

A Gene for Lindane + Cyclodiene Resistance in the Red Flour Beetle (Coleoptera: Tenebrionidae)

RICHARD W. BEEMAN AND JEFFREY J. STUART

U.S. Grain Marketing Research Laboratory, USDA-ARS,
Manhattan, Kansas 66502

J. Econ. Entomol. 83(5): 1745-1751 (1990)

ABSTRACT A field-collected strain of *Tribolium castaneum* (Herbst) resistant to lindane was genetically purified to make it isogenic with a laboratory-susceptible strain. Cross-resistance extended to the cyclodienes dieldrin and oxychlordan but not to methoxychlor, avermectin, malathion, or chlorpyrifos methyl. Backcrossing revealed that resistance was monogenic. Linkage analysis and genetic mapping showed that the cyclodiene resistance gene (*lin-R*) is located on the far left end of the third linkage group. Karyotype analysis of a T(Y;3) translocation confirmed that this linkage group is located on the longest autosome. *Lin-R* is the second insecticide resistance gene to be successfully mapped in *T. castaneum*.

KEY WORDS Insecta, lindane resistance, genetics, *Tribolium castaneum*

RESISTANCE to the stored-product protectant lindane is a worldwide phenomenon. In particular, lindane resistance in *Tribolium castaneum* (Herbst) is virtually universal, having been recorded in no fewer than 75 countries (Champ & Dyte 1977). Both polygenic and monogenic types have been reported (Champ & Campbell-Brown 1969, Kumar & Bhatia 1982). Lindane resistance often confers cross-resistance to the cyclodiene group of insecticides including dieldrin, chlordane, and endosulfan (Brown & Pal 1971). This type of resistance probably results from target site insensitivity (Kadous et al. 1983), specifically a modified GABA receptor-chloride ionophore complex at CNS synapses. Lindane + cyclodiene-specific resistance has been found in *T. castaneum* in India (Barwal & Kalra 1982), but it was not analyzed genetically.

The red flour beetle is an excellent experimental subject for analysis of the genetics and population behavior of insecticide resistance factors (Beeman 1983, Beeman & Nanis 1986). Previously, we showed that most of the field-derived resistance to malathion in *T. castaneum* was due to alleles of an esterase locus on the sixth linkage group (Beeman 1983, Haliscak & Beeman 1983, Beeman & Nanis 1986). In this paper, we describe the cross-resistance spectrum, linkage relationships, and probable chromosomal location of lindane + cyclodiene resistance in this species.

Materials and Methods

Strains. The lindane-resistant (R) strain was a field-collected strain (origin unknown) supplied by

the Slough Laboratories, Ministry of Agriculture, Fisheries and Food, England. The strain was highly resistant to both lindane and malathion when collected. The wild-type strains "laboratory susceptible" (Lab-S) and GA-1 were described previously (Beeman 1983). For linkage analysis, we used three linkage tester stocks (designated MASRRA, ABCS, and TLAP), each homozygous for several recessive markers. The MASRRA stock includes the following autosomal recessive markers (abbreviation and linkage group [LG] in parentheses): *missing abdominal sternites* (*mas*, 2), *aureate* (*au*, 3), *sooty* (*s*, 4), *ruby* (*rb*, 5), the wild-type (susceptibility) allele of *Resistance to malathion* (*Rmal*⁺, 6), and *antennapedia* (*ap*, 8). The ABCS stock includes *alate prothorax* (*apt*, 2), *black* (*b*, 3), *chestnut* (*c*, 7) and *short antennae* (*sa*, 7). The TLAP stock includes *tawny* (*b'*, 3) (allelic to *b*), *light ocular diaphragm* (*lod*, 3), *aureate* (*au*, 3), and *pearl* (*p*, 2). The TLAP stock was constructed specifically to map genes on LG 3. We incorporated the LG 2 marker "pearl" to enhance the expression of *lod*. Two other LG 3 markers, T(Y;3) and "Blunt abdominal and metathoracic projections" (*Bamp*²⁷), were described previously (Beeman 1986, Beeman et al. 1986). The former is a translocation between LG 3 and the Y chromosome, and the latter is an apparent point mutation on LG 3.

