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Genetics of the Sex Ratio Anomaly in *Drosophila* Hybrids of the *Virilis* Group

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Summary. A study was made of the effect of genotype and temperature (25 and 17°C) on sex ratio in the hybrids *D. virilis* Sturt. × *D. littoralis* Sokolov. A genetic system has been found controlling sex-differential viability. In the F_1 of the reciprocal hybrids *D. virilis* × *D. littoralis* the sex ratio is normal, though at 17°C females are slightly excessive. The abnormal sex ratio is observed only in the progeny of test crosses.

The major gene causing the death of female progeny of the cross ♀ [♂ (♀, ♂ *D. virilis* × ♂, ♀ *D. littoralis*) × ♀ *D. virilis*] × ♂ *D. littoralis* is located on chromosome 2 of *D. virilis*. It is expressed as a lethal if chromosome 5 is heterogeneous *virilis-littoralis*. Chromosome 3 of *D. virilis* bears a modifier-enhancer and chromosome 5, a suppressor, of this lethal found in chromosome 2. This genetic system has a maternal effect and functions at 25°C, interacting with the X-chromosome of *D. littoralis*. If the maintainance temperature is lowered to 17°C, the progeny of the cross 'hybrid ♀ FB_1 × ♂ *D. littoralis*' is predominantly female. Partial death of males is accounted for by a disturbance in the interaction between the genes of X-chromosome in certain combinations with the *D. virilis* autosomes and the Y-chromosome of the paternal species *D. littoralis*.

Sex-differential mortality in the hybrids *D. virilis* × *D. littoralis* is one of the isolating factors between these species which does not appear to act until the second and subsequent F_1 generations due to the formation of the recombination load.

Key words: Genotype – Material effects – Mortality – Sex ratio – Recombination load – *Drosophila* hybrids

Introduction

According to Haldane's rule (Haldane 1922), in the hybrid progeny from interspecific crosses it is most often the heterogametic sex that die or suffer developmental abnormalities. In *Drosophila* these are the males. In experimental practice, however, the death of hybrid males is less frequent than, for example, sterility. As shown by numerous examples of interspecific hybridization in the genus *Drosophila*, the sex ratio in the F_1 is often normal. (Patterson and Stone 1952).

Also, examples are known of deviations from the Haldane's rule in interspecific *Drosophila* hybrids. In hybrid progeny from crosses between ♀ *D. montana* and ♂ *D. americana texana* only males survive while homogametic females die; the reciprocal crosses give normal sex ratios. In *D. americana texana* the dominant lethal factor is located on the X-chromosome near the *ec* locus. In the subspecies *D. americana americana* no such factor has been found. In *D. montana* the factors operating through the egg cytoplasm proved recessive. Similar cases of the sex ratio anomaly was observed in hybrids of the subspecies of *D. aldrichi*. (Patterson and Griffen 1944; Crow 1942).

It can be seen even from these rare examples that either sex can be eliminated by the isolating mechanisms based on sex-differential mortality, though most often it is the male sex that dies.

The analysis of genetic mechanisms underlying sex-differential mortality is important for both the theory of evolution and for purely practical purposes since most of the up-to-date genetic methods of insect pest control are based on the elimination of one of the sexes.

The present paper describes the genetic mechanism responsible for the death of one of the sexes in the hybrids between the sibling species of *Drosophila*.

Materials and Methods

The experiments were carried out on two species of *Drosophila* of the *virilis* group: *D. virilis* with the recessive markers *broken (b), gapped (gp), cardinal (cd), peach (pe), glossy (gl)* in chromo-

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Table 1. Number of females obtained in hybrid progeny from reciprocal crosses

Cross	Temperature (°C)	No. of individuals studied	No. of experimental females, %	Theoretically expected no. of females, %
1) ♀ *D. virilis* ×	25	432	45.1-2.39	50.0-2.41
♂ *D. littoralis*	17	485	56.7-2.25	50.0-2.27
2) ♀ *D. littoralis* ×	25	441	49.2-2.38	50.0-2.38
♂ *D. virilis*	17	362	57.5-2.60	50.0-2.63

somes 2, 3, 4, 5, 6 respectively, and *D. littoralis* of wild type (Sokolov 1959). Reciprocal crosses as well as test- and backcrosses were made between the parental species. The progeny either developed from the egg stage to imago at 25°C, or the batches were kept at 17°C until hatching and then transferred to 25°C. A distortion of the sex ratio was estimated by the proportion of females in the progeny of each cross. Theoretically, 50% of the progeny were expected to be females.

