Heredity **69** (1992) 416–422

Received 17 December 1991

Loss of a paternal chromosome causes developmental anomalies among *Drosophila* hybrids

JOHN M. BRAVERMAN, BEATRIZ GOÑI* & H. ALLEN ORR

*Center for Population Biology and *Department of Genetics, University of California, Davis, CA 95616, U.S.A.*

Hybrids between *Drosophila virilis* and *D. lummei* suffer from developmental anomalies. Previous reports also suggest that these hybrids lose the *D. lummei* sixth chromosome early in development. Genetic and cytological data presented here confirm the loss of the microchromosome from both the soma and the germ-line of these hybrids and provide strong evidence that this loss causes the hybrid developmental anomalies.

Keywords: chromosome loss, *Drosophila lummei, Drosophila virilis*, hybrid inviability, morphological anomalies, speciation.

Introduction

Hybrid sterility and inviability are important forms of reproductive isolation in nature but their physiological bases are usually unknown. One exception is the syndrome of anomalies in hybrids between *Drosophila virilis* and *D. lummei*, which includes such deleterious traits as reduced eyes, unequal wing lengths, twisted abdomens, missing or reduced thoracic bristles, incomplete sclerotization of the abdomen, and uninflated, broken, or incomplete wing veins (Sokolov, 1948, 1959). There is some evidence to suggest that these anomalies result from somatic loss of the *D. lummei* microchromosome (the 'dot' or sixth chromosome) from hybrids (Orr, 1990). Individuals that lose their microchromosomes, for example, show far more anomalies than those that do not. Although Heikkinen (1991) also observed this correlation, she recently concluded that the anomalies do not result from microchromosome loss.

This note has two purposes: (i) to confirm that the *D. lummei* dot chromosome is, in fact, lost among *D. virilis*–*D. lummei* hybrids, and (ii) to test directly whether microchromosome loss causes the hybrid anomalies.

Previous evidence that hybrids lose the microchromosome has been indirect, largely based on the behaviour of the microchromosomal marker *glossy* (*gl* 6–1.0) among hybrids. Although *glossy* is recessive, F_1 hybrids that have *D. virilis* mothers are often mosaic or completely glossy (Sokolov, 1948, 1959; Evgen'ev & Sidorova, 1976; Orr 1990; Heikkinen, 1991). These observations have been interpreted as the result of a loss of the wild-type *D. lummei* chromosome from the hybrid embryo.

However, other explanations for the appearance of *glossy* are possible. For instance, hybrids might appear glossy if the normally-recessive *gl* allele acts dominantly among hybrids. Such reversals of dominance in species hybrids are well-known (Muller, 1942). Alternatively, the heterochromatic nature of the dot chromosome (Miklos, *et al.*, 1988; Ashburner, 1989, chapter 23) may predispose it to position-effect variegation, and this may result in glossy mosaicism.

Cytological evidence for loss of the *D. lummei* dot chromosome is also not strong. Evgen'ev & Sidorova (1976) claim that this chromosome is usually missing from salivary gland preparations of hybrids. However, the tiny dot chromosome is often entangled with the chromocentre and is difficult to see. More convincing evidence of chromosome loss would come from mitotic cells, where the number of microchromosomes can be scored more confidently.

The genetic and cytological data presented here demonstrate that the *D. lummei* dot chromosome is indeed lost from somatic tissues of hybrids. The temperature-sensitivity of chromosome loss, whether such loss occurs in the germ-line, and whether hybrids also lose other chromosomes, are also investigated. Finally, our data provide strong evidence that this loss causes the syndrome of developmental anomalies seen among these hybrids.

