Genetic Basis for Resistance to Gel Baits, Fipronil, and Sugar-Based Attractants in German Cockroaches (Dictyoptera: Blattellidae)

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ABSTRACT A gel bait-resistant *Blattella germanica* (L.) strain (Cincy) was collected in Cincinnati, OH, in 2003. This strain exhibited strong behavioral resistance to Avert (0.05% abamectin), Maxforce FC (0.01% fipronil), and Pre-Empt (2.15% imidacloprid) gel baits. Reciprocal mass crosses and back crosses between the Cincy strain and a susceptible strain (Jwax) were made and tested for their inheritance of resistance to Avert, Maxforce FC, and Pre-Empt gel baits. Topical assays comparing the parental and reciprocal-heterozygous strains indicated the resistance to fipronil was incompletely recessive. LD_{50} and LD_{90} values of the Jwax♂ × Cincy♀ strain were not significantly different from the Jwax♀ × Cincy♂ strain, suggesting no sex linkage in physiological fipronil resistance. Feeding assays revealed that F_1 reciprocal crosses were significantly less responsive to blank Avert and Maxforce FC baits (without active ingredients) than the susceptible strain. The Jwax♀ × Cincy♂ strain did not display significantly greater consumption of blank Avert and Maxforce FC baits relative to the Jwax♂ × Cincy♀ strain. In feeding assays with agar containing D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, and D-sucrose, the crosses showed an intermediate feeding response to glucose compared with the Cincy and Jwax strains, and a similar response to other sugars compared with the Jwax strain. The Jwax♂ × Cincy♀ strain was significantly less responsive to glucose than the Jwax♀ × Cincy♂ strain. Mortality induced by Avert, Maxforce FC, and Pre-Empt gel baits against the F_6 Jwax♂ × Cincy♀ strain was 44.2 ± 6.8, 92.9 ± 2.1, and 78.7 ± 5.2%, respectively, indicating the resistance to Avert and Pre-Empt gel baits inherited by Cincy females was extremely stable. The F_6 Jwax♂ × Cincy♀ strain was significantly more resistant to Avert, Maxforce FC, and Pre-Empt than the F_6 Jwax♀ × Cincy♂ strain. These findings suggest that behavioral resistance to gel baits has weak sex-linkage, with a greater degree of the resistance trait being inherited by female cockroaches. Alternatively, physiological resistance to fipronil has no sex-linkage, but it is nonetheless important to the complete resistance phenotype.

KEY WORDS *Blattella germanica*, insecticide resistance, gel bait, food aversion, inheritance

INSECTICIDE RESISTANCE IN THE German cockroach, *Blattella germanica* (L.), has been widely reported (Cochran 1995, Espinosa-Islas et al. 2002, Pai et al. 2005). Resistance typically evolves to detectable levels as a result of extended periods of application of certain insecticides (Scharf et al. 1998b). Resistance mechanisms included increased esterase and cytochrome P450-dependent monooxygenase activity, decreased sodium channel sensitivity, and decreased cuticular penetration (Dong et al. 1998; Scharf et al. 1998a, b, 1999; Wu et al. 1998; Valles and Strong 2001; Tan et al. 2002). Behavioral resistance to bait products was first reported in isolated *B. germanica* populations in the early 1990s (Silverman and Bieman 1993). From 1999, resistance to gel baits (i.e., gel bait aversion) was noted by some pest management professionals in scattered *B. germanica* populations in many locations in the United States (Harbison et al. 2003, Morrison et al. 2004, Wang et al. 2004, Liang 2005, Miller and McCoy 2005). The most interesting characteristics of this resistance are that it is mainly caused by behavioral avoidance to food ingredients in the baits, such as sugars (Wang et al. 2004), which are the most common ingredients used in gel baits. Thus, this resistance mechanism apparently affects all gel baits in the market, regardless of the active ingredients contained in the baits.