Results

The proportion of females in the F_1 of reciprocal hybrids *D. virilis* × *D. littoralis* is close to that theoretically expected (Table 1). At a maintainance temperature of of 17°C females somewhat outnumber males, though the difference is unreliable. Also, a series of experiments were run to elucidate the role of different combinations of *D. virilis* and *D. littoralis* chromosomes in sex-differential mortality. The individuals under study were homozygous only for the chromosomes of *D. virilis*, with *D. littoralis* chromosomes always in heterozygous condition.

In the first experiment females of *D. virilis* marked with recessive mutations were crossed to *D. littoralis* males; the resulting hybrid males were crossed to females of the marked line of *D. virilis*. Among the progeny of this cross males of 16 phenotypic classes were selected. In all these classes the males were homozygous for one or several *D. virilis* autosomes and heterozygous for *D. littoralis* autosomes. In addition, these individuals had homozygous *D. virilis* chromosomes 1 and 6. Males and females produced from the crosses between *D. littoralis* females and males from the 16 phenotypic classes were tested for the 'sex ratio' character.

As seen from Figure 1a, when crossing males bearing heterogeneous chromosomes (autosomes) to *D. littoralis* females, in none of 16 crosses at 25°C was a sex ratio distortion observed. At the low temperature of maintainance (17°C), the character was also not affected (Fig. 1b). Crosses of males of the genotypes *b, gp*, and *cd, pe* produced few progeny. Males of the genotype *b, gp, pe* were almost sterile and produced only 5 individuals (2 females and 3 males). The low percentage of females in the crosses ♂ *b, gp* × ♀ *D. littoralis* is unreliable due to the small number of progeny obtained.

When crossing hybrid females of all 16 classes to *D. littoralis* males, a unique pattern of sex ratio distortions was obtained which varied with female genotype and temperature of maintainance. At 25°C females bearing *D. virilis* chromosomes 2; 2 and 3; 2 and 4; 2, 3, 4 in

