HYBRIDIZATION BETWEEN *DROSOPHILA SETIFEMUR* AND *D. SPINOFEMORA*

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Summary

Drosophila setifemur Malloch and *D. spinofemora* Sturtevant can be hybridized in the laboratory without difficulty. From the cross *spinofemora* × *setifemur*, hybrids of both sexes are fertile, but in the reciprocal cross the male hybrids are sterile. In the salivary-gland chromosomes of the hybrids there is no indication of any gross structural differences. Maternal inheritance is suggested as a possible explanation for some physiological differences observed between parent species and the hybrids.

A revised systematic description of *D. setifemur* is presented.

I. Introduction

Interspecific hybridization in *Drosophila* has proved to be a valuable approach to problems of comparative genetics. Although up to 1934 only two cases of hybridization within this genus were known, since then a considerable amount of experimental work has been carried out and the number of cases stands at well over 100 (Patterson and Stone 1952). Besides providing an instance of hybridization between two members of a species group not previously studied in this way, the example to be described here illustrates some points of physiological interest.

D. setifemur Malloch and *D. spinofemora* Sturtevant belong to the *immigrans* species group of the subgenus *Drosophila*, a species group containing about 18 described forms, predominantly from Oriental regions. Since Malloch (1924) first described *D. setifemur*, no further reports of collections of this species seem to have been made. In July 1951, we were able to obtain a few individuals from *Drosophila* collections made with fermenting banana bait in the vicinity of Cairns, Qld. A single impregnated female was isolated and a stock established. *D. spinofemora*, described by Sturtevant (1942), appears to be fairly common in the Hawaiian islands but it does not seem to have spread across the western Pacific to any extent. The stock used in the present work was sent to us by Dr. Marshall R. Wheeler from the collection of *Drosophila* species maintained in the Zoology Department at the University of Texas.

II. General Biology

Both *D. setifemur* and *D. spinofemora* can be maintained in the laboratory on standard culture medium over a temperature range from 18 to 25°C. However, owing to the slow maturation of the female in both species, there is usually a considerable delay before fertilized eggs are laid in new cultures; hence it is essential to include some inhibitor of mould, such as 0·2 per cent. propionic acid, in the culture medium. The use of a mould inhibitor also serves to retard the growth of

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yeast used to seed the cultures, which is advantageous, for prolific yeast growth tends to render the surface of the culture too soft and moist. In neither species do the larvae climb far above the surface of the medium when pupation is about to take place, hence if the culture medium is too soft, many may die.

Both species may be anaesthetized very rapidly with ether. In common with *D. immigrans*, they show a tendency to extrude a drop of urine upon etherization, and this can be troublesome when large numbers of flies are being examined together, owing to one individual adhering to another.

Delay in the development of cultures of *setifemur* and *spinofemora* is primarily the result of the immature state of the females upon eclosion. Both sexes will mate within 24 hr of emergence, but the ovaries are quite immature at this time and fertilized eggs are not laid until the females are about 6 days old, the sperm being stored meanwhile in the spermathecae. Maturation of the ovaries and deposition of eggs can be delayed up to 10 days if the females are deprived of fresh yeast, but owing to the precocious mating, virgin females must be collected every 10 or 12 hr if genetical investigations are being made.

Courtship in *setifemur* is prolonged. In 15 cases in which copulation was observed to take place, the preliminary courting extended over more than $\frac{3}{4}$ hr. When initiating courtship, the male circles with wings extended and vibrating. Moving in front of the female and with wings making occasional scissor-like movements, he approaches and taps her fore legs. If mating is to be refused, the female lowers her abdomen, flutters the wings rapidly, and moves away. If the courtship is to be accepted, the female herself shows scissor-like movements of the wings. Before finally allowing the male to mount, she extends the wings and holds them motionless at right angles to the body while she rubs her genital region with one of the hind legs. The male meanwhile circles behind her, usually but not invariably licks her genitalia, then mounts and clasps her thorax beneath the wings with his fore legs. The duration of copulation is usually about 35 min the observed range being from 20 to 43 min.

On several occasions in which two or more males were courting the one female, it was observed that after the female had suddenly changed position and moved away, the two males would continue their courtship but directed to each other. There were the customary circling and vibrating movements, but as soon as they had tapped each other on the fore legs, the courtship was immediately broken off until a female was again encountered. The observations suggest that contact with the fore legs leads to recognition of another individual as being of the same or opposite sex.

The mating behaviour of *spinofemora* does not seem to differ in any observable way from that of *setifemur*, nor were there any apparent deviations from the usual pattern of courting behaviour when heterogamic matings were under observation.

III. Reproductive Isolation and Hybridization

The heterogamic pair-mating method was used to detect the occurrence of sexual isolation. Before the tests, both males and females were collected as virgins and aged separately on yeasted culture medium for 6 days. Single pairs of flies were then placed without etherization into culture tubes for 48 hr, after which the

spermathecae of the females were examined for the presence of sperm. Of 43 *spinofemora* females, 14 proved to have been inseminated, while of 46 *setifemur* females, 12 were found to have been inseminated. Thus under these conditions, mating between the two species will take place without much difficulty. When reared on standard culture medium at 25°C, successful single pair-matings gave the mean counts of offspring as set out in Table 1. In no case is there a significant deviation from an expected 1 : 1 sex ratio.