Before we determined the cross-resistance spectrum, *lin-R* was introduced into the the Lab-S genetic background by nine consecutive generations of crossing and backcrossing (including elimination of field-derived X and Y chromosomes) as described by Beeman & Nanis (1986). Resistant beetles for each backcross were obtained by selection with a discriminating dose (0.93 µg per beetle) of topically applied dieldrin, because the *lin-R* beetles were intensely cross-resistant to that insecticide.

Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

Table 1. Cross-resistance spectrum of *lin-R* strain of *T. castaneum* with reference to isogenic Lab-S strain^a

Insecticide	Strain	No. tested	Slope \pm SE	LD ₅₀	95% CL	R factor
Lindane	S	423	3.59 \pm 0.40	0.50	0.40-0.61	
	R	266	NE	>15		>30
Dieldrin	S	559	2.36 \pm 0.56	0.078	0.054-0.094	
	R	311	NE	>15		>190
Oxychlorthane	S	355	7.80 \pm 1.71	0.24	0.09-0.50	
	R	295	NE	>15		>60
Methoxychlor	S	400	3.90 \pm 2.27	0.91	NE	
	R	200	4.51 \pm 0.50	0.86	0.78-0.93	0.9
Avermectin	S	3,500	4.52 \pm 1.14	0.14	NE	
	R	3,500	3.64 \pm 0.51	0.13	0.07-0.22	1.1
Malathion	S	500	4.94 \pm 0.47	0.075	0.069-0.082	
	R	400	3.05 \pm 1.32	0.13	NE	1.7
Chlorpyrifos methyl	S	350	8.71 \pm 7.45	0.027	NE	
	R	300	4.39 \pm 1.67	0.033	NE	1.2

^a LD₅₀ doses are given in μ g per beetle for all insecticides except avermectin. Avermectin doses are given in ppm in flour. NE, not estimated.

The resulting isogenic *lin-R* strain was then rendered homozygous by several generations of intense selection of virgin adults with dieldrin (20 μ g/beetle). This treatment took advantage of the incomplete dominance of resistance to kill most heterozygotes while sparing homozygotes.

Rearing and Bioassay. All strains were reared in continuous darkness at 30°C and about 70% RH on wheat flour fortified with 5% (wt/wt) brewers' yeast. Three bioassay procedures were used. The first was the impregnated filter paper method of Stringer (1949) with modifications described by Beeman (1983) and Halisak & Beeman (1983). This method was used for linkage analysis. The second method was direct topical application of

test chemicals in 0.3 μ l of 2-pentanone to the adult pronotum using a microapplicator (Model M, ISCO, Lincoln, Nebr.). Each insecticide was tested in duplicate using five to seven doses and 30-50 beetles per test at each dose. This procedure was used for genetic purification and backcrossing, and for determining the cross-resistance spectrum for all insecticides except avermectin B₁. For the first two methods, we used adults 2-3 wk old.

The third method (dietary exposure) was used only for avermectin B₁, because this pesticide was expected to be less active topically than by ingestion. Avermectin B₁ was incorporated into a diet consisting of a 3:1 mixture (wt/wt) of flour/ground wheat. The ground wheat was sieved to a particle diameter range of 0.85-2 mm and was included to facilitate mixing on a rotary mixer. Acetone solutions of avermectin B₁ were added to the diet at a solvent concentration of 3% vol/wt with thorough mixing. After the acetone was allowed to evaporate overnight in vacuo, 250 eggs from the *lin-R* or S isogenic stocks were added. The F₁ adult progeny were counted after 6 wk. Each strain was tested with seven doses in duplicate. Bioassay data were analyzed using the probit procedure of Finney (1971).

Genetic Analysis. To determine the mode of inheritance, isogenized *lin-R* males were mass-crossed to Lab-S females. Dose-mortality tests were done with the two parent strains as well as with their F₁ progeny. Dieldrin was topically applied. To test for segregation of resistance factors, resistant F₁ males were selected at a discriminating dose of 0.93 μ g of dieldrin, then backcrossed for two consecutive generations to isogenic Lab-S females. Dose-mortality bioassays with both backcross generations were also done. Because of the shallow slope values (see below), no dose of dieldrin perfectly discriminated between F₁ heterozygotes and either of the two homozygous parents, *lin-R/lin-R* or Lab-S/Lab-S. However, doses in the range of 0.9-5 μ g per beetle killed all homozygous suscep-