Table 1 Glossy phenotypes of F_1 hybrids. Mosaics are classified as 1/2-eye glossy (Class 2), 1-eye glossy (Class 3), or 1-1/2 eye glossy (Class 4)

Genotype	Temperature	Both-eyes wild-type	Mosiac Class 2	Mosiac Class 3	Mosiac Class 4	Both-eyes glossy	Total
VL	22°C	224	101	262	92	247	926
LV	22°C	239	0	0	0	0	239
VL	18°C	0	0	7	4	876	887

Materials and methods

The fly stocks used were *D. virilis peach; glossy* [*pe*: 5-203; *gl*: 6-1.0; map positions from Alexander (1976)] and *D. lummei* Finland. These stocks were obtained from the Bowling Green Species Stock Center. Unless otherwise indicated, all crosses were performed at 22°C as described by Orr (1990).

We examined the karyotypes of *D. virilis, D. lummei* and *D. virilis-D. lummei* hybrids in somatic and germ cells. Mitotic cells from cerebral ganglia of third instar larvae or prepupae were karyotyped. Gonial and meiotic cell divisions from the testes of newly emerged adults were also karyotyped. Cytological preparations were made following the air-drying method of Imai *et al.* (1988), without colchicine treatment. This method produces C-banded metaphase karyotypes. All cytological preparations were made from single flies and data was collected only from those cells in which the full chromosome complement was clearly visible.

To abbreviate the description of crosses, the following notation is used: V= *D. virilis* and L= *D. lummei*; in hybrids and backcrosses the maternal parent is always listed first. Thus (VL)L represents the backcross of F_1 hybrid females that have *D. virilis* mothers, to *D. lummei* males.

Results

Is the D. lummei *microchromosome lost from somatic tissues of hybrids?* As expected (Sokolov, 1948, 1959; Evgen'ev & Sidorova, 1976; Orr, 1990), F_1 hybrids from the cross *D. virilis pe;gl* × *D. lummei* are usually glossy or glossy mosaics (Table 1, line 1). Many of these flies show a weak Minute phenotype [the dot chromosome of most, if not all, *Drosophila* species carries a Minute locus (Ashburner, 1989, chapter 23)]. The reciprocal cross, however, does not produce glossy offspring (line 2). Glossy phenotypes among F_1(VL) hybrids are much more common at lower temperatures (line 3), as previously reported (Evgen'ev & Sidorova, 1976). The important question is whether this glossy phenotype reflects somatic microchromosomes loss or whether it reflects one of the alternative explanations described in the Introduction.

Table 2 Cytological observations in somatic (cerebral ganglia) cells in *D. virilis, D. lummei* and F_1 (VL) reared at 18°C

Genotype	Individuals	Mitotic cells	Haplo-6 cells
D. virilis pe;gl			
Male	11	53	0
Female	4	12	0
D. lummei			
Male	12	59	0
Female	4	17	0
F_1(VL)			
Male	9	43	43
Female	7	34	34

To test the chromosome loss hypothesis, the karyotypes of cerebral ganglia cells from both pure species and hybrid larvae were examined (all flies raised at 18°C). The data are shown in Table 2 and are illustrated in Fig. 1 a-d. The chromosome complement of the two species consists of five pairs of rods and a pair of dot (or sixth) chromosomes (Throckmorton, 1982). All pure species individuals are diplo-6, while all hybrid individuals are haplo-6. Thus microchromosome loss does frequently occur among F_1(VL) hybrids, and the glossy phenotype can be used as a convenient indicator of this chromosome loss.

Is the microchromosome lost from the germ-line? There has been some confusion about whether chromosome loss also occurs in the germ-line of hybrids. Evgen'ev & Sidorova (1976), who constructed a special stock carrying the *D. lummei* dot chromosome in an otherwise *D. virilis* genome, reported that germ-line loss within this stock either does not occur or is very rare.