Historical data have shown that insecticide resistance in *B. germanica* can be either controlled by a single gene or a group of genes (Cochran 1995). No sex-linkage of resistance has been observed in German cockroaches, and resistance has been observed as being both dominant and recessive. Previously, Silverman and Bieman (1993) reported that glucose aversion was controlled by an autosomal and incompletely

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dominant trait. The genetics of the newly emerged bait resistance have not been studied. Understanding the genetic nature of bait resistance may help predict future resistance development and suggest approaches to resistance management. In this article, we report our findings on various aspects relating to the genetics of bait resistance in *B. germanica.*

Materials and Methods

Cockroaches. The gel bait resistant Cincy (C) strain was collected in May 2003 from eight apartments in Cincinnati, OH. They were exposed to gel bait treatments for at least 5 yr before collection. This strain showed strong behavioral resistance to gel baits (Wang et al. 2004). Jwax (J) is a standard susceptible strain that has been maintained in the laboratory for >30 yr. Reciprocal mass crosses between Jwax and Cincy, i.e., J♂ × C♀ and J♀ × C♂, were made within 7 mo after collection of Cincy strain from the field. Backcross progeny were obtained from mass crosses between Jwax males and F_1 females from J♂ × C♀ crosses. For each mass cross, 100–200 females of one strain were mixed with 100–200 males of the other strain. Mass crosses provided enough offspring for all reported bioassays. Generations F_2–F_6 were allowed to randomly inbreed in each successive generation. All of the strains were provided Harlan Teklad rodent diet (Harlan Teklad, Madison, WI), peanut butter, and mixed fruit jelly (J. M. Smucker Co., Orrville, OH) before the experiments. They were reared in 40.5- by 28.0- by 14.5-cm plastic boxes in walk-in environmental chambers at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

Insecticides. Fipronil [technical grade, 96.8% (wt:wt)] and blank Maxforce FC cockroach gel bait were provided by Bayer Environmental Science (Raleigh, NC). Maxforce FC Professional Insect Control Roach Bait (0.01% fipronil), Pre-Empt gel bait (2.15% imidacloprid), and Avert cockroach gel bait (0.05% Abamectin B1) were purchased from a commercial distributor. Blank Avert cockroach gel bait was provided by Whitmire Micro-Gen Laboratories (St. Louis, MO). Test solutions of fipronil were freshly prepared in HPLC grade acetone before topical assays.

Efficacy of Gel Baits. The efficacy of Avert, Maxforce FC, and Pre-Empt cockroach gel baits were evaluated against cockroaches of the Jwax, Cincy, and F_1–F_6 generations of the reciprocal crosses. Avert and Maxforce FC also were evaluated against the backcross between Jwax♂ and J♂ × C♀. For each strain, 10 to 20 1–4-wk-old adult male cockroaches were counted and placed in assay boxes (18.7 by 13.3 by 9.5 cm) 1 d before exposure to bait treatments. The number of cockroaches per experimental box was determined by the availability of cockroaches. The inner upper portion of the boxes was lightly greased with a mixture of petroleum jelly and mineral oil (2:3) to prevent escaping. Each box contained a cotton plugged water vial and a 10- by 10-cm cardboard "tent" as a harborage. Approximately 0.4 g of gel bait was applied in a 0.7-ml centrifuge vial was placed in each cockroach box on the assay start date. Each box also received a piece of rodent diet as alternative food. The control box was provided with rodent diet only. Each treatment was applied to three to five boxes. Cockroach mortality was recorded daily until 10 d. Moribund insects (defined by inability to walk) were considered dead in these and all other experiments. Experiment units were kept in a walk-in environment chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