Attempts to cross *setifemur* and *spinofemora* with *immigrans* failed. No courtship was observed when *immigrans* males were placed with *spinofemora* and *setifemur* females, while conversely, *setifemur* and *spinofemora* males made no attempts to copulate with *immigrans* females.

TABLE 1

SINGLE PAIR-MATINGS BETWEEN D. SETIFEMUR AND D. SPINOFEMORA

Cross	No. of Pairs	Mean Offspring per Pair	
		Female	Male
setifemur × *setifemur*	10	36	35
spinofemora × *spinofemora*	5	33	30
setifemur × *spinofemora*	10	9	10
spinofemora × *setifemur*	6	17	24

From the cross *spinofemora* × *setifemur*, hybrids of both sexes are fertile and interbreed readily. In the reciprocal cross, however, while the F_1 females are fertile the males are invariably sterile. It may be presumed, therefore, that a *setifemur* *X*-chromosome requires a *setifemur* *Y*-chromosome as well to ensure a full complement of fertility genes. On the other hand, a *setifemur* *Y*-chromosome is sufficient with either a *setifemur* *X* or a *spinofemora* *X*. This could be explained by supposing that during the evolution of these two species, fertility genes present on the differential part of the *Y*-chromosome of *setifemur* have become transposed to the *X*-chromosome of *spinofemora*. That the autosomes are probably not concerned in fertility is suggested by the fact that if a *setifemur–spinofemora* hybrid female is crossed to a *spinofemora* male, approximately one-half of the male offspring are fertile, the other half being sterile.

In both *setifemur* and *spinofemora*, the chromosome complement consists of a large V-shaped element, two medium sized rods, and a small dot. Miss M. M. Gunson (personal communication) finds that in larval salivary glands of hybrids, there is complete synapsis and no indication whatever of any gross structural differences between the euchromatic regions of the chromosomes of the two species.

IV. PHYSIOLOGICAL DIFFERENCES BETWEEN PARENT SPECIES AND HYBRIDS

Apart from the more yellowish and robust appearance of *spinofemora*, there are no marked morphological differences between the two parent species. There

are, however, a few physiological differences that are observable without difficulty. The most conspicuous is the rate of development. At 25°C, the life cycle of *setifemur* is remarkably short, about 8 or 9 days, whereas that of *spinofemora* at the same temperature is of about 15 days duration. The developmental rate of hybrids corresponds closely to that of the female parent species. Thus, the *setifemur–spinofemora* hybrids emerge about 10 days after egg laying, whereas hybrids from the reciprocal cross do not emerge until about the 16th day. There is a sex difference in developmental rate of hybrids from a *spinofemora* × *setifemur* cross, the males developing more rapidly, but in the reverse cross both sexes develop at about the same rate. In the parent species, no sexual differences in developmental rate are apparent.

TABLE 2

SENSITIVITY OF D. SPINOFEMORA AND D. SETIFEMUR SPECIES AND HYBRIDS AT 20°C TO PURE CARBON DIOXIDE PREVIOUSLY SATURATED WITH WATER VAPOUR

Material	No. Recovered after 2 Hr Exposure	No. Failed to Recover
spinofemora	44	9
setifemur	61	39
spinofemora–setifemur hybrids	46	4
setifemur–spinofemora hybrids	36	14

spinofemora *setifemur*	$\chi^2 = 6{\cdot}8^{**}$	*spinofemora–setifemur* hybrids *setifemur–spinofemora* hybrids	$\chi^2 = 5{\cdot}4^{**}$
spinofemora *spinofemora–setifemur* hybrids	$\chi^2 = 2{\cdot}0$	*spinofemora* *setifemur–spinofemora* hybrids	$\chi^2 = 1{\cdot}7$

** Significantly different at the 1 per cent. level.

A second physiological difference between *setifemur* and *spinofemora* concerns sensitivity towards carbon dioxide narcosis. This sensitivity is not as marked as that which characterizes the carbon dioxide-sensitive strains of *melanogaster* (L'Heritier 1951) but it is sufficiently pronounced to be readily demonstrable. After a 2-hr exposure to pure carbon dioxide saturated with water vapour, approximately 80 per cent. of the adults of *spinofemora* will show complete recovery, whereas little more than 50 per cent. of *setifemur* individuals will tolerate the treatment The difference is statistically significant. In hybrids, a maternal influence is again apparent, for the *spinofemora* × *setifemur* offspring are the more resistant (see Table 2).

As a final example of a physiological difference between the two species, data on the frequency of wing beat will serve. In conjunction with measurements of wing dimensions, beat frequency has been used on numerous occasions to demonstrate racial or specific differences between various drosophilines (Reed, Williams, and Chadwick 1942; Stalker and Carson 1947). As far as wing dimensions are concerned, there are no statistically significant differences between *setifemur* and *spinofemora*, as reared under laboratory conditions, but there is a difference between the two

species with regard to beat frequency. However, in contrast to the preceding physiological differences showing a pronounced maternal inheritance, wing-beat frequencies of the hybrids were found to be intermediate between those of the parent species (Table 3). This is in agreement with the views of Reed *et al.* (1942) who believe that the inheritance of wing-beat frequency is of the multifactorial type.