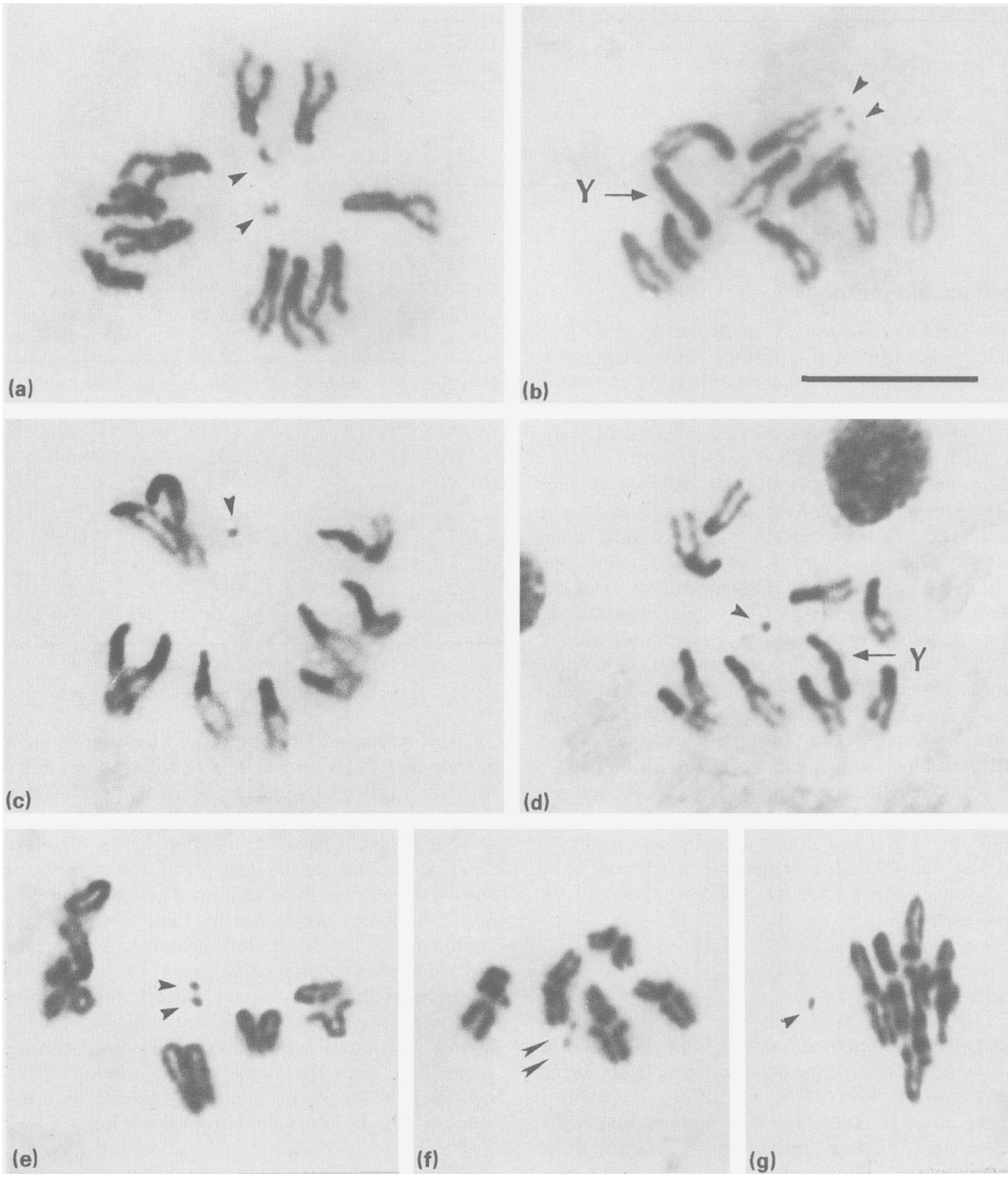

Fig. 1 Sample micrographs of *D. virilis*, *D. lummei*, and F_1(VL) hybrid chromosomes in C-band mitotic metaphase (a–d) and meiotic metaphase I and anaphase I (e–g): (a) *D. virilis* female, (b) *D. lummei* male, (c) F_1(VL) female, (d) F_1(VL) male, (e) *D. virilis* male, (f) *D. lummei* male and (g) F_1(VL) male. The dot chromosomes are indicated by arrow heads. The heterochromatic Y-chromosome is indicated in (b) and (d). Bar = 10 μm.

