
			3 A-B1d	F(ss)	F			
			4 E4d-G3a+					
			4 A-B4b	S	S			
			4 B1a-D1e+					
			4 G1c-H	S	F(*)			
			3 F3c-H+					
			4 C2b-F1f	S	S			
			3 F2a-H+					
			4 A-A5b	S	S			
			3A-B1e+					
			4 B4c-D3b	F(ss)	F			
			3 B5d-D4a+					
			4 F1b-H	S	S			
			3 A1a-H+					
			4 A1a-H	S	S			
			3 F3c-H+					
			4 E2b-H	S	S			

[a] The parentheses refer to uncertainties due to the imprecise determination of the extremes of the introgressed fragments.
[b] Some fertile and some sterile males. The fertile males can be recombinants.
The asterisks mark those results that do not coincide. S, sterile; F, fertile; F(ss), semisterile.

portant conclusions have been drawn from these studies. First, the genetic basis of this sterility is polygenic and the number of factors in the *X* chromosome is relatively high (Wu and Beckenbach 1983; Naveira and Fontdevila 1986; Coyne and Charlesworth 1989; Naveira 1992; Wu *et al.* 1993; Perez *et al.* 1993; Cabot *et al.* 1994; Palopoli and Wu 1994; Wu and Palopoli 1994). Second, these *X* factors interact. Sterility is produced only when two or more factors are present together, while each of them alone does not produce any effect. To explain this fact, epistatic interactions have been suggested (Cabot *et al.* 1994; Palopoli and Wu 1994; reviewed in Wu and Palopoli 1994). Two main reasons explain why the study of autosome-mediated sterility has been generally neglected. First, most of these studies have been performed in closely related species where autosome-mediated sterility, if present, is not as striking as *X*-mediated effects. Second, the main focuses of the research on sterility have been on isolating sterility genes or understanding Haldane's rule. In both cases, studying the effect of the *X* chromosome was either more promising or simply mandatory. The extensive works of Naveira and Fontdevila (1986, 1991a,b) and Naveira *et al.* (1984, 1989) are thus particularly interesting, because they describe autosome-mediated sterility with as much detail as the best works on *X*-linked sterility in other pairs of species. The basic conclusion of these studies, namely that autosome-mediated sterility in this pair of species has also a polygenic basis, is beyond doubt. The results of these and other works demonstrate that the basic model of single genes producing interspecific sterility is inappropriate in most cases (reviewed in Wu and Palopoli 1994). However, the exact number of sterility factors and how they interact remains to be determined.

In this work, I have developed procedures for estimating the number of autosomal sterility factors under the assumption of epistatic interactions between pairs of genes, and obtained values of a few per chromosome in *D. buzzatii-D. koepferae* hybrids. Furthermore, it has

TABLE 5

Fragments of *Drosophila buzzatii* introgressed in *D. koepferae*

Introgressed fragments	Phenotypes		Introgressed fragments	Phenotypes	
	Obs.	Exp.		Obs.	Exp.
3 A-D3a	S	S	4 B1a-H	S	S
3 A-D1b	S	S	4 B1a-G3d	S	S
3 A4f-D4b	S	S	4 B3d-G1d	S	S
3 E2g-H	F(ss)	F	4 B4b-G1d	S	S
3 B1c-E1c	S	S	4 B4d-F4f	S	F(*)
3 A-C4b	F(ss)	F	4 E1d-H	S	S
3 A4g-D2d	F(ss)	F	4 D1a-F4a	S	F(*)
3 E5b-H	F	F	4 B3d-E3e	F(ss)	F
3 A-C1e	F(ss)	F	4 B4c-E4d	F(ss)	F
3 A5f-D1b	F	F	4 E3f-H	F(ss)	F
3 B4c-D4b	F	F	4 E4a-H	F(ss)	F
3 B3f-D5a	F	F	4 C1a-E4c	F(ss)	F
3 A-B5b	F	F	4 D4c-F4f	F(ss)	F
3 F3b-H	F	F	4 E1d-G1a	F(ss)	F
3 B3a-D1b	F	F	4 F1a-H	F(ss)	F
3 E3e-G1d	F	F	4 F1d-H	F(ss)	F
3 B5d-D3d	F	F	4 F2a-H	F(ss)	F
3 C3a-E1e	F	S(*)	4 D4b-F1f	F(ss)	F
3 D1b-D5d	F	F	4 C3e-E3a	F	F
			4 B1d-D2b	F	F
3 E1a-E3d	S	S	4 B4e-D4d	F	F
3 D1a-E1h	S	S	4 D1a-E3a	F	F
3 C3a-E1h	S	S	4 E1d-F2b	F(ss)	F
3 B4f-E1h	S	S	4 A3c-C1a	F	F
3 B3a-E1h	S	S	4 A-B2e	F	F
3 E1b-H	S	S	4 E4a-F4e	F	F
3 D3c-H	S	S	4 B2c-D1a	F	F
3 C4c-H	S	S	4 F2a-G3d	F	F
3 C1c-H	S	S	4 F4e-H	F	F
3 B1a-H	S	S	4 D3b-E2f	F	F
3 A1a-H	S	S			

