Sterility of male and female hybrids of *Drosophila virilis* and *Drosophila lummei*

ERJA HEIKKINEN & JAAKKO LUMME

Department of Genetics, University of Oulu, SF 90570 Oulu, Finland

The sterility of interspecific hybrids between the sibling species *Drosophila virilis* and *D. lummei* was tested in reciprocal F_1 s and different second generation combinations of cytoplasm, sex chromosomes and autosomes. Males with motile sperm and females with at least one mature egg were scored as fertile. When *D. virilis* was the mother, about 5 per cent of the F_1 male progeny was sterile, the reason being that the X of *D. virilis* was weakly incompatible with the heterozygous second, fourth and fifth chromosomes. The F_1 males carrying the X chromosome of *D. lummei* were all fertile. The X chromosome of *D. lummei*, and in particular its species-specific double inversion *In(1)a + b*, nevertheless caused sterility in 70 per cent of males in the presence of homozygous autosomes of *D. virilis*. Sterility was rare among the females, although some weakly disadvantageous X versus autosome and autosome versus autosome interactions were detected. The male and female hybrid sterilities were based on different genetic systems. The results emphasize the central role of the X chromosome in hybrid male sterility.

Keywords: evolution, postzygotic isolation, speciation.

Introduction

In a wide survey of species hybrids in *Drosophila*, Coyne & Orr (1989) concluded that male sterility is one of the earliest indications of speciation. This conforms with the rule of Haldane (1922) that 'when in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex'. Despite many examples to reinforce this conclusion, the genetic basis for male hybrid sterility is known in detail only for a few pairs of species. Because male sterility usually develops first, it is clear that the genetic basis for hybrid female sterility is known in still fewer cases (Orr, 1987; Orr & Coyne, 1989).

Another generalization concerning postzygotic isolation is Coyne's rule, that it is almost invariably the X chromosome that plays the main role in both hybrid sterility and hybrid weakness. It is always incompatible with something else. Sterility and subvitality indicate failing interaction either with genes in another chromosome or with some cytoplasmic component.

Our aim here was to measure the relative fitness of heterospecific combinations of different categories of genetic elements: sex chromosomes, autosomes and cytoplasmic factors, as potential carriers of mutually incompatible interacting genes. It may be hypothesized that the evolutionary forces leading to postzygotic

isolation may also produce analogous incompatible systems which are not exposed in F_1 , but only in later generations (hybrid breakdown). The analysis of such interactions can also help us to understand the development of isolating interactions. We also aimed to answer the question of whether male and female postzygotic isolation usually result from the action of different genes (Orr, 1989b).

Materials and methods

Flies

The *D. lummei* stock 1101S was started from several females collected at Överkalix, Sweden in 1970. In some experiments we also used another stock of *D. lummei*, 1143 from Hokkaido, Japan.

As a wild type *D. virilis*, we used the isofemale wild stock 1422 from Gröningen, The Netherlands, collected in 1976. The marker stock 126 (*b; gp; cd; pe*) was from the N. K. Koltzov, Institute of Developmental Biology, Moscow. The markers used and their map positions (Alexander, 1976) are broken (*b* 2-188.0), gapped (*gp* 3-118.0), cardinal (*cd* 4-32.0) and peach (*pe* 5-203.0). Another marker stock used here was MM (Majors Marked, *w; b; gp; cd; pe*), which was made from 126 and a white-eyed *D. virilis* strain (white, *w* 1-

105.0). The *D. virilis* marker stock 139 has the X chromosomal markers yellow (*y* 1-2.9) and apricot (*ap* 1-136.0). Both *w* and *ap* are within the area included in the inversion *In(1)a + b*, which is fixed in *D. lummei*. The breakpoints of the inversion *a* are Qb-c and Ye-f and those of *b* Nf-g and Vc-b in the cytological map of *D. virilis* produced by Hsu (Patterson & Stone, 1952, p. 439).

We also used the HETEX line, in which the females were heterozygous for the inversion *In(1)a + b* but otherwise pure *D. virilis*. The inversion is marked with the *w*⁺ allele: *In(1)a + b/w; b; gp; cd; pe*. The HETEX line was combined from *D. virilis* stock MM and a wild type *D. lummei* male (1101S), and maintained by continued backcrossing of *w*⁺/*w* females with MM. The females used in the backcrosses were from generations 35-41 and 52-55. Three replicate lines with a different origin of X^{lu} were also studied.

Scoring fertility

The flies were reared in glass bottles on a malt medium (Lakovaara, 1969) in continuous light at 19°C and 70 per cent relative humidity. After adult eclosion, they were aged for 20-26 days at 19°C or 14 days at 25°C under continuous illumination, to be sure that their possible reproductive diapause was terminated (Lumme & Keränen, 1978). They were then dissected and their internal genitalia inspected in 0.67 per cent NaCl. A male was scored as sterile if no sperm motility was seen, and the females were considered not to be sterile if they had at least one fully developed egg in their ovaries. These definitions of sterility are not strictly correct, but they serve for the sake of convenience. The proportion of sterile flies was used as a measure of the sterility of a genotype.