This does not necessarily mean, however, that germ-line loss does not occur among F_1(VL) hybrids.

The karyotypes of gonial and meiotic cells from the testes of both pure species individuals and hybrid flies were examined. The microchromosome was always missing from the germ-line of hybrids (Table 3). Sample micrographs appear in Fig. 1e–g.

To verify that the *D. lummei*, and not the *D. virilis*, microchromosome is missing from germ cells, *individual* 'whole-eye glossy' F_1(VL) males — which lack a *D. lummei* microchromosome in most or all of their somatic tissues — were backcrossed to *D. virilis pe;gl* females. If a male has also lost the *D. lummei* dot chromosome from its germ-line, then all of its progeny should be gl (progeny were reared at 22°C to minimize zygotic chromosome loss). The absence of gl^+ progeny could, however, have another cause: the *D. lummei* microchromosome on a largely *D. virilis* genetic background may simply result in inviability. To control for this possibility, individual gl^+ F_1(VL) males — which have not lost the *D. lummei* dot chromosome from their soma — were backcrossed to *D. virilis pe;gl* females. These males will produce gl^+ progeny unless the *D. lummei* sixth on a *D. virilis* background causes inviability. In the experimental cross (glossy fathers), only three of 15 hybrid males produced any gl^+ progeny. In the control cross (wild-type fathers), 26 of 32 males produced gl^+ progeny ($\chi^2 = 16.2$, d.f. = 1, $P < 0.0001$; data included only if parental male produced seven or more offspring). Thus the absence of the *D. lummei* dot among the progeny of experimental males is not an artifact of hybrid inviability. Instead, the *D. lummei* dot chromosome is often missing from the germ cells of F_1(VL) males. This may well reflect loss of the chromosome before the germ-line is set aside in early embryogenesis [in *D. melanogaster*, pole cells form in the ninth division (Ashburner, 1989, p. 170)].

Are other chromosomes lost from hybrids? Evgen'ev & Sidorova (1976) suggest that there is nothing special about the dot chromosome: loss of other *D. lummei* chromosomes from hybrids might be common. They argue, however, that the inviability of embryos that lack a major chromosome would prevent easy detection of such chromosome loss. Loss of the dot chromosome, on the other hand, is easily detected among adult hybrids because haplo-dot chromosome flies are viable in *Drosophila* (Lindsley & Grell, 1968).

As a partial test of this hypothesis, we scored the presence of the Y chromosome among hybrid males. This is the only other chromosome whose loss can be tolerated in *Drosophila* [XO flies are viable in *D. virilis* (Alexander, 1976)]. Cytological data from 18 F_1(VL) males reared at 18°C revealed no other chromosome aneuploidy; the Y chromosome was present in all cases (Fig. 1d). This result demonstrates that all *D. lummei* chromosomes do not have equal chances of loss, and it suggests that the microchromosome may be particularly prone to loss. It remains possible, however, that the Y chromosome is particularly resistant to loss.

Does microchromosome loss cause the developmental anomalies among hybrids? To determine if microchromosome loss causes the hybrid anomalies, we produced two hybrid genotypes which differed, on average, only at the microchromosome: the first genotype inherited a *D. lummei* dot (which could then be lost early in development) and a *D. virilis* dot, while the second genotype inherited only *D. virilis* dot chromosomes (which are not lost). If hybrid anomalies result from loss of the *D. lummei* dot, the first genotype should frequently show anomalies, while the second should never (or very rarely) show anomalies.