NAVEIRA and FONTDEVILA (1991a,b); Stocks: B0/K0. Abbreviations as in Table 4.

been possible to map these factors, and show that three to six per autosome, acting in pairs, can explain ≥95% of the results obtained in these species (NAVEIRA and FONTDEVILA 1986, 1991a,b). There are several possible explanations for the exceptions. First, some specific pairs of factors may be genuinely unable to interact, explaining some of the cases where sterility is expected but fertile males actually appear. A second reason for these exceptional cases could be differences in the positions of the sterility factors in the different stocks or even individual variability. Finally, another reason could be errors due to the limitations of the technique used in assigning the breakpoints of the introgressed segments. Although the chromosomal asynapsis is a good marker of the introgressed zones (NAVEIRA *et al.* 1986), the method failed to exactly map the only gene whose position has been independently determined by *in situ* hybridization in these species. LABRADOR *et al.* (1990) localized the Alcohol dehydrogenase gene three to seven bands away from the position deduced using asynapsis by NAVEIRA *et al.* (1986). Considering the introgressions of *D. koepferae* in *D. buzzatii*, this problem does not seem to have seriously affected the mapping of factors in the chromosome *3*, but it could be important in their assignment to chromosome *4*. All except two of the available results for this chromosome would be explained postulating a different set of positions, adding a new factor: 4A-A4b, (4 C1a-d), (4D3c), (4F1b), (4G3a) (the parentheses refer to approximate positions). However, postulating these positions requires that the determination of the extremes of a few fragments was slightly misplaced, one to three bands.

Finally, it is still possible that some of these results require more complicated explanations. First, the zones deduced to have strong influence on sterility may carry clusters of closely linked factors instead of single genes. In this sense, the estimates of the number of factors are minimal. Second, when semisterility is considered it is found that, although the individual introgression of the same zones is neccessary for the production of semisterile phenotypes, some introgressions carrying one of these zones are apparently completely fertile. This re-

sult implies that other adjacent genes modulate the effects of the introgression of single main factors. Maybe they also contribute in some cases to the complete sterile phenotypes. Although it is likely that future experiments will demonstrate that the basic model of pairs of interacting factors is insufficient to explain every single case, the fact that practically all of the >200 different introgressions performed can be explained by this model is a strong indication that the genetic basis of the sterility in these species is much simpler than previously assumed. The main conclusion is that postulating higher order interactions among a large number of sterility factors is not required.

In the introduction to this work, I summarized the two hypotheses that have been proposed to explain the high resolution results on hybrid male sterility. The analysis presented here suggests that the "epistatic model" is the simplest explanation for autosome-mediated hybrid male sterility in the pair of species *D. buzzatii* and *D. koepferae*. The three main results used to develop the "threshold model" were: the high number of factors deduced from the existence of a threshold size for sterility (NAVEIRA and FONTDEVILA 1986, 1991a); the apparent additive effect of progressively larger fragments on the sterile phenotype of the hybrid males (NAVEIRA *et al.* 1984, 1989; NAVEIRA and FONTDEVILA 1991b); and the fact that introgressions of different chromosomal zones have similar phenotypic effects (NAVEIRA *et al.* 1989; NAVEIRA and FONTDEVILA 1991b). However, all this evidence can be contested or explained in a different way. First, the "threshold model" was established to explain why sterility appeared only when the introgressed zone was above a critical size. This critical size was postulated because the "minimum size for sterility" and the "maximum size for fertility", that is the sizes of the smaller sterilizing fragment and the larger fragment that allows fertility when introgressed, were similar when whole chromosomes were considered. However, supposing that the factors are randomly positioned, some zones of the chromosomes will have a higher density of sterility factors than others. Thus, the extreme values for the length of fertile and sterile fragments will necessarily come from different zones and their comparison is not relevant to the existence of a threshold size in any particular zone. It is more rigorous to compare pairs of fragments, the smaller allowing fertility, the larger causing sterility, where the big fragment contains the small one. No threshold is then apparent. The sterile fragments are, with a single exception, ≥23% (generally >40%) larger than the fertile ones (NAVEIRA and FONTDEVILA 1986, 1991a,b). At least tens, and frequently hundreds, of genes have to be introgressed to produce any noticeable effect on fertility.