Statistical analysis

The results of segregating generations were analysed as a factorial experiment (Snedecor & Cochran, 1967). In backcross progeny, each of the four *D. virilis* autosomes can be either homozygous (in eight phenotypes of each sex) or heterozygous (in another eight phenotypes). The total numbers of fertile and sterile flies were counted for homozygotes and heterozygotes with respect to each chromosome and the difference between the weighted means was defined as the contribution of the respective chromosome and tested with the *t*-test. Interaction terms were calculated in an analogous way, first-degree interaction between chromosomes as a comparison of similar (both heterozygous or homozygous) versus different (one homozygous, the other heterozygous) sets of respective

chromosomes. The grouping of genotypes to calculate the interactions is presented by Lumme & Heikkinen (1990). This method artificially increases the contributions of chromosomes if the numbers of observations for the genotypes vary widely. We also tested the contributions by using unweighted means, which over-emphasize the chance variation in small samples. The results are essentially identical, however.

Student's *t*-test for a binomial distribution was used to test the statistical significance of the differences in the proportions of sterile flies in the factorial effect totals. The same test was used to compare pairs of results.

Results

Male sterility

Parental generation. The results are presented in Table 1. Out of the 2133 pure *D. virilis* males dissected, 2.3

Table 1 Proportions of males with immotile sperm and females with no eggs in the ovaries in stocks, F₁ and BC₁. Numbers of flies dissected are given in brackets. Crosses indicated with * are analysed in greater detail elsewhere

	Proportion sterile (%)	
	Females	Males
P 1422 (wild type <i>virilis</i>)	0 (143)	2.0 (152)
126 (<i>b; gp; cd; pe virilis</i>)	0 (100)	1.0 (100)
MM (<i>w; b; gp; cd; pe virilis</i>)	0.6 (167)	5.9 (153)
139 (<i>y ap virilis</i>)	0 (103)	0 (96)
1101S (wild type <i>lummei</i>)	2.4 (127)	0.8 (120)
1143 (wild type <i>lummei</i>)	3.7 (108)	11.2 (89)
F ₁ <i>vi</i> × <i>vi</i> 126 × 1422	0.8 (132)	0.7 (143)
<i>vi</i> × <i>lu</i> 126 × 1101S	0.6 (164)	3.2 (62)
<i>vi</i> × <i>lu</i> MM × 1101S	0 (249)	6.1 (132)
<i>vi</i> × <i>lu</i> 139 × 1101S	0 (115)	10.3 (107)
<i>vi</i> × <i>lu</i> 139 × 1143	0 (67)	0 (24)
<i>lu</i> × <i>vi</i> 1101S × 126	0 (140)	0 (142)
<i>lu</i> × <i>vi</i> 1101S × MM	3.2 (126)	0 (119)
Backcrosses		
<i>vi</i> × (<i>vi</i> × <i>vi</i>)*	3.6 (1490)	2.3 (1489)
<i>vi</i> × (<i>vi</i> × <i>lu</i>)*	8.9 (1208)	7.4 (937)
<i>vi</i> × (<i>lu</i> × <i>vi</i>)*	4.1 (1538)	3.7 (1448)
<i>lu</i> × (<i>vi</i> × <i>lu</i>)	0 (59)	0 (83)
<i>lu</i> × (<i>lu</i> × <i>vi</i>)	0 (269)	10.7 (214)
(<i>vi</i> × <i>lu</i>) × <i>vi</i> *	1.0 (396)	41.8 (385)
(<i>vi</i> × <i>lu</i>) × <i>lu</i> *	3.6 (278)	6.4 (392)
HETEX × (<i>vi</i> × <i>lu</i>)*	0.7 (413)	32.5 (332)
HETEX × (<i>lu</i> × <i>vi</i>)*	3.2 (1498)	29.0 (687)
<i>In(1)a + b</i> in <i>virilis</i> *		30.4 (395)

per cent had immotile sperm. This proportion holds good over all stocks and control crosses. In *D. lummei*, as many as 5.3 per cent of the 209 males dissected were sterile, most of them in the Japanese stock 1143.

First hybrid generation. In the first interspecific hybrid generation 6.5 per cent of the 325 sons of *D. virilis* mothers had immotile sperm (Table 1). No sterile males at all were found among the 261 reciprocals.

D. virilis × (*D. virilis* × *D. virilis*) (Cross 1 in Fig. 1). An intraspecific backcross *b; gp; cd; pe* × (*b; gp; cd; pe* × 1422) was made to check that no unexpected sterility was caused by novel combinations of recessive markers of *D. virilis* stock 126. The results for 16 phenotypes are displayed in Fig. 1. The total proportion of sterile males in the control backcross was 2.3 per cent ($N = 1489$), which is of the same order of magnitude as the proportion of sterile males in the *D. virilis* stocks.

D. virilis × (*D. virilis* × *D. lummei*) (Cross 2 in Fig. 1). The marker stock 126 of *D. virilis* was used: *b; gp; cd; pe* × (*b; gp; cd; pe* × 1101S). This cross should reveal the reasons for the 5 per cent sterility in F_1 (*D. virilis* × *D. lummei*), because the sex chromosomes and egg cytoplasm here were similar, and the major autosomes were either heterozygous *virilis/lummei* or homozygous *virilis* (16 combinations). The male progeny of this cross had the sex chromosome combination X^{vi}/Y^{lu} . Thus the males in the phenotype class +; +; +; + are identical to F_1 (*D. virilis* × *D. lummei*) males. The one exception is the unmarked tiny sixth (dot) chromosome, which can be either homozygous or hemizygous for *D. virilis*, or heterozygous, or even mosaic, due to the elimination of the sixth chromosome of *D. lummei* in hybrids (e.g. Sokolov, 1959; Mitrofanov & Sidorova, 1979).

The results are displayed in Fig. 1. The total proportion of sterile males in this backcross progeny was 7.4 per cent ($n = 937$).

