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Viability of first and second generation hybrids of *Drosophila virilis* and *Drosophila lummei*

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F_1 hybrids of *D. virilis* and *D. lummei* survived significantly worse than the parents. When *D. virilis* was the mother, larger proportion of hybrids failed to emerge from pupal case, but overall survival rate of reciprocals was about the same. Viability of different heterospecific combinations of sex chromosomes and autosomes was studied in backcrosses. All the sex chromosomes survived well on the background of all-heterozygous autosomes. Hemizygous X was reciprocally subvital with homozygous alien autosomes. The reduced viability was male sex limited. The X-linked factor causing this effect was localized in the area of the large, phylogenetically ancient double inversion *In*(1)*a* + *b*. All four large autosomes were heterotic in backcross hybrids, more strongly in females. Five out of six possible first degree interactions between the autosomes were negative, indicating incompatibility between heterospecific autosomes. This effect was stronger in females. The genetic basis for hybrid subvitality is sex-specific.

INTRODUCTION

Species hybrids are often less viable than pure species. As a metaphor, this is said to be due to the breakdown of co-adapted genetic system in hybridization. Subvitality of hybrids has been under genetic analysis in very few cases, much more seldom than hybrid sterility (Coyne and Orr, 1989*a*, 1989*b*). An important exception is the case of *Drosophila melanogaster* and its siblings. Among them, the viability of hybrids can be rescued by some mutants. The gene *Lhr* (*L*ethal *h*ybrid *r*escue) was detected by Watanable (1979) in *Drosophila simulans*. Another mutant, *Hmr* (*H*ybrid *m*ale *r*escue) was found by Hutter and Ashburner (1987) in *Drosophila melanogaster*. The results of the further analysis are very important with respect to the understanding of the mechanisms of speciation in general (Hutter *et al.*, 1990). In the species group observed in this paper, Patterson and Griffen (1944) studied in detail the inviability of female progeny from the cross of *D. montana* females to *D. texana* males. A gene within *echinus–crossveinless* interval of the *texana* X chromosome was lethal in combination with the *montana* egg "protoplasm". Several other cases of hybrid lethality described in older literature were reviewed in Patterson and Stone (1952).

As a part of our investigations on the genetic differentiation of two allopatric sibling species *Drosophila virilis* and *Drosophila lummei*, we present here an analysis of the viability of different heterospecific combinations of sex chromosomes, and autosomes.

The model of speciation applicable to the present pair of taxa is through geographic isolation. Throckmorton (1982) presented the still valid phylogenic and biogeographical scheme for their separation. The geographical isolation continues: *D. lummei* shows a palaearctic boreal distribution, with suitable adaptations like better cold resistance (Heino and Lumme, 1989) and photoperiodic diapause (Lumme and Keranen, 1978). *D. virilis* has a more southern holarctic distribution, and it comes to the areas common with *D. lummei* only as aided by man.

The isolation of the taxa in question, expressed as subvitality of different heterospecific genotypes is rather weak. This fact was well known to us before beginning. The idea of studying weak isolation can be founded as follows. When the fertility and viability of F_1 hybrids is good, a comprehensive genetic analysis is possible. As a disadvantage

We dedicate this paper to Academician Esko Suomalainen, on the occasion of his 80th birthday.

there is not much to be analyzed. However, we believe that we may find such incompatibilities between the genetic components, which are not expressed in F_1, but in later generations. For example, incompatibility between an alien X chromosome and a homozygous autosome is not visible in F_1. Furthermore, it is reasonable to believe that genetic incompatibilities leading to postzygotic isolation accumulate with time, also after the completion of species formation. In the hybrid, they all interact, and details of the syndrome become more and more difficult to grasp when the degree of isolation, *i.e.*, the number of incompatible interacting systems increases. It is to be expected that the genetically controlled traits which express themselves as disadvantageous (subvitality, lethality, sterility) in hybrids, have some positive role in pure species. When the disadvantage is weak, it is perhaps possible to find out the normal function of the respective genes.

Among the virilis species group, the hybrids of *D. virilis* and *D. lummei* are the most viable and fertile of all. Yet, their relatedness is not so close. The phylogenetic tree of the virilis group has been constructed on the basis of salivary gland chromosomes (Throckmorton, 1982) and confirmed by the biochemical techniques (MacIntyre amd Collier, 1986; Coyne and Orr, 1989*a*). In the tree, the diversification of *D. virilis* from the common ancestor of *D. americana*, *D. novamexicana* and *D. lummei* is ancient. The other two species have developed considerable postzygotic isolation towards *D. virilis*. What is different in *D. lummei*?

MATERIAL AND METHODS

Fly stocks and crosses

We used in most experiments *Drosophila virilis* marker stocks 126 (*b*; *gp*; *cd*; *pe*) and MM (Majors Marked, *w*; *b*; *gp*; *cd*; *pe*). The stock 126 is from The Institute of Developmental Biology, USSR Academy of Sciences, Moscow. Stock NEW also contains *b*; *gp*; *cd*; *pe*. It is actually 126 refreshed through crossing with wild type 1422. MM was made through introduction of *w* from a stock *Bx w* into 126. Also 139 (*y ap*) was used in one experiment.

The recombination map positions of markers used in this work are as follows: *yellow* 1-3, *apricot* 1-136, *white*, 1-105, *broken* 2-188, *gapped* 3-118, *cardinal* 4-32, and *peach* 5-203 (Alexander, 1976). All large chromosomes are thus recessively marked. Dot chromosome is very small, comprising 0·1 per cent of the length of the recombination map. *D. virilis* stock 1422 from Groeningen, The Netherlands was used as a wild type, as well as stock Batumi *A* from Batumi, Georgia, U.S.S.R.

To represent *Drosophila lummei*, we used stock number 1101S, wild type, originating from Overkalix, northern Sweden, and stock number 1143, also wild type, from Hokkaido, Japan. Two other stocks, 1100 (Kuopio, Finland) and luJapFu (Hokkaido, Japan) served as donors of one X chromosomal inversion.

