

Genetic Mapping of a Major Locus Controlling Pyrethroid Resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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ABSTRACT *Tribolium castaneum* (Herbst) strain QTC279 is highly resistant to deltamethrin and other synthetic pyrethroids. This strain was shown to carry at least 1 resistance gene, *PyR-1*, on linkage group 9, ~20 map units from the visible mutant marker, *pearl*. Three-point mapping involving *pearl* and another visible mutant marker, *cola*, indicated a gene order of *pearl-cola-PyR-1*. Evidence of a 2nd LC9-linked resistance factor (R) mapping in the gene order R-*p-co* was also observed. Other resistance factors were clearly present in QTC279, but were not genetically mapped. Piperonyl butoxide, an inhibitor of cytochrome P450-mediated oxidative metabolism, significantly increased the toxicity of deltamethrin to a strain derived from QTC279 that carries *PyR-1*, strain pR. Compared to susceptible beetles, QTC279 and pR had elevated and comparable levels of cytochrome P450 protein. The significance of pyrethroid resistance in *T. castaneum* is discussed.

KEY WORDS genetic mapping, pyrethroid resistance, *Tribolium castaneum*

GENETIC CHARACTERIZATION OF insecticide resistance is necessary to develop an understanding of resistance mechanisms and for the development of resistance detection and management tools. This has been accomplished most readily when resistance has arisen in insect species that are amenable to genetic analysis (Beeman et al. 1992, French-Constant et al. 1993, Feyereisen 1995). Moreover, once the genetic basis of resistance has been characterized, it has been possible to identify similar mechanisms in important pests that are less amenable to genetic and biochemical manipulation (Brun et al. 1995, Miyazaki et al. 1996, Dong 1997).

We have been interested in the pyrethroid resistance present in strain QTC279 of the red flour beetle, *Tribolium castaneum* (Herbst). This insect is both an important pest of stored grain and grain products, and amenable to genetic analysis. It has a short generation time, a relatively small genome (Brown et al. 1990), a number of useful genetic markers, and has proven to be an excellent model for studies in quantitative and population genetics (Bell 1983).

T. castaneum strain QTC279 originated in Malu, Queensland, Australia where it was collected from stored grain that had a long history of frequent treatment with pyrethroid and other insecticides. In its initial characterization, QTC279 had substantial resistance to pyrethroid (e.g., 950-fold resistance to deltamethrin) and carbamate (e.g., 86-fold resistance to

carbaryl) insecticides (Collins 1990). It was most resistant to cyanopyrethroids, particularly deltamethrin. It had <10-fold resistance to organophosphate insecticides. In a subsequent analysis, increased glutathione S-transferase activity was found to be associated with organophosphate insecticide resistance in QTC279 (Reidy et al. 1990).

Our objectives in the current study were to determine the mode of inheritance of pyrethroid resistance in QTC279, map resistance relative to genetic markers, and test the hypothesis put forward by Collins (1990) that cytochrome P450 metabolism is associated with the pyrethroid resistance observed in this strain.

Materials and Methods

Insects. The resistant strain, QTC279, was provided by P. J. Collins. Resistant beetles were initially collected from stored grain that had been treated 6 mo earlier with 2 mg kg⁻¹ cyfluthrin and then selected for cyfluthrin resistance in the laboratory for 10 generations (Collins 1990). Lab-S is a susceptible laboratory strain originally developed by Beeman and Nanis (1986) for investigations of insecticide resistance in *T. castaneum*.

Genetic mapping was initiated with 3 *T. castaneum* multiply marked tester strains: MMS (Beeman et al. 1986) which is homozygous for the recessive morphological mutations *missing abdominal sternites* = *mas*, linkage group (LG) 2, *aureate* (*au*, dense setae, LG3), *sooty* (*s*, sooty body color, LG4), *ruby* (*rb*, red eyes, LG5) and *antennapedia* (*ap*, LG8); baptsac (Beeman et al. 1986), which is homozygous for the recessive morphological mutations *black* (*b*, black body color,