The proportion of sterile males in the phenotype class +; +; +; + was 9.5 per cent ($n = 95$), which is a little higher than the value in the corresponding F_1 (3.2 per cent, $n = 62$), but not significantly so ($t = 1.51$, $P > 0.05$).

All the autosomal main effects were non-significant. The only significant factorial effects were the first-degree interactions 3×4 (negative) and 4×5 (positive sign). This was due to two aberrant phenotypes. The proportion of sterile flies was as high as 17 per cent ($n = 100$) when the third chromosome was heterozygous and the fourth and fifth were homozygous

(phenotypes *b; +; cd; pe* and +; +; *cd; pe*). Comparing this value with similar autosomal phenotypes in cross 3, it may be concluded that this sterility indicates the incompatibility of Y^{lu} and the heterozygous third chromosome with homozygous *D. virilis* chromosomes 4 and 5.

D. virilis × (*D. lummei* × *D. virilis*) (Cross 3 in Fig. 1). This backcross also employed the markers of 126: *b; gp; cd; pe* × (1101S × *b; gp; cd; pe*). The proportions of sterile males in each of the 16 phenotype classes are displayed in Fig. 1. The male progeny of this cross carried a conspecific sex chromosome set X^{vi}/Y^{vi} , the only difference with respect to the cross described above being the origin of the Y chromosome. The total proportion of the sterile males was lower than in the previous cross (3.7 per cent, $n = 1448$). The proportion of sterile individuals was highest in the phenotype class +; +; +; + (13 per cent, $n = 113$). This sterility was due to the heterozygous autosomes 2, 4, and 5 interacting with X^{vi}/Y^{vi} and/or the *D. virilis* egg cytoplasm.

Chromosomes 4 and 5 also showed significant non-additive interaction, but this seems to be an artefact caused by a scaling difficulty that affects data of this kind: because all the males in both phenotypes +; *pe* and *cd; +* were fertile, the phenotype *cd; pe* cannot do much better, and therefore, the main effects of the fourth and fifth chromosome are not 'additive'.

The results show which elements of *D. lummei*, when heterozygous, were incompatible with X^{vi} . They were located in the second, fourth and fifth autosome, and were more or less equally weak and additive in their effect.

D. lummei × (*D. virilis* × *D. lummei*). We had no useful markers for the backcross to *D. lummei* to analyse the autosomes, but no sterile cases were detected among the progeny of 1101S × (*b; gp; cd; pe* × 1101S) ($n = 83$). Here the sex chromosomes were conspecific with each other (X^{lu}/Y^{lu}), and also with those autosomes which were homozygous. As the F_1 (*D. lummei* × *D. virilis*) was completely fertile, no sterility was to be expected here. The result shows that heterozygous *D. virilis* autosomes are all compatible with *D. lummei* sex chromosomes separately and together.

D. lummei × (*D. lummei* × *D. virilis*). The males from the cross 1101S × (1101S × *b; gp; cd; pe*) had the sex chromosome combination X^{lu}/Y^{vi} but the maternal cytoplasm and autosomes were like those in the previous cross. The proportion of sterile individuals was 10.7 per cent ($n = 214$, tested against the cross above $t = 3.10$, $P < 0.01$). Because the sex chromo-

Fig. 1 Proportions of males with immotile sperm in the backcrosses (left) and the contributions of autosomes and their interactions to the sterility of hybrids (right). (1) Control cross *D. virilis* $\times$ (*D. virilis* $\times$ *D. virilis*) (2) *D. virilis* $\times$ (*D. virilis* $\times$ *D. lummei*) (3) *D. virilis* $\times$ (*D. lummei* $\times$ *D. virilis*) (4) HETEX $\times$ (*D. virilis* $\times$ *D. lummei*) (5) HETEX $\times$ (*D. lummei* $\times$ *D. virilis*). Bars below the number of the cross illustrate the sex chromosome combination. White bars denote for *D. virilis* and black bars *D. lummei*. Solid bars are X chromosomes, bars with extensions are Y chromosomes. Columns represent the differences between homozygous and heterozygous flies. Positive value: heterozygotes more sterile. For primary interactions, a positive value means that there is a smaller proportion of sterile flies in a class with similar genotypes in both chromosomes than if the genotypes are different. Significance levels of the contributions: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

somes were compatible in $F_1(D. lummei \times ? . virilis)$, the sterility seemed to be caused by an interaction between the Y of *D. virilis* and homozygous autosomes of *D. lummei*. This case cannot be analysed any further with respect to the autosomes, until markers are obtained in *D. lummei*.

(*D. virilis* $\times$ *D. lummei*) $\times$ *D. virilis*. The possible incompatibility of the X chromosome of *D. lummei* with homozygous autosomes from *D. virilis* was studied in the backcross progeny of F_1 females to *D. virilis*. This cross employed stock 139 of *D. virilis*, which has the X-chromosomal markers *y* (1-2.9) and *ap* (1-136). The overall mean proportion of sterile males from these crosses was high, 42 per cent. Detailed results are given in Table 2. The proportion of sterile males carrying the *D. lummei* inversion *In(1)a + b* (marked with *ap*⁺) was 72.6 per cent (pooled, $n = 201$). This high percentage indicates that *In(1)a + b* was incompatible with several homozygous *D. virilis* autosomes, because each of them can be expected to be homozygous in only half of the flies. It is also possible that *In(1)a + b* may be incompatible with egg cytoplasm produced by hybrid females.