First generation hybrids were obtained reciprocally between 126, MM, and 1101S. To produce novel combinations of the genetic elements, F_1 males were crossed back to females of marker strain *D. virilis*. This always produced 16 combinations of heterozygous or homozygous autosomes, together with sex chromosome set depending on the direction of cross in P generation. Through repeated backcrossing to MM stock, the inversion *In*(1)*a* + *b* from *D. lummei* was introduced into *D virilis* chromosome. The inversion contains wild type allele of *white*, and it was maintained in heterozygous condition in females. Such heterozygous females were crossed with F_1 males to construct flies having a part of X chromosome from *D. lummei*, and autosomes either heterozygous, or homozygous for *D. virilis*. The role of X chromosome was further studied through crossing the *y ap* stock with *D. lummei*, and backcrossing the F_1 females to both parental species.

Survival from egg to adult

To measure the survival of parental stocks and hybrids, two females and four males were put in plastic vials on 5 ml of malt medium (Lakovaara, 1969). They were allowed to lay eggs 24 hours at 25°C. The eggs laid were counted. We did not make any estimates of the proportion of unfertilized eggs. The vials with developing larvae were kept at 17°C or 25°C, and the emerging adults were counted. When the eclosion had been ceased for two days, the number of pupal cases was counted. Mouldy, bacterially infected, or dried tubes were discarded.

Statistical methods

The contribution of chromosomes and their interactions to the survival of different phenotypes in backcross generations were calculated through a modification of analysis of factorial experiments (Snedecor and Cochran, 1967). The grouping of data for the analysis is presented in table 1, because it may help to understand figs 2 and 4 in the Results.

Table 1 Grouping of phenotype N values used to sum up the factorial effect totals, for the main effects and interactions between the chromosomes (expanded from Snedecor and Cochran, 1967; 6th edn, p. 360). N values are summed into groups a and b, and the factorial effect total (=difference between means) is $\Sigma_a - \Sigma_b$. Statistical tests are conducted between groups a and b. If the sex is added, the table would be four times as large

Chromosome	Phenotype Marker: homozygous *virilis* wild: heterozygous *virilis*/*lummei*																
2nd	+	b	+	b	+	b	+	b	+	b	+	b	+	b	+	b	
3rd	+	+	gp	gp	+	+	gp	gp	+	+	gp	gp	+	+	gp	gp	
4th	+	+	+	+	cd	cd	cd	cd	+	+	+	+	cd	cd	cd	cd	
5th	+	+	+	+	+	+	+	+	pe	pe	pe	pe	pe	pe	pe	pe	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	2nd
	a	a	b	b	a	a	b	b	a	a	b	b	a	a	b	b	3rd
	a	a	a	a	b	b	b	b	a	a	a	a	b	b	b	b	4th
	a	a	a	a	a	a	a	a	b	b	b	b	b	b	b	b	5th
	a	b	b	a	a	b	b	a	a	b	b	a	a	b	b	a	2×3
	a	b	a	b	b	a	b	a	a	b	a	b	b	a	b	a	2×4
	a	b	a	b	a	b	a	b	b	a	b	a	b	a	b	a	2×5
	a	a	b	b	b	b	a	a	a	a	b	b	b	b	a	a	3×4
	a	a	b	b	a	a	b	b	b	b	a	a	b	b	a	a	3×5
	a	a	a	a	b	b	b	b	b	b	b	b	a	a	a	a	4×5
	a	b	b	a	b	a	a	b	a	b	b	a	b	a	b	a	2×3×4
	a	b	b	a	a	b	b	a	b	a	a	b	b	a	b	a	2×3×5
	a	b	a	b	b	a	b	a	b	a	b	a	a	b	a	b	2×4×5
	a	a	b	b	b	b	a	a	b	b	a	a	a	a	b	b	3×4×5
	a	b	b	a	b	a	a	b	b	a	a	b	a	b	b	a	2×3×4×5

As a statistical test, we have used G-test for comparing observed and expected segregation ratios.

RESULTS

Survival from egg to pupariation and to adult in P and F_1

In average, the mortality of the eggs of *D. virilis* at 25°C before pupariation was 22·8 per cent (N = 3047), and the mortality within pupal case was 5·7 per cent (N = 2351). At 17°C, the mortality values were 30·8 per cent (N = 1928) and 5·8 per cent (N = 1335), respectively. Table 2 displays in detail the results of all stocks used, as percentages of eggs surviving at both steps of development. The weakest stock was 126, which is old, inbred marker stock. Best was MM, which has recently gone through crossing with wild type and new purification of markers.

In *D. lummei*, 38·0 per cent of the 573 counted eggs kept at 25°C died before pupariation, and 8·5 per cent of pupae failed to emerge. At 17°C, *D. lummei* survived better during early development: 23·4 per cent of 474 eggs died before pupariation, but 22·9 per cent of pupae died before emerging to adults.

The total fitness of reciprocal hybrids was about the same, and much lower than in parental generation. Reciprocal hybrids show clearly different developmental profiles. From *D. virilis* female × *D. lummei* male hybrids (data at 25°C, excluding cross NEW × 1143, 3864 eggs), 47·0 per cent died before pupariation, and 24·9 per cent of pupae failed to emerge. At 17°C, the mortality values were 50·0 per cent (N = 3476), and 17·4 per cent (N = 1739). In the reciprocal cross, *D. lummei* females × *D. virilis* males, a slightly larger proportion of the progeny died (66·8 per cent, 1587 eggs), but of them, 65·0 per cent of 1587 eggs failed to pupariate, and only 5·0 per cent of pupae died before adult emergence. The values at 17°C are very similar: 66·2 (N = 1845) and 7·2 per cent (N = 624).

The hybrids of stocks NEW of *D. virilis* and 1143 of *D. lummei* differed clearly from the others. Survival of eggs mothered by *D. virilis* was much better. Their mortality within pupal case was especially low (5·3 per cent, N = 414). [This is correlated with the low proportion of developmental disorders among these hybrids. Only 0·8 per cent of them suffer from eye syndrome (Heikkinen and Lumme, in preparation), which occurred in other hybrid progenies of *D. virilis* at a frequency of almost 10 per cent.] On the other hand, the

Table 2 Number of eggs counted, and proportion (percentage) of them surviving until pupariation (P) and adult eclosion (A), in two rearing temperatures

	Temperature					
	25°C			17°C		
Genotype	Eggs	P	A	Eggs	P	A
virilis 1422	116	71·6	69·8	80	56·3	53·8
virilis 126	358	61·7	57·3	167	69·5	66·5
virilis MM	273	93·4	86·8	190	72·6	61·6
lummei 1101S	454	65·0	56·2	474	76·6	59·1
lummei 1143	119	58·8	58·8	—		
126×1422	2300	77·9	73·6	1491	69·5	66·1
126×1101S	1589	47·2	36·9	1521	38·1	34·3
1101S×126	857	34·2	31·9	1155	32·2	30·2
MM×1101S	2275	57·0	41·9	1955	59·3	46·8
1101S×MM	730	35·9	34·8	690	36·5	33·3
NEW×1143	649	63·8	60·4	—		
1143×NEW	116	4·2	3·0	—		

survival of 1143 × NEW eggs was much worse than that of other hybrids (table 3). This observation was made after completing most of the experiments described here, and this special case will be analyzed later in detail.

