

Chromosome rearrangements in *Tribolium castaneum*

ABSTRACT: An efficient method for isolating chromosome rearrangements in the red flour beetle, *Tribolium castaneum*, is described. One or more viable rearrangements were detected in 46 percent of all chromosomes screened after γ -irradiation of males. Fertility of male rearrangement heterozygotes was measured for 35 unique rearrangements, and breakpoint distribution among eight of the nine autosomal linkage groups was determined for 33 of them using 2- and 3-point crosses. We measured the effects of most of the rearrangements on crossing over in the vicinities of their breakpoints. Autosomal linkage group 9, which was probably translocated onto the X-chromosome of *T. castaneum* during the speciation of the congeneric *T. confusum*, is shown to be a subset of linkage group 2.

R. W. Beeman
T. R. Johnson
S. M. Nanis

THE RED FLOUR BEETLE, *Tribolium castaneum* (Herbst), (Tenebrionidae), is a common and widespread pest of stored products. It has been one of the most extensively researched organisms from the viewpoint of ecology and population genetics¹¹. Nine linkage groups (LGs) have been defined and over 100 visible mutations have been described, including ca. 30 that are completely penetrant and easy to score^{10,11}. No chromosome rearrangements have been described, with the exception of a crossover suppressor on LG 4 with a visible phenotype known as *Spatulate*⁶. Our interest in *T. castaneum* is threefold. We are using this species in a genetic analysis of insecticide resistance, in a genetic study of development, and as an experimental model of genetic suppression of populations. To aid in these investigations we are attempting to construct balancer chromosomes and other genetic tools that are used routinely in *Drosophila*, but have been unavailable in almost all other insects. In this report we describe the isolation, characterization and breakpoint distribution of chromosome rearrangements in *T. castaneum*.

Materials and Methods

All beetles were reared in wheat flour/brewers' yeast (20:1). All crosses and incubations were carried out in glass vials at 30°C. Most of the mutant strains used were described by Sokoloff^{10,11}. The *Rmal* mutant was described by Beeman².

Irradiation of males and measurement of semisterility

To induce chromosome breakage and rearrangement, 100 7- to 12-day-old adult virgin *Rmal* males that had been starved for 24 hours were γ -irradiated at room temperature with 4 krad at a rate of 390 rads/min using a Gammacell 220 Co⁶⁰ irradiator. Because spermatogenesis in *Tribolium* is poorly understood, we adapted a brood fractionation method that has been successful with *Drosophila*⁸. After irradiation the males were immediately confined en masse with 100 1-week-old adult virgin sooty females in a jar of medium. The sooty mutation was used to facilitate identification of sex in the adult. After four days the medium and females were discarded because of our expectation (based on analogy to *Drosophila*) that immature sperm may be more sensitive to radiation than mature sperm⁸. The males were then confined with 100 8- to 16-day-old adult virgin sooty females in fresh medium. These beetles were allowed to mate and oviposit for 48 hours, after which time the males were discarded and the females (inseminated 4-6 days post-irradiation) were transferred to fresh medium. The F₁ male progeny were screened for the presence of rearrangements. The dose of radiation used produced ca. 75-80 percent dominant lethality in the ejaculate 4-6 days post-irradiation.

For both initial detection and subsequent linkage analysis we treated all rearrange-

The authors are affiliated with the U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, KS 66502, and the Department of Entomology, Kansas State University, Manhattan, KS 66506. Contribution No. 86-104-J from the Department of Entomology, Kansas Agricultural Experiment Station, Manhattan, KS 66506.

© 1986, American Genetic Association.

ments as dominant Mendelian factors for semisterility. During initial screening we used a rapid, semiquantitative measure based on progeny weight. In this method each F₁ male to be tested was confined with two 15- to 30-day-old virgin sooty females for seven days. The two females were then transferred (together) onto 3 g of medium for three days. They were then removed and the medium was incubated for 20 days, after which time the progeny were collected on a 25 mesh sieve and weighed. The assumptions (confirmed by experiment prior to this work) are that the male inseminates both females, that the oviposition rate is constant, and that the weight of 20-day-old progeny is proportional to their number. Males suspected of carrying a rearrangement based on progeny weight were paired singly with virgin females and the fertility of each female (percent hatch of eggs laid after insemination) was measured quantitatively³.

