Heredity **66** (1991) 357–365

Received 7 August 1990

Wrinkling of the eye in hybrids between *Drosophila virilis* and *Drosophila lummei* is caused by interaction of maternal and zygotic genes

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Hybrids of *Drosophila virilis* females and *Drosophila lummei* males have visible developmental anomalies in external adult structures. Reciprocal hybrids are normal, and the anomalies are not found in other interspecific F_1 hybrids of the *virilis* subgroup. Antibiotic treatments with ampicillin, streptomycin and tetracycline did not cure the syndrome. The genetic basis of the wrinkling of the compound eye was analysed in detail. Both male and female F_1 progeny of *D. virilis* mothers were affected, indicating that wrinkling of eyes is maternally determined. F_1 hybrid females produced healthy progeny. Backcross hybrid females (*D. virilis* × F_1) with 16 different combinations of autosomes, either heterozygous or homozygous for *D. virilis*, were crossed with *D. lummei* males. It was found that homozygous *D. virilis* chromosomes 2 and 5 *together* were necessary to induce the maternal effect. The hybrid zygote responded to the maternal effect when the chromosomes 2, 4 and/or 5 were heterozygous. Elimination of the small sixth (dot) chromosome of *D. lummei* from the hybrids was correlated with the wrinkling of eyes, but was not the cause, as the two phenomena had a different genetic basis and temperature response. Furthermore, the eyes were wrinkled in flies which presumably had had no *D. lummei* dot chromosome to lose.

Keywords: evolution, postzygotic isolation, speciation.

Introduction

When *Drosophila virilis* females and *Drosophila lummei* males are crossed, the majority of the first hybrid generation adult flies suffer from external structural anomalies. This has already been noted by Sokolov (1948, 1959). Recently, Orr (1990) re-examined the phenomenon. He confirmed the maternal origin of the anomalies and excluded any effect of the X chromosome, which usually plays a large role in the postzygotic isolation mechanisms (Coyne & Orr, 1989). Sub-lethal morphological anomalies are not rare in species hybrids. Sturtevant (1920) described them in his first paper concerning interspecific hybridization in *Drosophila*. He studied hybrids between *D. melanogaster* and *D. simulans*, which often had abnormally banded abdomens, broken or missing crossveins and certain bristles absent. Anomalies were obtained in both reciprocals. Coyne (1985) re-analysed anomalies in hybrids of *D. simulans* and *D. melanogaster*, and also studied combinations with *D. mauritiana*. Weisbrot (1963) reported that in hybrids between *D. pseudoobscura* females and *D. persimilis* males, 0.2 per cent of the progeny had abnormal tergites.

In this paper the anomalies of *D. virilis* × *D. lummei* hybrids and their genetic basis are investigated. *D. virilis* and *D. lummei* are weakly isolated, geographically allopatric, sibling species. The genetics and biology of the *Drosophila virilis* group is described in detail by Throckmorton (1982).

Materials and methods

Flies

Two marker stocks of *D. virilis* were used. Stock 126 has the major autosomes recessively marked: *b*;*gp*;*cd*;*pe*. The map positions (Alexander, 1976) are: broken (*b* 2-188.0), gapped (*gp* 3-118.0), cardinal (*cd* 4-32.2), peach (*pe* 5-203). *D. virilis* marker stock 162 was *b*;*gp*;*cd*;*pe*;*Gp gl* (Gap, *Gp* 6-0.4, glossy, *gl* 6-1.0). The stocks 126 and 162 were from the Institute of Developmental Biology, USSR Academy of Sciences, Moscow. For control crosses, the wild type *D. virilis*

stock 1422 was used (Groeningen, The Netherlands, 1976).

In genetic analyses extending to the second and third generation, *D. lummei* stock 1101S was used (Överkalix, Sweden, 1970). In addition, wild caught *D. lummei* males from Kemi, Finland were crossed individually to single females from the *D. virilis* marker stock 126.

F_1 hybrids were inspected from all possible combinations between at least two stocks of each taxa *D. virilis*, *D. lummei*, *D. novamexicana*, *D. americana americana* and *D. americana texana* (The National Drosophila Species Resource Center, Bowling Green, Ohio, USA).

The stocks were reared on malt medium (Lakovaara, 1969) at 19°C under continuous illumination and 70 per cent relative humidity.

Antibiotic treatments

To test whether the wrinkling of the compound eye was caused by some symbiotic micro-organism, culture medium was supplemented to a final concentration of 0.03 per cent with ampicillin, streptomycin, tetracycline, or both ampicillin and streptomycin (both 0.03 per cent), following Hoffmann *et al.* (1986). Tetracycline bottles were lined with aluminium foil, to keep them dark. Sodium chloride, up to a final concentration of 0.67 per cent, was used as a control.

D. virilis marker stock 126 and wild type *D. lummei* 1101S were reared on the antibiotics supplemented medium in P and F_1 hybrid generations, and scored for the wrinkled eye syndrome.