The sex ratios of the survived hybrid adults are presented in table 3. In *D. virilis* × *D. lummei* progenies grown at 25°C, there were significantly fewer males than females, in both replicates. At 17°C, also the control cross between *D. virilis* stocks produced less males.

Segregating generations

In the following, we compare the viabilities of different chromosomal combinations obtained through crossing the F_1 males to marker stock *D. virilis*. Only relative viabilities can be measured, since the markers used can be scored only in adult flies.

In fig. 1 we present the proportions of males and females in pooled data comprising of three different types of crossing, including the control cross between marker stock and wild type *D. virilis*. All crosses produce 16 autosomal combinations, with various combinations of sex chromosomes. The sex chromosome set of course depends on the direction of cross made in the parental generation. The statistical analysis of the results is depicted in fig. 2.

Control virilis × (virilis × virilis)

Fig. 1 displays combined results from several crosses, using as marker stocks either 126 or NEW, and as the wild type, 1422 or *A*. There was no significant inhomogeneity among the results.

The total deviation from expected phenotype frequencies is small, hardly significant ($G_{(31)} = 48{\cdot}45$, $P < 0{\cdot}05$). The sex ratio is even: 1890 males

Table 3 Sex ratios of the control cross and F_1 hybrids at two different temperatures

	Temperature					
	25°C			17°C		
Genotype	Males	Females	$G_{(1)}$	Males	Females	$G_{(1)}$
126×1422	841	851	0·059	457	529	5·262*
126×1101S	246	340	15·144***	229	292	7·637**
1101S×126	150	123	2·675	163	186	1·517
MM×1101S	418	536	14·633***	421	493	5·677*
1101S×MM	119	135	1·009	120	110	0·435

* $P < 0{\cdot}05$.
** $P < 0{\cdot}01$.
*** $P < 0{\cdot}001$.

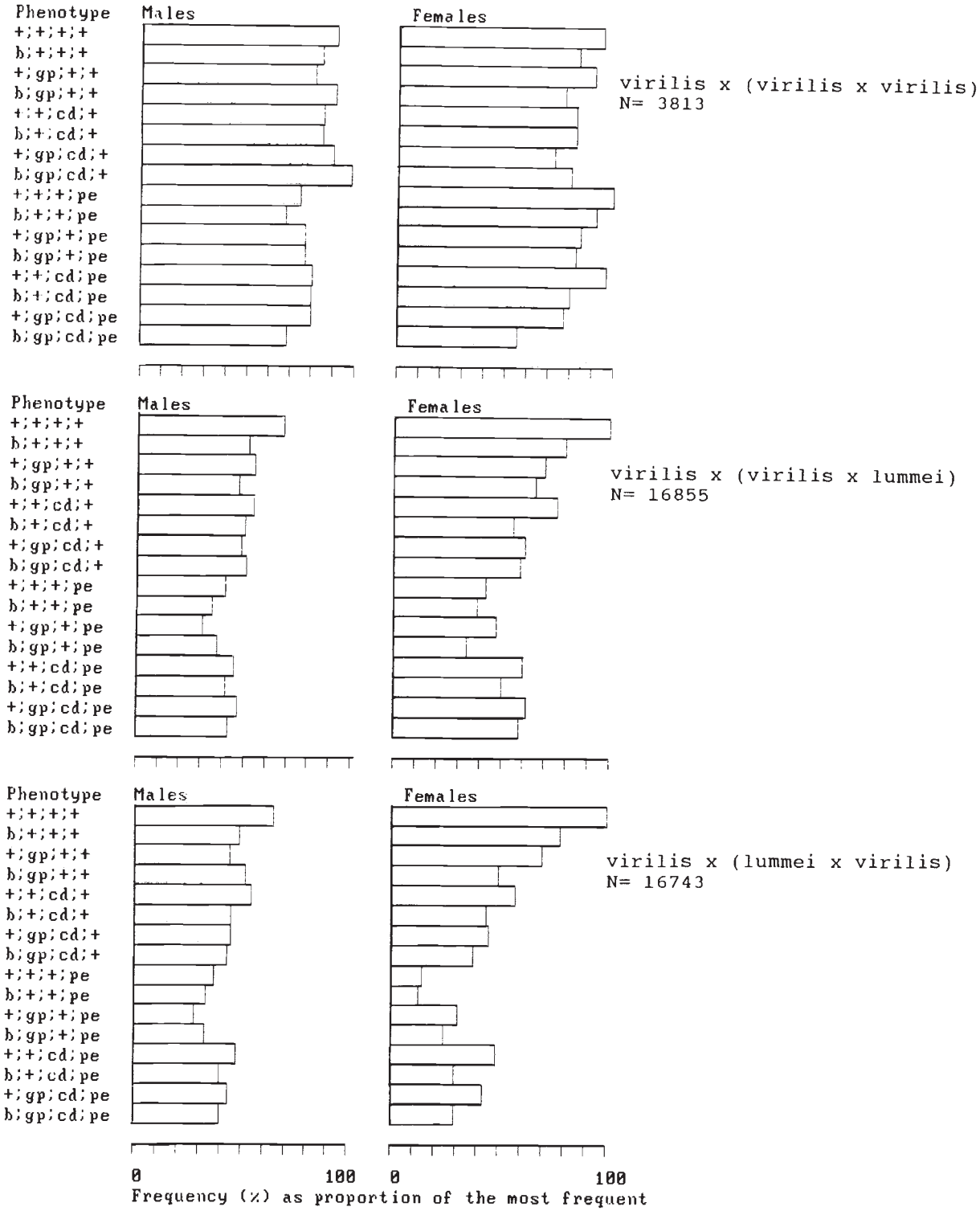


Figure 1 Relative frequencies of phenotypes from control and hybrid backcrosses. *D. virilis* marker stock was *b*; *gp*; *cd*; *pe*. *D. lummei* and *D. virilis* in controls were of wild type. Marker phenotype then indicates homozygous *D. virilis* chromosome; + is for heterozygous. Among each progeny, the most frequent phenotype (in hybrids, +; +; +; + female) is set to have the column of 100 units.