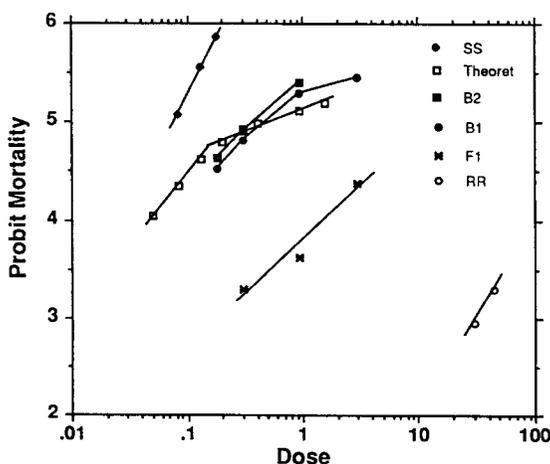


Fig. 1. Inheritance and segregation of *lin-R*. SS and RR denote isogenic Lab-S (susceptible) and homozygous *lin-R* strains, respectively. All crosses and backcrosses to the SS strain employed resistant males. The theoretical backcross line for monogenic inheritance was calculated from the observed SS and F₁ data. Dieldrin dosage (applied topically in 0.3 μ l of 2-pentanone) is expressed in units of micrograms per beetle. Mortality was determined after 24 h.

Table 2. Repulsion test of linkage of cyclodiene resistance (*lin-R*) with autosomal markers in *T. castaneum*

Marker	LG	Observed no. of progeny of given phenotype (corrected no.) ^a				χ^2	P	Exp ^b	% Recombination
		R,m	+,m	R,+	+,+				
apt	II	108 (157)	171 (170)	137 (170)	190 (157)	0.88	>0.05	164	48
mas	II	89 (164)	284 (256)	156 (256)	264 (164)	40.3	<0.0001	210	39
b	III	75 (90)	248 (233)	170 (233)	113 (90)	128	<0.0001	162	28
au	III	103 (225)	235 (230)	142 (230)	313 (225)	0.02	>0.05	228	49
s	IV	72 (296)	149 (276)	173 (276)	399 (296)	1.40	>0.05	286	52
rb	V	80 (190)	247 (276)	165 (276)	301 (190)	31.8	<0.0001	233	41
c	VII	115 (162)	169 (160)	130 (160)	192 (162)	0.02	>0.05	161	50
ap	VIII	102 (261)	202 (228)	143 (228)	346 (261)	4.74	>0.05	244	53

^a R, resistant; m, visible mutant marker phenotype; +, susceptible or wild type.

^b Exp, expected number of progeny in each class under assumption of independent assortment or 50% recombination.

tible individuals and spared most heterozygotes. This meant that resistance behaved as a dominant, incompletely penetrant trait with doses of dieldrin within this range; i.e., some heterozygous-resistant individuals were misclassified as susceptible, but susceptible ones were never misclassified as resistant. Thus, linkage analysis was performed using *lin-R* as a dominant, incompletely penetrant trait. Testcrosses were of the type AaBbCc . . . ♂♂ × aabbcc . . . ♀♀. Linkage data were corrected first for differential preadult survival of mutant and non-mutant classes, then for incomplete penetrance of resistance (see Appendix). Up to five recessive markers could be tested simultaneously for linkage to *lin-R* with the tester stocks described above (*Rmal* was not used). For mapping the position of *Bamp*²⁷ on the third linkage group, we used the four-point testcross *Bamp*²⁷, +, +, +/+ , *b*¹, *lod*, *au*; *p*/+ X +, *b*¹, *lod*, *au*/+ , *b*¹, *lod*, *au*; *p*/*p*. The *pearl* mutation was included to facilitate scoring for *lod*. Linkage data were subjected to χ^2 analysis for significant deviation from the 1:1:1:1 ratio of phenotypes predicted for independent assortment of two traits.