Statistical analysis

In testing the proportions of damaged flies, a standard *G*-test, *G*-test for independence, and Student's *t*-test were used (Sokal & Rohlf, 1981). A log-linear model and the *G*-statistic were used to analyse the progeny families of wild-caught flies (Sokal & Rohlf, 1981). The contributions of chromosomes and their interactions on the expression of the eye syndrome were treated as a modification of the analysis of factorial experiments (Snedecor & Cochran, 1967; Lumme & Heikkinen, 1990).

Results

External structural anomalies in the F_1 generation

External structure anomalies are illustrated in Fig. 1.

Abdominal cuticula. All the emerged F_1 adults from crossing *D. virilis* females to *D. lummei* males had abnormalities in the abdominal chitinization, i.e. in sternites (Fig. 1f) and tergites (Fig. 1h). The eighth tergite, derived from the genital disc, was invariably normal. Tergites 1–7 are derived from the abdominal histoblasts. Tergites 2 and 3 were most often and most badly misshapen, and their bristle patterns irregular. The joining of hemitergites had frequently failed (Fig. 1h). This type of developmental disorder was rare in pure stocks, and still less frequent in the reciprocal cross *D. lummei* × *D. virilis*.

Other anomalies were less frequent. Out of 2975 emerged hybrids of *D. virilis* × *D. lummei*, 2430 had no other external anomalies except abnormalities in abdominal cuticula. The frequency and pairwise co-occurrence of anomalies in eyes, legs, wings, antennae and halteres are presented in Table 1. In a few cases, three or four of these organs were affected in one individual. Data in Table 1 are pooled over temperatures and sexes.

Wrinkling of the eye. In total, 8.7 per cent of the 2975 F_1 flies from the cross *D. virilis* × *D. lummei* had wrinkled eyes (Fig. 1b–d). As a control, 2719 pure *D. virilis* were studied; nine of them (0.33 per cent) had wrinkled eyes. Out of the 1118 flies studied from the reciprocal cross, only one had the wrinkled eye syndrome.

There were significantly more wrinkled eyes in the flies cultured at 25°C than at 17°C (10.1 per cent, $n = 1540$ vs. 6.5 per cent, $n = 1435$; $t = 3.5$, $P < 0.001$).

Males and females were almost equally affected. There were no significant differences in the numbers of affected males and females (at 17°C: males 5.1 per cent, $n = 650$, females 7.6 per cent, $n = 785$, $t = 1.9$, $P > 0.05$; and at 25°C: males 12.0 per cent, $n = 664$, females 9.1 per cent, $n = 876$, $t = 1.9$, $P > 0.05$).

Only the right eye was wrinkled in 123 cases, only the left one in 130 cases. Both eyes were wrinkled in six individuals, when the expected number was 5.4 ($G_{(1)} = 0.090$, $P > 0.75$). The left and the right side of an individual thus seemed to be independent.

Misshapen legs. Legs were considered as abnormal, if any of the six was considerably shorter than its pair; sometimes a leg was missing, and one fly with an extra leg was observed. The frequency of leg abnormalities was 5.4 per cent. Leg anomalies occurred significantly more often in flies that also had misshapen eyes (25 observed, 13.9 expected, $G_{(1)} = 8.518$, $P < 0.01$).

Wing anomalies. Wings were missing, or sometimes comprised of two blades. Small vein defects were not counted. Altogether, 1.9 per cent of the flies were scored with abnormal wings.