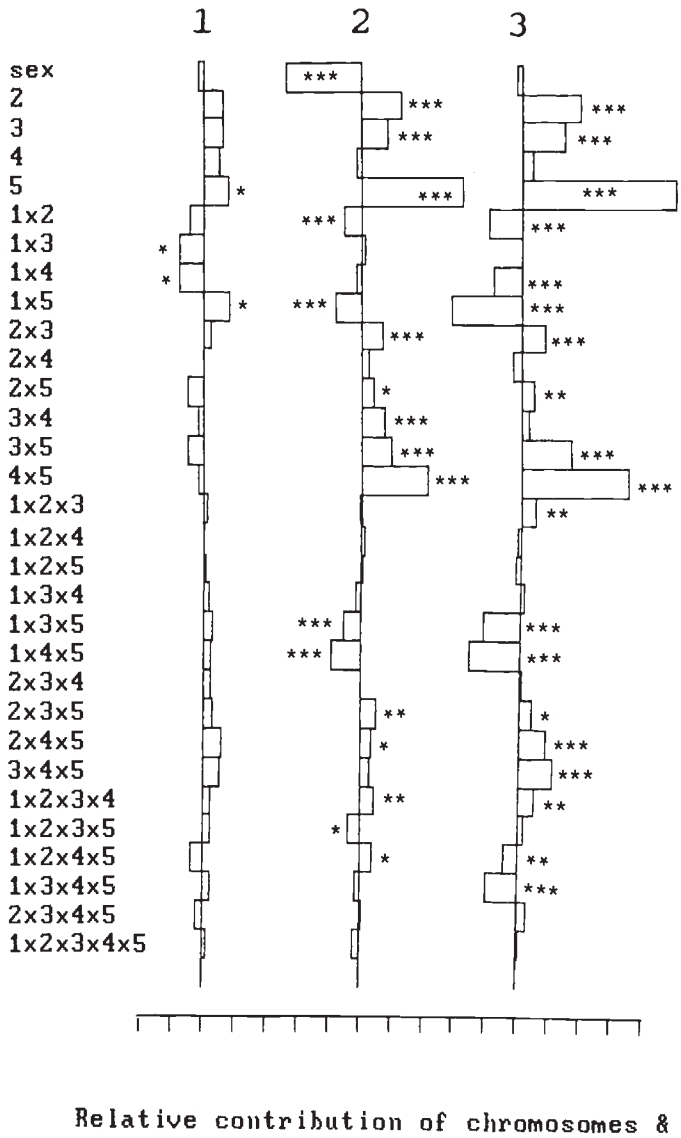


Figure 2 Main effects and interactions of genetic elements (Analysis of data in fig. 1). The contributions are scaled to be proportional to the expected number $[(\Sigma a - \Sigma b)/(N/2)]$ (See table 1.). One division = 0·1. 1. Control *virilis* × (*virilis* × *virilis*), 2. *virilis* × (*virilis* × *lummei*), 3. *virilis* × (*lummei* × *virilis*). The significance was tested by *G*-test (* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$).

and 1923 females were counted ($N_m/N_f = 0{\cdot}983$).

In the total material, there were 1980 *pe*/+ heterozygotes and 1833 *pe*/*pe* homozygotes. This difference is to be understood as the main effect of the fifth chromosome showing subviability of mutant homozygotes. Deviation from the expected 1:1 segregation is significant ($G_{(1)} = 5{\cdot}214$, $P < 0{\cdot}05$). Interactions 1×3, 1×4, and 1×5, where 1 means the sex, are significant at the 5 per cent level, $G_{(1)}$-values being from 5·3 to 5·7. This is due to the small differences in the frequencies of the corresponding phenotypes among males and females.

Heterospecific virilis × (virilis × lummei)

The total data depicted in fig. 1 contains altogether 16 845 individuals, collected from different combinations of stocks during diverse experiments. As *D. virilis*, both 126 and NEW have been used, and as *D. lummei*, wild type stocks 1101S and 1143.

No inhomogeneity was observed, and therefore we present the pooled results pooled over all replicates.

Males from this backcross carry heterospecific sex chromosome combination X^{vi}/Y^{1u}, as in the F_1 with more aberrant sex ratio, *D. virilis* × *D. lummei*. Females carry homozygous combination X^{vi}/X^{vi}, which is fittest together with all-heterozygous autosomes in phenotype (+; +; +; +).

Among the backcross progeny, the sex ratio was significantly aberrant ($N_m/N_f = 0{\cdot}788$, $N = 16{,}845$, $G_{(1)} = 236$, $P \ll 0{\cdot}001$). This is expressed in fig. 2 as the large negative main effect of "sex". The weighted mean sex ratio in F_1 (*D. virilis* × *D. lummei*) (table 3) was very closely the same, 0·791 ($N = 2975$, $G_{(1)} = 40{\cdot}57$, $P < 0{\cdot}001$).

The frequencies of the different autosomal combinations deviate significantly from the expected. All 16 classes should be equally frequent. Among females, the deviations are wider than among the males, but the patterns resemble each other. The main effects of the autosomes 2, 3, and 5 are highly significant, and in all cases, there are more heterozygous than homozygous flies in the progeny. The interactions 1 × 2 and 1 × 5 are highly significant, which means that the relative shortage of *b*/*b* and *pe*/*pe* homozygotes is much worse among the females. This is not due to X-autosome incompatibility, since both X chromosomes of the females arised from *D. virilis*.

Between the autosomes, the interactions 2 × 3, 3 × 4, 3 × 5, and 4 × 5 are significant at the level of $P < 0{\cdot}001$, and all of them have positive sign (fig. 2, table 4). This means that chromosomes of *similar* origin are functioning better together. As a consequence, the phenotype *b*; *gp*; *cd*; *pe* is not the weakest one, even if it contains all the homozygous large autosomes. The relative fitnesses of autosomal combinations *similar*/*different* origin are calculated in table 4.