Topical Assays. Adult males from J♂ × C♀ and J♀ × C♂ crosses, 1–4 wk old, were removed from rearing containers and deprived of food 1 d before insecticide treatment. Their weight was measured on a Mettler AE100 balance (Mettler-Toledo Inc., Columbus, OH) immediately before treatment. Solutions of fipronil in acetone were applied to individual cockroaches by using a Burkard Auto Microapplicator (Burkard Manufacturing Co. Ltd., Hertfordshire, England) equipped with a 1-ml glass syringe. One microliter of insecticide solution was applied onto the first abdominal sternite of each CO_2 anesthetized cockroach. After application, the cockroaches were placed in groups of 10 in 100- by 25-mm plastic petri dishes with a water vial and cardboard harborage. The dish lid had a 2.5-cm-diameter screened opening for ventilation. Each insecticide concentration was applied to 30 cockroaches. The concentrations of fipronil were Jwax, 0.5, 1, 1.5, 2, and 2.5 ppm; Cincy, 1.2, 3.6, 10.8, 32.4, and 97.2 ppm; J♂ × C♀, 1.5, 3.6, 4.5, and 5 ppm; and J♀ × C♂, 1.5, 2.5, 3.6, 4.5, 5, and 6 ppm. At least four concentrations tested caused 1–99% mortality for each strain. After treatment, insects were held in a walk-in environmental chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h. Mortality was scored 72 h after treatment.

Consumption of Blank Gel Baits. Blank Avert and Maxforce FC baits were evaluated against Jwax, Cincy, J♀ × C♂, and J♂ × C♀ strains of *B. germanica* to determine the inheritance of cockroach aversion behavior to inert ingredients in the bait matrices. Fifteen adult males and 15 nongravid adult females were placed in each plastic box along with harborage and a water vial. After 1 d of starvation, a 0.7-ml centrifuge vial containing blank gel bait and a piece of rodent diet were added to each box. The weight of the vials was recorded after 2-d exposure. Three vials containing known amount of bait were placed in an empty box for estimation of the natural weight loss because of evaporation. Data beyond 2 d was not analyzed because low levels of natural mortality occurred in some of the boxes. A vial containing each of the two baits was placed in a box without cockroaches for estimation of water loss. Consumption of bait was calculated by the following formula: $W_0 - W_n / (1 - \text{water}\%)$, where W_0 and W_n are the weight before and after exposure to cockroaches, respectively, and water% is the percentage of water loss in the control vial. Experiment units were kept in a walk-in environmental chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

Table 1. Efficacy of three cockroach gel bait products against five genetic variant strains of *B. germanica*

Cockroach strain	Avert		Maxforce FC		Pre-Empt	
	n	Corrected mortality at 7 d (mean ± SE)	*n*	Corrected mortality at 7 d (mean ± SE)	*n*	Corrected mortality at 7 d (mean ± SE)
Jwax	100	100.0 ± 0.0a	30	100.0 ± 0.0a	30	100.0 ± 0.0a
J♂ × C♀	100	51.0 ± 6.5c	75	80.0 ± 4.7b	75	32.0 ± 3.9b
J♀ × C♂	100	52.0 ± 6.0c	75	85.3 ± 2.5b	75	42.7 ± 3.4b
J♂ × F_1 (J♂ × C♀) ♀	75	73.2 ± 5.1b	75	97.1 ± 2.9a		
Cincy	30	3.3 ± 3.3d	30	30.0 ± 17.3c	30	10.7 ± 6.4c

Mortality was corrected by the formula in Abbott (1925). Means within the same column followed by different letters indicate significant differences between strains ($P < 0.05$, LSD).

Sugar Feeding Assay. The goal of this experiment was to examine the inheritance of sugar avoidance behaviors. Sugar-agar diet of each of the sugars D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, and D-sucrose were made by mixing sugar [15% (wt:wt)] and agar [1% (wt:wt)] in deionized water, bringing it to a boil, and then pouring the sugar water–agar mixture into sterile petri dishes. The diet was stored at 4°C before use. Mixed age populations of Jwax, J♂ × C♀, J♀ × C♂, and Cincy strains were tested. They were maintained in 40.5- by 28.0- by 14.5-cm plastic boxes. Each box contained 200–1,000 individuals. Food was removed 48 h before feeding trials to facilitate a rapid response to the diets. A cube of each diet was placed in a weighing container and weighed. Seven diets (six sugar–agar diets and one agar diet) were placed in each cockroach rearing box. Four to six boxes of each strain were assayed. The diets were weighed after 24 h of exposure. A set of diets was placed in a box without cockroaches for estimation of water loss. Consumption of diets was calculated by the same method used in the blank bait consumption experiment. Consumption indices were calculated as $(Ws - Wa)/T$, where Ws and Wa are consumption of sugar–agar diet and agar diet (without sugar), and T is the total consumption of the seven diets in each box (Silverman and Bieman 1993, Wang et al. 2004). A positive number indicates that the sugar stimulates feeding. A negative number indicates that the sugar deters feeding. Experimental units were kept in a walk-in environmental chamber at 26°C, 60% RH, and a photoperiod of 12:12 (L:D) h.