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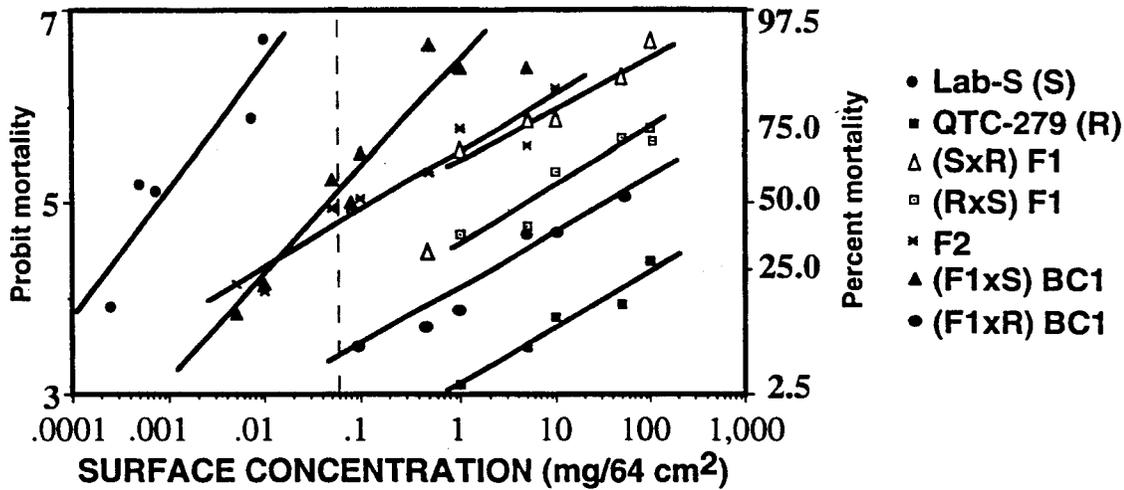


Fig. 1. Dose-response curves for *T. castaneum* adults resistant (QTC-279) and susceptible (Lab-S) to deltamethrin, and dose-response curves for their hybrid (F₁), F₂, and backcross (BC₁) progeny. Vertical dashed line indicates the dose that was used to discriminate between the resistant and susceptible types in mating experiments.

LG3), *chestnut* (*c*, reddish eye color, LG7) and *short antenna* (*sa*, LG undetermined); and *Rdmcp* (J.J.S., unpublished data) which is homozygous for the dominant mutation *Reindeer* (*Rd*, malformed antennae, LG2), and the recessive mutations *microcephalic* (*mc*, LG5) and *pearl* (*p*, white eye color, LG9).

Three additional strains, 'pearl', 'cola', and 'pearl-cola', which are homozygous for 1 or both of the recessive LG9 mutations *p* and *cola* (*co*, cola body color), were selected for use in additional mapping experiments. All strains and matings were maintained in total darkness at 30–33°C and >50% RH on standard *T. castaneum* media (95% wheat flour and 5% brewer's yeast). Strains were maintained in glass 1-pint Mason jars containing 1/3 pint of media. Each jar was covered with 25- μ m wire mesh and filter paper that fit into the metal band of the jar. Matings were made by placing virgin beetles into 20-ml glass creamer vials containing \approx 10 ml of media. Vials were capped with paper lids. Strains and matings were subcultured weekly.

Insecticide Assays. Beetles were exposed to filter paper surfaces treated with varying amounts of technical grade deltamethrin. Deltamethrin was applied to the filters as a solution in either ethanol or acetone. The beetles were confined on this surface for a predetermined period of time with stainless steel rings (75 mm in diameter) that *T. castaneum* are unable to climb. Beetles were classified as dead if they were unable to move in a completely coordinated fashion and unable to freely bend the joints of both metathoracic legs as they walked on the treated surface.

Matings. To evaluate the inheritance of the deltamethrin resistance observed in QTC279, reciprocal matings were made between QTC279 (R) and Lab-S (S) beetles; S \times R and R \times S, where the 1st letter indicates the females in the mating. The F₁ progeny of these matings were collected as pupae, and the sexes were separated to ensure their virginity. (R \times S) F₁

were intermated to produce an F₂ generation, and (R \times S) F₁ males were backcrossed to both R and S females to produce 2 backcross populations. Individuals from each generation were collected and assayed for resistance at the doses shown in Fig. 1 in the manner described above (2-h exposures). Fifty beetles were used for each concentration of deltamethrin and each concentration was replicated 3 times.