As males hemizygous for the *D. virilis* gene arrangement (*ap*) showed only 7.2 per cent sterile individuals ($n = 180$), the main effect of the inversion is thus 65.4 per cent ($t = 12.93$, $P < 0.001$). The marker of the distal tip of the chromosome (*y*) was not linked to any sterility factor. The proportions of sterile males were

42.7 per cent ($n = 199$) and 40.9 per cent ($n = 186$) for the *y*⁺ and *y* phenotypes, respectively. The main effect of *y* is 1.8 per cent ($t = 0.36$, $0.9 > P > 0.05$). These values also show that there was no significant non-additive interaction between the distal tip of X and the area of *In(1)a + b*. The interaction term was calculated to be 5.1 per cent ($t = 1.01$, $0.4 > P > 0.2$).

(*D. virilis* $\times$ *D. lummei*) $\times$ *D. lummei*. *D. virilis* marker stock 139 *y ap* was used. This cross tested the interaction of parts of the *D. virilis* X with homozygous *D. lummei* autosomes. The results are presented in Table 2. The sample size was too small, but no extreme sterility interactions were indicated.

In(1)a + b of *D. lummei* in a *D. virilis* genetic background. The above crosses showed that the part of *D. lummei* X, including the *ap* locus, is incompatible with homozygous *D. virilis* autosomes. It is known from unpublished experiments that *w* (location 1-105) and *ap* (1-136) do not recombine in the hybrid X chromosome, so that they are probably included within the *In(1)a + b* that separates the species. This fragment of the X chromosome of *D. lummei* was introduced into an otherwise *D. virilis* genetic background through repeated backcrossing to marker stock MM and maintained in heterozygous form in females. After 50 generations of backcrossing, we ascertained from salivary gland chromosomes that the females of these lines still carried the inversion *In(1)a + b w*⁺ of *D. lummei*.

Table 2 Proportions of sterile males and females in F_1 and different sex chromosome recombinant classes. Numbers of flies studied are given in brackets. *y ap* are the visible markers on the X chromosome in *D. virilis* stock 139 and (+ +) for wild type stocks of *D. lummei*, 1101S and 1143

Cross and phenotype	<i>D. lummei</i> stock			
	1101S		1143	
	Females	Males	Females	Males
<i>y ap</i> $\times$ + +	0 (115)	10.3 (107)	0 (67)	0 (24)
(<i>y ap</i> $\times$ + +) $\times$ <i>y ap</i>				
(+ +)	2.2 (45)	67.6 (37)	0 (76)	79.1 (67)
(<i>y</i> +)	6.1 (33)	62.1 (29)	0 (65)	72.4 (72)
(+ <i>ap</i>)	0 (31)	6.9 (29)	0 (56)	7.6 (66)
(<i>y ap</i>)	3.8 (26)	12.5 (16)	0 (62)	5.8 (69)
(<i>y ap</i> $\times$ + +) $\times$ + +				
(+ +)	0 (200)	6.7 (119)	12.8 (78)	7.1 (14)
(<i>y</i> +)		5.9 (102)		18.8 (16)
(+ <i>ap</i>)		5.3 (76)		11.8 (17)
(<i>y ap</i>)		3.0 (33)		0 (15)

An attempt was made to make the lines pure breeding. This succeeded in two lines only, because w^+ males are subvital and frequently sterile.

The proportions of sterile males among the inversion hemizygotes are presented in Table 3. On average, 30.4 per cent of the 395 males carrying $In(1)a + b$ were sterile, while 3.4 per cent of their 387 white-eyed brothers were sterile. The lines differ greatly, e.g. in that the two lines founded from stock 1101S had 81.5 per cent ($n=108$) and 5.9 per cent ($n=51$) sterile males respectively. The reason for this variability remains unknown, but one possibility is recombination between the $In(1)a + b$ and sterility factors.

The sterility may be due to an interaction either within the X chromosome, or between $In(1)a + b$ and homozygous autosomes of *D. virilis*. This is analysed in the following.

HETEX × (*D. virilis* × *D. lummei*) (Cross 4 in Fig. 1). The markers of the MM stock were used in the cross: $In(1)a + b/w; b; gp; cd; pe \times (w; b; gp; cd; pe \times 1101S)$. In the progeny, the heterologous X chromosome was combined with all 16 major autosome combinations (and Y chromosomes of both species, next cross).

Half of the male progeny presumably received intact X^{vi} , marked with w (not shown in Fig. 1), but the proportion of sterile individuals among them was 21.0 per cent ($n=181$, crosses 4 and 5 pooled). As they had the w allele masking the eye colour markers of the fourth and fifth chromosomes, not all 16 phenotypes were testable. Wing vein markers of the second and third chromosomes were inspected in a very small sample. The results from crosses 4 and 5 were pooled. The proportions of sterile males were $w; +; +$ 22.7 per cent ($n=22$), $w; b; +$ 13.3 per cent ($n=30$), $w; +; gp$ 22.2 per cent ($n=36$), and $w; b; gp$ 2.1 per cent

($n=47$). These values should be compared with results from cross 2, where the sex chromosome set is the same X^{vi}/Y^{lu} , but X is without a marker (Fig. 1). The proportions of sterile males were $+; +; +$ 10.3 per cent ($n=263$, compared with the above results $t=1.77$, $P<0.05$), $+; b; +$ 6.4 per cent ($n=236$, $t=1.38$, $P>0.05$), $+; +; gp$ 5.2 per cent ($n=210$, $t=3.35$, $P<0.001$), and $+; b; gp$ 7.0 per cent ($n=228$, $t=1.27$, $P>0.05$). The high proportion of sterile individuals among the HETEX progenies carrying X^{vi} probably indicates that the heterozygous X of these females is the cause of sterility in the sons, as a maternal effect. It is also possible that there may be genetic variability in the content of X.