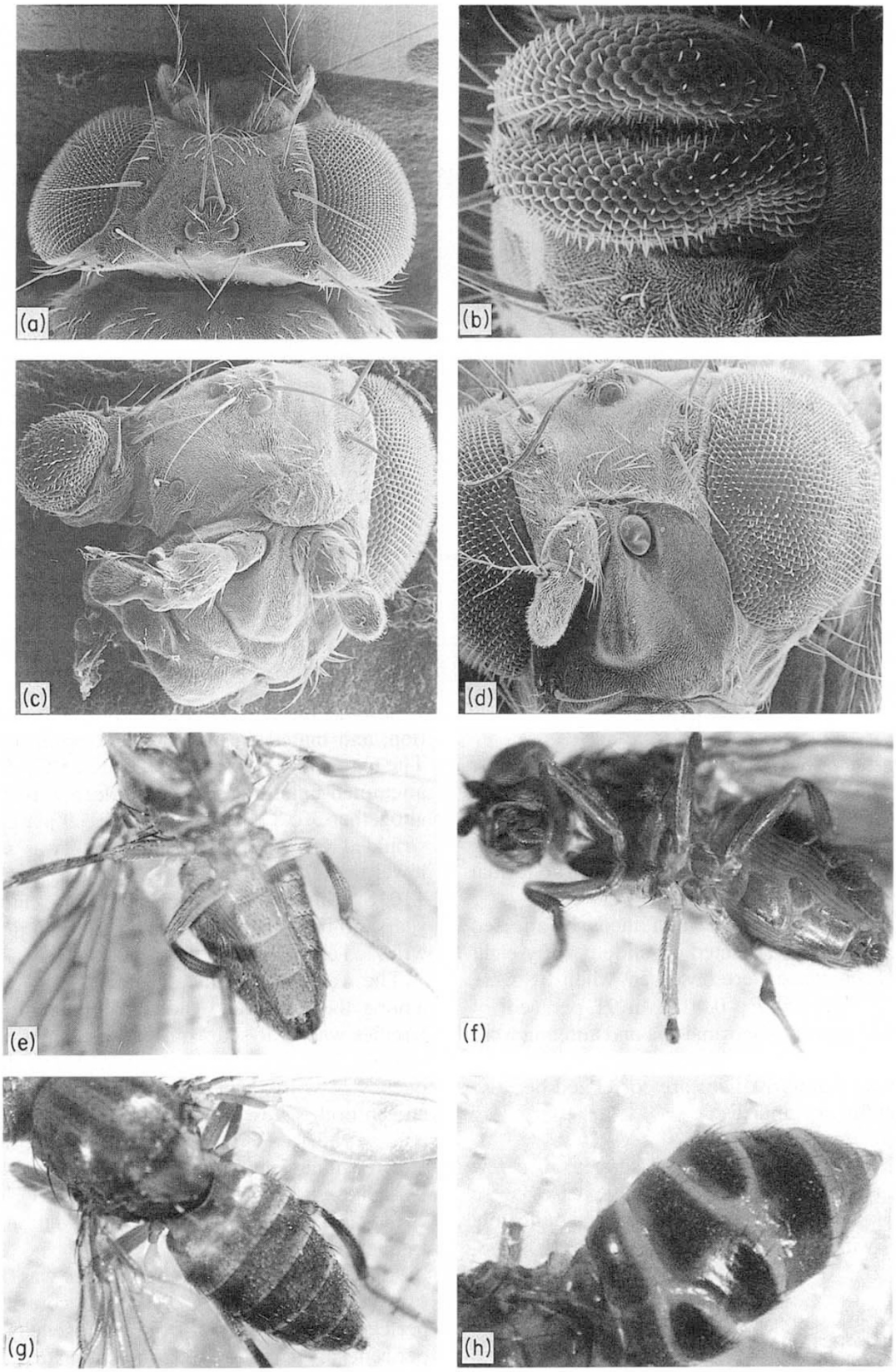

Fig. 1 Morphological anomalies in hybrids between *D. virilis* females and *D. lummei* males. (a) Compound eyes of normal *D. lummei*. (b) Mildly wrinkled eye in F_1 hybrid. (c and d). Eye–antennae anomalies in F_1 hybrids. (e) Sternites of a normal *D. lummei* male. (f) F_1 male with abnormal sternites. (g) Tergites of a normal *D. lummei* female. (h) Abnormally joined hemitergites in an F_1 female.

Table 1 Pairwise co-occurrence of abnormalities in the first hybrid generation between *D. virilis* females and *D. lummei* males. Along the diagonal are the numbers of flies with only one organ damaged. The off-diagonal cells show the numbers of flies with anomalies that occur together. Data are pooled over temperatures and sexes, $n = 2975$

	Eye	Leg	Wing	Antenna	Halter	Total
Eye	162	25**	12**	58***	2	259
Leg		126	8**	1	0	160
Wing			33	1	0	54
Antenna				7	0	67
Halter					3	5
Healthy						2430

*$P<0.05$, **$P<0.01$, ***$P<0.001$. Test of independence using G-Statistic, d.f. = 1. All four significant deviations are positive, i.e. the anomalies occurred together more often than might be expected at random.

The eye and the wing were defective simultaneously more frequently than might be expected at random (12 flies, 4.7 expected, $G_{(1)} = 9.262$, $P < 0.01$). In eight flies, leg and wing were simultaneously affected and this is significantly more than the random expectation (2.9 expected, $G_{(1)} = 6.723$, $P < 0.01$).

Antennal anomalies. Rudimentary antennae were most common, and sometimes antennae with leg-like appendages (Fig. 1c and d) were found. Two flies with three antennae and one with both antennae missing were recorded. The flies scored with abnormal antennae amounted to 2.3 per cent.

Antennae and eyes were simultaneously affected much more often than expected at random. Fifty-eight cases of co-occurrence were observed, when 5.8 were expected ($G_{(1)} = 244.5$, $P \ll 0.001$). In 91 per cent of the 58 cases observed, abnormal eye and antenna were on the same side of the fly. This demonstrates that the eye and antennal abnormalities are correlated because of their common imaginal disc.

Halteres. Halteres were seldom misshapen; in a few cases they resembled wings. Only five cases were observed. Haltere anomalies were not significantly correlated with other anomalies ($G_{(1)} = 4.098$, $P < 0.05$).