A 2-h exposure to 50 μ g of deltamethrin on 64-cm² filter paper surfaces killed 50% of the (F₁ \times S) BC₁ beetles (Fig. 1) and caused 100% mortality among the beetles in each of the multiply marked tester strains. Therefore, unless indicated otherwise, that treatment was used to discriminate between heterozygous-resistant and homozygous-susceptible beetles in all subsequent experiments.

Genetic Mapping. To test for linkage between morphological markers and resistance in repulsion (*trans*), QTC279 beetles were mated with females derived from the multiply-marked tester strains MMS, baptsac and *Rdmcp*. F₁ progeny from each of these matings were intercrossed and backcrossed to the parental strains to obtain both F₂ and backcross (BC₁) progeny. BC₁ and F₂ beetles were selected for resistance as described above. BC₁ mortality was >50%. This indicated that the penetrance of resistance was incomplete (i.e., some heterozygous-resistant individuals were dying at the discriminating dose), but homozygous susceptible individuals never survived. Therefore, to avoid misclassifying resistant types as susceptible, we excluded all dead beetles from our recombinational analyses. Linkage data were subjected to chi-square analysis for significant deviation from the 1:1 (3:1, *Rd*) ratio of phenotypes predicted for independent assortment of a single trait.

To test for linkage between morphological mutations and resistance in coupling (*cis*), BC₁ individuals that were both resistant and homozygous for either

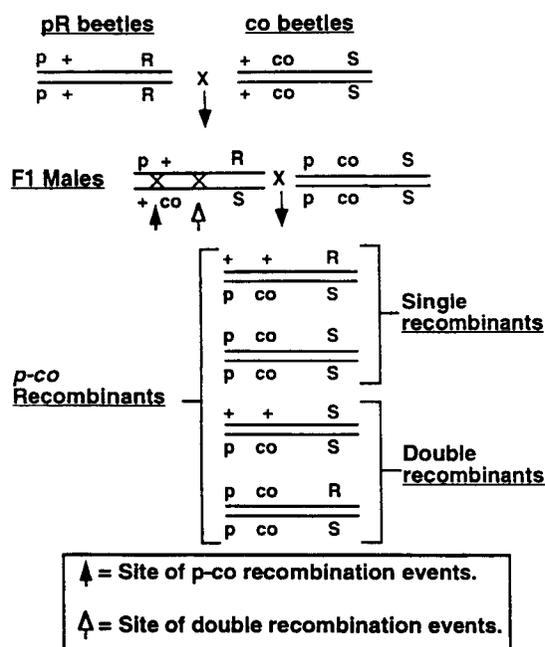


Fig. 2. Diagram showing the mating used to determine the relative gene orders of *pearl* (*p*), *cola* (*co*) and pyrethroid resistance (*R*). Diagram indicates the result expected if the gene order is *p-co-R*: Among the resistant types, single *p-co* recombinants ($+ + R/p co S$) will outnumber double recombinants ($p co R/p co S$). Among susceptible types, single *p-co* recombinants ($p co S/p co S$) will outnumber double recombinants ($+ + S/p co S$).

mc, *ap*, or *p* were selected. These were presumed to carry a chromosome which had a resistance factor and 1 of the 3 marker mutations in *cis*. In an effort to construct stocks that were homozygous for these chromosomes, we inbred individuals within each line and selected their progeny for the marker in question and deltamethrin resistance (2-h exposures to 500 μ g of deltamethrin on 64-cm² surfaces) for 5 generations. We then selected 4 resistant males with each marker for matings to Lab-S females. F₁ males were collected from among the progeny of each of these matings and these were crossed to susceptible females derived from marker strains. The progeny of these matings were then selected for resistance, and the morphological phenotypes of the resistant beetles were determined. Linkage data were subjected to chi-square analysis for significant deviation from the 1:1 ratio of phenotypes predicted for independent assortment of a single trait.