The first genotype was produced by backcrossing pe^+; gl^+ F_2(LV) males, which carry at least one *D. lummei* microchromosome, to *D. virilis pe;gl* females. Zygotes resulting from this cross were gl^+/gl or gl/gl. The second genotype was produced by backcrossing pe^+; gl F_2(LV) males, which carry two *D. virilis* dot chromosomes, to *D. virilis pe;gl* females. All progeny from this cross were gl/gl. Because all F_2 males were derived from an initial LV (not VL) cross, these F_2 males have experienced almost no chromosome loss and are almost all diplo-6. The genetic background was

Table 3 Cytological observations in germ cells in *D. virilis*, *D. lummei*, and F_1(VL) reared at 18°C

Genotype	Males	Gonial cells	Meotic cells	Haplo-6 cells
D. virilis pe;gl	10	14	13	0*
D. lummei	12	27	13	0
F_1(VL) pe^+;gl	16	27	13	40

*One gonial mitotic cell had $2n = 14$ (including one pair of dot chromosomes and a Y chromosome), instead of the usual $2n = 12$.

Table 4 Test of dependence of hybrid anomalies on microchromosomal genotype. 'Eye', 'wing' and 'vein' refer to types of hybrid anomalies. The total per cent anomalous is a weighted average. See text for details

Parental male	Offspring	Normal	Eye	Wing	Vein	Total	Per cent anomalous
$pe^+; gl^+$	gl	509	11	1	0	521	2.30
	gl^+	38	0	18	4	60	36.67
	gl-mosaic	64	27	20	6	117	45.30
	Total	611	38	39	10	698	12.46
$pe^+; gl$	gl	1068	0	0	3	1071	0.28

partly controlled by using only F_2 males that carried at least one *D. lummei* fifth chromosome (i.e. all males were pe^+). The frequency and type of anomalies was scored for each eye phenotype produced in each cross.

Table 4 reports the results from each cross. In the first cross, over 12 per cent of all progeny showed an anomaly. In the second cross (where hybrids do not inherit a *D. lummei* dot) only 0.28 per cent showed any anomaly (χ^2 pooling anomalous types = 129.9, d.f. = 2, $P < 0.0001$). Out of over 1,000 hybrids scored, no eye or wing-length anomalies ever appeared in this second cross (three subtle wing venation variants were seen), although these anomalies were common among the progeny of the first cross. Thus, among diplo-6 zygotes, appearance of these dramatic anomalies requires inheritance of the *D. lummei* microchromosome. Subtle wing venation variations may, however, have a different or additional genetic cause.

Table 4 provides further evidence of microchromosome involvement in the anomalies. Among the progeny of the first cross, about 40 per cent of the gl^+ and gl-mosaic flies (which definitely inherited a *D. lummei* dot) showed anomalies, while only about 2 per cent of the gl flies (most of which did not inherit a *D. lummei* dot) showed anomalies ($\chi^2 = 194.4$, d.f. = 2, $P < 0.0001$). The low frequency of anomalies among gl flies presumably reflects the small fraction of gl flies which are *gl/0*.

An additional detail from these crosses indicates that microchromosome loss causes the hybrid anomalies. While gl-mosaic flies show frequent wing-length, wing venation and eye anomalies, gl^+ flies show no eye anomalies, although they frequently exhibit other anomalies (Table 4). These two phenotypes differ in only one respect: although they begin with identical zygotic genotypes, gl-mosaics have lost the *D. lummei* dot from patches of eye tissue, while gl^+ flies have not (they may, however, have lost the dot from patches of other tissues). The fact that flies that have lost the dot from eye tissue show eye anomalies, while flies that have not lost the dot from eye tissue do not show eye anomalies, represents strong evidence that loss of the *D. lummei* microchromosome is the cause of the hybrid anomalies.

Discussion

The *D. lummei* microchromosome is frequently lost from both the soma and the germ-line of *D. virilis–D. lummei* hybrids. The results presented here also confirm that this chromosome loss is temperature-sensitive and suggest that other *D. lummei* chromosomes (at least the Y chromosome) are not lost as frequently from hybrids.