Data Analysis. Where necessary, bioassay data were corrected for control mortality (Abbott 1925). Mortality (arcsine of the square root transformed), blank bait consumption (original or log transformed), and consumption index data were analyzed by analysis of variance (ANOVA) by using SAS software (PROC GLM, SAS Institute 2001). Topical assay data were analyzed by probit analysis (PROC PROBIT, SAS Institute 2001). The resistance ratio for each strain was estimated by dividing the LD_{50} value for the resistant strain by the LD_{50} value for the susceptible strain. The degree of dominance for LD_{50} was calculated as in Bourget et al. (2000): $D = (X_2 - X_3)/(X_1 - X_3)$, where $X_2 = \log (LD_{50}$ J♀ × C♂) or $\log (LD_{50}$ J♂ × C♀), $X_1 = \log (LD_{50}\ C)$, and $X_3 = \log (LD_{50}\ J)$. The D value ranges from 0 to 1. A value of 0 reflects recessivity, and a value of 1 reflects dominance.

Results

Genetics of Behavioral Resistance to Gel Baits. Feeding upon Avert, Maxforce FC, and Pre-Empt gel baits resulted in 100.0% mortality to the Jwax strain and ≤30.0% mortality to the Cincy strain, indicating that Jwax was susceptible and Cincy was resistant to the three gel baits (Table 1). The susceptibility of the two F_1 crosses to gel baits was intermediate compared with Jwax and Cincy. Both reciprocal crosses showed high levels of resistance to Avert and Pre-Empt baits (≤52% mortality). The F_1 reciprocal cross strains had similar levels of resistance for each of the three baits ($P > 0.05$, least significant difference [LSD]). The backcross, J♂ × F_1 (J♂ × C♀) ♀, showed significantly lower levels of resistance to Avert and Maxforce FC gel baits than the either of the two F_1 J × C reciprocal crosses ($P < 0.05$, LSD).

Genetics of Physiological Resistance to Fipronil. Dose–mortality responses to fipronil and probit analysis results of the parental susceptible, resistant, and reciprocal cross strains are presented in Fig. 1 and Table 2. F_1 offspring from the two reciprocal crosses were significantly more resistant to fipronil than to Jwax. The two crosses overlapped in the ranges of their estimated LD_{50}, LD_{90}, and slopes, indicating that physiological resistance to fipronil is autosomal. The LD_{50} of F_1 progeny from mass crosses yielded D values for J♀ × C♂ and J♂ × C♀ of 0.31 and 0.42, respectively (Table 2). These values indicate that resistance was generally inherited as an incompletely recessive trait.

Genetics of Feeding Behavior. In blank gel bait feeding assays, the mean consumption of blank Avert and Maxforce baits by both F_1 reciprocal cross off-

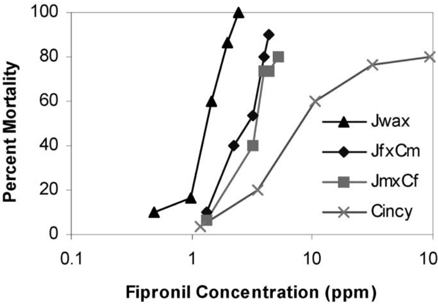

Fig. 1. Dose–mortality plot of fipronil toxicity to *B. germanica* strains as determined by topical assays.