Linkage analysis

To detect pseudolinkage between rearranged chromosomes and to assign rearrangement breakpoints to linkage groups (LG) we constructed two tester strains homozygous for recessive markers on a total of eight of the nine autosomal LGs. The markers (abbreviation and LG in parentheses) were: missing abdominal sternite (*mas*, 2), black (*b*, 3), aureate (*au*, 3), sooty (*s*, 4), ruby (*rb*, 5), malathion susceptibility (*Rmal*⁺, 6), chestnut (*c*, 7), antennapedia (*ap*, 8) and alate prothorax (*apt*, 9). The two strains were *mas au s rb Rmal*⁺ *ap* and *b c apt*, hereafter referred to as MASRRA and BCA, respectively. With these two strains we could in principle detect and map interchromosomal rearrangements involving any of eight autosomal LG and the Y chromosome. X-linked rearrangements were not recovered in this work, since only males were irradiated and only their sons were screened for the presence of rearrangements. In addition to pseudolinkage analysis, rearrangement breakpoints were tested for linkage to the nine markers by using semisterility as the dominant phenotype of each rearrangement. Every generation, males were sampled and scored as "fertile" or "semisterile" by measuring percent hatch of eggs laid after single-pair matings of test males or karyotypically wild-type females. The results of such fertility tests were seldom ambiguous, because wild-type beetles were usually >90 percent fertile and because each rearrangement was associated with a characteristic and rather precise level of sterility (Tables II and III).

For each pseudolinkage/linkage test a sin-

gle male doubly heterozygous for *Rmal* and one of the rearrangements was crossed with nine females from either of the two homozygous linkage tester strains. A single F₁ male heterozygous for the rearrangement and for all markers was backcrossed to a harem of nine tester females. To avoid bias all progeny (ca. 200) produced by each backcross in two weeks were reared to adulthood and scored for each mutant trait. This precaution was taken only for crosses involving the MASRRA tester strain. For crosses involving BCA only 25 male progeny were scored. Twenty-five of the male progeny from each testcross were also scored for the presence of the rearrangement by fertility measurement. Linkage was inferred if fewer than five recombinants were found in the sample of 25 ($P < 0.002$, binomial distribution).

For rearrangements with breakpoints showing linkage to markers on LG 2, 3, 4 or 6 we did 3-point crosses to map breakpoints and to measure crossover suppression. We assumed that interference was complete. The 3-point sequence could then be deduced after finding any two of the three possible crossover types *R-ab*, *Ra-b* and *Rb-a* among the F₁ progeny of *R++/+ab* × *+ab/+ab* harem (*RA++/+ + B* × *++++/++++* for LG 6), where *R* is a breakpoint linked to the recessive markers *a* and *b* (dominant in the case of LG 6). In all testcrosses the male was heterozygous and the females homozygous. The pairs of markers used were *mas* and pearl (*p*) for LG 2, tawny (*b'*) and light ocular diaphragm (*lod*) for LG 3, *s* and hazel (*h*) for LG 4 and *Rmal* and Microphthalmic (*Mo*) for LG 6. For LG 3, all beetles were made homozygous for the eye-color mutation *p*, since *lod* is difficult to score against wild-type (black) eye color. In scoring the progeny for crossover suppression the same precaution was taken to avoid bias as described

previously. For LG 4 we arbitrarily scored only the *s/+* progeny of the 3-point testcross, *R++/+sh* × *+sh/+sh*. We first separated *s-h* recombinants (phenotypically *s⁺h* from *s-h* nonrecombinants (phenotypically *s⁺h⁺*). We then scored a sample of 10–30 males of each type for semisterility. A similar procedure was used for LG 3 and LG 6. For LG 3 we scored only the *b'/+* progeny of the 3-point testcross, *p; R++/+b⁺lod* × *p; +b⁺lod/+b⁺lod*, because *lod* is difficult to score against a *b'/b'* background. For LG 6 we scored only the phenotypically *Mo* (genotypically *Mo/+*) progeny of the 3-point testcross, *R Rmal+/+++Mo* × *++++/++++*, because *Mo* is incompletely penetrant.