These results also provide strong evidence that loss of the *D. lummei* dot chromosome causes the developmental anomalies observed among these hybrids. The most direct evidence is based on a comparison of two hybrid genotypes that differ only in the species origin of the dot chromosomes. Although hybrids that inherit a *D. lummei* microchromosome often suffer from developmental anomalies, those which inherit only *D. virilis* microchromosomes show almost no anomalies. In addition, while backcross hybrids showing chromosome loss in eye tissues often suffer eye anomalies, hybrids not showing chromosome loss from the eye do not suffer eye anomalies (also see Orr, 1990). This pattern is evidence that the appearance of hybrid anomalies involves loss of the *D. lummei* microchromosome.

Heikkinen (1991), however, recently concluded that loss of the *D. lummei* microchromosome does not cause the hybrid anomalies. Her conclusion is primarily based on the claim that chromosome loss and the eye anomalies have different genetic bases. Specifically, Heikkinen found that the eye anomaly depends primarily on maternal genotype at chromosomes 2 and 5 (with 2 having the largest effect, see her fig. 3).

Mitrofanov & Sidorova (1979), however, found that microchromosome loss involves a large effect of maternal chromosome 2 and smaller effects of all other major autosomes (all results from 25°C). Further comparison of Heikkinen's data with Mitrofanov & Sidorova's (1979) caused Heikkinen to conclude that chromosome 4 has a smaller effect on the hybrid anomalies than on chromosome loss, while chromosome 5 shows the reverse pattern.

Although these data do suggest some subtle differences between the genetics of hybrid chromosome loss and hybrid anomalies, note that these studies were performed many years apart using different strains in different laboratories. Considering the uncertainty in such an uncontrolled comparison, the similarities in genetic basis seem more significant than the differences mentioned above. Both microchromosome loss and the hybrid anomalies occur non-reciprocally (*D. virilis* mothers in both cases). Both involve maternal effects. In both cases, the maternally acting genes from *D. virilis* act recessively. Finally, in both cases, maternal chromosome 2 has the largest effect.

In addition, Heikkinen's claim that the two characters have different genetic bases seems contradicted by her own data. Heikkinen (1991, table 3) found, as did Orr (1990), that there is an extremely stong correlation between those F_1(VL) hybrids that show chromosome loss and those that show the anomalies. Given that all F_1 hybrids have identical zygotic genotypes (i.e. a haploid complement of chromosomes from each species) and identical maternal genotypes, the two characters cannot have separate genetic bases. If these characters had different genetic bases, it is unclear why flies of identical zygotic and maternal genotype would show either both characters or neither.

Heikkinen's direct test of the effect of the microchromosome on the hybrid anomalies also has some problems. In this test, Heikkinen compared the frequency of anomalies among the progeny of F_1(LV) versus F_1(VL) males backcrossed to *D. virilis* females. Under the chromosome loss hypothesis, the progeny of F_1(LV) males will often inherit (and subsequently lose) a *D. lummei* dot, and so will show anomalies. However, because many of the reciprocal F_1(VL) males have already lost their dot chromosome, Heikkinen (p. 365) argued that their progeny will not inherit a *D. lummei* dot and so should not show anomalies. Because the progeny of both types of male show roughly equal frequencies of anomalies. Heikkinen (p. 357) concludes that 'the role of the sixth chromosome (in producing the eye anomaly) is not decisive (p. 361).

However, the prediction that the progeny of F_1(VL) males which have already lost their *D. lummei* dot, should not show anomalies assumes that a hybrid zygote must inherit and then lose a *D. lummei* dot in order to show anomalies. It is not clear, however, why a hybrid, which is haplo-6 from the moment of fertilization (i.e. one resulting from a nullo-6 sperm from a F_1(VL) male) would not also show anomalies. If so, then one would expect the progeny of both F_1(VL) and F_1(LV) males to show anomalies, as observed. In any case, the present data clearly show that the sixth chromosome does, in fact, play a decisive role in the hybrid anomalies.