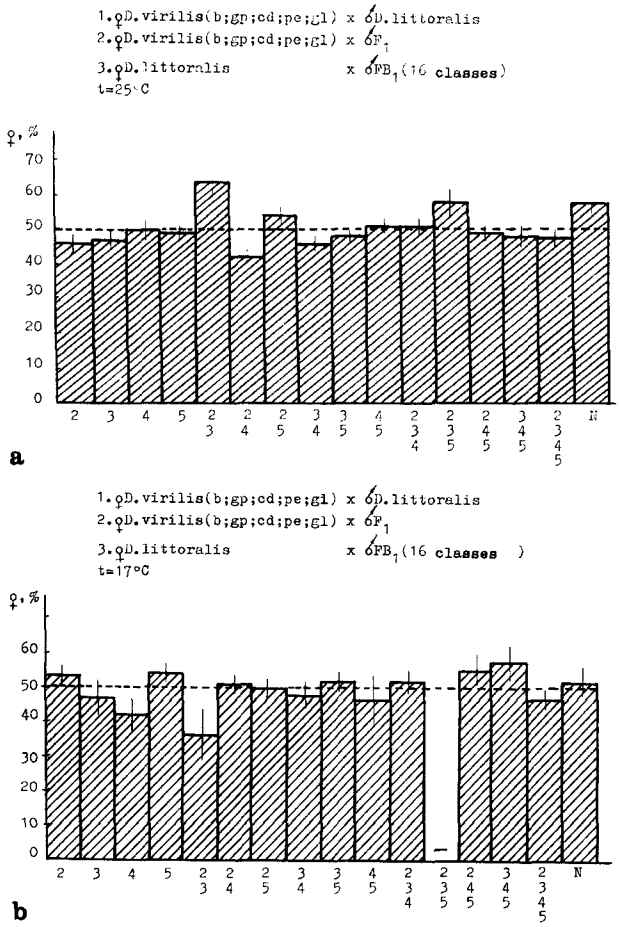

Fig. 1a and b. Number of female offspring obtained from crossing ♀ *D. littoralis* × ♂ FB_1 homozygous by different chromosomes of *D. virilis*, at 25° and 17°C. On the abscissa: 2-5 homozygous and heterozygous (N) *D. virilis* chromosome combinations under analysis

homozygous condition gave mostly male progeny (Fig. 2a), the sex ratio distortion being especially sharp in the latter case with combination of all three chromosomes of *D. virilis*.

The major gene (or genes) responsible for the death of females is linked with chromosome 2 of *D. virilis*. The gene in chromosome 3 appears to be its enhancer since in combination with chromosome 2 it is especially effective in decreasing the number of female progeny. The gene of chromosome 4 is relatively neutral with regard to the gene of chromosome 2, but in combination with chromosomes 2 and 3 it produces the minimal number of female progeny (2.2%). The gene (genes) of chromosome 5 suppresses all lethal effects of other chromosome combinations and can be thought of as a suppressor of the lethal action of chromosome 2. Only in combination with chromosomes 2 and 3 is the death of females high enough to differ significantly from that theoretically expected.

If the development of eggs begins at 17°C the percentage of female progeny from almost all crosses increases. This shift in the equilibrium sex ratio at the expense of male deficiency is observed with most of the different combinations of *D. virilis* chromosomes (Fig. 2b). Chromosomes 2 and 5, as well as combinations of *D. virilis* chromosomes 2 and 4; 3 and 4; 3 and 5; 4 and 5; 2, 4 and 5; 3, 4 and 5 increase the number of females (or decrease the number of males) by 12-15% on the average compared with the theoretically expected value. In females fully homozygous or heterozygous for *D. virilis* chromosomes the sex ratio is 1:1; Also normal is the sex ratio in the progeny of females *gp; cd; b, pe; b, gp, pe; b, gp; b, gp, cd.*

When crossing females from the 16 phenotypic classes not to *D. littoralis* males but to *D. virilis* from the marked line the sex ratio is normal in all progenies from all crosses at all temperatures of maintainance studied (Fig. 3a, b).