Construction of double rearrangement heterozygotes

Rearrangements that were either male-linked, closely linked to a visible marker, or that had a visible phenotype were combined to determine their compatibility in doubly heterozygous strains. The rearrangements used were the two male-linked translocations *T(Y;3)1* and *T(Y;4)*, *T(4;8)*, which has a breakpoint between *s* and *h* on LG 4 ca. one map unit from *s*, and *R(7)Fas*, which is associated with partial fusion of the antennal segments (*Fas*). Two types of double rearrangement heterozygotes were constructed. For the first type we crossed sooty males hemizygous for one of the Y-linked translocations to *+R/s+* females, where *R* = *T(4;8)*. Neglecting *R-s* recombination, doubly heterozygous F₁ male progeny were indicated by their *s⁺* phenotype (wild-type body color). For the second type we crossed males hemizygous for a Y-linked translocation to females heterozygous for *R(7)Fas*. Doubly heterozygous F₁ male progeny were indicated by their *Fas* phenotype.

Table I. Distribution of rearrangement breakpoints among eight autosomal linkage groups and the Y chromosome in *Tribolium castaneum*

Tester strain	No. tested	LG	Marker	No. linked (%)	No. unique types (%)
MASRRA	89	2	<i>mas</i>	16 (18)	9 (10)
		3	<i>au</i>	0(0)	0 (0)
		4	<i>s</i>	13 (15)	9 (10)
		5	<i>rb</i>	1(1)	1 (1)
		6	<i>Rmal</i> ⁺	12 (13)	7 (8)
		8	<i>ap</i>	8 (9)	7 (8)
		Y	male	1(1)	1 (1)
		Y	male	1(1)	1 (1)
BCA	59	3	<i>b</i>	26 (43)	11 (19)
		7	<i>c</i>	13 (23)	6 (10)
		9	<i>apt</i>	13 (22)	7 (12)
		Y	male	3 (5)	1 (2)
		Y	male	3 (5)	1 (2)

Results and Discussion

Distribution of rearrangement breakpoints among 8 autosomal LGs and the Y chromosome

Three-hundred-eighty-six F_1 males derived from day 4-6 inseminations (as well as 406 nonirradiated controls) were screened for reduced fertility. Of the 386, 181 (47 percent) were semisterile as judged by the semiquantitative method compared to 15.4 (4 percent) of the 406 controls. Of the 181, 122 were retested by quantitative measurement of fertility. Of the 122, 114 were confirmed semisterile (1-76 percent hatch), three were fertile (>95 percent hatch) and five were lost before testing was completed. The 114 confirmed semisterile males were presumed to carry chromosome rearrangements. Of the 114 lines, 101 were subjected to pseudolinkage/linkage analysis using the MASRRA tester strain. Eighty-nine of the 101 analyses were successfully completed. Of these 89, 64 were further tested using the BCA linkage tester and 59 analyses were successfully completed.

Of the 89 rearrangements for which linkage tests were completed using the

MASRRA strain, 44 (49 percent) proved to be linked to one or two of the seven LGs tested (i.e., six autosomal + Y), including 37 linked to only one LG and seven linked to two LGs. Of the subsample of 59 rearrangements further tested using the BCA strain, 47 (80 percent) proved to be linked to one or two of the four LGs tested (three autosomal + Y) including 40 linked to only one of the four LGs and seven linked to two LGs. All rearrangements involving two identified LGs are assumed to be simple reciprocal translocations. No rearrangement was linked to more than two LGs with the exception of six "triples" (including four unique types), all of which involved both LG 2 and LG 9 (see below for further discussion). Combining the data for both tester strains (but ignoring LG 2-LG 9 pseudolinkage), 54 of the 59 rearrangements tested proved to be linked to one or two of the nine LGs, including 27 linked to only one LG and 27 linked to two LGs.