In cross 4 (Fig. 1), the sex chromosome combination of the w^+ male progeny is written as $X^{vi} In(1)a + b^{lu}/Y^{lu}$, and the total proportion of sterile males was 32.5 per cent ($n=332$, unweighted mean over phenotypes 34.7 per cent). This value is clearly higher than in any of the first generation hybrid progeny. In backcross 2 the whole X was from *D. virilis* and the proportion of sterile males was only 7.4 per cent ($n=937$). In F_1 between *D. lummei* females and *D. virilis* males, the whole X was from *D. lummei*, and the males were perfectly fertile. Here the cytoplasm was made by the homozygous hybrid X chromosome $X^{vi} In(1)a + b^{lu}$, which may explain the higher proportions of sterile cases in both backcrosses to HETEX than in the backcrosses to *D. virilis*.

The frequency of sterile cases was, in crosses using the markers y and ap , much higher among the carriers of $In(1)a + b$ than the mean observed here (Table 2). One explanation could be that in the crosses, ($y ap \times + +$) $\times + +$, the whole X of the hybrid mothers was heterozygous, not only $In(1)a + b$, and therefore a larger proportion of the sons was sterile, due to interaction between the maternal effect and the X chromo-

Table 3 Proportions of sterile flies in inversion substitution lines. The lines are named after the *D. lummei* stock used as the source of $In(1)a + b$ (HETEX is from stock 1101). The numbers of flies studied are given in brackets

Line	Eye w^+		Eye w	
	Females	Males	Females	Males
HETEX heterozygous	0 (158)	81.5 (108)	4.6 (109)	0 (103)
1101*				
Heterozygous	0 (106)	5.9 (51)	5.6 (108)	1.0 (101)
Pure breeding	0 (187)	2.9 (35)		
1100				
Heterozygous	4.3 (47)	0 (18)	4.9 (61)	3.8 (80)
Pure breeding	2.9 (204)	17.3 (104)		
1uJapFu heterozygous	0 (103)	12.7 (79)	1.9 (107)	8.7 (103)

some of the sons. Recombination within autosomes could also explain the higher proportion of sterile progeny in this cross.

HETEX × (*D. lummei* × *D. virilis*) (Cross 5 in Fig. 1). The markers in this cross were *In(1)a + b/w*; *b*; *gp*; *cd*; *pe* × (1101S × *w*; *b*; *gp*; *cd*; *pe*). The only difference from the previous cross was the X^{vi} of the males. Of the progeny of this cross, the white-eyed phenotype with intact X^{vi} has already been discussed above. The total proportion of males with heterospecific X that was sterile was 29.0 per cent ($n=687$, unweighted mean over phenotypes 28.4 per cent).

Males in phenotype class +; +; +; + were chromosomally identical to F_1 males from the cross *D. lummei* × *D. virilis* with the exception of a heterogenic X chromosome, the tip of which now came from *D. virilis*. In F_1 (*D. lummei* × *D. virilis*) progeny all the males had motile sperm (Table 1). In the backcross phenotype class +; +; +; +, the proportion of sterile males was 31 per cent ($n=51$), a higher figure than in the corresponding F_1 and comparable to the +; +; +; + males from cross 4: 32 per cent ($n=31$). The Y chromosome seemed to have no effect at all in the presence of all-heterozygous autosomes. The main effect of the fourth chromosome was significant and very large, which indicates that this chromosome of *D. virilis*, when homozygous, did not work well with *In(1)a + b*. The effect of the fifth chromosome seems to be the opposite.

Hybrid cytoplasm made by combined X chromosomes probably played the same role here as in the previous cross, maternally inducing sterility in the sons.

Female sterility

Parental generation and first generation reciprocal hybrids. The females of the first hybrid generation were almost 100 per cent fertile, and the heterospecific X chromosomes were compatible. Reciprocals were identical, and there was no maternally determined female sterility.

Table 1 shows that some sterile females were observed in later generations, even though the fractions always remained much lower than in the males. The details of the segregating generations are analysed in the following.

D. virilis × (*D. virilis* × *D. virilis*) (Cross 1 in Fig. 2). The proportion of sterile females in the control backcross was surprisingly high (3.6 per cent, $n=1490$). The results are displayed in Fig. 2.

Among the different phenotypes, *peach* (fifth chromosome marker) homozygotes were sterile slightly

less often than heterozygotes on an otherwise heterozygous background. The second chromosome homozygotes (*b*) were *not* any more often sterile than the heterozygotes, as appears in hybrid crosses 2, 3, and 5.

D. virilis × (*D. virilis* × *D. lummei*) (Cross 2 in Fig. 2). The females had homozygous sex chromosomes X^{vi}/X^{vi} , which were slightly incompatible with the heterozygous fourth and fifth chromosomes. The mean proportions of sterile females in different fourth-fifth chromosome phenotypes were as follows: in phenotype +; + 17.1 per cent ($n=351$), +; *cd* 9.5 per cent ($n=304$), +; *pe* 5.1 per cent ($n=255$), and *cd*; *pe* 1.7 per cent ($n=298$). All steps are statistically significant ($t=2.83$, $P<0.01$, $t=1.97$, $P<0.05$, $t=2.58$, $P<0.05$, respectively).

Females with the second chromosome homozygous *D. virilis*, more often had undeveloped ovaries than the second chromosome heterozygotes (13.9 per cent, $n=564$ versus 6.3 per cent, $n=644$, $t=4.41$, $P<0.001$). This seemed to be due to incompatibility between *D. virilis* chromosome 2 and heterozygous *D. lummei* autosomes, especially the fifth chromosome. The 2 × 5 interaction pointed in this direction but was not significant.