TABLE 3

WING-BEAT FREQUENCY OF D. SPINOFEMORA AND D. SETIFEMUR SPECIES AND HYBRIDS AS DETERMINED WITH A STROBOFLASH

Material	Mean Frequency of Complete Beats (hundreds/min)	No. of Determinations	*t*	*P*
spinofemora	82·41	14	3·7	0·01
setifemur	91·74	14		
spinofemora–setifemur hybrids	88·22	15	1·8	0·05
setifemur–spinofemora hybrids	83·70	15		

V. DISCUSSION

From the relative ease with which these two species may be crossed, as well as from the close similarity of form, it is clear that divergence has not proceeded very far. The only other member of the group available for hybridization tests was *immigrans* itself, but in view of the considerable morphological differences between this species and the other two, it is not surprising that, under the conditions of the laboratory test, successful heterogamic inseminations between *immigrans* and either *setifemur* or *spinofemora* could not be obtained. More promising perhaps would be attempted hybridization between *setifemur* and *spinofemora*, on the one hand, and *hexastriata* on the other. The chromosomal configuration of the latter species appears to be similar to that of *spinofemora* and *setifemur* (Tan, Hsu, and Sheng 1949).

The most interesting fact revealed by this study is the demonstration of a strong maternal influence in the determination of some of the physiological characteristics of the hybrids. The similarity between the developmental rates of the hybrid and the female parent species has its counterpart in the differences in cleavage rates that Moore (1933) observed in some echinoderm hybrids, where maternal influences were also found to be of importance. No evidence has yet been obtained to suggest that the sensitivity of *setifemur* towards carbon dioxide is related to the presence of a cytoplasmic viroid, hence maternal inheritance is to be regarded provisionally as the most likely explanation for this physiological difference as well. It is probable that if a systematic search were made, other examples of maternally- or cytoplasmically-inherited characteristics of a physiological nature would be discovered readily in *Drosophila*. Rasmuson (1952) has found that the differences in the resistance of various strains of *melanogaster* to narcotics, such as ether, appear to depend, at least in part, on an autonomous cytoplasmic system. However, no

evidence for differences in sensitivity towards ether has been obtained in the cases of *setifemur* and *spinofemora* or for their hybrids.

VI. Revised Description of Drosophila setifemur Malloch

The original description of this species, while adequate for the identification of the form, fails to make reference to certain morphological features that have gradually been accepted by *Drosophila* systematists as being reliable and useful. Therefore a revised description has been prepared, based upon material taken from the laboratory stock maintained in this Department.

Male.—Arista plumose, about 12 branches. The antenna pale yellow, the 3rd segment somewhat darker. Front dark yellow, pollinose. Middle orbital nearer to upper orbital and about half as long as the latter. Second oral bristle nearly as long as the 1st. Palps very pale yellow, 1 prominent terminal bristle, 2 prominent lateral bristles. Carina light yellow, narrow, and only slightly broadened below. Cheeks pale yellow, the greatest width about one-eighth the diameter of the eye. Eyes bright red with light pile.

Acrostichal hairs in 8 rows; prescutellar bristles absent. Anterior scutellars convergent; sterno-index 0·8; mesonotum dark, amber coloured, unmarked in young individuals but with a very faint trident pattern appearing in older individuals. Legs yellow, the apical bristles on the 2nd tibia very large. A row of about 12 short, stout, microscopic setae on the apical half of the anteroventral surface of the fore femur. Short, closely placed bristles on the ventrolateral surface, the apical 2 or 3 distinctly longer than the remainder.

Abdomen pale yellow, each segment with a narrow, brown, apical band fading away before reaching the lateral margin. The bands show a narrow but distinct interruption in the mid line.

Wings dusky with conspicuous clouding along the posterior crossvein. Costal index about 3·4; 4th vein index 1·4; the 4*c* index about 0·65 and the 5*X* index about 1·2. Apex of the 1st costal section with 2 well-developed bristles of similar size. Third costal section with heavy bristles on the basal half. Length of body 3·0 mm, wings 2·8 mm.

Female.—Abdominal bands darker. Body 3·2 mm, wings 2·9 mm.

Eggs.—0·56 mm long, 0·19 mm in diameter. Four filaments, the anterior pair thin, about 0·53 mm long, and the posterior pair thick, about 0·74 mm long, and finely branching distally.

Pupa.—Brown, transparent. Anterior spiracles have 18–20 branches.

Chromosomes.—One large V-shaped element, 2 medium sized rods, and a small dot.

Male genitalia.—The genital arch broad above. The lower part with 8 or 9 long bristles, the upper part with 3 or 4 bristles near the posterior margin. Anal plate with 12–15 short bristles at the tip. The claspers have a rounded process above and are provided with 8 or 9 primary teeth and 4 to 6 very stout marginal bristles.

Affinities.—Belongs to the *immigrans* group.

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