

SEX AND CROSSING OVER IN *TRIBOLIUM CASTANEUM*

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T*RIBOLIUM castaneum* Herbst is a tenebrionid flour beetle which, along with *T. confusum*, has been intensively investigated for over 30 years, with the result that these two are probably the best ecologically documented species in existence. Because of the ease in maintenance of these species in the laboratory, and the vast ecological information available, the two species have been used in various population genetics and eco-genetics problems (see for example BRAY, KING and BELL 1962; DAWSON and LERNER 1962; LERNER and DEMPSTER 1962; LERNER and Ho 1961; McDONALD 1963; SOKAL and KARTEN 1964).

Basic genetic information on *Tribolium* began in 1937, with the discovery by PARK of the mutant pearl. But from 1937 to 1955 only five mutants had been described for the two species named above. Thanks to the fact that these beetles are apparently highly mutable, a large number of spontaneous, as well as a few possibly induced, mutations have been found in the last five years with the result that, at present, eight of the ten possible linkage groups in *T. castaneum* have markers, and maps bearing from two to twelve points are available for seven linkage groups. (For complete references to this work see SOKOLOFF and DAWSON 1963; SOKOLOFF 1964a.)

With the determination of linkage relationships between some of the genes, it has been possible to investigate the role of sex on crossing over in *Tribolium*. Preliminary work (LASLEY 1960; SOKOLOFF 1963) seemed to indicate that crossing over was equal in the two sexes, conforming with the findings of SCHUURMAN (1937), who found equal recombinations between two autosomal genes in the two sexes of another tenebrionid, *Tenebrio molitor*.

The purpose of this communication is to record the fact that, at least for a portion of the seventh linkage group, crossing over is not equal for the two sexes of *T. castaneum*. Furthermore, the data to be presented violate HALDANE's (1922) rule that crossing over is reduced or prevented in the heterogametic sex.

MATERIALS AND METHODS

Eleven genes, most of them of spontaneous occurrence, have been identified with the seventh linkage group for which Short antenna, *Sa*, serves as a marker (SOKOLOFF, DAWSON and ENGLERT 1963), but eight of these have proved to be allelic (DAWSON and SOKOLOFF 1964). For this reason this study has included only the following mutants: two alleles of Short antenna, *Sa-2* and *sa* (figured in DAWSON and SOKOLOFF 1964); Fused tarsi and antennae, *Fta*, (figured in SOKOLOFF 1964); blistered elytra *ble* which, as the name implies, produces a permanent blister of varying size in various places of one or both of the elytra; and chestnut, *c*, a reddish eye-color mutant independently reported by EDDLEMAN and BELL (1963) and SOKOLOFF (1963a) as being

on the same autosome as Short antenna. *Fta* and *Sa* are dominants with recessive lethal effects and their interaction results in a dominant synthetic lethal (SOKOLOFF 1964). The *sa* allele of *Sa-2* is incompletely recessive, some heterozygotes being identifiable by slight fusions of some of the funicular segments of the antenna. The *ble* gene is incompletely penetrant, since individuals with normal elytra or with one elytron normal, and the other blistered, occur with a high frequency in the *ble* stock. However, the degree of penetrance of *ble* in some crosses is high, making it possible to map this gene on the seventh linkage group.

All crosses were carried out in single pairs in creamers containing standard flour-beetle medium consisting of 95 percent by weight wholewheat flour and five percent brewers' yeast. The creamers were placed in a dark incubator maintained at 29°C and 70 percent relative humidity.

Samples of all the mutations used in this investigation are on deposit at the Chicago Natural History Museum, Chicago, Illinois, American Museum of Natural History, New York, N.Y., and U.S. National Museum, Washington, D.C.

RESULTS

Table 1 shows the (essentially) backcross data of various combinations of genes associated with linkage group VII in *T. castaneum*. The different blocks of data include different gene combinations. Crossing over in some cases was tested with genes in coupling and repulsion in both sexes, in other cases in coupling in one sex and in repulsion in the other sex.