Not all of the rearrangements referred to in the preceding two paragraphs are necessarily unique. Since the males originally picked for semisterility screening were the F_1 progeny of a mass-mating of irradiated fathers, it is possible that two or more isolated rearrangements may have proliferated from a single event in a primary spermatocyte. Of the 54 mapped rearrangements, 35 (including 16 linked to only one LG and 19 linked to two LGs) were found to be unique based on fertility, linkage relationships, effects on

crossing over, visible phenotype or a combination of these criteria. The distribution of all mapped rearrangements among eight autosomal LG and the Y chromosome is shown in Table I. The data for the complete set of 54 and for the subset of 35 unique types are listed separately in the table. Unless specifically mentioned the 19 redundant types are not considered further.

Each LG is scored independently in Table I. Rearrangements that were linked to more than one LG are counted separately in each LG. For example, the reciprocal translocation $T(2;6)$ is counted among the 16 LG 2 rearrangements and among the 12 LG 6 rearrangements. It also is one of the nine unique LG 2 rearrangements as well as one of the seven unique LG 6 rearrangements. Thus, the total number of mapped rearrangements (54, including 35 unique types) cannot be calculated by adding the individual values in Table I.

A list of the 35 unique rearrangements and their levels of sterility are given in Tables II and III. For the two autosomal translocations $T(2;4)2$ and $T(4;6)$, females were much more fertile than males (Table II). This sex difference (for which we can offer no explanation) was observed in sibgroups irrespective of the sex of the rearranged parent. For species in which recombination is restricted to one sex, such a sex difference in the fertility of an autosomal rearrangement heterozygote could mean that an inversion is associated with the rearrangement¹. However, recombination occurs with nearly equal frequency in both sexes of *T. castaneum*.

Table II. Linkage group assignments and fertilities of 19 identified reciprocal translocations in *Tribolium castaneum*

Translocation	Percent hatch \pm SD (N)	
	male	female
$T(2;3)1$	42.4 \pm 9.3(15)	
$T(2;4)1^{*†}$	51.5 \pm 7.0(15)	61.6 \pm 9.9(7)
$T(2;4)2^{*†}$	25.3 \pm 9.0(18)	60.9 \pm 12.0(19)
$T(2;4)3^{*†}$	45.2 \pm 5.9(15)	38.5 \pm 10.6(2)
$T(2;6)^{*†}$	41.7 \pm 9.1(9)	32.9 \pm 8.7(9)
$T(3;4)1$	34.8 \pm 8.0(11)	34.8 \pm 8.7(10)
$T(3;4)2^{\ddagger}$	32.3 \pm 6.9(12)	
$T(3;6)1$	38.6 \pm 13.1(8)	
$T(3;6)2$	70.0 \pm 6.8(21)	
$T(3;7)^{\dagger}$	32.1 \pm 6.4(9)	
$T(3;8)1$	27.4 \pm 7.7(11)	
$T(3;8)2$	55.8 \pm 5.4(13)	
$T(4;6)^{\dagger}$	35.9 \pm 9.9(13)	66.9 \pm 8.3(9)
$T(4;7)$	37.3 \pm 6.4(12)	
$T(4;8)^{\dagger}$	44.5 \pm 6.7(18)	45.0 \pm 7.1(4)
$T(6;7)$	38.5 \pm 7.6(14)	
$T(6;8)^{\dagger}$	38.8 \pm 10.7(6)	40.6 \pm 10.4(14)
$T(Y;3)1$	24.1 \pm 7.3(15)	94.0 \pm 4.5(16)
$T(Y;4)^{\dagger}$	36.5 \pm 9.0(19)	93.8 \pm 3.8(10)

* These rearrangements also were linked to LG 9; see text for explanation

[†] Pseudolinkage between the indicated LGs was confirmed by direct measurement using either the MASRRA or BCA strains; in all other cases, pseudolinkage was inferred from linkage of both indicated LGs to semisterility