Effect of antibiotics on the wrinkling of the eyes

The frequencies of hybrids with wrinkled eyes from the vials containing the antibiotics are given in Table 2. In comparison to the control, treatment with tetracycline, ampicillin, or streptomycin decreased the frequency of wrinkled eyes significantly. However, the combined effect of ampicillin and streptomycin was not significant.

Table 2 Proportion of F_1 hybrid flies with wrinkled eyes (%, n within brackets), when parental and F_1 generations had been reared on antibiotics. Student's t-test compares the effect of each treatment with the control

Drug	%	n	t	
Control (0.67% NaCl)	7.8	(217)		
Ampicillin (0.03%)	3.5	(288)	2.126	$P<0.05$
Streptomycin (0.03%)	3.5	(764)	2.711	$P<0.01$
Tetracycline (0.03%)	2.8	(145)	1.995	$P<0.05$
Ampicillin + streptomycin	5.2	(862)	1.474	NS

Quantitative data on the defects of the abdominal chitinization were not collected but very obviously they were not cured by antibiotic treatment. Moreover, all other external structural anomalies were also observed in the treated flies.

Wrinkled eyes in the progenies of wild-collected D. lummei *males*

D. lummei males were collected from the wild population, and mated pairwise with *D. virilis* 126 females. The progenies were reared at 25°C. Out of 51 attempted families, 35 were fertile, and they produced altogether 3088 hybrids, of which 5.9 per cent had the wrinkled eye syndrome. This value was significantly lower than that obtained with the males from the stock 1101S, which had been in laboratory culture for 20 years (1540 hybrids reared at 25°C, 9.76 per cent with wrinkled eye, $t = 4.866$, $P < 0.001$).

The variation in the frequency of wrinkled eye among the males and the females in the 35 full-sib families was analysed as a log-linear model, using G-statistic. The null hypothesis tested was that the frequency of wrinkled eyes was the same in all families and in both sexes. The total deviation was significant ($G_{(69)} = 185.1$, $P < 0.001$). Males were more often affected than females (7.8 per cent, $n = 1481$ vs. 4.9 per cent, $n = 1607$, $G_{(1)} = 8.572$, $P < 0.01$). This difference was 4.6 per cent of the total deviation from even frequencies.

The variation between the families was also significant ($G_{(34)} = 132.9$, $P < 0.001$) and contributed 71.8 per cent to the deviation from homogeneity. This 71.8 per cent can be considered as an estimate of heritability in the wild population of *D. lummei* and in the marker stock of *D. virilis*.

The family × sex interaction was not significant ($G_{(35)} = 43.615$, $P > 0.05$), and the contribution value

23.6 per cent of the interaction can be considered as an error estimate for the heritability.

Elimination of the D. lummei *dot chromosome from the F_1 hybrids*

According to Sokolov (1959), in hybrids between *D. virilis* females and *D. lummei* males the sixth chromosome (dot) of *D. lummei* is eliminated during early embryogenesis, which has been confirmed by Sidorova (1974). If it is eliminated in the first mitotic cleavage, the entire hybrid is monosomic for the dot chromosome. The later this elimination occurs, the larger will be the proportion of tissues heterozygous for *D. lummei/D. virilis* dot chromosomes. By using the visible *D. virilis* marker, glossy eye (*gl* 6-1.0) the elimination of the *D. lummei* dot chromosome can be detected on the surface of the compound eye.

D. virilis stock 162 *b;gp;cd;pe;Gp gl* females were mated with *D. lummei* 1101S males. The eyes of the first hybrid generation were scored for wrinkled eye and glossy eye surface phenotypes. The results are displayed in Table 3. The interdependence of the two phenotypes is strong and highly significant ($G_{(2)} = 270.4$, $P \ll 0.001$).

Response to the maternal effect in the backcross D. virilis × F_1

In order to analyse the inheritance of the wrinkling of the eyes in more detail, reciprocal F_1 males were backcrossed to the marker stock 126 of *D. virilis*. The progenies were reared at 25°C.

D. virilis × (*D. virilis* × *D. lummei*), (b;gp;cd;pe) × [(b;gp;cd;pe) × 1101S]

Females from this cross had a conspecific set of sex chromosomes, X^{vi}/X^{vi}. The frequency of wrinkled eyes was 1.9 per cent ($n = 1930$). The sex chromosome set of males was X^{vi}/Y^{lu}, and the frequency of wrinkled eyes 0.9 per cent ($n = 1597$).

D. virilis × (*D. lummei* × *D. virilis*), (b;gp;cd;pe) × [1101S × (b;gp;cd;pe)]

Table 3 Co-occurrence of wrinkled and *glossy* eye phenotypes in *D. virilis* 162 × *D. lummei* 1101S crosses. The figures are numbers of eyes, not of individuals. The total number is uneven because one female had only one eye

Phenotype	Normal	Wrinkled
Whole eye *glossy*	2	46
Mosaic + /*glossy*	229	25
Eye wild type	614	9

Female progeny had heterospecific X chromosomes, X^{lu}/X^{vi}. Of these 1.6 per cent had wrinkled eyes ($n = 2218$). The sex chromosomes of males were X^{vi}/Y^{vi}, and the frequency of the eye anomaly 0.4 per cent ($n = 1993$).

