

Linkage analysis of genes affecting scale color, eye color, and resistance to malathion in the Indianmeal moth

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ABSTRACT: Golden (*g*), a spontaneous mutation affecting scale color in the Indianmeal moth, *Plodia interpunctella* (Hübner), is described. The golden gene is a simple Mendelian autosomal recessive. In affected moths the normal coppery red color of scales covering most of the body and forewings is completely absent; instead, these scales are gold or ochre yellow. Golden showed complete penetrance and no linkage to any of the previously described autosomal recessive mutants, white-eye, chestnut-eye, or copper. Golden was incompletely hypostatic to the sex-linked recessive gene, melanic, and was noninteractive with copper. The autosomal dominant gene *R* (malathion resistance) is not linked to any of the above-mentioned markers.

MUTANT MARKERS in insects are useful in studies of chromosome mapping; chromosomal inversions, translocations and breakages; chromosomal evolution and speciation; sperm precedence; and in studies of competition, dispersal and other aspects of population dynamics. The Indianmeal moth, *Plodia interpunctella* (Hübner), is an easily reared and economically important species for which approximately eight

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visible mutant markers have been described²⁻⁷. In this communication I present linkage data for two new mutant markers: an autosomal recessive affecting scale color and an autosomal dominant conferring resistance to the insecticide malathion.

Materials and Methods

Stocks of the marker strains white-eye (*w*), chestnut-eye (*ch*), melanic (*m*), and copper (*cu*) were obtained from the Stored Product Insects Research and Development Laboratory, Agricultural Research Service, USDA, Savannah, GA. Methods of rearing and genetic analysis were essentially similar to those described by Brower^{2,3} except that all stages were held in dark incubators, and no attempt was made to regulate photoperiod.

Golden moths were found to be numerous in the malathion-resistant strain from Illinois (strain R) described by Beeman and Schmidt¹. These were subcultured and a true-breeding strain *g* was established. Since the original discovery, golden moths also have been observed in malathion-resistant and susceptible strains collected in Kansas, Georgia, and South Dakota. In wild-type moths the distal two-thirds of the forewing appears coppery red. Close inspection reveals that reddish scales cover much of the head, thorax, and forewings. Golden mutant moths are devoid of reddish coloration, the affected scales instead being gold or ochre yellow in color. Normally black scales are unaffected in mutant moths. The mutation may be identical with or allelic to the gelbfärbung mutation described by Schwartz⁶.

Results and Discussion

Reciprocal crosses between homozygous golden and wild-type moths produced only wild-type offspring. Heterozygous F₁ progeny were self-crossed or backcrossed to the recessive parental type. All observed phenotypic ratios were consistent with monohybrid Mendelian inheritance of an autosomal recessive allele, based on chi-square analysis of the data (Table I). The mutation appeared to have uniform expression and complete penetrance, and there was no significant loss of fecundity, viability, or overall

vigor. All sex ratios were near unity, and no spontaneous reversions to wild type were detected.

Linkage of golden to white-eye, chestnut-eye, and copper was tested using standard dihybrid crosses. Golden was apparently not linked with any of these, since the chi-square test showed no significant deviation from the expected ratio of 9:3:3:1 in any case (Table II).

The golden mutation affects only scales or portions of scales that are normally reddish, whereas the copper mutation affects only scales or portions of scales that are normally black. The two genes were noninteractive in their effects on scale pigmentation. Moths doubly homozygous for these two alleles expressed both traits and were distinguishable from either singly homozygous progenitor, having an orange cast on gross examination (color class *or* in Table II). Evidence that *or* moths were doubly homozygous for golden and copper was obtained by backcrosses to either of the singly homozygous stocks. Crosses between *or* and golden produced only golden progeny, whereas crosses between *or* and copper produced only copper progeny.