[‡] $T(3;4)2$ is distinguished from $T(3;4)1$ by the position of the LG 4 breakpoint (see Figure 1)

Table III. Linkage group assignments and male fertilities of 16 unique chromosome rearrangements (R), each with one mapped breakpoint

Rearrangement ^{*†‡}	% Hatch \pm SD (N)
$R(2)1$	21.0 \pm 8.5 (27)
$R(2)2$	12.6 \pm 8.3 (23)
$R(2)3$	66.3 \pm 6.3 (15)
$R(2)p^m$	35.0 \pm 6.9 (12)
$R(3)1$	23.5 \pm 4.7 (11)
$R(3)2$	35.5 \pm 5.4 (11)
$R(3)3$	55.2 \pm 9.1 (23)
$R(4)1$	36.6 \pm 8.6 (26)
$R(5)$	39.8 \pm 11.7 (9)
$R(6)1$	31.8 \pm 9.8 (26)
$R(7)1$	41.0 \pm 7.9 (23)
$R(7)2$	66.0 \pm 3.9 (5)
$R(7)Fas$	25.8 \pm 6.1 (18)
$R(8)1$	11.0 \pm 6.4 (11)
$R(8)2$	33.0 \pm 7.9 (22)
$R(8)3$	71.4 \pm 6.7 (22)

* All rearrangements were tested for linkage to both MASRRA and BCA

[†] $R(2)1$, $R(2)2$ and $R(2)3$ were also linked to LG 9; see text for explanation

[‡] $R(2)1$ and $R(2)2$ are distinguished by their effects on *mas-p* crossover (see Table IV)

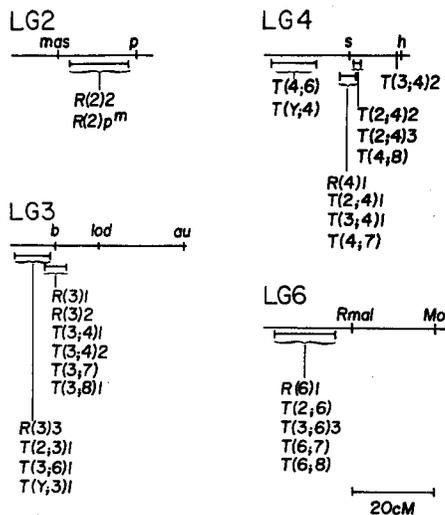


FIGURE 1 Breakpoint positions of 25 chromosome rearrangements involving LG 2, 3, 4, and 6. Maps are based on 4-52 crossovers from 100-200 total progeny from each 3-point testcross harem, with the assumption that single crossovers outnumbered double crossovers.

Smith⁹ proposed that an X-autosome fusion occurred during the speciation of the derived species *T. confusum* ($1N = 9$) from the more primitive *T. castaneum* ($1N = 10$). Sokoloff¹⁰ suggested that autosomal LG 9 in *castaneum* is homologous to a group of X-linked genes in *confusum*. Our discovery that LG 2 and LG 9 are linked means that substantially more described loci can be examined to test Sokoloff's hypothesis.

Three-point mapping and crossover suppression by rearrangements on LG 2, 3, 4, and 6

The reported *mas-p* crossover value is ~20 percent (see Sokoloff et al.¹³). However, when we measured *mas-p* crossover in nine different LG 2 rearrangement strains, we observed free recombination in all but three (Table IV). We also measured free recombination between *mas* and *p* in two unrelated nonrearranged strains. We therefore propose that the strain used by Sokoloff may have contained an inversion, and that in the normal karyotype, *mas* and *p* recombine freely. Two of the rearrangements tested, namely *R(2)2* and *R(2)p^m*, were good crossover suppressors for LG 2. Both of these were independent of all other LGs and thus, may be simple inversions. For most of the LG 2 rearrangements 3-point mapping could not be accomplished using *mas* and *p* since single and double crossovers could not be distin-