Contributions of the zygotic chromosomes on the wrinkling of the eye are given in Fig. 2. The males and the females had different frequencies of the eye anomaly (pooled over two crosses, females 1.74 per cent, $n = 4148$ vs. males 0.64 per cent, $n = 3590$, $t = 4.362$, $P < 0.001$).

The two crosses were not different, however, in spite of the qualitative differences in the sex chromosomes of the progenies. The similarity extended to the autosomal phenotypes. Furthermore, the similarity also indicates that the role of the sixth chromosome is not decisive. The two crosses are pooled in Fig. 2, where the role of the four large autosomes is analysed.

The highest proportion of females with wrinkled eyes, 4.2 per cent ($n = 569$), was in the autosomal phenotype class +;+;+;+, which resembled F_1. This

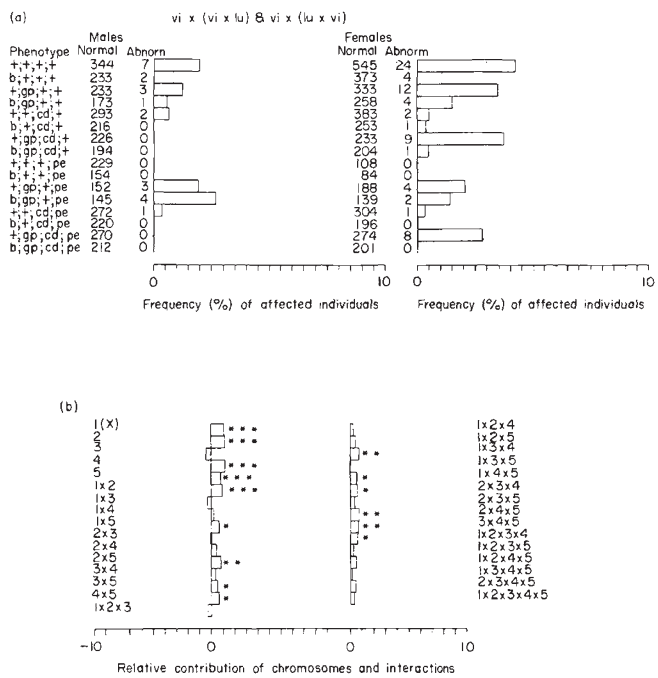

Fig. 2 Contribution of the zygotic chromosomes on the eye wrinkling. (a) Proportions of flies with wrinkled eye in the pooled backcrosses. (b) The analysis of factorial effects of the autosomes 2–5 and sex (1) on the frequency of wrinkled eye. A positive value for the main effect of an autosome means that heterozygous flies had more frequently wrinkled eyes than homozygous (for *D. virilis* chromosome). For primary interactions, a positive value means that flies with a similar origin of respective autosomes are less frequently damaged than those with different. Significance levels of the contributions: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

frequency was significantly lower than in the F_1 females at 25°C (9.1 per cent, $n=876$: $t=3.5$, $P<0.001$). It is to be pointed out that the frequency of wrinkled eyes was higher in females carrying X^{vi}/X^{vi} (13 out of 211) than in those exactly like F_1: X^{lu}/X^{vi} (11 out of 358). Therefore, it seems safe to conclude that the zygotic X chromosomes have no role in eye wrinkling in females.

In males, the largest proportion of abnormals, 2.7 per cent ($n=149$) was in phenotype class *b;gp;+;pe*. In phenotype class +;+;+;+, the proportion of affected flies was 2.0 per cent ($n=351$). This proportion differs significantly from the proportion in F_1 (12.0 per cent, $n=664$: $t=5.4$, $P<0.001$). F_1 and BC_1 +;+;+;+ males had the same genotype for chromosomes 2–5. F_1 males of the affected cross had the sex chromosome set X^{vi}/Y^{lu}. Backcross males had either the same combination (then 4 out of 157 wrinkled) or both sex chromosomes derived from *D. virilis* (three out of 194 wrinkled). This leads to the conclusion that the zygotic sex chromosomes have no role in the wrinkling of eyes in males, either. The difference between males and females has no chromosomal explanation.

Chromosomes 2, 4 and 5, when heterozygous in zygote, increase the incidence of wrinkled eyes. They had significant main effects and also significant primary (2×5, 4×5) and secondary interactions ($2\times4\times5$). The role of heterozygous chromosome 4 in males was especially clear: 20 males out of 23 having the wrinkled eye syndrome were heterozygous for chromosome 4 (+/*cd* 1.2 per cent, $n=1683$ vs. *cd/cd* 0.2 per cent, $n=1906$, $t=3.7$, $P<0.001$).