Because the various genes have differential viabilities and various degrees of manifestation with possibility of misclassification, the recombination fraction has been determined by various methods recommended by BAILEY (1961): when differential viability was involved, the method outlined on pages 50 and 51 has been used, depending on whether only one gene or both genes had differential viability; with one gene exhibiting partial manifestation, the method outlined on page 74 was used. A summary of the recombination fractions and standard errors of the various genes involved, and the degree of misclassification (if any) with its standard error is given in Table 2. In one instance, Cross F, no standard errors are given. This is because the proportion of *ble* could not be computed directly

TABLE 1

Backcross progeny of various linkage group VII genes in coupling or repulsion

Cross	Numbers and phenotypes of progeny	Number of successful matings
A. $++/Fta\ ble\ \delta \times ble\ \text{♀}$	527 +; 45 <i>Fta</i> ; 36 <i>ble</i> ; 469 <i>Fta ble</i>	8
B. $Fta\ +/+ble\ \delta \times ble\ \text{♀}$	43 +; 186 <i>Fta</i> ; 147 <i>ble</i> ; 10 <i>Fta ble</i>	4
C. $++/Fta\ ble\ \text{♀} \times ble\ \delta$	879 +; 149 <i>Fta</i> ; 16 <i>ble</i> ; 708 <i>Fta ble</i>	8
D. $Fta\ +/+ble\ \text{♀} \times ble\ \delta$	68 +; 211 <i>Fta</i> ; 195 <i>ble</i> ; 4 <i>Fta ble</i>	4
E. $++/Sa\ ble\ \delta \times ble\ \text{♀}$	96 +; 97 <i>Sa</i> ; 69 <i>ble</i> ; 54 <i>Sa ble</i>	3
F. $Sa\ +/+ble\ \text{♀} \times Sa\ ble/+ble\ \delta$	130 +; 526 <i>Sa</i> ; 223 <i>ble</i> ; 97 <i>Sa ble</i>	11
G. $++/Fta\ c\ \delta \times c\ \text{♀}$	186 +; 163 <i>Fta</i> ; 172 <i>c</i> ; 151 <i>Fta c</i>	12
H. $Fta\ +/+c\ \text{♀} \times c\ \delta$	158 +; 226 <i>Fta</i> ; 207 <i>c</i> ; 138 <i>Fta c</i>	7
I. $++/sa\ c\ \delta \times sa\ c\ \text{♀}$	61 +; 58 <i>c</i> ; 99 <i>sa</i> ; 89 <i>sa c</i>	3
J. $Sa-2\ +/+c\ \text{♀} \times c\ \delta$	74 +; 110 <i>Sa-2</i> ; 121 <i>c</i> ; 77 <i>Sa-2 c</i>	5
K. $sa\ +/+c\ \text{♀} \times sa\ c\ \delta$	129 +; 180 <i>c</i> ; 203 <i>sa</i> ; 118 <i>sa c</i>	8

TABLE 2

Recombination fractions between various linkage group VII genes in coupling or repulsion

Cross* and heterozygous sex	Recombination fraction	SE of recombination fraction	Proportion of misclassified phenotypes	SE of proportion of misclassified phenotypes
A. male	.0655	.0101	.0236	.0161
B. male	.0619	.0182	.1753	.0340
C. female	.0212	.0052	.1560	.0136
D. female	.0245	.0113	.2400	.0296
E. male	.5390	.0353	.2242	.0547
F. female	.210320
G. male	.4985	.0193
H. female	.4060	.0182
I. male	.5086	.0358	.2242	.0556
J. female	.3953	.0250
K. female	.3915	.0200	.0074	.0389

* For genes involved in these crosses see Table 1.

from that cross. It was assumed that misclassification was of the same order of magnitude as in Cross E, and $\hat{\lambda}$ was given the value of 0.2.

The data in Table 2 clearly indicate that the recombination fraction of any given pair of genes whether in coupling or repulsion is not significantly different within a sex, but is significantly different between sexes. The recombination values may differ only slightly (but significantly) between the sexes, as shown in the first block in Table 2, or they may differ considerably as shown by the recombination fractions in crosses involving *Sa* and *ble* (second block in the table).

The recombination values in the male are so greatly increased, that they approach 50 percent giving the erroneous impression that the genes are not linked. The female data, on the other hand, indicate that the genes are linked.

DISCUSSION

These results are at variance with recombination values obtained for the two sexes involving genes in the fourth and fifth linkage groups. LASLEY (1960) determined that jet and split were linked and backcrosses in every possible mating combination gave a recombination of 6 to 17 percent for the two sexes.