Melanic is a sex-linked recessive mutation described by Brower² that confers a uniformly black pigmentation to most of the forewing. Crosses between melanic and golden were set up to determine any gene interactions. Melanic females were mass-crossed to golden males. The F₁ progeny were self-crossed (three single pairs). Of a total of 410 F₂ females, 209 were melanic, suggesting that melanic is epistatic to golden (expected no. = 205). However, closer examination of the F₂ melanic females revealed that they could be easily classified as phenotypically mutant or wild type with respect to the golden allele with the aid of a binocular microscope. This was possible because not all of the normally red forewing scales are affected by the melanic mutation. Of the 209 melanic F₂ females, 53 were golden (expected no. = 52.2). This diagnosis was verified by backcrossing the golden, melanic females to males from the singly homozygous golden stock. All progeny from such crosses were golden. Thus, melanic is partially epistatic to golden. The epistasis appears to be complete for scales affected by both genes, but some scales are affected only by the golden mutation.

Table I. Segregation of phenotypes in crosses between golden and wild-type Indianmeal moths

Genotypes of parents†		Mean no. progeny	N	Phenotypes of progeny				χ ²
♂	♀			total no. observed		total no. expected		
				golden	wild type	golden	wild type	
Golden	golden	214	3	643	0	643	0	
Golden	wild type	194	11	0	2133	0	2133	
Wild type	golden	203	10	0	2034	0	2034	
F ₁ (<i>g</i> /+)	F ₁ (<i>g</i> /+)	264	7	459	1390	462.2	1386.8	0.029*
F ₁ (+/g)	F ₁ (+/g)	290	6	429	1312	435.2	1305.8	0.117*
Golden	F ₁ (+/g)	297	6	896	887	891.5	891.5	0.046*
F ₁ (+/g)	golden	280	9	1294	1225	1259.5	1259.5	1.89*

† Sex ratios were determined for each color class within each set of crosses. All sex ratios were near unity (range = 0.93-1.08)

* *P* > 0.05

Analyses of linkage between the autosomal dominant gene *R* (malathion resistance) and the autosomal recessive genes white-eye (*w*), chestnut-eye (*ch*), golden (*g*), and copper (*cu*) were performed as follows:

***R-w* linkage.** White-eyed, malathion-sensitive females were mass-crossed with males homozygous for malathion resistance and normal (dark brown) eye color. *F*₁ females were mass-backcrossed to white-eyed, sensitive males. Fifth-instar progeny of this backcross were separated into white-eye and normal-eye phenotypes based on the pleiotropic effects of the *w* mutation on larval pigmentation³. One-hundred-thirty white-eyed and 140 normal-eyed fifth instar progeny were individually typed as malathion resistant or sensitive by selection with a discriminating dose of malathion¹. The observed frequencies of the malathion-susceptible phenotype were 0.431 and 0.521 for white-eye, and normal eye phenotypic classes, respectively. Neither value is significantly different from 0.5, the frequency predicted by independent assortment of *R* and *w* ($P > 0.05$, two-tailed *t* test).

***R-ch* linkage.** Chestnut-eyed, malathion-sensitive males were mass-crossed with females homozygous for malathion resistance and normal eye color. *F*₁ females were mass-backcrossed to chestnut-eyed, susceptible males. Approximately 200 fifth-instar progeny of this backcross were selected with malathion. The 99 survivors (resistant) were eye-color typed as adults. The observed frequency of the chestnut-eye phenotype was 0.535. This is not significantly different from the value of 0.5 predicted by independent assortment of *ch* and *R* ($P > 0.05$, two-tailed *t* test).

***R-cu* linkage.** The experimental protocol for analysis of *R-cu* linkage was the same as that just described for *R-ch* linkage. Copper, malathion sensitive males were mass-crossed with females homozygous for malathion resistance and normal scale color. *F*₁ progeny were mass-backcrossed (both reciprocal crosses) to the copper, sensitive strain. Approximately 1000 backcross progeny were selected with malathion and color-typed as described previously. The observed frequency of the copper phenotype among the 486 survivors

was 0.506 ($P > 0.05$ for non-linkage, two-tailed *t* test).