Karyotype Analysis. Spermatogonial metaphase or metaphase I cells from pupae were used for

karyotype analysis. Testes were dissected from midstage, male pupae (2–3 d after pupation at 30°C) in a hypotonic saline solution (0.4% NaCl, 0.1% CaCl, 0.1% KCl, 0.1% NaHCO₃) and then transferred to the same solution containing 0.05% colchicine in a 1.5-ml Eppendorf tube. After incubating in the solution for 30–45 min at room temperature, the cells were pelleted by centrifugation and the supernatant was discarded. The cells were then immediately fixed by adding methanol/acetic acid (3:1) to the tube. After 10–15 min of fixation, the cells were pelleted by centrifugation at 2,000 revolutions per minute, the fixative was discarded, and the cells were washed with the fixative. After washing, the cells were pelleted by centrifugation, the fixative was discarded, and two drops of 45% acetic acid per testis were added to the tube. The cells were then dispersed in the acetic acid by mixing on a vortex mixer, and chromosome preparations were made by placing individual drops of the 45% acetic acid solution onto clean glass microscope slides. The slides were placed on a slide warmer at 45°C, and the drops were allowed to dry. The preparations were then stained with 2% Giemsa in 1/5 M phosphate buffer at room tem-

Table 3. Coupling test of linkage of cyclodiene resistance (*lin-R*) with autosomal markers in *T. castaneum*

Marker	LG	Observed no. of progeny of given phenotype (corrected no.) ^a				χ^2	P	Exp ^b	% Recombination
		R,m	+,m	R,+	+,+				
mas	II	120 (207)	395 (308)	171 (308)	322 (207)	38.9	<0.0001	258	60
b	III	96 (387)	302 (158)	54 (159)	492 (387)	190	<0.0001	273	29
rb	V	158 (246)	322 (282)	200 (282)	328 (246)	4.91	>0.05	233	53

^a R, resistant; m, visible mutant marker phenotype; +, susceptible or wild type.

^b Exp, expected number of progeny in each class under assumption of independent assortment or 50% recombination.

Table 4. Four-point linkage analysis for visible markers on LG 3 in *Tribolium castaneum*

Progeny phenotype ^a	No. progeny
+ + p Bamp ²⁷ +	109
+ au p + lod	24
+ au p Bamp ²⁷ +	16
+ + p + lod	1
+ + p Bamp ²⁷ lod	0
+ au p + +	24
+ + + Bamp ²⁷ X	80
+ au + + X	38
+ au + Bamp ²⁷ X	9
+ + + + X	1
b ^t au X + X	95
b ^t + X + X	36
b ^t + X Bamp ²⁷ X	34
All others	0
Total	467

^a lod could be scored accurately only in pearl-eyed beetles with wild-type (non-b^t) body color. X, not scored.

perature for 20–30 min. Chromosomes were examined under a coverslip suspended in immersion oil on an Olympus BH2 microscope. Cells were photographed with Kodak technical pan film.

Results and Discussion

Cross-Resistance Spectrum. After nine generations of backcrossing to genetically isolate the lindane resistance factor(s), followed by intense selection to reestablish homozygosity, the purified *lin-R* strain was >30 times more resistant to lindane at LD₅₀ than the isogenic Lab-S strain. Intense cross-resistance extended to the cyclodiene insecticides dieldrin (>190 times) and oxychlorane (>60 times), but not to either of two organophosphate insecticides (malathion and chlorpyrifos methyl), the DDT analog methoxychlor, or to the macrocyclic lactone avermectin B₁ (Table 1). Before genetic purification was done, the *lin-R* strain had been more than 100 times resistant to malathion (data not shown). The malathion resistance gene(s) apparently recombined with or segregated away from factor(s) controlling lindane + cyclodiene re-

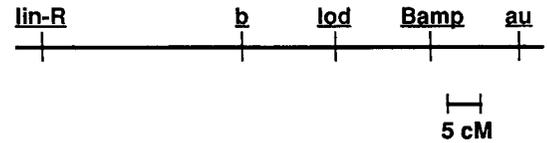


Fig. 2. Map of linkage group 3 of *Tribolium castaneum*. Horizontal scale bar indicates 5% recombination (5 centimorgans, cM).

sistance. This observation simultaneously demonstrated the genetic independence of *Rmal* and *lin-R* and illustrated the effectiveness of the backcrossing scheme.

Mode of Inheritance. Resistant males passed the *lin-R* trait equally to sons (XY) and daughters (XX), indicating an autosomal mode of inheritance (data not shown). Resistance was semidominant (Fig. 1). Backcrossing resistant hybrid males to the Lab-S strain for two consecutive generations produced dose-response curves similar to the one predicted by monogenic inheritance (Fig. 1), suggesting that resistance is controlled primarily by a single, semidominant allele or closely linked set of alleles.