SOKOLOFF (1963b), in preliminary studies of factors that might affect crossing over, used Bar eye and sooty (fourth linkage group). He found that at 32° C and 70 percent relative humidity the two sexes give nearly identical recombination values: $1492/5957 = 25.05$ percent in the male and $633/2539 = 24.93$ percent in the female. These values could be modified somewhat by such factors as age, temperature and X rays, but the effect was different in the two sexes: age and X rays increased the rate of crossing over slightly in the male (to about 27 percent); cold reduced crossing over in the female (to about 22 percent) but had no effect in the male (for complete data see SOKOLOFF 1963b).

It is well known that crossing over may be unequal in the two sexes. The best

known case is that of *Drosophila melanogaster* where crossing over in the male does not take place except under unusual circumstances (WHITTINGHILL 1947). The same is true for *Bombyx mori* except that in this organism crossing over takes place only in the male (review in TANAKA 1953). Where crossing over has been found to occur in both sexes in animals and plants, now one sex, now the other may exceed the opposite sex in crossover values, or the values may be equal (see review by SWANSON 1957).

HALDANE (1922) has pointed out that where differences in crossing over are found in the two sexes, it is the heterogametic sex in which crossing over is lowered in frequency or is absent. The data presented above appear to be an exception to HALDANE'S rule. SMITH (1950, 1952) has shown that the male *T. castaneum*, possesses an X as large as one of the larger autosomes and a small, parachute-shaped Y, hence it is the heterogametic sex. Therefore, it might be expected that recombination values in the male would be smaller. Instead, these values are so magnified as to give the impression that the genes are not linked.

The situation described here is opposite to that described by NABOURS (1925) for the tettigid grouse locust *Paratettix texanus* (= *P. cucculatus* Burm., according to HENDERSON 1961). He found that crossing over of genes involving body color occurred at a frequency of 25.34 percent in the male and 47.58 in females. Here, clearly, it is the female which gives exaggerated recombination values.

Cytological studies of Coleoptera by SMITH (1950) indicate that in beetles, as a rule, the frequency of chiasmata is one per chromosome arm. Although it would be extremely difficult to prove cytologically, it is highly probable that the discrepancy in crossover values in the two sexes of *T. castaneum* results from a difference in the distribution of a single chiasma. The possibility that the aberrant results in *Tribolium* might be explained in this manner is not unreasonable: HENDERSON (1961) has noted that (with one exception) distal localization of chiasmata results in a reduction in crossing over in *Paratettix*, or complete absence in *Apotettix* males and an increase in crossing over in the females. On the other hand, when chiasmata were located subterminally (at male meiosis) crossing over was at the same level in both sexes. Recent studies by WATSON and CALLAN (1963) have pointed out that different species may behave differently: in the newt *Triturus helveticus* they found that chiasmata are not restricted in their distribution in oocyte bivalents (but they are in spermatocyte bivalents since here the chiasmata are proterminally localized). On the other hand, in three subspecies of *T. cristatus* the chiasmata are procentrally localized in oocyte bivalents but in spermatocyte bivalents they are not restricted in their distribution. Thus, in *T. helveticus* meiosis, it is the female which is mainly responsible for genetic recombination, whereas in *T. cristatus* this situation is reversed.

It is not known whether the phenomenon described here for, presumably, one arm of the linkage group in *T. castaneum* is a general one for the other autosomes, and it is not known whether a similar situation may be found in *T. confusum*. However, it is clear that in linkage studies, at least in *T. castaneum*, it is necessary to set up crosses both ways, otherwise linkage relationships might be missed.

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SUMMARY

Data are presented to show that recombination values of a number of genes located on the seventh linkage group (Short antenna, Fused tarsi and antennae, blistered elytra, and chestnut), whether in coupling or repulsion, are not equal in the two sexes of *Tribolium castaneum*. The male values may be slightly or much inflated, resembling those that would be obtained when genes are not linked. Since the male is heterogametic, these data violate HALDANE'S rule that crossing over is reduced or prevented in the heterogametic sex. Although this material has not been examined cytologically, evidence from other organisms seems to indicate that such results are obtainable when the two sexes differ in the distribution of a single chiasma.

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