RECOMBINATION DIFFERENCES WITH RECIPROCAL CROSSES IN *TRIBOLIUM CASTANEUM*¹

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IN 1922 HALDANE proposed his empirical rule that recombination is reduced or absent in the heterogametic sex. Contrary to this generalization SOKOLOFF (1964) reported that in *Tribolium castaneum* Herbst recombination between certain genes of linkage group VII was greater in males than in females. This is a report on the stability of this unusual recombination pattern with increasing parental age and with parental exposure to various temperatures. A recombination pattern for linkage group IV is reported which agrees with HALDANE's rule and is likewise stable with increasing parental age and under various temperatures.

MATERIALS AND METHODS

Markers used in this study are the linkage group VII mutants Fused tarsi and antennae (*Fta*) and chestnut colored eyes (*c*), and the linkage group IV mutants Bar eyes (*Be*) and sooty body (*s*). Reciprocal crosses with these mutant genes in coupling linkage were cultured at 29°C and 60% relative humidity in half-pint bottles containing a mixture of 95% wholewheat flour and 5% brewer's yeast.

To test the influence of age on female recombination, ten bottles were established for each linkage group with 30 pairs of newly hatched adults per bottle. Each week the parents were transferred to fresh medium until the weekly production of progeny was less than 100 in any bottle. The influence of age on male recombination was tested by setting up four bottles for each linkage group with 20 pairs of newly hatched adults per bottle. Each week the heterozygous males were transferred to bottles containing fresh medium and 20 virgin females homozygous for *c* or *s*. The homozygous *c* or *s* females used in the crosses of the previous week were discarded to eliminate subsequent use of their stored sperm. The study of recombination with increasing male age was discontinued when only three and eight males remained for linkage groups VII and IV, respectively.

Beetles which had developed at 29°C were used to test the influence of temperature on recombination. For each sex and linkage group two bottles with ten pairs of newly hatched adults per bottle were established at 21°, 25°, 29°, 33°, and 37°C and 60% relative humidity. Each week for six weeks the immature progeny were removed and placed at 29°C to complete their development. In crosses where male recombination was being studied, the inseminated females homozygous for *c* or *s* were replaced each week by virgin females of the same types.

RESULTS

Data given in Figure 1 were based on the weekly classification of 100 progeny

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FIGURE 1.—Recombination between *Fta* and *c* of linkage group VII and between *Be* and *s* of linkage group IV with increasing parental age. Solid lines show male recombination, and broken lines show female recombination. Each point is based on 1000 beetles.

from each of the ten bottles representing female recombination, and 250 progeny from each of the four bottles representing male recombination. Tests of homogeneity were made to determine the significance of week to week and bottle to bottle variation in the number of offspring per phenotypic class. Using the 0.05 level, the number of significant differences in the number of beetles per phenotypic class was not greater than expected by chance when the weekly samples from the same bottles were compared or the weekly samples from different bottles of the same crosses were compared. Likewise, the data from the temperature experiments (Table 1) were found to be homogeneous.

Recombination data shown in Figure 1 are corrected for viability differences as outlined by BAILEY (1961, on pages 50 and 51). Average recombination between *Fta* and *c* was 43.5% in males and 40.6% in females. Between *Be* and *s* the average recombination values were 22.6% in males and 25.5% in females. Results of reciprocal crosses with *Be* and *s* in repulsion demonstrated that the pattern shown in Figure 1 for linkage group IV is correct and repeatable. Figure 1 illustrates the stability of recombination with increasing parental age for the *Fta-c* segment of linkage group VII and the *Be-s* segment of linkage group IV. However, the differences in recombination between the sexes were highly significant.

Figure 2 shows that temperatures ranging from 21° to 37°C were ineffective in significantly altering recombination in the segments under study. On the other hand, recombination differences between the sexes for each segment were highly significant.

The viability of recombinant offspring with mutant genes was greatly influenced by the sex of the heterozygous parent (see Table 1). Crosses between *Fta* *+/+* *c* females and *+/+* *c* males produced fewer *Fta c* offspring than the reciprocal cross at all temperatures studied. Crosses between *Be* *+/+* *s* males and *+/+* *s* females produced fewer *Be s* progeny than the reciprocal cross. This difference was more pronounced at higher temperatures.