Localization of the maternal effect on D. virilis *chromosomes*

To localize the maternal effect, the second generation backcross hybrid females (at least 10 females of each of the 16 phenotypes) were crossed with *D. lummei* males and the frequency of wrinkled eyes was scored in their progenies reared at 25°C. Females with genotype *b;pe/b;+* were difficult to breed, and phenotypes *b;gp;+;+* and *b;+;cd;+* produced 5 and 0 flies, respectively, in spite of a much increased effort.

[*D. virilis* × (*D. virilis* × *D. lummei*)] × *D. lummei*,
[(b;gp;cd;pe) × ((b;gp;cd;pe) × 1101S)] × 1101S

The backcross mothers in this cross had conspecific X chromosomes, X^{vi}/X^{vi}. Among their progenies, 1.3 per cent had wrinkled eyes (weighted mean over all phenotypes, even if phenotype frequencies are arbitrary, $n=4823$).

[*D. virilis* × (*D. lummei* × *D. virilis*)] × *D. lummei*,
({(b;gp;cd;pe) × [1101S × (b;gp;cd;pe)]}) × 1101S

The mothers in this cross had heterospecific X chromosomes, X^{lu}/X^{vi}. The frequency of wrinkled eyes was 1.1 per cent, $n=3988$. This was not significantly different from the value in the first cross ($t=0.85$, $P\gg0.1$). Not only were the overall means identical, but also the occurrence of wrinkled eyes among the female autosomal phenotypes were similar irrespective of the X chromosome. It is to be concluded that the female X chromosomes do not contribute to the maternal effect.

Among the progenies of pooled crosses, the males and the females were equally affected ($t=0.26$, $P>0.05$, n of males 3974 and n of females 4837).

The proportion of flies with wrinkled eyes in the progenies of females of each of the 16 different backcross phenotypes are given in Fig. 3. The data were pooled over the two crosses (different female X chromosomes) and the sexes of the progeny.

It is obvious that if the mother was homozygous for the *D. virilis* chromosomes 2 *and* 5, the progeny contained significantly more flies with wrinkled eyes than otherwise. The proportion of abnormals among the progenies of *b;pe/b;pe* mothers was 6.4 per cent ($n=1353$), whereas the weighted mean of abnormals among all other phenotypes was 0.2 per cent ($n=7458$). In fact, out of the 105 handicapped individuals of this generation, 87 were born from *b;pe/b;pe* mothers. The analysis in Fig. 3b is based on a comparison of weighted means. If unweighted means are used, the contributions of the second and fifth chromosomes are equal. Chromosome 4 plays no role in the wrinkling of eyes unlike the sixth chromosome of *D. lummei*.

Discussion

The experiments reported here describe maternally determined external structural anomalies of species hybrids. The case was originally described by Sokolov (1948, 1959).

In the progenies of wild caught *D. lummei* males the total frequency of flies with wrinkled eye was slightly lower than in the progenies of laboratory stock males. However, the trait was frequent enough to imply that the anomaly was not due to strains being kept for a long time in the laboratory (>20 years). The low frequency was due to several pairs not producing handicapped progeny. The experiment revealed that there is genetic variation in *D. lummei* in the response to the maternal effect. This variation is also manifested as variation between *D. lummei* stocks (data not shown).

During this study, several stocks of *D. virilis* were tested, and there seems to be a consistent maternal effect (data not shown). All other possible hybridizations between the species in the *virilis* subgroup have

