

Genetic Studies of a Behavioral Mutant, Glucose Aversion, in the German Cockroach (Dictyoptera: Blattellidae)

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Glucose aversion (Glu) is a naturally occurring behavioral mutant of the German cockroach, Blattella germanica. Earlier work suggested that Glu is a semi-dominant autosomal trait. Further study was undertaken to place the mutant gene in the linkage map of the species and expand the information on its genetic basis. Linkage was investigated in test crosses with morphologic markers for 7 of the 11 autosomal linkage groups. Assays for amounts of glucose ingested per individual confirmed the expected low consumption of Glu heterozygotes (Glu/+) and that continuous selection pressure on the parental strain had resulted in near- and probably complete homozygosity for Glu. Glu genotypes were identified by means of a discriminating ingestion that separated heterozygotes from wild type (Glu+). Variations in expression indicate that genetic factors influence food consumption in B. germanica. Positive results were obtained in linkage tests of Glu with ruby eye (ru), indicating that the Glu locus lies in linkage group VIII (chromosome 9), a group notable for mutants that apparently result in the expression of latent, highly primitive development pathways.

KEY WORDS: cockroach: *Blattella germanica*; behavior; glucose aversion; linkage.

INTRODUCTION

Behaviors are generally assumed to have a genetic basis, but those controlled by single genes and hence amenable to demonstrating genetic control of behavior are rare (Mathews and Mathews, 1978). One apparent exception, glucose aversion (*Glu*), has been found in the German cockroach, *Blattella germanica* (Sil-

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verman and Bieman, 1993). It is the first occurrence of aversion to a sugar that generally stimulates feeding in insects. Failure of an initially effective toxic bait to control two localized populations of the German cockroach, *Blattella germanica* (L.), led to the discovery that cockroaches were avoiding a component of the bait base, D-glucose, rather than the toxicant itself (Silverman and Bieman, 1993). Selection pressure exerted by use of the toxic bait had enhanced the ability of the populations to avoid lethal effects of the bait. The occurrence fits Lockwood and co-workers' (1984) definition of behavioral resistance, except that it was the combination of the active ingredient with D-glucose that led to the development of resistance. Haynes (1988) used a somewhat broader definition in that he referred to reduced contact with a toxic material, which, of course, could include toxic baits as well as conventional insecticides.

Glucose aversion is of particular interest to behaviorists because it occurs naturally, unlike many mutants studied in *Drosophila*. Selection experiments indicated that *Glu* was present in several field-collected strains from diverse geographic localities (Silverman and Ross, 1994).

Glu is apparently controlled by a single semidominant autosomal gene (Silverman and Bieman, 1993). Not only is a behavioral trait with a comparatively simple inheritance pattern unusual, but its occurrence in a cockroach may ultimately provide a tool for further unraveling of the complex taxonomic problems in the classification of the Blattaria. Therefore, we have expanded research on *Glu* to include additional data on inheritance and to study its linkage relationship to other *B. germanica* mutants.

MATERIALS AND METHODS

Insects

Glucose-avoiding cockroaches were drawn from the T-164 strain collected in 1989 from Gainesville, FL. It is maintained at the Clorox Technical Center, where it has been kept under constant selection pressure for several years by rearing in the presence of a toxic bait and dog chow (Purina). The active ingredient of the bait was hydramethylnon. Glucose was present in the bait base. We assume that the strain is homozygous for glucose aversion (*Glu/Glu*); bioassay indicates that continuous selection pressure eliminated most, and probably all, *Glu* heterozygotes, producing a pure *Glu/Glu* population (Silverman, unpublished). Other strains were mutant markers maintained at the Genetic Stock Center for the German Cockroach at Virginia Polytechnic Institute & State University. Markers and linkage groups (LG) were as follows: balloon wing (*ba*), LG II; rose eye (*ro*), LG III; orange body (*or*), LG IV; black body (*Bl*), LG VI; ruby eye (*ru*), LG VIII; hooded pronotum (*hd*), LG IX; and yellow-body (*y*), LG X.