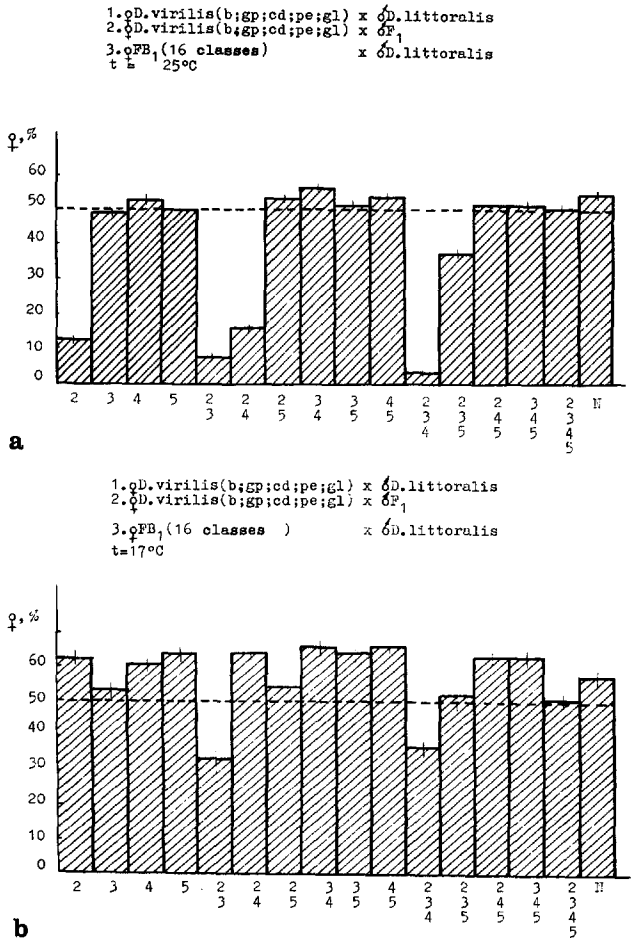

Fig. 2a and b. Number of female offspring obtained from crossing ♀ FB_1 × ♂ *D. littoralis* homozygous by different chromosomes of *D. virilis*, at 25° and 17°C. On the abscissa: 2-5 homozygous and heterozygous (N) *D. virilis* chromosome combinations under analysis

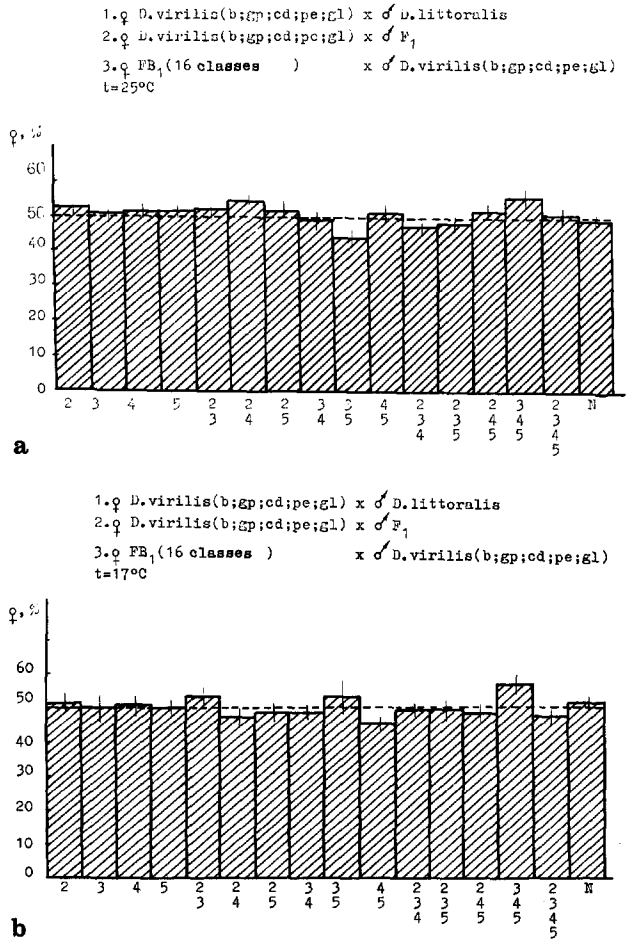

Fig. 3a and b. Number of female offspring obtained from crossing ♀ FB_1 × ♂ *D. virilis* homozygous by different chromosomes of *D. virilis*, at 25° and 17°C. On the abscissa: 2-5 homozygous and heterozygous (N) *D. virilis* chromosome combination under analysis