***R-g* linkage.** A more complicated protocol had to be used to test for *R-g* linkage, since the homozygous golden strain also was homozygous for *R*. Females of this strain were mass-crossed with wild-type males. *F*₁ adults were self-crossed. Virgin *F*₂ adults were collected as they emerged and 120 males of golden phenotype were mass-backcrossed to wild-type females. Five-hundred-and-five fifth-instar progeny of this backcross were phenotyped as malathion resistant or susceptible. The observed frequency of the susceptible phenotype was 0.570. The predicted value is $f = 0.5$ for independent assortment and $f = 1.0$ for tight linkage of *g* and *R*. If the data are subjected to the two-tailed *t* test, the observed ratio of 0.570 is found to be significantly different from 0.5 ($P = 0.002$), tending to suggest linkage, albeit loose. However, this simple analysis does not account for the probable error in the actual ratio of *R* genotypes in the sample of golden *F*₂ segregants used for the final backcross. Analysis of this source of error (see following paragraph) reveals that the observed frequency of the susceptible phenotype in the backcross ($f = 0.570$) is consistent with nonlinkage of *g* and *R*.

The predicted ratio of genotypes for the *F*₂ in the above protocol is 1:2:1 for both *R/R/R/+*:*+/+* and *g/g/g/+*:*+/+*. If *g* and *R* assort independently, then each genotypic class for wing color (in particular *g/g*) should consist of the same 1:2:1 ratio of the three *R* genotypes. When golden *F*₂ are mass backcrossed to the susceptible strain, the overall genotypic ratio in the offspring is predicted to be 1:1 for *R/+*:*+/+* (i.e., the predicted frequency of the susceptible phenotype is 0.5) assuming independent assortment of *g* and *R*. However, if the actual ratio of *R* genotypes in the sample of *F*₂ used for backcross deviates slightly from 1:2:1, then the predicted frequency of the susceptible phenotype for their offspring will deviate from 0.5. In such a case, $f = 0.5$ would not be a valid assumption for the two-tailed *t* test referred to above.

The actual number of *F*₂ used for the backcross was 120. Considerable chance deviation from the true ratio of 1:2:1 can be expected

in a sample of this size. As an arbitrary example, there is a better than even chance ($P > 0.61$) that the observed ratio in a random sample of 120 will deviate by as much as 0.9:2:1.1. In this example the frequency of the susceptible phenotype in the subsequent backcross is predicted to be 0.533 rather than 0.5. If the assumption $P = 0.533$ is made, rather than $P = 0.5$, then the observed frequency of susceptible insects in the backcross ($f = 0.570$, $N = 505$) agrees with prediction at the 5 percent level of significance (two-tailed *t* test). Thus, the significant deviation of the observed frequency from 0.5 can be attributed to random error in sampling the *F*₂ for backcross.

The data are therefore consistent with non-linkage of *g* and *R*.

Summary

Golden is a useful recessive marker that can be combined with other markers, including other scale-color mutants, without significant interaction that could mask the true genotype. Evidence indicates that golden and malathion resistance assort independently of each other and of three other autosomal markers.

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Table II. Segregation of phenotypes in crosses between golden (*g*) and copper (*cu*), white-eye (*w*), and chestnut eye (*ch*) mutants of the Indianmeal moth

Genotypes of parents†		Mean no. progeny	N	Phenotypes of progeny								χ ²
♂	♀			total no. observed				total no. expected				
<i>g+/g+</i>	<i>+cu/+cu</i>	175	7	<i>g</i>	<i>cu</i>	<i>or</i>	<i>+</i>	<i>g</i>	<i>cu</i>	<i>or</i>	<i>+</i>	
<i>+cu/+cu</i>	<i>g+/g+</i>	291	3	0	0	0	1224	0	0	0	1224	
<i>g+/+cu</i>	<i>g+/+cu</i>	211	8	0	0	0	872	0	0	0	872	
				310	303	102	975	316.9	316.9	105.6	950.6	1.51*
<i>g+/+ch</i>	<i>g+/+ch</i>	264	7	<i>g</i>	<i>ch</i>	<i>g,ch</i>	<i>+</i>	<i>g</i>	<i>ch</i>	<i>g,ch</i>	<i>+</i>	
				346	316	113	1074	346.7	346.7	115.6	1040.1	3.94*
<i>g+/+w</i>	<i>g+/+w</i>	262	3	<i>g</i>	<i>w</i>	<i>g,w</i>	<i>+</i>	<i>g</i>	<i>w</i>	<i>g,w</i>	<i>+</i>	
				140	155	55	437	147.6	147.6	49.2	442.7	1.52*

† Overall sex ratio for each cross was near unity (range = 0.93-1.11)

* $P > 0.05$ for independent assortment