The apparent additivity and interchangeability of the effects of the introgressed zones on the phenotypes admits several simple explanations: 1) the products of the sterility factors may form protein complexes. The introgressed proteins would cause poisoning or unstability of these complexes. 2) These products may act through specific interactions with a particular gene or sets of genes. 3) Constrains on the possible phenotypes: the analysis of male sterile mutations in *D. melanogaster* has shown that spermatogenesis has two characteristics that cause that mutations in different genes produce similar phenotypes (reviewed in FULLER 1993). First, different processes in spermatid differentiation occur through independent pathways. Affecting one of them does not necessarily stop the others, so the final aberrant phenotype can be similar in spite of the completely different nature of the original alterations. Second, several genes together regulate critical checkpoints, major decisions at the cellular level. Mutations in any of these genes arrest development at similar stages. In conclusion, to deduce the number of genes involved or the way they interact from the observed phenotypes is impossible in the absence of molecular data.

There are reasons to think that sterility induced through factors in heterozygosis requires an infrequent type of gene interaction. Sterility in Drosophila often follows Haldane's rule (HALDANE 1922; reviewed n WU and DAVIS 1993), that is, hybrids of the heterogametic sex suffer sterility more frequently. According to MULLER (1940) this would be so because the effects of the *X*-linked sterility factors are recessive and thus only evident in the heterogametic sex. ORR (1993) has refined this hypothesis showing that it holds only if a negative covariance exists between the degree of dominance of the factors in the hybrid and their effects in homozygosis on the phenotype. If, as this model suggests, both *X*- and autosome-linked sterility genes are as a rule very recessive in the hybrids, a predominant effect of the *X* chromosome in backcross hybrids is to be expected. This hypothesis is supported by the fact that fragments or whole chromosomes that do not produce any effect in heterozygosis induce sterility when homozygous (VIGNEAULT and ZOUROS 1986; PANTAZIDIS and ZOUROS 1988; PANTAZIDIS *et al.* 1993; ORR 1992; see also DAVIS *et al.* 1994 concerning hybrid female sterility). In fact, the results obtained for *D. buzzatii* and *D. koepferae* contrast with those found for other Drosophila species, where complete autosomes can be introgressed without apparent effect (ORR 1987; ORR and COYNE 1989). Moreover, the phenotypic abnormalities observed in F_1 *buzzatii-koepferae* hybrids (NAVEIRA *et al.* 1989; NAVEIRA and FONTDEVILA 1991b) are much more severe than those described in other cases, where only the postmeiotic differentiation is affected (PEREZ *et al.* 1993). These results could be explained by the fact that *D. buzzatii* and *D. koepferae* belong to a group of species that hybridize frequently, often producing fertile females, in spite of extensive genetic differentiation (SÁNCHEZ 1986; MARÍN *et al.* 1993). All these data suggest that the number of au-

tosome-linked sterility factors may be even smaller in other cases. An interesting evolutionary consideration is that the small sterilizing effect of the autosomes in crosses between closely related species, and especially in intersubspecific crosses, argues against a major contribution of autosome-mediated sterility (*vs.* *X*-linked sterility) in the first stages of interspecific reproductive isolation.

Two final considerations. The epistatic model for autosome-mediated sterility allows us to establish testable hypotheses about the number and positions of the sterility factors, something that previous interpretations did not provide. For example, the mapping of factors on chromosomes 3 and 4 of the *D. buzzatii-D. koepferae* hybrids can be used as a starting point for their isolation and characterization. Finally, some authors have suggested that multiple genes with epistatic interactions could explain the emergence of reproductive isolation more easily than single genes or multiple genes with additive effects (discussed in CABOT *et al.* 1994; WU and PALOPOLI 1994; ORR 1995). These theoretical models suggest that the differences that we find studying species hybrids may reflect the genetics of the speciation process itself.

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