Although chromosome loss is well-documented among plant hybrids [e.g. in barley, see Bothmer *et al.* (1991) and Bennett *et al.* (1976)], it is unknown how common it is among animals. It remains possible that chromosome loss is fairly common among animal hybrids and that it is a frequent cause of hybrid inviability. However, because early loss of a major chromosome would presumably be embryonic lethal, hybrid chromosome loss would usually go undetected (we know of no case other than *D. virilis–D. lummei* in which chromosome loss has been looked for among hybrid embryos). Whether chromosome loss among *D. virilis–D. lummei* hybrids represents a rare, isolated incident or some more common phenomenon will remain unknown until additional animal hybridizations are studied.

Acknowledgements

We thank J. Coyne, C. Langley, M. Orr, and T. Prout for helpful discussion and comments. This work was supported by a Sloan Post Doctoral Fellowship to HAO.

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.

ASHBURNER, M. 1989. *Drosophila: a Laboratory Handbook*. Cold Spring Harbor Laboratory Press, New York.

BENNETT, M. D., FINCH, R. A. AND BARCLAY, I. R. 1976. The time rate and mechanism of chromosome elimination in *Hordeum* by hybrids. *Chromosoma*, **54**, 175–200.

BOTHMER, R. VON, SALOMON, B. AND LINDE-LAURSEN, I. 1991. Cytogenetics in hybrids of *Hordeum jubatum* and *H. tetraploidium* with cultivated barley (*H. vulgare* l.). *Hereditas*, **114**, 41–46.

EVGEN'EV, M. B. AND SIDOROVA, N. V. 1976. Genetic regulation of chromosome behavior in interspecific hybrids of *Drosophila*. *Theor. Appl. Gen.*, **48**, 55–61.

HEIKKINEN, E. 1991. Wrinkling of the eye in hybrids between *Drosophila virilis* and *Drosophila lummei* is caused by interaction of maternal and zygotic genes. *Heredity*, **66**, 357–365.

IMAI, H. T., TAYLOR, R. W., CROSLAND, M. W. J. AND CROZIER, R. H. 1988. Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. *Jpn J. Genet.*, **63**, 159–185.

LINDSLEY, D. L. AND GRELL, E. H. 1968. *Genetic Variations of* Drosophila melanogaster. Carnegie Inst. Wash. Publ. 627. Washington, DC.

MIKLOS, G. L. G., YAMAMOTO, M. -T., DAVIES, J. AND PIRROTTA, V. 1988. Microcloning reveals a high frequency of repetitive sequences characteristic of chromosome 4 and the *B*-heterochromatin of *Drosophila melanogaster. Proc. Natl. Acad. Sci.*, U.S.A., **85**, 2051–2055.

MITROFANOV, V. G. AND SIDOROVA, N. V. 1979. Phenogenetic analysis of chromosome behavior during mitosis in female *Drosophila virilis* X male *Drosophila lummei* hybrids. *Soviet Genetics*, **15**, 807–811.

MULLER, H. J. 1942. Recessive genes causing interspecific sterility and other disharmonies between *Drosophila melanogaster* and *simulans. Genetics*, **27**, 157.

ORR, H. A. 1990. Developmental anomalies in *Drosophila* hybrids are apparently caused by loss of microchromosome. *Heredity*, **64**, 255–262.

SOKOLOV, N. N. 1948. Elimination of chromosomes in interspecific hybrids of *Drosophila* and the problems of distant hybrids. *Dokladi Academii Nauk*, **59**, 163–166. (In Russian).

SOKOLOV, N. N. 1959. The interaction of nucleus and cytoplasm in distant hybrids. *Moscow: Izdatelstvo Academii Nauk*, 1–148 (In Russian).

THROCKMORTON, L. H. 1982. The *virilis* species group. In: Ashburner, M., Carson, H. L. and Thompson, J. N. Jr. (eds) *The Genetics and Biology of Drosophila*. Vol. 3b, Academic Press, London, pp. 227–296.