TABLE 1

Recombination data for linkage groups VII (upper block) and IV (lower block)

Temperature(°C)	Heterozygous parent	Numbers and phenotypes of progeny				Corrected percent recombination
Cross: <i>Fta</i> $+/+c \times +c/+c$						
21	female	205	294	134	197	39.8
21	male	226	240	160	219	45.4
25	female	585	755	370	535	40.4
25	male	719	707	554	575	44.2
29	female	1495	2104	981	1420	40.0
29	male	1678	1703	1270	1349	43.6
33	female	1481	2180	1020	1670	42.3
33	male	1753	1865	1340	1675	45.0
37	female	122	230	87	174	42.3
37	male	654	642	444	585	44.0
Cross: <i>Be</i> $+/+s \times +s/+s$						
21	female	412	416	146	193	28.9
21	male	676	594	182	245	25.0
25	female	1114	1264	404	432	26.0
25	male	1382	1422	364	497	22.3
29	female	1754	1718	580	679	26.5
29	male	2622	2719	582	1037	22.5
33	female	1779	1856	605	701	26.4
33	male	2567	2153	440	1122	23.5
37	female	332	383	122	160	28.2
37	male	1011	750	162	563	25.7

DISCUSSION

SOKOLOFF (1963) found that recombination between *Be* and *s* was 25.1% (1492/5957) in the male and 24.9% (633/2539) in the female. Data of the present study indicate that recombination between *Be* and *s* was 22.6% (30,000 offspring) for males and 25.5% (16,000 offspring) for females. The reason for this discrepancy in male recombination is unknown.

In *Drosophila*, crossing over between genes that span common centromeres can be greatly increased with age (BRIDGES 1915, 1929; PLOUGH 1921) or temperature (PLOUGH 1917). STERN (1926) demonstrated an effect of age and tem-

FIGURE 2.—Recombination between *Fta* and *c* of linkage group VII and between *Be* and *s* of linkage group IV with parental exposure to temperatures ranging from 21° to 37°C. Solid lines show male recombination, and broken lines show female recombination.

perature on crossing over for proximal segments of the first chromosome in *D. melanogaster*. If the results shown in Figures 1 and 2 were based on *Drosophila* studies, it would be concluded that the segments *Fta-c* and *Be-s* either do not include a centromere or are not proximally located. When the positions of the centromeres are known for linkage groups VII and IV, it will be interesting to compare these results with *Tribolium* and the results of similar *Drosophila* studies.

Through cytological studies of Coleoptera, SMITH (1950) observed that usually only one chiasma was formed per chromosome arm. Based on this finding, SOKOLOFF (1964) suggested that sex differences in recombination between *Fta* and *c* exist because of a difference in the distribution of a single chiasma. According to this suggestion it follows that recombination for other segments of linkage group VII will be higher in females than males. Thus, it isn't surprising that SOKOLOFF (1964a) found that recombination was higher in females than males between *c* and *ble* (blistered elytra).

The differences in recombination with reciprocal crosses presented in this paper can be explained with a more definite statement of SOKOLOFF's hypothesis. More specifically, his hypothesis would be that crossing over in one sex is more likely to occur in proximal regions, and crossing over in the other sex tends to occur in distal regions. Thus, it is predicted that one of the segments studied will have a much more proximal location than the other segment.

SUMMARY

Different recombination patterns were observed with reciprocal crosses involving the *Fta-c* segment of linkage group VII and the *Be-s* segment of linkage group IV. Recombination between *Fta* and *c* was higher in males (43.4%) than in females (40.4%); on the other hand, recombination between *Be* and *s* was lower in males (22.6%) than in females (25.5%). These highly significant differences in recombination were stable with increasing parental age and parental exposure to temperatures ranging from 21° to 37°C.

Recombination patterns in *Tribolium castaneum* are not satisfactorily explained with HALDANE's rule that recombination is reduced or absent in the heterogametic sex. To account for the different recombination patterns found with sex in this study, it is proposed that crossing over in one sex is more likely to happen in proximal regions, and crossing over in the other sex is more probable in distal regions.

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