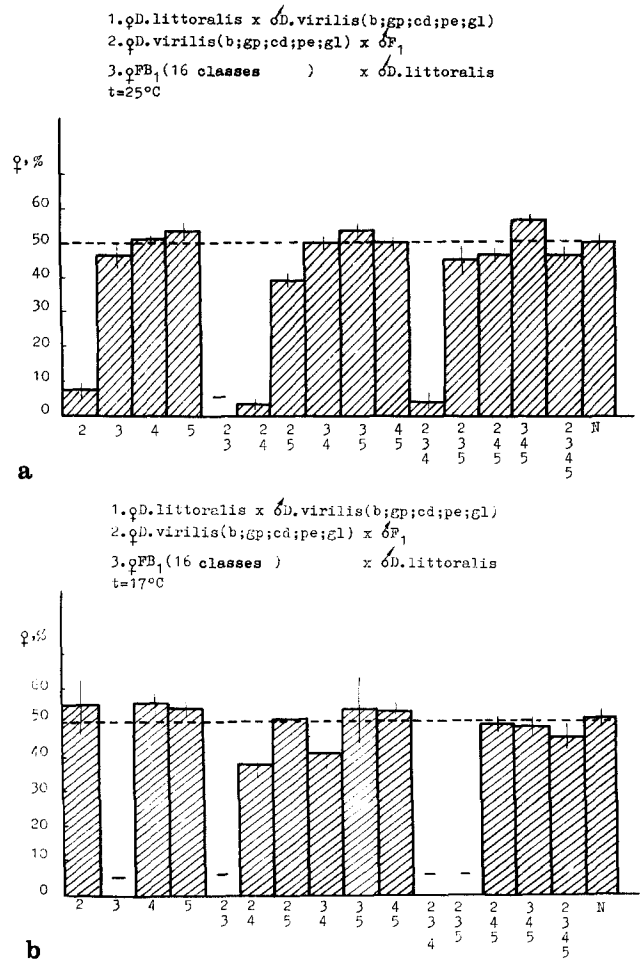

Fig. 4a and b. Number of female offspring obtained from crossing ♀ FB_1 × ♂ *D. littoralis* homozygous by different chromosomes of *D. virilis*, at 25° and 17°C. On the abscissa: 2-5 homozygous and heterozygous (N) *D. virilis* chromosome combination under analysis

To determine the influence of the X-chromosome of *D. virilis* the females to be analyzed were obtained according to the above given scheme, though in the initial cross *D. littoralis* females were used as a maternal line. As a result, females of all 16 classes had the heterogeneous, *virilis-littoralis*, X-chromosome. These females were crossed to *D. littoralis* males. At 25°C hybrid females bearing *D. virilis* chromosome 2 in the homozygous condition produced mostly male progeny (Fig. 4a). Unfortunately, females bearing the combination of *D. virilis* chromosomes 2 and 3 are mostly sterile, and the role of the gene of chromosome 3 is unclear. The low percentage of female progeny obtained from individuals with combinations of *D. virilis* chromosomes 2 and 4 and 2, 3, 4 point to the important role of the gene of chromosome 4 as a modifier. Death of the daughters from female progeny with a heterogeneous X-chromosome is expressed stronger here than in the preceding experiment. On the whole, the genetic system responsible for the differential female viability remains the same. The gene of chromosome 5 fully suppresses the deleterious effect of all other combinations of *D. virilis* chromosomes.

At a low temperature of maintainance (17°C) many hybrids with various chromosome combinations are sterile or have very low fertilities (Fig. 4b). All other crosses give normal sex ratios. As judged by these results, the above mentioned partial death of males at 17°C can be attributed to realization in the hybrid genome of lethals (probably, incomplete ones) of the X-chromosome of *D. virilis*.

Discussion

In the present study an analysis has been made, though only at the chromosome level, of the genetic mechanisms underlying the differential male and female lethality, as well as of the relationship between the expression of these genes and temperature.

A lethality of this kind can be considered as one of the causes of the evolutionary divergence of *D. virilis* and *D. littoralis*. The unique character of this isolating mechanism resides in the fact that it acts not in the first generation, as believed by Haldane, but in subsequent ones, with combinations of various chromosomes of related species whose genes are realized through the egg cytoplasm, i.e. those with distinct maternal effect. In this case recombination load acts as the isolating mechanism. Though the genes controlling sex-differential lethality are localized in chromosomes of *D. virilis* they are expressed as lethals only in combination with chromosomes of *D. littoralis*. Otherwise, the anomalous sex ratio would be observed as early as in the first generation because of their maternal effect. However, in the first generation these genes are not lethal, at least they cause no preferential death of one or the other sex. Within the species these genes have no lethal effect either. In other words, the discovered genetic system involves a set of genes which under normal conditions control some characters determining male or female viability. As a result of hybrid inbalance, they are incapable of normal functioning, and the genes of chromosome 2 are expressed as lethals against the background of heterozygosity of the rest of chromosomes. Restoration of normal sex ratio requires the presence of chromosome 5 of *D. virilis* in the homozygous condition. Equal viability of sexes is also ensured by a fully heterogeneous genome.