Table IV. Crossover suppression by LG 2 rearrangements in *Tribolium castaneum*

Rearrangement	++	+p	mas+	mas p	% Recombination
control 1	65	79	78	69	54 ± 3
control 2	194	173	182	185	48 ± 2
<i>R(2)1</i>	41	46	41	42	51 ± 4
<i>R(2)2</i>	70	10	10	65	13 ± 3
<i>R(2)3</i>	179	130	25	113	35 ± 2
<i>R(2)p^m</i>	88*	17	8*	138	10 ± 2
<i>T(2;3)1</i>	63	62	66	52	53 ± 3
<i>T(2;4)1</i>	109	110	92	97	50 ± 2
<i>T(2;4)2</i>	43	52	58	38	58 ± 4
<i>T(2;4)3</i>	80	79	60	102	43 ± 3
<i>T(2;6)</i>	78	102	80	80	54 ± 3

* Eighty-one of the 88 ++ and seven of the eight *mas* + were mosaic for *p*

Table V. Crossover suppression by LG 3 rearrangements in *Tribolium castaneum*

Rearrangement	No. progeny of indicated phenotype*		% Recombination
	++	+ <i>lod</i>	
control 1	161	16	9.0 ± 2.2
control 2	130	20	13.3 ± 2.8
<i>R(3)1</i>	73	12	14.1 ± 3.8
<i>R(3)2</i>	207	5	2.4 ± 1.0
<i>R(3)3</i>	131	18	12.1 ± 2.7
<i>T(2;3)1</i>	41	8	16.3 ± 5.3
<i>T(3;4)1</i>	178	18	9.2 ± 2.1
<i>T(3;4)2</i>	119	30	20.1 ± 3.3
<i>T(3;6)1</i>	109	22	16.8 ± 3.3
<i>T(3;7)</i>	48	13	21.3 ± 5.2
<i>T(3;8)1</i>	98	13	11.7 ± 3.1
<i>T(Y;3)1</i>	75	9	10.7 ± 3.4

* Only rust-red (*b⁺/+*) progeny were used, since *lod* is difficult to score against the *b⁺* background

Table VI. Three-point map determinations for LG 3 rearrangements

Rearrangement	Sequence	No. crossovers of indicated type	
		<i>R-b⁺lod</i>	<i>Rb⁺-lod</i>
<i>R(3)3</i>	<i>Rb⁺lod</i>	3	4
<i>T(2;3)1</i>	<i>Rb⁺lod</i>	2	4
<i>T(3;6)1</i>	<i>Rb⁺lod</i>	1	13
<i>T(Y;3)1</i>	<i>Rb⁺lod</i>	3	9

Table VII. Crossover suppression by LG 4 rearrangements in *Tribolium castaneum*

Rearrangement	No. progeny of indicated phenotype				% Recombination
	++	+h	s+	sh	
control 1	41	12	6	50	16.5 ± 3.6
control 2	112	20	11	70	14.6 ± 2.4
<i>R(4)1</i>	116	10	10	113	8.0 ± 1.7
<i>T(2;4)1</i>	106	11	7	112	7.6 ± 1.7
<i>T(2;4)2</i>	63	6	11	55	12.6 ± 2.9
<i>T(2;4)3</i>	91	5	5	85	5.4 ± 1.7
<i>T(3;4)1</i>	51	4	6	48	9.2 ± 2.8
<i>T(3;4)2</i>	88	6	5	63	6.8 ± 2.0
<i>T(4;6)</i>	79	10	5	85	8.4 ± 2.1
<i>T(4;7)</i>	100	5	6	66	6.2 ± 1.8
<i>T(4;8)</i>	107	16	15	106	12.7 ± 2.1
<i>T(Y;4) male</i>	107	18	0	5	13.6 ± 2.1
female	14	0	15	84	

guished. However, in the case of $R(2)2$ the 3-point sequence was determined to be $mas-R-p$. Since $R(2)p^m$ caused variegation (mosaic) at the p locus in a large fraction of $R+/+p$ beetles (Table IV), we did not attempt to do 3-point mapping using this rearrangement.