To map resistance relative to the LG9 markers *p* and *co*, we performed experimental matings as diagrammed in Fig. 2. Beetles that were both phenotypically *pearl* and deltamethrin resistant (pR) were collected from the *cis* linkage test, inbred and selected for resistance (2-h exposures to 500 μ g of deltamethrin on 64-cm² surfaces) for 6 generations. We then mated 10 resistant pR males ($p +/p +$) to 10 susceptible cola females ($+ co/+ co$). Thirty F₁ male progeny ($p +/+$

co) were then collected and mated to 60 susceptible pearl-cola beetles ($p co/p co$) in each of 4 replicates (A, B, C, and D). A total of 84 *pearl-cola* (*p-co*) recombinants (including both $+ +/p co$ and $p co/p co$ genotypes, hereafter referred to as $+ +$ and *p co* recombinant types, respectively) was recovered from among the testcross progeny. Fifty-seven of these recombinants were each mated separately with pearl-cola beetles to establish *p-co* recombinant families. Each of these 57 families was then tested at least 3 times on a discriminating dose of deltamethrin for the presence of resistance (*R*) within the family. Greater numbers of resistant types were expected to be $+ +$ recombinants if the gene order was *p-co-R* (as diagrammed in Fig. 2), whereas greater numbers of resistant types were expected to be *p co* recombinants if the gene order was *R-p-co*.

As shown below, we observed resistance in some of both the $+ +$ and the *p co* recombinant families. We suspected that either double recombination or unlinked resistance factors in the pR parental beetles were responsible for this result. Therefore, to test for the presence of an unlinked resistance factor, 3 of the most resistant $+ +$ recombinant families and 2 of the most resistant *p co* recombinant families were selected for further analysis. Each of these families were inbred and selected for resistance to deltamethrin for 3 generations. We then tested each family for LG9-linked resistance as follows: 2 resistant males ($+ +/+ +$) from each $+ +$ recombinant family were mated separately to 3 pearl-cola females ($p co/p co$). Likewise, 2 resistant males ($p co/p co$) from each *p co* recombinant family were mated separately to 3 Lab-S females ($+ +/+ +$). Single F₁ males ($p co/+ +$) from each mating were then mated separately with 3 virgin pearl females ($p +/p +$). The progeny of these matings were then assayed separately for resistance. Resistant progeny were collected and scored for eye color. Linkage data were subjected to chi-square analysis for significant deviation from the 1:1 ratio of phenotypes predicted for independent assortment of a single trait. We reasoned that if resistance resulted from the action of an unlinked resistance factor, *p*-linked resistance would not be observed.

QTC279, pR Resistance Comparison. The presence of a mapped resistance gene in pR beetles did not preclude the possibility that additional resistance factors are present in strain QTC279. To test that possibility, we compared the deltamethrin tolerance of the pR beetles with QTC279 and susceptible pearl (pS) beetles. In these comparisons, beetles from each strain were exposed for 20 h to sublethal and lethal doses of deltamethrin, both with and without 100 mg of piperonyl butoxide (PBO), on the treated filter paper. Differences were determined by analysis of variance (ANOVA) using the arcsine percent mortality transformation. Confidence limits were derived from the standard deviation of arcsine transformed percent mortality.

Microsome Preparations and Total P450 Measurements. For each of 3 replicates of Lab-S, QTC279, pS, and pR strains, separate microsomal preparations were

made using 0.5 g of *T. castaneum* adults. Beetles were 1st homogenized on ice with a Vertis homogenizer in 5 ml of cold 100 mM potassium phosphate buffer (pH 7.4) containing 0.15 M KCl, 2.5 M sucrose, 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 mM dithiothreitol (DTT), 1 mM EDTA, 1 mM phenylthiourea (PTU), and 10% glycerol. Homogenates were then centrifuged for 20 min at 12,000 × *g* at 4°C. The supernatant was then collected and recentrifuged for 60 min at 100,000 × *g* at 4°C to pellet microsomes. Approximately 2 mg of microsomal protein was recovered from each stain as determined by the method of Bradford (1976) and resuspended in 0.1 M potassium phosphate buffer (pH 7.4) with 0.15 M KCl, 1 mM PMSF, 0.1 mM DTT, 1 mM EDTA, 1 mM PTU, and 30% glycerol. To estimate total P450 protein in each microsomal preparation, carbon monoxide difference spectra were obtained from dithionite reduced microsomes (~0.5 mg/ml) according to the method of Omura and Sato (1964) using a Perkin Elmer Lambda-2 dual beam spectrophotometer.