Analysis of Linkage. The results of linkage tests with markers on autosomal groups 2–5 and 7–8 (*lin-R* in repulsion with the autosomal markers) are given in Table 2. Significant deviation from the predicted ratio of 1:1:1:1 for independent assortment occurred in test crosses involving the recessive markers *mas*, *b*, and *rb*. To confirm which (if any) of the three markers was truly linked to *lin-R*, we repeated the three linkage tests in coupling. The results (Table 3) demonstrated that only *b* is truly linked to *lin-R* and that the cyclodiene resistance gene is located on the third linkage group. Corrected values for percentage crossover between *b* and *lin-R* were 28% in repulsion (the two traits on homologous chromosomes) and 29% in coupling (the two traits on the same chromosome). The *rb* mutation assorted independently from *lin-R* in the coupling test, whereas *mas* recombined with *lin-R* at an apparent frequency of 60%, significantly greater than the theoretical maximum of 50% for

Table 5. Tests of linkage of *lin-R* to LG 3 markers in *T. castaneum*^a

Marker	Observed no. of progeny of given phenotype (corrected no.) ^b				χ^2	P	Exp ^c	% Recombination
	R,m	+ ,m	R,+	+,+				
lod (c)	53 (248)	163 (256)	128 (256)	377 (249)	0.26	>0.05	252	49
lod (r)	24 (176)	496 (344)	42 (344)	426 (176)	109	<0.0001	260	34
Bamp ²⁷	42 (89)	63 (119)	111 (119)	97 (89)	8.6	>0.03	104	43
b ^t	35 (60)	88 (130)	118 (130)	72 (60)	52	<0.0001	95	32

^a Test crosses were *lod lin-R*/+ + X *lod* +/lod + (coupling = c); *lod* +/+ *lin-R* X *lod* +/lod + (repulsion = r); and + b^t Bamp²⁷/*lin-R* + + X + b^t +/+ b^t +. Data for Bamp-*lin-R* and b^t-*lin-R* linkage were calculated separately.

^b R, resistant; m, visible mutant marker phenotype; +, susceptible or wild type.

^c Exp, expected number of progeny in each class under assumption of independent assortment or 50% recombination.