To make this point clear, we display in table 5 the observed relative fitnesses of each autosomal phenotype combination in females. The data is pooled over both backcrosses. The phenotype frequencies were transformed to relative fitness by dividing the number of each class by the class containing most of the flies (invariably, the heterozygous or double heterozygous phenotype).

Heterospecific virilis × (lummei × virilis)

As can be seen in figs 1 and 2, the relative proportions of different phenotypes follow the same pattern as in the previous cross, but the variation of the viabilities among phenotypes was clearly amplified, especially among the females. Here the males carry homospecific set of sex chromosomes, X^{vi}/Y^{vi}. Females are heterozygous X^{vi}/X^{1u}. The overall sex ratio is close to unity ($N_m/N_f = 0{\cdot}984$, $N = 16{,}743$, $G_{(1)} = 1{\cdot}1$, $P \gg 0{\cdot}1$).

The deviation of female phenotype frequencies from the expected was wider than in the previous cross, where the females were homozygous X^{vi}/X^{vi}. The variability in viabilities of the various autosomal combinations is due to some second degree X/autosome/autosome-interaction, which can be expressed by saying that the negative interaction of heterozygous to homozygous autosomes is

Table 4 Relative fitness (w) of flies homozygous for each autosome in backcrosses (for heterozygotes w = 1·0), or flies carrying one pair homozygous, other pair heterozygous autosomes (in flies having both homozygous + both heterozygous, w = 1·0). Data is pooled over replicates, different stocks, and sexes

Chromosome	*vir* × (*vir* × *lum*) $N = 16855$	*vir* × (*lum* × *vir*) $N = 16743$	Total $N = 33625$
2	0·880***	0·829***	0·833***
3	0·917***	0·873***	0·896***
4	1·015	0·964*	0·991
5	0·719***	0·607***	0·660***
Interaction			
2 × 3	0·935***	0·929***	0·931***
2 × 4	0·980	1·026	1·001
2 × 5	0·961*	0·958**	0·961***
3 × 4	0·927***	0·974	0·948***
3 × 5	0·906***	0·852***	0·880***
4 × 5	0·808***	0·707***	0·758***

* $P < 0{\cdot}05$.
** $P < 0{\cdot}01$.
*** $P < 0{\cdot}001$, tested with $G_{(1)}$.

Table 5 Relative fitness (w) of homozygous or heterozygous autosomes 2 to 5, and their pairwise combinations in female progenies in *virilis* × (*virilis* × *lummei*) and *virilis* × (*lummei* × *virilis*) backcrosses ($N = 17895$). For testing the interaction, double homozygotes and double heterozygotes were pooled to have w = 1·0 and compared with sum of $A^{vi/vi}B^{vi/lu}$ and $A^{vi/lu}B^{vi/vi}$

A	B	$A^{vi/vi}B^{vi/vi}$	$A^{vi/vi}B^{vi/lu}$	$A^{vi/lu}B^{vi/vi}$	$A^{vi/lu}B^{vi/lu}$	A/B
2		0·803***			1·000	
3		0·887***			1·000	
4		0·946***			1·000	
5		0·581***			1·000	
2	3	0·717	0·778	0·860	1·000	0·954**
2	4	0·759	0·806	0·948	1·000	0·997
2	5	0·464	0·813	0·589	1·000	0·957**
3	4	0·843	0·841	0·899	1·000	0·944***
3	5	0·544	0·775	0·487	1·000	0·801***
4	5	0·605	0·706	0·386	1·000	0·681***

* $P < 0·05$.
** $P < 0·01$.
*** $P < 0·001$; $G_{(1)}$ test.

amplified in the presence of X^{lu}, or because of the heterozygosity of X.

Recombinant X-chromosome: Inversion substitution lines

D. lummei has in X chromosome a fixed double inversion *In*(1)*a*+*b* in comparison to X of *D. virilis*. We constructed inversion substitution lines, originally to obtain *D. virilis* with photoperiodic diapause. For this purpose, MM females (*w*; *b*; *gp*; *cd*; *pe*) were crossed with single *D. lummei* males. F_1 daughters were then backcrossed to MM males. From the next generation, *w*/+ females were backcrossed to MM males, and this backcrossing was continued. During the first few generations, the autosomal markers *b*, *gp*, *cd* and *pe* were picked to be homozygous. After 50 generations of backcrossing, the lines were maintained by *w*/+ × *w* crossings within the line. An attempt was made to make the lines homozygous for w^+-allele (and *In*(1)*a*+*b*), but in only two of the four lines this succeeded, because of the high degree of sterility of the w^+-hemizygous males (Heikkinen and Lumme, in preparation).

In table 6, we present the phenotype frequencies in four inversion substitution lines. What is relevant for the present topic is that the viability of w^+ males was strongly reduced. Summed over lines and generations from table 6, 44·3 per cent of females were heterozygous. It deviates significantly from the expected 1:1 segregation ($N = 3874$, $G_{(1)} = 50·08$, $P < 0·001$). In males, the deviation is very much larger. Only 12·6 per cent were hemizygous w^+ ($N = 2577$, $G_{(1)} = 1706$, $P \ll 0·001$).

Table 6 Phenotype frequencies in inversion substitution lines. The backcrossing of heterozygous *w*/+ females to MM *w* males was continued until BC_{50}. After this, the lines were maintained through *w*/+ × *w* crosses within the line. Total sums of phenotypes are: *w*/*w* 2157, *w*/+ 1717, *w*/Y 2301, +/Y 276

Origin of *In*(1)*a*+*b*	Phenotype	Time of inspection BC_{9-10}	BC_{50}	G_{10}
1101*	*w*/*w*	148	102	208
	w/+	138	58	151
	w	221	114	252
	+	34	9	28
1101	*w*/*w*	265	81	175
HETEX	*w*/+	276	49	164
	w	280	92	175
	+	46	7	23
*lu*JapFu	*w*/*w*	251	110	270
	w/+	219	53	165
	w	266	107	223
	+	26	3	39
1100	*w*/*w*	192	107	248
	w/+	141	113	190
	w	193	109	242
	+	10	27	24

The total sex ratio is aberrant because of the missing w^+ males ($N_m/N_f = 0·665$, $G_{(1)} = 262$, $P \ll 0·001$). This indicates that hemizygous *In*(1)*a*+*b* from *D. lummei* causes poor survival when implemented into *D. virilis* genome.