Crossing Procedures

Parental crosses were reciprocal mass matings between presumptive *Glu/Glu* and mutant homozygotes for the markers listed above. Test crosses used single-pair matings. In two instances, double-mutant stocks were used to test for two linkage groups simultaneously. They were *ro/ro,ba/ba* and *y/y,hd/hd*. Because *Glu* is semidominant, non-glucose avoiding insects (+) are readily separated from heterozygotes for glucose aversion (*Glu*/+) (Silverman and Bieman, 1993). This made it possible to use backcrosses to test for linkage with recessive markers. F₁ hybrids from *Glu/Glu* × *Bl/Bl* were crossed to wild type because *Bl*, like *Glu*, is an autosomal, semidominant mutant.

The order of crosses depended on cockroach availability. When linkage was found, no additional crosses were made to markers for the remaining linkage groups, i.e., groups V, VII, XI, and XII.

Assays of Glucose Ingestion

Individual *B. germanica* adult males from 12 or more replicates of each test cross were assayed for glucose intake using the procedure described by Silverman and Bieman (1993). Backcross male progeny from single-pair matings were deprived of food and water for 48 h, then offered 0.5 ml of a 2 M glucose solution prepared with 8 mM amaranth. This concentration was selected because it provided the greatest F₁ versus parent discrimination and the least variation. The dye solution was left in place for 5 min, after which water was also provided for an additional 15 min. Individual insects were extracted twice: first in ethanol:water (1:1), then in acetone. The supernatant was measured at A_{520nm} and dye (glucose) intake was determined by comparing the absorbance reading from each insect against a calibration curve, established by feeding known volumes of 8 mM amaranth to individual cockroaches. In addition to assays of individuals from test crosses, males drawn randomly from mutant stocks and F₁ hybrid males from crosses of *Glu/Glu* to mutant markers were tested to confirm the expected higher glucose consumption by the mutants than the heterozygotes.

Analysis of Linkage

A specific amount of ingested glucose was used to discriminate between heterozygotes for glucose aversion (*Glu*/+) and wild type in the test crosses. The choice was based on a combination of data that separated insects drawn from the mutant stocks and F₁ heterozygotes with data that separated test-cross progeny into two groups, presumably *Glu* heterozygotes (*Glu*/+) and nonavoiders (+/+). The segregants were tested with a χ^2 goodness-of-fit test for independent segregation (1:1:1:1 ratio). When backcrosses were to double-mutant stocks, segregation was analyzed separately for each mutant. Data from recip-

rocal crosses were pooled because there were no significant differences between them.

When linkage was found, the standard error of the recombination fraction (p) was estimated by the standard formula: $s_p = \text{the square root of } p \times (1 - p) \text{ divided by } n$ (Mather, 1957).

RESULTS

In the wild-type and mutant strains used here, the sex ratio is 1:1. In single-pair matings, at least 80% of the nymphs usually survive into the adult stage (approximately 35–38 adults). Based on these figures, it appears that survivorship was excellent in all crosses, except that involving y . The average number of adults from each test cross, estimated as $2 \times$ the number of adult males, ranged from 37 in the test cross with Bl to 40 in the ru crosses. The only exception was the estimate of 34 (average 17 males per cross) in the backcross to y . The slightly lower figure was not unexpected because minor deleterious effects occur in y mutants (Ross and Cochran, 1966).

Small variations occurred in the expression of Glu in F_1 hybrids and in test crosses. In F_1 hybrids from crosses to or , aversion was greater than in the F_1 from crosses to any other mutant, for example, Bl (Figs. 1A and B, respectively). However, in both crosses, an ingestion of $\leq 3.5 \mu\text{l}$ per individual gave a clear separation of genotypes. Distribution was bimodal in all test crosses, as expected. In test crosses with or (Fig. 2A), as well as those with Bl , ba , and ro , consumption of $\leq 3.5 \mu\text{l}$ again gave a good separation of genotypes. In F_1 hybrids from $Glu/Glu \times y/y,hd/hd$, ingestion was $> 4.5 \mu\text{l}$, but consumption by three individuals was $> 3.5 \mu\text{l}$ ($n = 21$). Therefore, ingestion of $\leq 4.5 \mu\text{l}$ was used as a discriminating ingestion in the test crosses. This gave a reasonably clear separation of genotypes, for example, in the test cross with y (Fig. 2B).