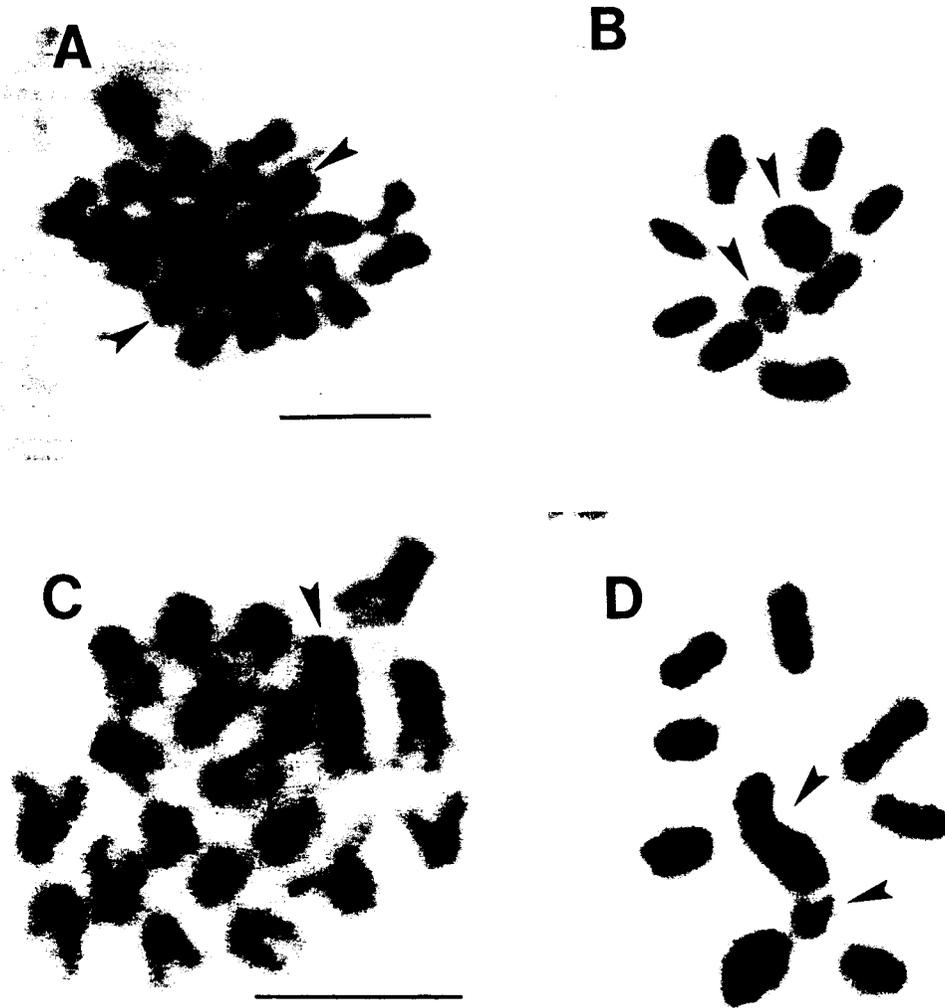


Fig. 3. Spermatogonial metaphase and metaphase I chromosomes from wild-type translocation-bearing *T. castaneum* pupae. (A) Spermatogonial metaphase of wild-type pupae with the large pair of metacentric chromosomes (long arrows) and the Y chromosome (short arrow) indicated. Scale bar indicates 5 μ m in A and B. (B) Wild-type metaphase I with the large metacentric bivalent and the XY parachute indicated. (C) Spermatogonial metaphase of T(Y;3) heterozygotes with the single large metacentric indicated. Note lack of its homologue as well as absence of Y, and their replacement by two new intermediate-sized chromosomes. Scale bar indicates 5 μ m in C and D. (D) Metaphase I of T(Y;3) heterozygotes, with the quadrivalent (composed of the largest bivalent and the XY parachute) indicated.

independent assortment. The reason for this anomalous result may be that *mas* homozygotes are hypersensitive to dieldrin because of a large area of exposed, nonsclerotized cuticle on the ventral abdomen that is associated with the mutation. This defect may provide a surface for more rapid penetration of insecticide through the cuticle. Such an effect is consistent with the abnormally low apparent recombination observed in the repulsion cross and with the abnormally high apparent recombination observed in the coupling cross.

Genetic Mapping. Before *lin-R* was mapped on LG 3, we improved the existing map by positioning

the *Bamp* mutation. This was done by a series of 2-, 3-, and 4-point crosses, one of which is summarized in Table 4. This cross, confirmed by several independent crosses not shown, demonstrated that *Bamp* is located roughly equidistant between *lod* and *au*. Genetic distances (percent recombination) calculated from the appropriate subsets of the data in Table 4 are: *au-Bamp* (13), *Bamp-lod* (14), *lod-b'* (14), *au-lod* (24), *au-b'* (39), *Bamp-b'* (26). These data, in combination with results of the previous experiment (see above) demonstrating that *lin-R* and *au* are unlinked, indicate that *lin-R* is located on the left of *b* and away from *lod*, *Bamp*,

and *au*. To verify this gene order, we performed additional two- and three-point crosses involving *lin-R*, *lod*, *b'*, and *Bamp*. Results of a three-point cross involving *lin-R*, *Bamp*, and *b'* and two-point coupling and repulsion crosses between *lin-R* and *lod* are shown in Table 5. Data for *lin-R-b'* and *lin-R-Bamp* linkage are tabulated separately. The discrepancy between the repulsion and coupling tests of linkage between *lod* and *lin-R* in Table 5 may reflect hypersensitivity of *lod* beetles to diel-drin, as in the case proposed for *mas*. Taken together, these data confirm that *lin-R* lies to the left of *b* and away from *Bamp*. The complete map of LG 3 is shown in Fig. 2. Attempts to determine the relative positions of *lin-R* and the T(Y;3) translocation (a translocation involving the Y chromosome and LG 3 [see Beeman et al. 1986]) were unsuccessful, apparently because of the strong sex dependence of *lin-R* penetrance in the presence of T(Y;3), the possible complexity of the T(Y;3) rearrangement, and the uncertainties of sex determination in beetles. Although tenebrionid beetles have a sex chromosome system in which females are XX and males XY, the precise mechanism of sex determination is unknown. Results of independent analyses of *lin-R* (this work) and T(Y;3) (Beeman et al. 1986) suggest that the two are closely linked to the left of the *b* locus.