The sex ratio in the two pure breeding *In*(1)*a*+*b* substitution lines (females homozygous) remained uneven in the first few generations after making them homozygous (1101*: $N_m/N_f = 32/189 = 0·169$, $G_{(1)} = 124$, $P \ll 0·001$)); 1100: $N_m/N_f = 76/257 = 0·296$, $G_{(1)} = 104$, $P \ll 0·001$).

This demonstrates that the observed hybrid weakness is a male sex-limited character. Meiotic drive as an explanation for the under-representation of the $In(1)a+b$ males is also excluded. The genetic basis was analysed as follows.

Heterospecific recombinant X in combination with alien autosomes

Heterozygous $w/+$ females from inversions substitution line 1101 (HETEX) were crossed with F_1 (1101S×MM) and F_1 (MM×1101S) males. The autosomal phenotype frequencies of the resulting male progenies are displayed in fig. 3.

For the analysis of the data, males from both crosses, which had different Y chromosome but showed similar autosomal phenotype frequencies, were pooled together. Fig. 4 displays the contribution of each homozygous *D. virilis* autosome to the weak viability of carriers of $In(1)a+b$. The contribution of fifth chromosome is twice as large as the contribution of the second chromosome. The interaction of the fifth and second does not deviate significantly from additive, but it is closer

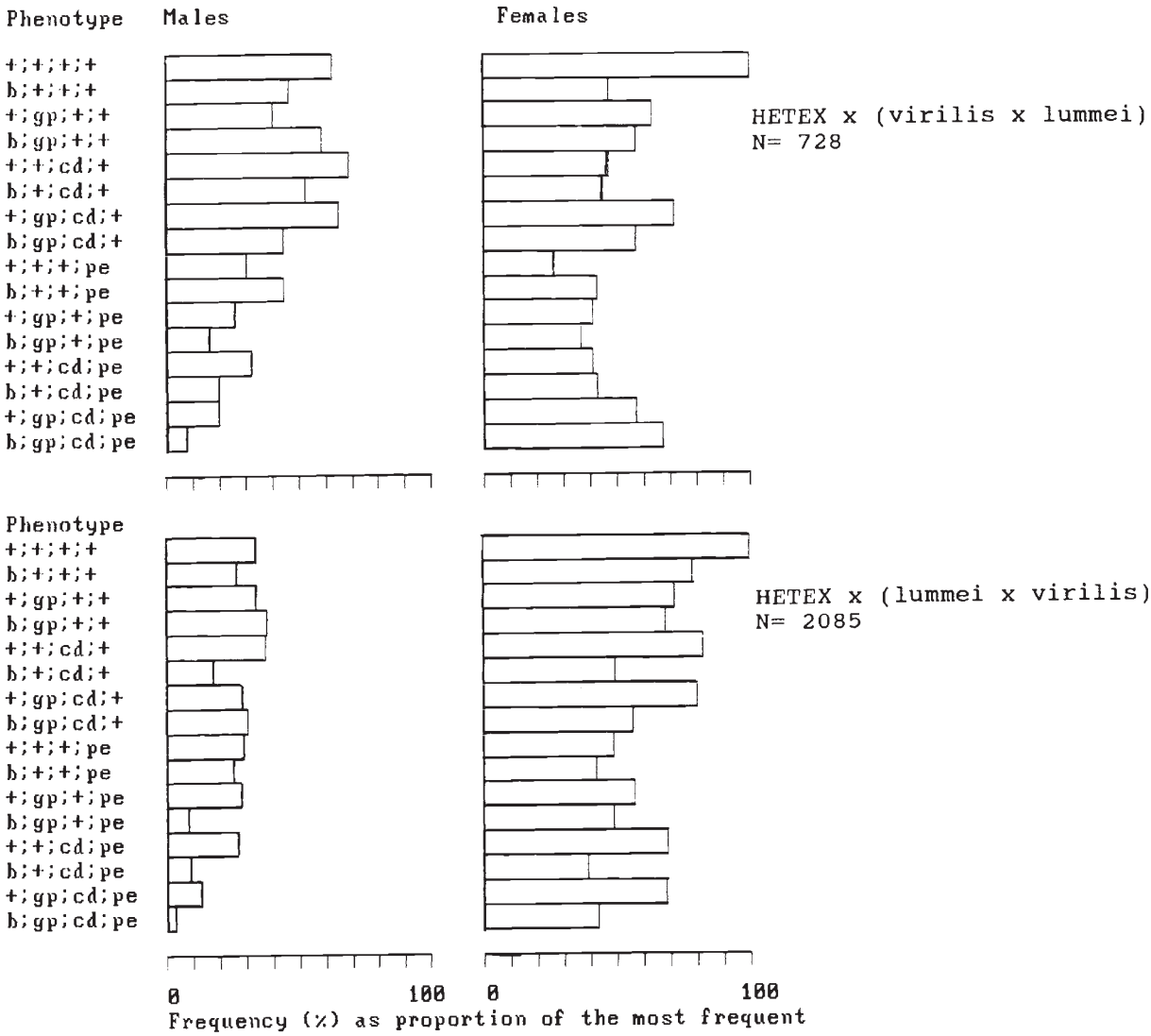
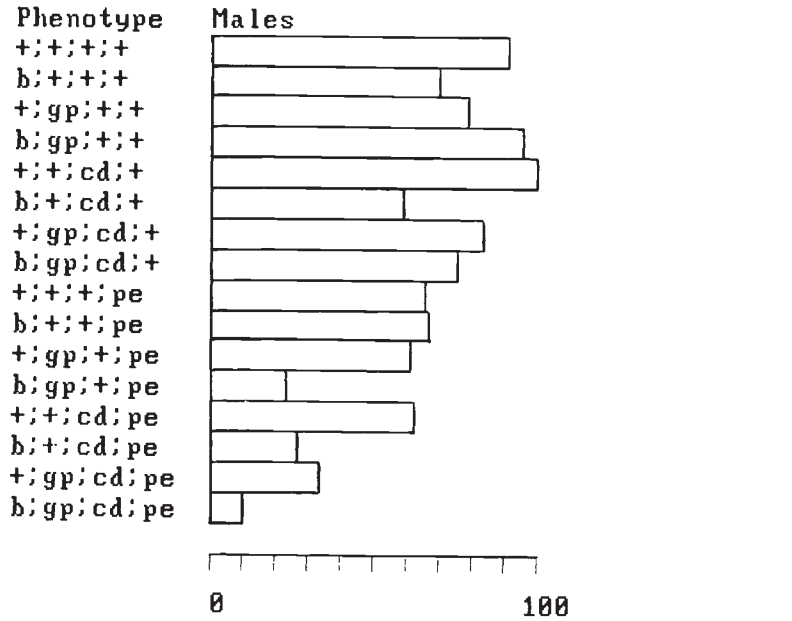