Another important aspect of the data obtained, an ontogenetic one, is that of the time when the genes controlling the viability of the sexes begin to operate. In *D. melanogaster* there is quite a number of mutations causing aberrant sex ratios. The sex-linked recessive gene

sonless (*snl*, 1-56,1) has a maternal effect and produces almost total death of males in the progeny of *snl/snl* females (Colaianne and Bell 1970, 1972). The males die during embryonic or early larval development.

The second chromosome of *D. melanogaster* bears the recessive mutation *maleless* (*mle*). This mutation has no maternal effect, and the stage at which *mle/mle* males die varies with the genotype of parental females. In the progeny of *mle/mle* mothers, the males die during the third larval instar, the sons of *mle/+* females survive to the pupal stage, though their differentiation is abnormal (Fucunaga et al. 1975; Tanaka et al. 1976). In this chromosome two more mutations, *km* (2)A (2-45) and *km* (2)B (2-20), have been found which kill males at the very early stages of embryogenesis (Pierre 1972).

There is also the mutation *daughterless* (*da*, 2-41,5) in *D. melanogaster*. It has a maternal effect and causes the death of females (Bell 1954). In the progeny of *da/da* females only males develop, regardless of the male parent's genotype. All known aspects of the expression of this mutation are temperature-sensitive. If parental females are kept at 18°C during the last 60 hours of oogenesis and the progeny are kept at this temperature during the first 3 hours of development, the females survive, though with abnormal cuticular structures (Cline 1976). The mutation is suppressed by the dominant gene *Suppressor daughterless* (*Su*, 1-19) which acts as a lethal in *XY* and *XO* males (Cline 1977).

The genetic system of sex-differential lethality in the *virlis-littoralis* hybrids operates only through the hybrid egg cytoplasm, just as the *snl* and *da* genes of *D. melanogaster*. This might indicate that some factors controlling sex-differential lethality are in operation as early as the first cleavage divisions. It can be assumed that the gene products produced in oogenesis specifically interact with the paternal chromosomes of *D. littoralis*, with the X-chromosome in particular. It would appear that these cytoplasmic factors are capable of distinguishing between male and female chromosome sets as well as between the genomes of their own and foreign species.

From this it seems quite probable that the genes responsible for sex development are not functioning during the cleavage divisions; it might be that males and females have different replication patterns from the early development. Such differential replication takes place as a result of chromosomal or gene polymorphism and is the beginning of differentiation. Thus, differential replication may precede differential transcription.

Of special interest in this respect is the development of the hybrids *D. melanogaster* × *D. simulans*. In the progeny from the crosses of ♀ *D. melanogaster* × ♂ *D. simulans* only females survive. Reciprocal crosses give only males, though occasionally a small number of females survive (Sturtevant 1920). Here one can distinctly trace the interaction between the cytoplasm and sex chromosomes.

In the *virilis-littoralis* hybrids the major gene responsible for the differential death of females in the hybrid progeny is located in the second chromosome of *D. virilis* and acts through the egg cytoplasm. It has a modifier-enhancer in the third chromosome and a suppressor in the fifth chromosome. This genetic system functions at 25°C. At a low maintainance temperature, in the progenies of most crosses between the individuals with heterogeneous combinations of *D. virilis* and *D. littoralis* chromosomes, the number of females increases and that of males decreases. The death of males us due to the interaction between the genes of the X-chromosome of *D. virilis* in combination with autosomes 2; 5; 2, 4; 4, 5; 3, 5; 2, 4, 5; 3, 4, 5; and the Y-chromosome of the paternal species *D. littoralis*.

When comparing genetic systems controlling sex-differential lethality in *D. virilis* and *D. melanogaster* one can see certain similarities between them: preferential localization of genes in autosomes, maternal effect and temperature sensitivity. It seems likely that every species of *Drosophila* has a specific gene system responsible for sex-differential viability. In *D. virilis* and *D. littoralis* this system has been changed in the course of evolutionary divergence of the species, and the resultant variability can be detected by the given scheme of crosses.

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