Karyotype Analysis. The T(Y;3) translocation was used to determine the chromosomal location of the third linkage group (Fig. 3). The chromosome complement of *T. castaneum* includes nine pairs of autosomes and an XY pair of sex chromosomes (Smith 1952). In spermatogonial cells of wild-type pupae, two long metacentric chromosomes are always evident (Fig. 3A). These chromosomes are easily distinguished as the longest chromosomes in each cell and probably represent a pair of homologous autosomes. Also evident in wild-type spermatogonial cells is one minute chromosome (Fig. 3A), identified as the Y chromosome by Smith (1952). All other chromosomes are similar and intermediate in length. In spermatogonial metaphase cells of T(Y;3) pupae, only one long metacentric chromosome is present and no Y chromosome is visible (Fig. 3C). This suggests that the T(Y;3) translocation involves a reciprocal exchange between one long metacentric chromosome and the Y chromosome to create two new intermediate-sized chromosomes. The two chromosomes which resulted from this exchange have not yet been uniquely identified because they are similar in length to all but the remaining copy of the long metacentric.

The interpretation that the T(Y;3) translocation involves the large metacentric and Y chromosomes is supported by the chromosome morphologies of cells in metaphase I. Wild-type metaphase I cells have 10 bivalents. Among the bivalents, the largest one (a metacentric which probably corresponds to the pair of large metacentric chromosomes seen in spermatogonial metaphase) and the XY pair (XY

parachute [Smith 1952]) are easily identified (Fig. 3B). In contrast, metaphase I T(Y;3) cells have eight bivalents and one quadrivalent (Fig. 3D). The quadrivalent apparently involves the chromosomes normally associated with the large bivalent and the XY parachute. Such an arrangement would be expected if LG 3 is associated with the large metacentric chromosome. Independent evidence that the longest autosome may contain LG 3 is that LG 3 is twice as likely to undergo radiation-induced rearrangement as any other linkage group (Beeman et al. 1986). This argument presupposes that there are no chromosome breakage hotspots; i.e., that the proportion of breaks on a given chromosome is a function only of chromosome length. Taken together, these data strongly suggest that LG 3 is located on the long metacentric chromosome and indicate that through cytological study, the correlation of other linkage groups and their associated chromosomes in the red flour beetle may be possible where additional genetically identified rearrangements exist (Beeman et al. 1986, 1989).

References Cited

- Barwal, B. N. & R. L. Kalra. 1982. Cross-resistance characteristics of lindane resistant and susceptible strains of *Tribolium castaneum* (Herbst) (Col.: Tenebrionidae). *Entomol.* 7: 91-95.
- Beeman, R. W. 1983. Inheritance and linkage of malathion resistance in the red flour beetle. *J. Econ. Entomol.* 76: 737-740.
1986. Section on new mutants. *Tribolium Info. Bull.* 26: 83.
- Beeman, R. W. & S. M. Nanis. 1986. Malathion resistance alleles and their fitness in the red flour beetle (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* 79: 580-587.
- Beeman, R. W., T. R. Johnson & S. M. Nanis. 1986. Chromosome rearrangements in *Tribolium castaneum*. *J. Hered.* 77: 451-456.
- Beeman, R. W., J. J. Stuart, M. S. Haas & R. E. Denell. 1989. Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum*. *Dev. Biol.* 133: 196-209.
- Brown, A. W. A. & R. Pal. 1971. Insecticide resistance in arthropods. World Health Organization, Monograph Series 38, Geneva, Switzerland.
- Champ, B. R. & M. Campbell-Brown. 1969. Genetics of lindane resistance in *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *J. Stored Prod. Res.* 5: 399-406.
- Champ, B. R. & C. E. Dyte. 1977. FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Prot. Bull. 25: 1-40.
- Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, London.
- Haliscak, J. P. & R. W. Beeman. 1983. Status of malathion resistance in five genera of beetles infesting farm-stored corn, wheat and oats in the United States. *J. Econ. Entomol.* 76: 717-722.
- Kadous, A. A., S. M. Ghiasuddin, F. Matsumura, J. G. Scott & K. Tanaka. 1983. Difference in the picrotoxinin receptor between the cyclodiene-resistant and susceptible strains of the German cockroach. *Pestic. Biochem. Physiol.* 19: 157-167.