Results

Inheritance of Resistance. QTC279 had >100,000-fold greater resistance to deltamethrin than our standard susceptible laboratory strain Lab-S (Fig. 1). Resistance was incompletely dominant. Because of shallow dose-response slopes, segregation of major genes could not be detected in backcrosses. The dose-response of the reciprocal F_1 suggested a slight maternal effect (i.e., $[S \times R] F_1$ were slightly more sensitive to deltamethrin than $[R \times S] F_1$). However, male and female F_1 from reciprocal matings survived exposure to deltamethrin equally well (data not shown), indicating that the major gene(s) conferring resistance are autosomal.

Genetic Mapping. To identify genes of major effect that might be associated with pyrethroid resistance in QTC279, we looked for linkage between deltamethrin resistance and the morphological markers contained in the multiply-marked tester strains MMS, baptsac, and Rdmcp. Based on the response of the ($F_1 \times S$) BC_1 in the previous experiment (Fig. 1), we anticipated that 50% of the BC_1 in these experiments would be resistant. However, because some of the morphological mutations in the tester strains probably increase susceptibility to deltamethrin, the percentage of the BC_1 beetles that were resistant to the discriminating dose averaged only 20 ± 3 per experiment. Nonetheless, an analysis of the surviving BC_1 individuals suggested resistance might be linked to 1 or more of the loci *s*, *mc*, *ap*, *sa* and *p* (Table 1). The most likely possibility was linkage to *p*.

An analysis of the F_2 also indicated that resistance factors might be linked to *mc*, *ap* and *p*, but not to *s* or *sa* (data not shown). When tested in *cis*, linkage between resistance and *ap* and *mc* was not detected (data not shown), but linkage between resistance and *p* was confirmed (Table 2). The frequency of recombination between *p* and deltamethrin resistance in

Table 1. Tests in repulsion (*trans*) for linkage between resistance (R) and morphological mutations in *T. castaneum* where + indicates a wild-type phenotype and m indicates a mutant phenotype

Marker	R +	R m	% recombinant	χ^2 value	Prob.
<i>mas</i> (LG2)	98	109	52	0.585	0.445
<i>b</i> (LG3)	139	132	48	0.181	0.671
<i>au</i> (LG3)	101	106	51	0.121	0.728
<i>s</i> (LG4)	148	59	28	38.27	<0.0001
<i>rb</i> (LG5)	103	104	50	0.005	0.9448
<i>mc</i> (LG5)	214	132	38	19.434	<0.0001
<i>c</i> (LG7)	133	138	50	0.092	0.761
<i>ap</i> (LG8)	137	70	34	21.686	<0.0001
<i>sa</i> (LG?)	173	98	36	20.756	<0.0001
<i>p</i> (LG9)	282	64	18	137.353	<0.0001
<i>Rd</i> (LG2)	91	247	54	0.667	0.414

LC, linkage group.

both the *trans* and *cis* linkage tests indicated that the gene or genes conditioning pyrethroid resistance on LG9 map 11–33 cM from *p* (Tables 1 and 2).

To map this LG9-linked pyrethroid resistance (R) relative to the LG9 markers *p* and *co*, we conducted an experimental mating involving *pR*, *cola*, and *pearl-cola* beetles as described above (Fig. 2). Eighty-four *pearl-cola*-recombinant beetles (*p-co* recombinants) were recovered from a total of 8426 progeny scored (Table 3), indicating that the genetic distance between *p* and *co* is only 1 cM. With an estimated genetic distance of 11–33 cM between *p* and R, we suspected that R did not map between *p* and *co*. Moreover, we found that *co* could recombine with resistance without recombining with *p*: 70 resistant and genotypically + *co/p co* beetles were recovered among 1,045 testcross + *co/p co* progeny (=6.7% recombination). This observation was inconsistent with a *p-R-co* gene order.