D. virilis × (*D. lummei* × *D. virilis*) (Cross 3 in Fig. 2). These females were heterozygous for the X chromosome. The flies of the phenotype class +; +; +; + were chromosomally identical to both reciprocal F_1 females with the exception of the dot chromosome. The proportion of sterile females was 4.6 per cent ($n=151$), higher than the mean in F_1 (0.4 per cent, $n=304$, $t=3.18$, $P<0.01$).

In this cross, too, the females homozygous for the *D. virilis* second chromosome were significantly more often sterile than the heterozygotes. The other main effects and first-degree interactions were all non-significant. The difference between cross 2 and this cross was that the heterozygous chromosomes 4 and 5 had no negative effects on ovarian development in this case. Their effects in the previous cross thus indicated interaction with homozygous X^{vi} .

D. lummei × (*D. virilis* × *D. lummei*). All daughters had perfect ovaries when F_1 males were backcrossed to *D. lummei*, despite the X chromosome combination. Here it was X^{lu}/X^{vi} , and all 59 females inspected had eggs.

D. lummei × (*D. lummei* × *D. virilis*). These females were homozygous for sex chromosomes (X^{lu}/X^{lu}), and all 269 of them had eggs.

Fig. 2 Proportions of females with no eggs in their ovaries. Other explanations as in Fig. 1.

(*D. virilis* × *D. lummei*) × *D. virilis*. The *D. virilis* marker stock 139 y *ap*, and two wild-type stocks of *D. lummei* (1101S and 1143) were used in this cross. When the F_1 females were backcrossed with *D. virilis* marker stock males, four recognizable classes of X chromosome recombinants were obtained (Table 2). The progeny of 1143 grandfathers had all females fertile. Among the hybrid granddaughters of 1101S, the + *ap* females were all fertile, but the proportions of sterile individuals were also small in the other three phenotype classes.

(*D. virilis* × *D. lummei*) × *D. lummei*. The *D. virilis* used was 139 y *ap*, but because the *D. lummei* males were used in backcrosses, the markers were useless in the females (Table 2). With 1101S, all 200 females dissected had eggs in their ovaries but with 1143, 14 per cent were sterile ($n = 78$).

In(1)a + b of *D. lummei* in a *D. virilis* background. We also analysed the proportion of sterile females in six substitution lines which had *D. lummei* inversion, either homozygous or heterozygous (Table 3). In the lines originating from stock 1100 (Kuopio, Finland), a small proportion of the w^+ females were sterile among both the heterozygous and homozygous classes, but all the other females had eggs. The weighted average of sterile females was 1.0 per cent ($n = 805$). There were more steriles among the w females in the heterozygous inversion lines, 4.2 per cent ($n = 385$), which was slightly more than in the *D. virilis* stock MM used in the continuous backcrossing (0.6 per cent, $n = 167$).

HETEX × (*D. virilis* × *D. lummei*) (Cross 4 in Fig. 2). The markers of this cross were: *In(1)a + b/w*; *b*; *gp*; *cd*; *pe* × (*w*; *b*; *gp*; *cd*; *pe* × 1101S). In strong contrast to the males, the heterospecific X chromosome caused no sterility in the females (Table 1, Fig. 2).

The weak sterility of X^{lu} against the homozygous second chromosome of *D. virilis*, apparent in backcross 3, had disappeared together with the distal tip of X^{lu} . This was also obvious in the heterozygous inversion substitution lines (Table 3).

HETEX × (*D. lummei* × *D. virilis*) (Cross 5 in Fig. 2). The markers were used as follows: *In(1)a + b/w*; *b*; *gp*; *cd*; *pe* × (1101S × *w*; *b*; *gp*; *cd*; *pe*). The females had either X^{lu}/X^{vi} or X^{vi}/X^{lu} *In(X)a + b^{lu}/X^{lu}*, both combinations being expected to exist in equal frequencies. Thus the progeny was a mixture of flies identical to those from cross 3 and a new combination. The total proportion of sterile females in the backcross progeny was 3.2 per cent, the highest columns being the same as in cross 3. There was slight incompatibility of the distal of the

D. lummei X chromosome with homozygous *D. virilis* chromosomes 2 and 3, and this was made worse by the presence of a heterozygous chromosome 4. Chromosomes 3 and 4 also had significant non-additive interaction. When chromosome 3 was homozygous for *D. virilis* and chromosome 4 heterozygous, 9.6 per cent of the females were sterile ($n = 335$).

Discussion

This work is a continuation of our investigations into the genetic systems involved in the isolation between *Drosophila virilis* and *D. lummei*, and deals with hybrid sterility. Earlier analyses have focused on the male courtship sound (Hoikkala & Lumme, 1984), cold shock resistance (Heino & Lumme, 1989), viability (Lumme & Heikkinen, 1990) and developmental anomalies in hybrids (Heikkinen, 1991).

Male sterility

Incompatibility between X chromosome and autosomes. The isolating effect of F_1 male sterility proved to be relatively weak in crossings of *D. virilis* females to *D. lummei* males. The proportion of sterile males in the reciprocal cross was invariably zero. It may be recalled that the relative viability of F_1 progenies is much more decreased, especially in the progenies of *D. lummei* females (Lumme & Heikkinen, 1990).

The second generation analysis indicated that the sterility of the F_1 males from the cross of *D. virilis* females with *D. lummei* males was caused by negative interaction of X^{vi} with the second, fourth and fifth chromosomes of *D. lummei*. The autosome effect was dominant, being expressed when they were heterozygous (Fig. 1, cross 3).