On LG 3, black (b) and lod normally recombine in males at a frequency of 14 percent (see Sokoloff¹²). Using b' (allelic to b , see Beeman and Nanis⁴) we measured 9 and 13.3 percent in two beetles with unrearranged karyotype (Table V). Of ten rearrangements tested $R(3)2$ was the only good crossover suppressor for this region (Table V). In the presence of $R(3)2$, $b'-lod$ crossover was reduced to 2.4 ± 1.0 percent. The break-

points for all 10 of the rearrangements tested for crossover suppression were located to the left of lod towards b' (Figure 1). Three-point mapping was accomplished for four of the 10 rearrangements, and in every case the sequence was $R-b'-lod$ (Table VI). These findings are in agreement with the earlier finding that none of the original 89 rearrangements had a breakpoint linked to au on LG 3 (Table I). These 89 included 26 rearrangements (representing 11 unique types) that had a breakpoint linked to b' . Since au is located to the right of lod , away from b (see Sokoloff¹²), it follows that for most or perhaps all of the LG 3 rearrangements isolated in this work, the correct sequence is $R-b'-lod-au$.

On LG 4 s and h normally recombine at a frequency of 20 percent⁶. We measured 14.6 and 16.5 percent in two "control" beetles, one with unrearranged karyotype and the other carrying a rearrangement that was independent of LG 4 (Table VII). All 10 LG 4 rearrangements tested suppressed $s-h$ crossover. The maximum extent of crossover suppression was threefold in the case of $T(2;4)3$ (Table VII). The breakpoints of six of the 10 LG 4 rearrangements are mapped in Table VIII. Four of these had breakpoints between s and h , three just to the right of s and one just to the left of h . The other two rearrangements had breakpoints to the left of s away from h (Table VIII). Map distances can be estimated by comparing Tables VII and VIII. The remaining four LG 4 rearrangements were tightly linked to the s locus, and

thus 3-point determinations could not be made (see also Figure 1).

On LG 6 $Rmal$ and Mo recombine at a frequency of 20-25 percent² (unpub. obs.). The sample sizes analyzed in the 3-point crosses for LG 6 were too small for accurate measurement of crossover suppression, but 3-point mapping was completed for 5 rearrangements. In every case the sequence was $R-Rmal-Mo$ (Table IX and Figure 1).

The breakpoints of all 25 rearrangements tested are mapped in Figure 1. The indicated map distances are only approximations since many of the rearrangements exert varying degrees of crossover suppression, and since most sample sizes were large enough to establish 3-point sequences but were too small to reveal precise map positions. Brackets that encompass the visible mutant markers b and s on LG 3 and 4 indicate that no crossovers were recovered between marker and rearrangement.

Double rearrangement heterozygotes

All four possible double heterozygotes of the four rearrangements $T(Y;3)1$, $T(Y;4)$, $T(4;8)$, and $R(7)Fas$ were viable. Furthermore the observed male fertility of each double heterozygote was not significantly different from the expected value, obtained by taking the arithmetic product of the fertilities of the individual rearrangement heterozygotes (Table X).

Brower⁵ has discussed the potential for genetic manipulation of postharvest insect pests, including the use of single and multiple chromosome translocations for population suppression. *Tribolium castaneum* is an appropriate model for control trials since it has been well characterized from the viewpoint of formal genetics and population biology, and since a large number of chromosome rearrangements have now been isolated and characterized. Laboratory-scale tests of the population behavior of chromosome rearrangements in *Tribolium castaneum* are in progress in our laboratory.

References

1. BAKER, R. H., R. K. SAKAI, U. T. SAIFUDDIN, and R. W. AINSLEY. Translocations in the mosquito, *Culex tritaeniorhynchus*. *J. Hered.* 68:157-166. 1977.
2. BEEMAN, R. W. Inheritance and linkage of malathion resistance in the red flour beetle. *J. Econ. Entomol.* 76:737-740. 1983.
3. ——— and S. M. NANIS. Efficient methods for the detection of radiation-induced chromosome rearrangements in *T. castaneum*. *Tribolium Info. Bull.* 24:86-88. 1984.
4. ——— and ———. Multiple alleles at the black locus in *Tribolium castaneum*. *J. Hered.* 76:472-473. 1985.