An examination of 57 families derived from *p-co*-recombinant beetles found that the majority with resistance (22/32) had a wild-type phenotype, + + R (Table 3), whereas the majority with complete deltamethrin susceptibility (18/25) had a *pearl-cola* phenotype, *p co S*. Thus, the gene order appeared to be *p-co-R*, assuming that the + + S and *p co R* types arose through double recombination (as shown in Fig. 2). However, the possibility of a *R-p-co* gene order remained if the resistance among the + + R types was conditioned by a resistance factor that is not LG9-linked. Therefore, we tested 3 + + R recombinant families and 2 *p co R* recombinant families for *p*-linked resistance (Table 4). The resistant progeny in each replicate of each + + R family deviated from the

Table 2. Tests in coupling (*cis*) for linkage between resistance (R) and *pearl* (*p*) where *p* indicates a white-eyed phenotype and + indicates a wild-type phenotype

Mating	Total no. resistant	Progeny phenotypes		% recombination
		R, <i>p</i>	R, +	
1	71	63	8	11 ± 4
2	213	142	71	33 ± 3
3	189	160	29	15 ± 3
4	103	80	23	22 ± 4
Total	576	445	131	22 ± 2

Table 3. Total number of testcross progeny (TC₁) scored, total number and phenotypes of the *pearl-cola* recombinants (*p-co* recombinants) recovered among the TC₁, and the number and phenotypes (resistant = R and susceptible = S) of the recombinant families tested for resistance

Rep.	Total no. of TC ₁	No. of <i>p-co</i> recombinants ^a		No. and phenotypes of <i>p-co</i> recombinant families ^a			
		++	<i>p co</i>	++R	++S	<i>p co</i> R	<i>p co</i> S
A	2,155	10	6	6	2	3	2
B	1,942	10	14	6	2	5	9
C	2,265	11	10	8	2	1	6
D	2,064	12	11	2	1	1	1
Totals	8,426	43	41	22	7	10	18

^a ++ indicates a wild-type eye color and wild-type body color phenotype, and *p co* indicates a white-eyed (*pearl*) and dark body color (*cola*) phenotype.

expected 1:1 (*p*: wild-type) ratio. This confirmed that these ++R recombinant families carried at least 1 *p*-linked resistance factor, consistent with a *p-co*-R gene order. Although it is still possible that this resistance is conditioned by >1 gene, we will refer to it hereafter as *Pyrethroid resistance-1* (*PyR-1*).

The resistant progeny of at least 2 replicates of each *p co* R recombinant family also deviated from the expected 1:1 ratio. This observation was also consistent with a *p-co-PyR-1* gene order, assuming that these types arose via double recombination. Interestingly, recombination between resistance and *p* among the *p co* R families (32–42%) was much greater than that observed among the ++R families (7–23%), suggesting that the resistance in these *p co* R recombinant families may be modified or conditioned by a factor that is distinct from *PyR-1*. Thus, we observed evidence of at least 2 resistance factors among the parental pR beetles: (1) *PyR-1* which maps in the *p-co-PyR-1* gene order on LG9, and (2) a factor that is at least 32 cM from *p*, and if linked to *p*, maps in a R-*p-co* gene order.

Multiple Resistance Factors in QTC279. Although much more resistant than susceptible *pearl* beetles (*pS*), pR beetles were not as resistant as QTC279

(Table 5). Thus, we have additional evidence that the resistance in QTC279 is multifactorial, because resistance or modifying alleles, in addition to those carried by pR beetles, are apparently necessary for maximum resistance. Moreover, unlike strain QTC279, the resistance of the pR beetles was reduced by PBO to a level equivalent to the tolerance of pS beetles unexposed to PBO (Table 6). Therefore, it appeared that the deltamethrin resistance present in pR beetles may be solely cytochrome P450 based. This conclusion was supported by the amounts of total cytochrome P450 protein observed in these strains: QTC279 and pR microsomes had equivalent amounts of total P450 protein (2.4 ± 0.2 and 2.1 ± 0.4 nmol/mg total microsomal protein, respectively), whereas the total amount of P450 protein in both Lab-S and pS microsomes was below the limit of detection (<0.05 nmol/mg microsomal protein).