Orr & Coyne (1989) demonstrated that the incompatibility between the X chromosome and autosomes is the genetic reason for hybrid sterility in other pairs of species in the *virilis* group of *Drosophila*, and also in the present pair. They also demonstrated that the reason for this sterility was in the X chromosome of *D. virilis* and was 'fairly tightly linked to the *white* locus' (Orr & Coyne, 1989). Our results are in agreement with this. Cytological examination of the chromosome substitution lines confirmed that w^+ is a marker of the inversion *In(1)a + b*.

In addition to the F_1 male sterility system, the second generation data revealed that the X chromosomal inversion *In(1)a + b* from *D. lummei* was at least linked with a factor incompatible with homozygous autosomes from *D. virilis*. This sterility is not expressed in F_1 because the autosomal effects of *D. virilis* chromosomes are recessive. Of the autosomes, fourth seemed

to have the strongest effect but the analysis was not very clear (Fig. 1, crosses 4 and 5). The analysis was disturbed by the low viability of the male carriers of *In(1)a + b* (Lumme & Heikkinen, 1990).

Thus the X chromosomes, and especially the region of the *In(1)a + b* in both species, are incompatible with the autosomes of the opposite species. It is important to note that the asymmetry in F_1 (difference in reciprocals) is caused by the recessivity or dominance of the autosomal reaction. Autosomes of *D. lummei* showed a dominant reaction to X^{vi} , while autosomes of *D. virilis* cause sterility recessively with X^{lu} .

There are other examples of X-autosome incompatibility among *Drosophila*. Zouros *et al.* (1988) have demonstrated that the X of *D. arizonensis* is incompatible with autosomes of *D. mojavensis*, while Naveira & Fontdevila (1986), who studied the hybrids between *D. buzzatii* and *D. serido*, found that even the smallest segments of the X chromosome of *D. serido* caused male sterility when introgressed into the *D. buzzatii* genomic background. The autosomes had a size-dependent threshold effect.

Y-autosome sterility. Y^{lu} participated in a sterility system which involved the fourth and fifth chromosomes of *D. virilis* in homozygous condition and the third chromosome as heterozygous (Fig. 1, crosses 2 and 3). Seventeen per cent of the males from that combination were sterile. Vigneault & Zouros (1986) showed that in hybrids between *D. mojavensis baja* and *D. arizonensis*, the Y chromosome of the latter species is incompatible with homozygous *D. mojavensis* autosomes 3 and 4. Pantazidis & Zouros (1988) located the recessive autosomal factor, which was incompatible with the Y of *D. arizonensis*, to the fourth chromosome of *D. mojavensis*.

There was no evidence of incompatibility between X and Y chromosomes, which is one of the most common reasons for male sterility in the well analysed cases of *Drosophila* (Lancefield, 1929; Coyne, 1985; Coyne & Charlesworth, 1986; Orr, 1987, 1989a, 1989b).

Autosome/autosome interactions. We could not demonstrate any obvious negative interactions between autosomes alone in our data, although autosomes participated on both sides of the system including Y, as described above. No heterospecific homozygous combinations were produced in our crossing scheme. Schaefer (1978) who studied hybrids between *D. hydei* and *D. neohydei*, found females heterozygous for chromosome 3 and homozygous for *D. hydei* chromosome 4 to be completely sterile regardless of their sex chromosome combination.

There are some differences between our results and those of Orr & Coyne (1989) with regard to hybrid sterility in the virilis group. Backcrossing of *D. virilis* males to F_1 females gave a mean of 41 per cent sterile progeny in our experiments whereas Orr & Coyne (1989) report such males to be 95 per cent fertile. There is a possibility that there may be segregating sterility factors in these species.

Another difference between our results and those of Orr & Coyne is that according to them, 'within the virilis phylad, the autosomes have little or no effect on hybrid male fertility when heterozygous'. As we have shown, at least in hybrid male sterility between *D. virilis* and *D. lummei*, heterozygous autosomes have to play an interactive role with the X chromosome, and their total effect on male sterility is then exactly equal to the effect of X.

Female sterility

The present flies were reared in continuous light, because it is known that *D. lummei* has a photoperiodic reproductive diapause. This means that the female ovaries remain undeveloped under short-day conditions. The main difference between *D. virilis* and *D. lummei* is inherited in the X chromosome, linked with *white* within the *In(1)a + b* (Lumme & Keränen, 1978). We excluded the photoperiodic diapause here by the use of continuous light and a relatively high temperature. It is to be noted that Orr & Coyne (1989) do not mention the daylength of their experiments, and the possibility remains that some of the X-linked female sterility may be attributed to the diapause.

X chromosome/autosome incompatibility. It was obvious that heterozygous *D. lummei* autosomes 4 and 5 interacted negatively with the homozygous *D. virilis* X chromosome, causing sterility in some individuals (Fig. 2, cross 2). The effect of autosomes was fairly small, and additive. Orr & Coyne (1989) claim that females with homospecific X chromosomes are always fertile but our results differ slightly from this, possibly due to segregating sterility factors in the species.

The *D. lummei* X chromosome, when homozygous, was compatible with heterozygous autosomes, no sterile cases at all being detected among such progeny.

In conclusion, it may be said that no significant female sterility was detected here, even though we exposed some unusual combinations of chromosomes. It must be remembered that the criterion for sterility was simply the morphological development of ovaries. Some of the backcross female phenotypes homozygous for the *D. virilis* second chromosome are almost completely sterile, or 'daughterless', however (Mitrofanov

& Sidorova, 1981), we do not yet know whether this is due to the inviability of the progeny or to some other reason.