Figure 3 Relative frequencies of autosomal combinations in males and females carrying $In(1)a+b$ from crosses HETEX× (MM×1101S) and HETEX×(1101S×MM). In the first cross, white-eyed flies were discarded. In the latter cross, females are overrepresented, because $w/+$ and $+/+$ genotypes are not separable, and white-eyed males are not included. The markers in the stocks are: HETEX: $In(1)a+b\ w^+$/Standard *w*; *b*; *gp*; *cd*; *pe*. MM: *w*; *b*; *gp*; *cd*; *pe*. 1101S *D. lummei* +; +; +; +.



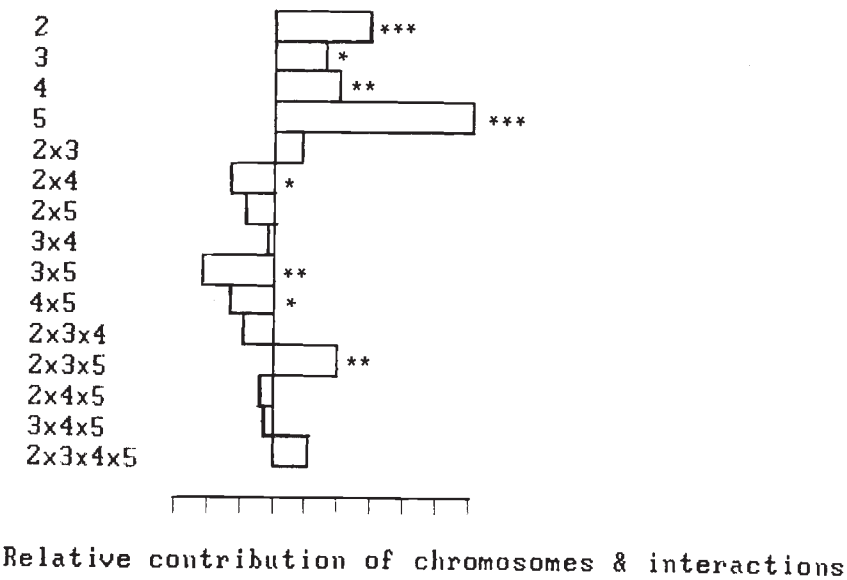


Figure 4 (Upper panel) Relative frequencies of autosomal combinations in males carrying hemizygous $In(1)a+b$ (N = 902) from crosses depicted in fig. 3. (Lower panel) Main effects and interactions of homozygous *D. virilis* autosomes causing the weak viability of carriers of $In(1)a+b$. The contributions are scaled to be proportional to the expected number $[(\Sigma a - \Sigma b)/(N/2)]$ (See table 1.). One division = 0·1. (* $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$, G-test).

to multiplicative model. When the fitness of +/+ is set to 1·000, the relative fitnesses of other combinations of the second and fifth chromosomes are for *b*;+0·849, +; *pe* 0·629, and *b*; *pe* 0·358. The multiplicative prediction for double homozygote is 0·534. It is to be expected that some of the autosome-autosome interactions detected even irrespective of the origin of intact X chromosome,

are acting here, too, but they cannot override the negative interaction of *In*(1)*a*+*b* and autosomes 2 and 5. Here *b*; *pe* phenotype survives badly, even if both autosomes are from *D. virilis*.

Recombination within the X chromosome

In the previous experiment, only a part of the X implemented into the genome of *D. virilis* was from *D. lummei*. Because there exists a possibility that the hybrid weakness is not caused solely by the incompatibility between hemizygous *In*(1)*a*+*b* and homozygous *D. virilis* autosomes, but by the incompatibility between distal and proximal parts of the X itself, the following cross was made. F_1 females from a cross *y ap* × wild-type *D. lummei* were backcrossed to *y ap* or to *D. lummei*. The allele *yellow* marks the distal tip of the X chromosome, and apricot is a marker of the part of the chromosome included in the *In*(1)*a*+*b*. The frequencies of phenotypes in BC_1 are presented in table 7. The overall recombination frequency of the markers is 0·489, indicating undisturbed crossing over outside of the inversion.

From the inspection of the results in table 7 it can be seen that none of the novel male combinations from backcrosses to *D. virilis* has much reduced viability. The reason is that the proportion of autosomes homozygous for *D. virilis* is still rather low in BC_1. Among the males (pooled over both stocks), the relative fitness of ap^+ phenotypes is $w = 0{\cdot}808$ ($G_{(1)} = 12{\cdot}4$, $P < 0{\cdot}001$), and that of y^+, $w = 0{\cdot}896$ ($G_{(1)} = 3{\cdot}3$, $P > 0{\cdot}05$), but the recombination within X seems not to be harmful. Thus, the subvitality of *In*(1)*a*+*b* in males having otherwise *D. virilis* background is caused by its incompatibility with several homozygous autosomes.

In table 7 we also present results of backcrossing F_1 to *D. lummei*. When crossed with stock 1101S, the *virilis* segment of X including the standard gene arrangement for *In*(1)*a*+*b* has a clear disadvantage with partially homozygous *lummei* autosomes. Additional reduction of fitness is achieved when the distal tip of the X also is from *D. virilis*, but the tip alone seems not to be incompatible. In cross involving Japanese stock 1143 the standard X of *D. virilis* is not subvital. This difference between the *D. lummei* stocks is autosomal, and will be analysed later.

DISCUSSION

Our studies about adaptive and non-adaptive genetic differences between *Drosophila virilis* and *D. lummei* have been mainly directed to traits other than viability. The data presented here have been collected when genetically analysing other traits which are or will be reported elsewhere (cold shock resistance: Heino and Lumme, 1989; male and female sterility, developmental disorders, and mate choice, Heikkinen and Lumme, in preparation).