Discussion

We discovered that pyrethroid resistance in *T. castaneum* strain QTC279 is conditioned by at least 1 gene on LG9. The resistance (R) that maps in the gene order *p-co*-R is probably conditioned by a single major gene, which we call *PyR-1*. These experiments suggest that *p* and *PyR-1* are separated by at least 7 map units on LG9. The position of the dominant mutation *Short elytra* (*SE*) relative to *p* and *co* (R.W.B., unpublished data) suggests that the gene order on LG9 is *SE-p-co-PyR-1*. Two other loci, *antenna bifurcata* (*ab*) (Vasquez and Castillo 1985) and the T(2;9) translocation *maxillopedia-Dachs-1* (*mxd^{Dch-1}*) (Beeman et

Table 4. Genetic linkage between *pearl* (*p*) and resistance (R) in each of five resistant *pearl-cola* recombinant families as measured by mating two F₁ males (++*p co*) derived from each family to *pearl* females (*p +/p +*), where + indicates wild-type alleles, and *p* and *co* indicate recessive mutant alleles at the *p* and *co* loci

Resistant recombinant family (rep.)	Progeny phenotypes ^b		% recombinant	χ ² value	Prob.
	R, <i>p</i>	R, +			
++ 14 (a)	2	25	7	19.6	<0.0001
++ 14 (b)	8	43	16	24.0	<0.0001
++ 16 (a)	15	54	22	22.1	<0.0001
++ 16 (b)	3	36	8	27.9	<0.0001
++ 21 (a)	9	41	18	21.1	<0.0001
++ 21 (b)	15	49	23	18.1	<0.0001
<i>p co</i> 5 (a)	32	15	32	6.1	0.0131
<i>p co</i> 5 (b)	19	12	39	1.6	0.2087
<i>p co</i> 8 (a)	43	21	33	7.6	0.006
<i>p co</i> 8 (b)	50	36	42	2.3	0.1311

^a ++ Denotes a wild-type recombinant family (dark eye color and wild-type body color), *p co* denotes a *pearl-cola* recombinant family (*pearl* eyes and *cola* body color).

^b + Indicates a wild-type eye color phenotype, *p* indicates a white-eyed (*pearl*) phenotype, and R indicates a resistant phenotype.

Table 5. Percent mortality of pS, pR and QTC279 beetles after a 20-h exposure to 4 doses of deltamethrin (3 replications, 50 beetles per replicate)

Dose (mg/64 cm ²)	Mean % mortality (95% CL)		
	pS	pR	QTC279
0.05	99 (98–100)a	2 (0–4)b	0b
0.5	100a	13 (9–17)b	0c
5.0	100a	13 (12–15)b	0c
50.0	100a	47 (27–57)b	0c

Means within rows followed by the same letter are not significantly different (ANOVA of arcsine transformed data and Tukey-Kramer multiple comparisons test; P > 0.05). Confidence limits were not shown if values for all 3 replicates were identical.

Table 6. Effect of piperonyl butoxide (PBO, 100 mg/64 cm²) on the mortality of resistant (QTC279 and pR) and susceptible (pS) strains given 20-h exposures of sublethal doses of deltamethrin (3 replications, 50 beetles/rep.)

Strain	Mean % mortality (95% CL)		
	0.0 mg/64 cm ² No PBO	0.05 mg/64 cm ² No PBO	0.05 mg/64 cm ² With PBO
QTC	0	0	0
pR	0	9 (3-15)	96 (85-100)
pS	0	98 (83-99)	100

Confidence limits are derived from the standard deviation of arcsine transformed percent mortality. Confidence limits are not shown if the values of all three replicates were identical.

al. 1986), reside 20–30 map units from *pearl* (Beeman et al. 1996). The relative positions of *mwp*^{Dch-1}, *ab* and *p* suggest that *mwp*^{Dch-1}, and *ab* are in close proximity to *PyR-1*. However, the discrimination between susceptible and resistant types in our bioassay was imperfect. Thus, linkage estimates between *p* and *PyR-1* ranged from 7 to 33 cM among the various experiments in this study. Our best approximation is a genetic distance of ~20 cM, but this may be an overestimate. A more accurate map position of *PyR-1* awaits experiments with closer markers and improved discrimination.