The results of linkage tests are shown in Table I. Glu was independent of markers that identify linkage groups II, III, IV, VI, IX, and X. The data from backcrosses to the group II marker, ba , were a rather poor fit to a 1:1:1:1 ratio, but they were not suggestive of linkage.

In 8 of 12 backcrosses to ru/ru , a discriminating ingestion of $\leq 4.5 \mu\text{l}$ of glucose per individual gave the best separation of genotypes. Each individual cross fit the expected 1:1 ratio of $Glu/+$ and $Glu+/Glu+$. The data were pooled, as follows: 93 $Glu/+$ and 88 $Glu+/Glu+$ ($\chi^2 = 0.138$, $P > 0.70$). In four crosses, the data did not fit a 1:1 ratio when ingestion of $\leq 4.5 \mu\text{l}$ was used to separate genotypes for glucose aversion (14, $\leq 4.5 \mu\text{l}$; 73, $> 4.5 \mu\text{l}$).

Segregation in the eight crosses where glucose ingestion of $\leq 4.5 \mu\text{l}$ per individual was used to discriminate genotypes indicated linkage (17 +, +; 81 Glu , +; 76 +, ru ; 7 Glu , ru) (Fig. 3A). Recombination was estimated as 13.3

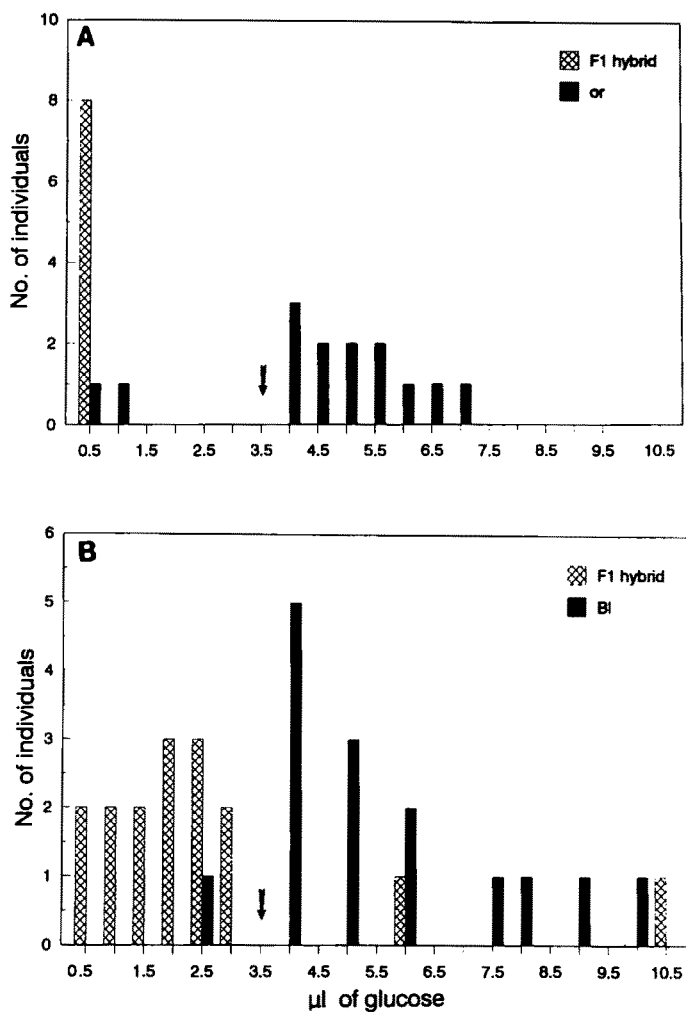


Fig. 1. Ingestion of 2 M glucose by mutant markers and F₁ hybrids: (A) orange-body (*or*) and F₁ hybrids from *Glu* × *or*; (B) black-body (*Bl*) and F₁ hybrids from *Glu* × *Bl*. Arrows mark a discriminating ingestion that separates genotypes for *Glu*.