The major findings of the present analysis can be summarized as follows.

F_1 was clearly weaker than parental stocks: fewer eggs survived until adults. In data pooled over all experiments, the relative fitnesses (w) were as follows. Out of *D. virilis* eggs, 69·8 per cent

Table 7 Phenotype frequencies in progenies from crosses between *D. virilis* 139 *y ap* and two wild type (++) stocks of *D. lummei*, 1143 and 1101S

	(*y ap* × ++) × *y ap*				(*y ap* × ++) × ++			
	1143		1101S		1143		1101S	
Phenotype	Female	Male	Female	Male	Female	Male	Female	Male
++	148	115	119	142	160	26	560	150
y +	105	112	108	118		39		148
+ *ap*	115	125	146	133		40		91
y ap	99	144	145	201		33		53
Sum	467	496	518	594	160	138	560	442
Sex ratio	1·062		1·147		0·863		0·789	
$G_{(1)}$	0·9		5·2*		1·6		13·9***	
Phenotype ratio $G_{(3)}$	11·8**	4·9	8·5*	25·4***		3·8		65·0***

* $P < 0{\cdot}05$.
** $P < 0{\cdot}01$.
*** $P < 0{\cdot}001$.

survived to adults (w = 1). Survival of *D. lummei* eggs was 57·7 per cent (w = 0·826), *D. virilis* × *D. lummei* hybrids 40·5 per cent (w = 0·581), and of *D. lummei* × *D. virilis* hybrids 32·2 per cent (w = 0·462). Altogether, 4008 adults emerged from the 5903 counted eggs of pure species, (w = 1), and 4081 adults from 10772 eggs from hybridizations (w = 0·558). We do not know how large a proportion of the eggs was unfertilized.

Reciprocal hybrid progenies have different sex ratio. *D. virilis* mothers had the sex ratio in their progeny: $N_m/N_f = 0{\cdot}791$ ($N = 2975$), *D. lummei* mothers had as many sons as daughters ($N_m/N_f = 0{\cdot}996$, $N = 1106$).

Reciprocal hybrids with *D. lummei* stock 1101S differed more with respect to the developmental profile of the lethality than of the total numbers of surviving eggs. The hybrids mothered by *D. virilis* survived slightly better, but a large proportion of their mortality occurred during adult metamorphosis within pupal case. Probably correlated with this, the emerging adults frequently suffered from visible developmental disorders in eyes, antennae, wings, and abdominal chitinization (Heikkinen and Lumme, in preparation). Many of them could survive only in the sheltered situation in laboratory. The Japanese stock 1143 was different from Swedish stock 1101S, in pupal mortality, and also in frequency of developmental disorders.

The *D. virilis* × *D. lummei* hybrids (especially males) are also more susceptible to cold shock than pure species or the progeny of the reciprocal cross (Heino and Lumme, 1989).

In backcross generations, the second, third and fifth chromosomes expressed heterosis. In all possible sex chromosome backgrounds, the relative viability of flies heterozygous for a given autosome was higher than that of flies homozygous for *D. virilis* autosome. This is not to be expected on the basis of comparing P and F_1 generations: flies having *all* autosomes as heterozygous (reciprocal F_1 values) were clearly less fit than parental species, when measured by egg to adult survival.

The role of maternal cytoplasm cannot explain this apparent controversy between first and second hybrid generation, because mothers of both generations were pure species. A similar unresolved situation was observed in a study of cold shock resistance (Heino and Lumme, 1989). There, the F_1 was weaker than the most similar backcross phenotype. The role of elimination of the tiny sixth chromosome was speculated (about the elimination, see Sokolov, 1948, 1959; Mitrofanov and Sidorova, 1979).

Any two of the autosomes work relatively better when they are identical, *i.e.*, both heterozygous, or both homozygous. Out of six possible combinations, 2 × 4 is the only exception (tables 4 and 5). Fitnesses of double homozygotes follow well estimates made according to multiplicative interaction, but genotypes with one chromosome homozygous and one heterozygous fall below this prediction. This is a very interesting finding. While in the species pair studied, the degree of postzygotic isolation is rather weak, polygenic accumulation of small effects in all chromosomes seems to be going on. Interestingly enough, this effect was much stronger in females than in males.

Inversion *In*(1)*a* + *b* from *D. lummei* is incompatible with the homozygous autosomes of *D. virilis*, causing strongly decreased viability of carrier males. All *D. virilis* autosomes take part in this incompatibility system, the role of fifth being strongest. Reciprocally, the standard X of *D. virilis* is incompatible with the homozygous autosomes of *D. lummei* (this interaction was, however, strain-dependent and thus polymorphic in *D. lummei*). The participating autosomes are not yet analyzed because of the lack of suitable markers in *D. lummei.*

Our results are not contradictory with the "two rules of speciation" (Coyne and Orr, 1989*b*), even if the isolation is weakly expressed in F_1. In this work, we investigated a quantitative and relative subvitality among hybrids. The few analyzed cases in the literature concern rather absolute inviability of males of females. The rules hold for quantitative viability, too.

Haldane's rule (1922) states that the heterogametic sex is affected first. In our case, the weak incompatibilities between heterozygous and homozygous autosomes were more pronounced among the females. This seems to be contradictory with Haldane's rule, but this effect was really rather weak. With this same pair of species, Mitrofanov and Sidorova (1981) demonstrated another autosomal lethal interaction, affecting only females. Certain backcross phenotypes gave no female progeny, when crossed again with *D. lummei* males.

In our results, the stronger male sex-limited viability interactions obeyed Haldane's rule. Even if we have been able to construct pure breeding *D. virilis* carrying *In*(1)*a* + *b* of *D. lummei*, only hemizygous males are subvital, not the homozygous females.

Coyne's rule says that X chromosome is responsible for the earliest and strongest postzygotic isolation. This was confirmed here, too. However, our analysis also reveals the elements

interacting with the X. In our case, proximal half of X included in the inversion $In(1)a+b$ of *D. lummei* is subvital with homozygous alien (*D. virilis*) autosomes, especially the fifth. Reciprocally, standard arrangement of the same part of X is subvital with combination of *D. lummei* autosomes (which of them, is to be analysed). The observed polymorphism in response of autosomes of *D. lummei* will be of experimental value in analysing this incompatibility.

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