- Kumar, J. & S. K. Bhatia. 1982. Inheritance of resistance to lindane in a laboratory-selected strain of *Tribolium castaneum* (Herbst). Pestic. Sci. 13: 513-516.
- Smith, S. G. 1952. The cytology of some tenebrionid beetles (Coleoptera). J. Morphol. 91: 325-363.
- Stringer, A. 1949. A simple method for assaying con-

tact toxicity of insecticides, with results of tests of some organic compounds against *Calandra granaria* L. Ann. Appl. Biol. 36: 213-224.

Received for publication 16 January 1990; accepted 17 April 1990.

Appendix

Method for correcting two-point linkage data when one of the two mutations (Resistance) is incompletely penetrant and the other (visible marker) has reduced viability.

Comments. The method is still applicable even if no viability reduction is associated with the mutant class. The method is explained for the particular (and most common) case where the incompletely penetrant (Resistance) gene is dominant (or functionally dominant for purposes of linkage analysis). The method applies to the observed phenotypic ratios obtained in the cross ($Rm^+/R^+m \times R^+m/R^+m$ (i.e., the trans-double heterozygote backcrossed to the homozygous susceptible, visible mutant). R, resistance; m, visible mutant linked to R.

Assumptions

1. The visible marker is completely penetrant.
2. Alleles at the R locus segregate normally and are equally viable. No assumption regarding fertility is needed; i.e., the method is applicable to recessive lethal or semisterile R factors.
3. The penetrance of R is the same in mutant and nonmutant classes. If, as may often be the case, the penetrance of R is lower for mutant than nonmutant classes, then the percentage crossover will be underestimated. This possibility should always be tested by performing the cross in both coupling and repulsion phases.

Method

1. Correct the observed data for any deficiency of the visible mutant class, while keeping the observed frequency of R constant in mutant and nonmutant classes. Do this by adding enough mutants to bring the mutant/nonmutant ratio to 1.0.
2. Calculate the penetrance of R after Correction 1 has been made. Penetrance, $2(\#R \div \#Total)$.
3. Correct for incomplete penetrance of R. For the mutant class, corrected $\#R = \text{observed } \#R \div \text{penetrance}$, and corrected $\#S = \text{total } \#mutant - \text{corrected } \#R$. A similar calculation is done for the nonmutant class.
4. Calculate percentage recombination.

Example	Phenotype	Raw data
	Rm	50
	Rm ⁺	300
	R ⁺ m	500
	R ⁺ m ⁺	450

where R⁺ is susceptible and m⁺ is nonmutant.

1. Correct for any deficiency of the mutant class. Total $\#m = 550$, total $\#m^+ = 750$. Thus, the m class is deficient; i.e., $750 - 550 = 200$ m insects failed to complete development. These 200 must be added to the observed data. Of the 550 m, 50 (9.09%) are R. Thus, of the 200 m to be added, 9.09% (or 18) must be R, and the remaining 182 must be R⁺.

Corrected data:	Rm	68	(50 + 18)
	Rm ⁺	300	
	R ⁺ m	682	(500 + 182)
	R ⁺ m ⁺	450	

2. Calculate the penetrance of R. Of a total of 1,500 insects scored, $68 + 300 = 368$ (24.5%) were R (expected value, 50%). Thus, R is incompletely penetrant. Penetrance = $2(368 \div 1,500) = 0.491$.
3. Correct for incomplete penetrance of R. Corrected Rm = $68 \div 0.491 = 138$. Because there are $68 + 682 = 750$ m, and 138 of these are R, the corrected value for R⁺m is $750 - 138 = 612$. Similarly for Rm⁺, $300 \div 0.491 = 611$, and for R⁺m⁺, $750 - 611 = 139$.

Corrected data:	Rm	138
	Rm ⁺	611
	R ⁺ m	612
	R ⁺ m ⁺	139

4. Calculate percentage recombination.

$$\begin{aligned} \text{Percentage recombination} &= \#recombinants \div \#total \\ &= (138 + 139) \div 1,500 \\ &= 18.5\% \end{aligned}$$

(Percentage recombination calculated for uncorrected data = $(50 + 450) \div 1,300 = 38.5\%$; i.e., greatly overestimated because of the incomplete penetrance of R. Percentage recombination calculated after correcting only for reduced viability of mutant class = 34.6%; i.e., still greatly overestimated.)