± 2.52%. In the four crosses characterized by high glucose consumption, ingestion of glucose by phenotypically wild-type individuals (*ru*/+) was less than that by *ru* homozygotes (*ru*/*ru*), as expected from linkage (Fig. 3B). In these crosses, an ingestion of ≤ 9.5 µl of glucose apparently gave a reasonably good

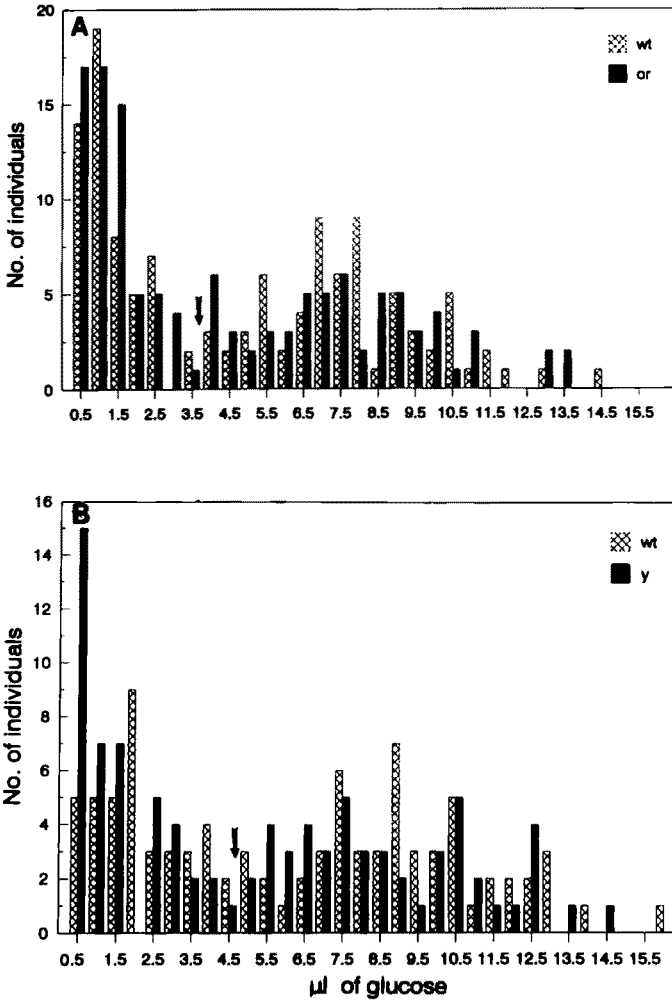


Fig. 2. Examples of 2 M glucose ingestion in test crosses: (A) ingestion in backcrosses of F_1 hybrids to orange-body (*or*); (B) ingestion in backcrosses of F_1 hybrids to yellow-body (*y*). Arrows indicate slightly different discriminating ingestions that were used to distinguish genotypes, $\leq 3.5 \mu\text{l}$ in A and $\leq 4.5 \mu\text{l}$ in B (see text).

separation of genotypes and was used arbitrarily for this purpose. The data fit the expected 1:1 ratio between presumptive *Glu* + and *Glu*+/+ (30 and 36 individuals, respectively). On this basis, segregation was 6 +, +; 25 *Glu*, +; 30 +, *ru*; and 5 *Glu*, *ru*. Recombination, estimated as $16.7 \pm 4.49\%$, did not