The results emphasize the central role of the X chromosome in hybrid male sterility. Only very weak female sterility was found but it bore many similarities to the male sterility systems: e.g. asymmetry and X-autosome interaction.

Acknowledgements

We would like to thank J. Coyne for instructive comments on the manuscript. This work was supported by the Natural Science Research Council (Project 1051049).

References

- ALEXANDER, M. L. 1976. The genetics of *Drosophila virilis*. In: Ashburner, M. and Novitski, E. (eds), *The Genetics and Biology of Drosophila*, Vol. 1c, Academic Press, London, pp. 1365–1427.
- COYNE, J. A. 1985. The genetic basis of Haldane's rule. *Nature*, **314**, 736–738.
- COYNE, J. A. AND CHARLESWORTH, B. 1986. The location of an X-linked factor causing sterility in male hybrids of *Drosophila simulans* and *D. mauritiana*. *Heredity*, **57**, 243–246.
- COYNE, J. A. AND ORR, H. A. 1989. Patterns of speciation in *Drosophila*. *Evolution*, **43**, 362–381.
- HALDANE, J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. *J. Genet.*, **12**, 101–109.
- HEIKKINEN, E. 1991. Wrinkling of the eye in hybrids between *Drosophila virilis* and *Drosophila lummei* is caused by interaction of material and zygotic genes. *Heredity*, **66**, 357–365.
- HEINO, R. AND LUMME, J. 1989. Inheritance of cold shock tolerance in hybrids of *Drosophila virilis* and *Drosophila lummei*. *Genetica*, **79**, 17–25.
- HOIKKALA, A. AND LUMME, J. 1984. Genetic control of the difference in male courtship sound between *Drosophila virilis* and *D. lummei*. *Behav. Genet.*, **14**, 257–268.
- LAKOVAARA, S. 1969. Malt as a culture medium for *Drosophila* species. *Drosoph. Inf. Serv.*, **44**, 128.
- LANCEFIELD, D. E. 1929. A genetic study of crosses of two races or physiological species of *Drosophila obscura*. *Z. Indukt. Abstamm. Vererbungsl.*, **52**, 287–317.
- LUMME, J. AND HEIKKINEN, E. 1990. Viability of first and second generation hybrids of *Drosophila virilis* and *Drosophila lummei*. *Heredity*, **00**, 000–000.
- LUMME, J. AND KERÄNEN, L. 1978. Photoperiodic diapause in *Drosophila lummei* Hackman is controlled by an X-chromosomal factor. *Hereditas*, **89**, 261–262.
- MITROFANOV, V. G. AND SIDOROVA, N. V. 1979. Phenogenetic analysis of chromosome behavior during mitosis in female *Drosophila virilis* × male *Drosophila lummei* hybrids. *Genetika*, **15**, 1221–1227. In Russian with an abstract in English.
- MITROFANOV, V. G. AND SIDOROVA, N. V. 1981. Genetics of the sex ratio anomaly in *Drosophila* hybrids of the virilis group. *Theor. Appl. Genet.*, **59**, 17–22.
- NAVEIRA, H. AND FONTDEVILA, A. 1986. The evolutionary history of *Drosophila buzzatii*. XII. The genetic basis of sterility in hybrids between *D. buzzatii* and its sibling *D. serido* from Argentina. *Genetics*, **114**, 841–857.
- ORR, H. A. 1987. Genetics of male and female sterility in hybrids of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics*, **116**, 555–563.
- ORR, H. A. 1989a. Genetics of sterility in hybrids between two subspecies of *Drosophila*. *Evolution*, **43**, 180–189.
- ORR, H. A. 1989b. Localization of genes causing postzygotic isolation in two hybridizations involving *Drosophila pseudoobscura*. *Heredity*, **63**, 231–237.
- ORR, H. A. AND COYNE, J. A. 1989. The genetic basis of post-zygotic isolation in the *Drosophila virilis* group. *Genetics*, **121**, 527–537.
- PANTAZIDIS, A. C. AND ZOUROS, E. 1988. Location of an autosomal factor causing sterility in *Drosophila mojavensis* males carrying the *Drosophila arizonensis* Y chromosome. *Heredity*, **60**, 299–304.
- PATTERSON, J. T. AND STONE, W. S. 1952. *Evolution in the Genus Drosophila*. The Macmillan Co., New York.
- SCHAEFER, U. 1978. Sterility in *Drosophila hydei* × *D. neohydei* hybrids. *Genetica*, **49**, 205–214.
- SNEDECOR, G. W. AND COCHRAN, W. G. 1967. *Statistical Methods*. The Iowa State University Press, Ames, Iowa.
- SOKOLOV, N. N. (1959) *Interaction of Nucleus and Cytoplasm in Remote Hybridizations of Animals*. Publishing House of the Academy of Sciences, USSR, Moscow (in Russian).
- VIGNEAULT, G. AND ZOUROS, E. 1986. The genetics of asymmetrical male sterility in *Drosophila mojavensis* and *Drosophila arizonensis* hybrids: interaction between the Y-chromosome and autosomes. *Evolution*, **40**, 1160–1170.
- ZOUROS, E., LOFDAHL, K. AND MARTIN, P. A. 1988. Male hybrid sterility in *Drosophila*: interactions between autosomes and sex chromosomes in crosses of *D. mojavensis* and *D. arizonensis*. *Evolution*, **42**, 1321–1331.