Table VIII. Three-point map determinations for LG 4 rearrangements*

Rearrangement	Sequence	No. crossovers of indicated type	
		$s-Rh$	$sR-h$
$T(2;4)2$	sRh	2	12
$T(2;4)3$	sRh	1	5
$T(3;4)2$	sRh	8	1
$T(4;8)$	sRh	1	13
		$R-sh$	$Rs-h$
$T(4;6)$	Rsh	2	7
$T(Y;4)$	Rsh	19	33

* Three-point sequences were deduced after determining the R phenotypes of samples of the ++ and +h progeny listed in Table VII

Table IX. Three-point map determinations for LG 6 rearrangements*

Rearrangement	Sequence	No. crossovers of indicated type	
		$R-RmalMo$	$RRmal-Mo$
$R(6)1$	$RRmalMo$	3	3
$T(2;6)$	$RRmalMo$	3	4
$T(3;6)3$	$RRmalMo$	3	1
$T(6;7)$	$RRmalMo$	8	5
$T(6;8)$	$RRmalMo$	1	4

* $T(3;6)3$ is indistinguishable from $T(3;6)1$

Table X. Fertilities (% hatch) of single and double rearrangement heterozygotes*

Pairing†	Fertilities (% hatch)				exp†
	A B	A	B	AB	
1,3		25.4 ± 7.8 (13)	28.6 ± 9.9 (11)	6.8 ± 5.4 (5)	7.3
1,4		25.4 ± 7.8 (13)	44.9 ± 6.7 (17)	11.2 ± 4.0 (19)	11.4
2,3		40.2 ± 8.5 (20)	28.6 ± 9.9 (11)	7.1 ± 3.6 (10)	11.5
2,4		40.2 ± 8.5 (20)	44.9 ± 6.7 (17)	13.1 ± 5.9 (20)	18.0

* Fertilities are expressed as percent hatch \pm SD (N)

† Identifications of rearrangements = 1 = $T(Y;3)1$, 2 = $T(Y;4)$, 3 = $R(7)Fas$ and 4 = $T(4;8)$

‡ Expected value equals the product of the values for the individual rearrangements

5. BROWER, J. H. Potential for genetic control of stored-product insect populations. Proc. 1st Int'l. Work. Conf. Stored Prod. Entomol. p. 167-180. 1975.
6. DAWSON, P. S. Linkage group IV of *Tribolium castaneum*. *Can. J. Genet. Cytol.* 14:675-680. 1972.
7. ———. Linkage of *Reindeer* and *alate prothorax* loci and sex differences in recombination frequency in linkage group IX of *Tribolium castaneum*. *Can. J. Genet. Cytol.* 27:341-344. 1985.
8. GLASS, B. A comparative study of induced mutation in the oocytes and spermatozoa of *Drosophila melanogaster*. I. Translocations and inversions. *Genetics* 40:252-267. 1955.
9. SMITH, S. G. The cytology of some Tenebrionid beetles. *J. Morphol.* 91:325-363. 1952.
10. SOKOLOFF, A. The genetics of *Tribolium* and related species. Adv. Genet. Suppl. 1, E. W. Caspari and J. M. Thoday, Eds. Academic Press, NY. 1966.
11. ———. The Biology of *Tribolium*, Vol. 3. Clarendon Press, Oxford. 1977.
12. ———. Sex and crossing over a linkage group III of *Tribolium castaneum*. *Can. J. Genet. Cytol.* 19:259-263. 1977.
13. ———, R. F. FERRONE, and R. MUNOZ. Genetic studies on maxillopedia, missing abdominal sternite, Dachs, and pearl in *Tribolium castaneum*. *Tribolium Info. Bull.* 23:122. 1983.