Table I. Segregation in Test Crosses for Linkage of Glucose Aversion (*Glu*)^a

Marker (group)	No. and phenotype of progeny				χ^2 ^b	P
<i>ba</i> (II)	85 ++	58 <i>Glu</i> , +	61 +, <i>ba</i>	67 <i>Glu</i> , <i>ba</i>	6.468	>0.05
<i>ro</i> (III)	69 ++	70 <i>Glu</i> , +	77 +, <i>ro</i>	55 <i>Glu</i> , <i>ro</i>	3.760	>0.10
<i>or</i> (IV)	60 ++	49 <i>Glu</i> , +	67 +, <i>or</i>	64 <i>Glu</i> , <i>or</i>	3.154	>0.30
<i>Bl</i> (VI)	78 ++	67 <i>Glu</i> , +	78 +, <i>Bl</i>	89 <i>Glu</i> , <i>Bl</i>	3.103	>0.30
<i>ru</i> (VIII)	17 ++	81 <i>Glu</i> , +	76 +, <i>ru</i>	7 <i>Glu</i> , <i>ru</i>	100.040	<0.001 ^c
<i>hd</i> (IX)	53 ++	49 <i>Glu</i> , +	46 +, <i>hd</i>	38 <i>Glu</i> , <i>hd</i>	2.601	>0.30
<i>y</i> (X)	53 ++	40 <i>Glu</i> , +	49 +, <i>y</i>	44 <i>Glu</i> , <i>y</i>	2.086	>0.50

^aIngestion of either ≤ 3.5 or ≤ 4.5 μ l of glucose per individual was used to discriminate genotypes (see text); *Glu* indicates the hybrid expression of the glucose aversion gene.

^bCalculated for fit to a 1:1:1:1 ratio.

^cData from 8 of 12 crosses. Four crosses with aberrantly high glucose consumption are omitted from the analysis (see text).

differ significantly from that estimated from the group of eight crosses (13.3%). In both data sets (Figs. 3A and B), *ru* is associated with cockroaches consuming the higher amounts of glucose, as expected from linkage of the two traits.

DISCUSSION

Although most insect behaviors are likely the result of long evolutionary histories, there is at least one exception. The use of insecticides has led to altered behaviors in *B. germanica* and undoubtedly in other insect pests. Modifications of insecticide-induced behavior in field-collected strains of *B. germanica* were reported by Wooster and Ross (1989), Ross (1992, and 1993a), and Ross and Cochran (1992). They include an altered response to a formulation base, suggesting that modifications may include polymorphisms that are independent of mechanisms of physiological/biochemical resistance. It is not known whether these behavioral differences affect cockroach control. However, Mahmood *et al.* (1993) indicated that behavioral avoidance was one of three mechanisms that contributed to failure of a synthetic pyrethroid to control a localized population of *B. germanica*. In contrast, selection for glucose aversion was the primary, and apparently sole, cause of a control failure associated with a toxic bait (Silverman and Bieman, 1993).

The bimodal distribution of glucose consumption data present in all test crosses confirmed the earlier report that *Glu* is a semidominant trait (Silverman and Bieman, 1993). Minor variation in ingestions used to discriminate genotypes in different crossing systems suggests the existence of genetic modifiers that affect *Glu* expression, rather than an influence of the environment. The lowest