Three lines of evidence were consistent with multifactorial resistance in QTC279. First, the dose-response curves associated with the progeny of QTC279 × Lab-S matings were shallow, and the BC₁ dose-response curves lacked the plateaus that are often associated with a single resistance gene. Second, pR beetles were significantly more susceptible to deltamethrin than QTC279 and showed greater response to the cytochrome P450 inhibitor PBO. If only a single resistance locus was involved, after 6 generations of inbreeding and selection for resistance to deltamethrin, the probability resistance becoming fixed in the pR beetles was ~99% (Falconer 1989). Obviously, the pR beetles were either continuing to segregate for resistance at multiple loci, or more likely, resistance alleles associated with additional loci in QTC279 were never introduced into the pR strain. Apparently *PyR-1*, or other resistance factors carried by both QTC279 and pR beetles, elevate total cytochrome P450 protein to comparable levels in both QTC279 and pR beetles. QTC279 probably has even greater pyrethroid resistance and greater tolerance to PBO because of additional genes that contribute to alternative resistance mechanisms.

The 3rd line of evidence for multifactorial resistance in QTC279 was observed during the initial genetic mapping experiments. These experiments indicated that other markers, in addition to *p*, might be linked to resistance. Although we were unable to demonstrate linkage between these markers and resistance, these experiments failed to eliminate that possibility. Most likely, the genetic distance between the *PyR-1* and *p*, and the penetrance and expression of the resistance phenotype associated with *PyR-1* favored the identification of this gene over others. The possi-

bility that another LG9 linked resistance factor exists may also have helped identify *PyR-1* over other loci. Other resistance genes may also depend on epistatic effects for complete expression, making it even more difficult to identify and map those genes.

Cytochrome P450 involvement in QTC279 pyrethroid resistance was first suggested by Collins (1990). He found that PBO increased deltamethrin toxicity almost 20-fold and reduced the resistance ratio 4-fold. Because resistance was incompletely reversed by PBO, multiple resistance mechanisms were proposed (Collins 1990, Reidy et al. 1990). We found that when *PyR-1* was isolated from other resistance factors in the QTC-279-derived pR beetles, PBO lowered deltamethrin tolerance to a level comparable to that observed in susceptible beetles. This strongly suggested that *PyR-1* is associated with cytochrome P450 based insecticide metabolism. Therefore, *PyR-1* is likely either a P450 gene itself or a locus that regulates P450 gene expression. Evidence that P450 based resistance is conditioned by regulatory genes has been observed in pyrethroid resistant houseflies, *Musca domestica* (Feyereisen et al. 1995, Liu et al. 1995).

Together with the cyclodiene resistance gene, *LinR* (LG3), and malathion specific resistance, *Rmal* (LG6), *PyR-1* makes the 3rd major locus conditioning insecticide resistance to be mapped in the *T. castaneum* genome. As was the case with *LinR* (Lin et al. 1993), we hope to use this information to identify the gene associated with resistance at the molecular level. In this context, it is exciting to note that recent experiments have suggested linkage between *pearl* and cytochrome P450 genes in *T. castaneum* (J.J.S., unpublished data).

The use of model organisms to investigate the mechanisms of insecticide resistance has been proposed and pursued for about a decade (Wilson and Fabian 1986, Beeman et al. 1992, French-Constant et al. 1993). When no previous resistance to an insecticide is available, the most amenable organism for analysis is the fruit fly, *Drosophila melanogaster*. Because of its facility for mutagenesis and a well mapped genome, such studies have proven to reveal the target site of certain insecticides (Wilson and Fabian 1987, French-Constant et al. 1991). These same types of studies, however, seem less likely to uncover the complexities of metabolic resistance mechanisms. To fully understand their evolution and mechanics, as well as the interactions between mechanisms, field-derived resistance in an insect amenable to genetic analysis is required. Thus, QTC279 is an extremely valuable resource as it apparently provides an opportunity to investigate the mechanisms associated with cytochrome P450 based resistance with a precision that has heretofore been available only in the housefly.

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