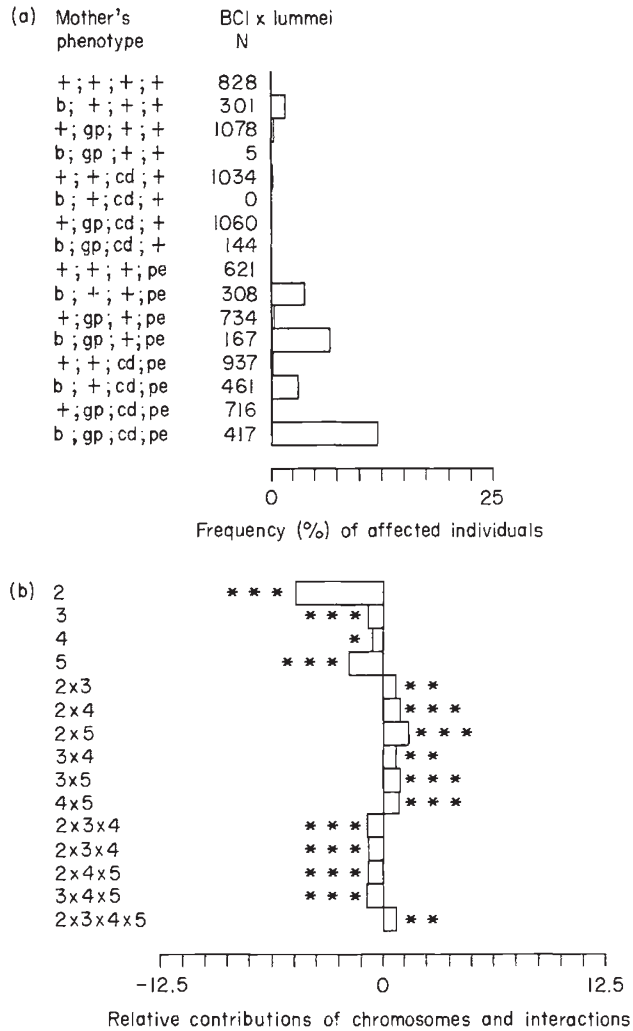

Fig. 3 Contributions of the maternal chromosomes on eye wrinkling. (a) The proportions of wrinkled eye individuals in the progenies of females of 16 different backcross phenotypes, crossed to *D. lummei*. Data are pooled over the X chromosome combinations. (b) The analysis of the data showing the relative autosomal contributions. The main effect is negative when the progenies of females homozygous for *D. virilis* chromosome were more affected than the progenies of heterozygous females. A positive value for a primary interaction means that the frequency of wrinkled eyes was lower in the progenies of females, which have a similar genotype in both chromosomes, than if the genotypes are different. Significance levels of the contributions: $*P<0.05$, $**P<0.01$, $***P<0.001$.

also been tested. No other crosses displaying structural anomalies were found.

It has been observed earlier that *D. virilis* × *D. lummei* F_1 hybrids failed to emerge from the pupal case more often than reciprocals, where almost all pupae developed to adult flies (Lumme & Heikkinen, 1990). The failure to emerge might be connected with external structural anomalies. The morphology of flies dying within the pupal case remains to be studied. Perhaps the abnormalities observed in this study are the least serious fraction of developmental defects.

The anomalies co-occurred in different organs of the same fly more often than expected at random. Simultaneous eye–leg, eye–wing, and leg–wing combinations were significantly more frequent than expected. However, the left and the right eye showed independent anomalies. The strongest correlation was between the compound eye and antenna, which were simultaneously anomalous 10 times as frequently as expected (and at the same side of the fly). The compound eye and antenna develop from the same imaginal disc, and the co-occurrence of defects indicates that the anomaly dates back to the imaginal disc determination.

There were abnormalities both in the anterior and the posterior parts of the body. Some large scale morphological maternal mutations in *Drosophila* are known, which disturb the global fate map of the egg (Davidson, 1986). The original function of these factors may be in some common cellular function, as in cell formation or multiplication. Some indications of separate maternal control for cephalic and caudalic cell specification are also known, such as *bicaudal*. Not many mutations with a maternal effect on the early zygote mitosis are known, even in *D. melanogaster*.

The role of the elimination of the microchromosome

During completion of this study, Orr (1990) published an analysis of the anomalies, concluding that the proximal cause of the defects is the elimination (equal to the absence of the sixth chromosome) of the microchromosome (sixth chromosome) of *D. lummei* in hybrids. Sokolov's (1959) conclusion was:

'If we look more carefully at the compound eye surface mosaics, which are caused by microchromosome elimination, we see that the process [microchromosome elimination] cannot cause all the anomaly types of eyes detected in the hybrids.'

The resemblance of the two phenomena is striking, and they are closely correlated. However, the results obtained during this study do not confirm this hypothesis. Mitrofanov *et al.* (1990) have showed that the elimination of the sixth chromosome involves not only a maternal effect, but also some structural and functional specialities of the genome of *D. lummei*, as the dot chromosome is sometimes eliminated from the *D. lummei* × *D. virilis* hybrids.

The proportion of wrinkled eyes was significantly lower at 17 than at 25°C. Orr (1990) observed more

anomalies (all types pooled) at 18 than at 22°C. The proportion of F_1 hybrids with monosomic *D. virilis* dot chromosome is 100 per cent at 17°C, much more than at 25°C (Sidorova, 1974). Evgen'ev *et al.* (1983), who studied the toromere of *D. lummei*, showed that the elimination of the sixth chromosome of the species increased if the culture temperature decreased and if the sixth chromosome had to replicate in *D. virilis* genetic conditions. If elimination would cause the wrinkling then the proportion of anomalous flies should increase at lower temperature. The temperature dependence thus remains controversial. A more detailed developmental profile is needed.

Orr (1990) crossed different taxa of the virilis subgroup and observed the co-occurrence of the elimination of the sixth (*glossy* phenotype) and developmental anomalies. *D. virilis* × *D. lummei* was the only combination where both phenomena were common. This is no strong evidence for a common basis. In the results presented here, the phenotypic correlation between the appearance of the visible marker of the sixth chromosome, *glossy*, and the wrinkling of the eye in F_1 is striking. Correlation between these phenomena does not mean that their relationship is causal. The conclusion is that at the individual phenotypic level, wrinkling occurs much more often in eyes which are haploid for the *D. virilis* sixth chromosome. This analysis will be extended to the genetic level.