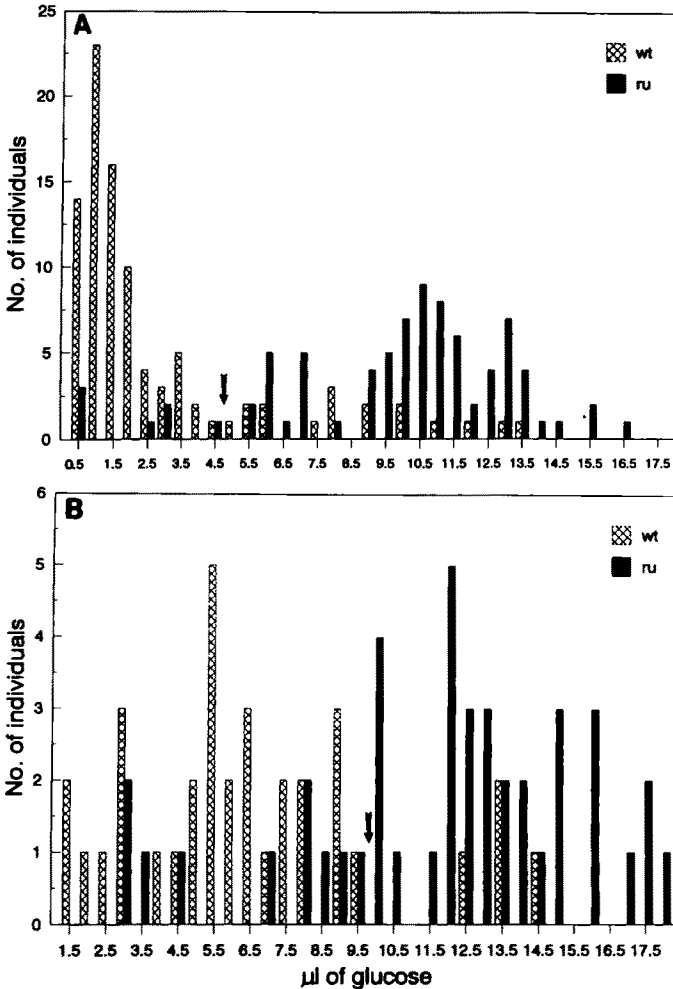


Fig. 3. Ingestion of 2 M glucose in backcrosses to ruby-eye (*ru*): (A) data from eight crosses with typical 2 M glucose ingestion of presumptive *Glu* hybrids and *Glu*+; (B) data from four crosses with unusually high glucose consumption. Arrows mark discriminating ingestions used to separate genotypes for *Glu* (see text).

ingestion of nonavoiders ($< 1.5 \mu\text{l}$ of glucose per individual) was in the orange-body mutants, and consequently, the results of crosses involving *or* were closer to those with the Orlando susceptible strain and F_1 heterozygotes reported by Silverman and Bieman (1993) than they were to crosses involving other mutant

markers. This is reasonably strong evidence that low glucose consumption had a genetic basis because *or* was originally isolated from the Orlando strain.

The most aberrant crosses were the four *ru* backcrosses, which were characterized by an exceptionally high glucose consumption of individuals assumed to be *Glu* heterozygotes and wild type. Cockroaches from these crosses were assayed at the same time as those from five crosses typical of the other *ru* backcrosses. Therefore, an influence of environmental factors was unlikely. A suppressor of *Glu* was also unlikely because the distribution pattern was bimodal. Instead, a gene(s) that originated in one of the *ru* stocks used in the crosses probably caused an overall unusually high consumption in the aberrant crosses. Evidence suggesting a genetic component in control of food consumption of *B. germanica* was found elsewhere. Up to twofold differences in food consumption during a specific time period were found in studies of field-collected strains (Silverman and Ross, 1994). Other evidence was found in choice experiments with a toxic bait in which the bait base differed from that containing glucose (Ross, 1993b). Cockroaches adapted to the bait by eating less food during the experimental period, both the toxic bait and a nontoxic food source.

Glu is assigned to linkage group VIII on the basis of linkage with *ru*. Group VIII lies on chromosome 9 (Ross and Cochran, 1971). It contains a group of closely linked mutants, of which several are probably the most primitive type of homeotic known in any insect (Ross and Keil, 1978; Ross and Tanaka, 1988). Whether the assignment of *Glu* to this group has evolutionary significance is unknown. The linkage distance, estimated as approximately 13 map units from *ru*, is greater than that which includes the nine other loci that make up the linkage group (all within approximately 5 map units). Certainly the presence of *Glu* in strains from diverse geographic localities suggests that it may have arisen in its original geographic locality, presumably southeast Asia (Roth, 1985). Perhaps glucose aversion is a behavioral trait that has been conserved but no longer has adaptive value, but to test this hypothesis information is needed on whether the trait occurs in related species. At present, genetic information on other cockroaches is nearly nonexistent, and indeed *B. germanica* is the only hemimetabolous insect for which a linkage map has been published (Heckel, 1993).

Glu heterozygotes and, of course, *Glu/Glu* homozygotes (Silverman and Bieman, 1993) are able to discriminate between glucose and nonglucose food sources, but the mechanism underlying this behavior is unknown. However, cockroaches in the T-164 strain were observed retreating following contact of the external mouthparts with glucose solutions. We suspect that the gene affects taste detection by possibly altering a deterrent receptor(s) to bind D-glucose or coding for aversive rather than phagostimulatory behavior within a chemosensory pathway.

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