Maternal effect is nuclear and autosomal (the second and the fifth chromosome of D. virilis*)*

The anomalies were found only in one of the reciprocal crosses and they were equally frequent in males and females, in accordance with Orr's (1990) findings. Because female progenies from both reciprocal crosses carried an identical set of heterozygous X chromosomes, these observations lead to the assumption of a maternal effect. This could be caused by three different mechanisms: nuclear genes, mitochondrial genes, or endosymbionts.

Barr (1980) showed that the incompatibility of two strains of *Culex pipiens* is due to a rickettsia-like bacterial symbiote. Hoffmann *et al.* (1986) and Hoffmann & Turelli (1988) found that a similar situation existed between some populations of *D. simulans*. The *D. virilis* × *D. lummei* cross was repeated on antibiotic supplemented medium in order to test the possibility that the anomalies are caused by a symbiotic micro-organism. Both males and females of the parental generation were treated with ampicillin, streptomycin, and tetracycline, from egg to adult, as well as the F_1.

Although the frequency of individuals with wrinkled eyes decreased following treatment by all the antibiotics, the anomalies were not otherwise cured. The compound effect of ampicillin and streptomycin was not significant. The vials were attacked by different kinds of molds, bacteria and *Histiostoma laboratorium* mites. In superinfected vials, a large amount of flies died within pupal cases. This probably affected the frequency of handicapped flies by killing a larger proportion of them than normal. Abnormal flies tend to eclose later than normal flies.

Orr (1990) found a higher proportion of externally abnormal hybrids from tetracycline-supplemented vials than from normal vials, but the proportion of eye anomalies also decreased in his experiment.

These results suggest that wrinkling of eyes in hybrids is not caused by a micro-organism but rather by the interactions of maternal and zygotic genes in the hybrids.

The maternal effect of *D. virilis* females was analysed by producing backcross females with different combinations of heterozygous and homozygous X and large autosomes. To keep the chromosomes intact, F_1 males were used for backcrossing, and therefore all female genotypes tested carried the mitochondrial genes of *D. virilis*. Among them, the maternal effect was solely caused by the simultaneous presence of the homozygous second and fifth chromosomes of *D. virilis*. In contrast to the reduced viability and fertility of interspecific hybrids, neither the X chromosome of the mother (*D. virilis*) nor the X chromosome of the zygote was found to play any significant role. Orr (1990) made crosses which demonstrated that the mitochondrial genes of *D. virilis* do not participate in the induction of zygotic anomalies.

Microchromosome elimination is also determined maternally by nuclear genes (Sokolov, 1959; Orr, 1990). Sidorova (1974) showed that at low temperatures the elimination of the dot chromosome occurs in the first mitotic cleavage. The maternal control of the elimination of the *D. lummei* dot chromosome was carefully analysed by Mitrofanov & Sidorova (1979). Their data consisted of 24,709 offspring of backcross females. At 25°C, *only the second* chromosome contributed to the maternal effect. Among the progeny of *b*/*b* females, 33.7 per cent ($n = 7065$) lose the microchromosome of *D. lummei*, when the proportion was 1.8 per cent in progeny of *b*/+ ($n = 10{,}032$). At 17°C, the frequency of elimination was 100 per cent in the progenies of all genotypes carrying *the fourth* chromosome as homozygous. Among the fourth chromosome heterozygotes, a heterozygous second chromosome was able to partially rescue the microchromosome. When the wrinkling of the eye was clearly controlled by simultaneous homozygous factors on *D. virilis*

chromosomes 2 *and* 5, it is to be concluded that the genetic basis of maternal effect is not identical for the two traits.

Zygotic response is polygenic, autosomal

The present results indicated that the second, the fourth and the fifth chromosomes of *D. lummei* when heterozygous in the F_1 facilitate the wrinkling of the eye. Their role is polygenic, with some mild non-additive interactions (Fig. 2). Thus, the sixth chromosome of *D. lummei* is not alone in responding to the maternal effect.

The similarity of the proportions of the wrinkled eyes in backcrosses to reciprocal F_1 does not give any new information about the role of the sixth chromosome in the wrinkling of eyes. If in F_1 (*D. virilis* × *D. lummei*) the sixth chromosome of *D. lummei* is already eliminated, the elimination cannot be the reason for wrinkling in *D. virilis* × F_1. Elimination is infrequent in the germ-line (Evgen'ev & Sidorova, 1976). On the other hand, if F_1 (*D. virilis* × *D. lummei*) males have lost the paternal dot chromosome in the first mitotic cleavage (Sidorova, 1974), they produce nullo-6 sperm also (Evgen'ev & Sidorova, 1976).

Acknowledgements

This work was supported by the Natural Science Research Council (Project 1051049). I thank Jaakko Lumme and Allen Orr for comments on the manuscript.

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