Table 2. Susceptibility of the four strains of *B. germanica* to topically applied fipronil

Cockroach strain	n^a	Model parameters[b]		Lethal dose (μg/g)[c]		RR^d_{50}	RR^e_{90}	Model fit			Degree of dominance
		Intercept ± SE	Slope ± SE	LD_{50} (95% FL)	LD_{90} (95% FL)			χ^2	df	P	
Jwax	180	−1.24 ± 0.35	8.23 ± 1.47	0.030 (0.026–0.033)	0.043 (0.039–0.050)			4.02	3	0.26	
J♀ × C♂	180	−2.63 ± 0.65	5.22 ± 1.08	0.058 (0.047–0.066)	0.102 (0.087–0.138)	1.94	2.39	2.33	3	0.51	0.31
J♂ × C♀	180	−2.85 ± 0.94	4.85 ± 1.38	0.073 (0.054–0.084)	0.135 (0.113–0.231)	2.47	3.17	1.86	3	0.60	0.42
Cincy	180	−1.57 ± 0.40	1.39 ± 0.31	0.26 (0.06–1.00)	2.13 (0.66–446.19)	8.6	49.9	6.70	3	0.08	

[a] Total number of insects used in bioassay, with 30 insects in each concentration.

[b] The intercept and slope parameters are for models in which the independent variable is natural logarithm of concentration (ppm).

[c] Dose (micrograms of insecticide per gram of insect) calculated based on body weights. Average body weights (mean ± SEM) per individual ($n = 30$) were Jwax, 0.048 ± 0.007 g; Cincy, 0.051 ± 0.001 g; J♀ × C♂, 0.049 ± 0.017 g; and J♂ × C♀, 0.050 ± 0.010 g.

[d] Resistance ratio based on LD_{50} values compared with Jwax.

[e] Resistance ratio based on LD_{90} values compared with Jwax.

spring was significantly lower than Jwax (blank avert: $F = 40.4$; df = 5, 15; $P < 0.001$; and blank Maxforce FC: $F = 6.25$; df = 3, 8; $P = 0.02$) (Table 3). The heterozygous offspring consumed significantly more blank Avert bait than Cincy, but less than Jwax, indicating an intermediate (incompletely dominant) response to blank Avert bait. Inbred F_2 heterozygotes also showed significantly lower consumption than Jwax ($P < 0.05$, LSD). There were no detectable differences in consumption between the two F_2 heterozygote strains; however, there was a trend in which the J♂ × C♀ strain tended to have lower consumption of blank baits compared with the J♀ × C♂ strain. Thus, the bait avoidance trait might have weak sex-linkage in the parental Cincy strain.

In sugar diet assays, the parental Cincy strain showed strong aversion to all of the tested sugars (Fig. 2). The Jwax strain responded positively to maltose, sucrose, glucose, and fructose but not to galactose and lactose. The feeding responses of the reciprocal F_1 heterozygotes to fructose, maltose, and sucrose were similar to the parental Jwax strain. The J♂ × C♀ strain had a significantly lower consumption index to sugars than the J♀ × C♂ ($F = 18.4$; df = 15, 64; $P < 0.001$). Thus, the inheritance of response to sugars is partially sex-linked. The J♂ × C♀ strain had an intermediate feeding response to glucose compared with Jwax and Cincy strains ($P < 0.05$, LSD), indicating that the F_1 offspring inherited the glucose aversion trait in an incompletely dominant manner.

Table 3. Consumption of blank gel baits by resistant, susceptible, and heterozygous strains of *B. germanica*

Cockroach strain	2-d Consumption (mg)[a] (mean ± SE)			
	n	Blank Avert	n	Blank Maxforce FC
Jwax	3	289 ± 12a	3	172 ± 21a
F_1 J♂ × C♀	3	56 ± 2b	3	12 ± 12b
F_1 J♀ × C♂	3	80 ± 35b	3	41 ± 12b
F_2 J♂ × C♀	5	72 ± 37b		
F_2 J♀ × C♂	5	119 ± 24b		
Cincy	3	0 ± 0c	3	8 ± 5b

[a] Means were based on consumption per box with 15 adult males and 15 nongravid females. Means within the same column followed by different letters indicate significant differences between strains ($P <$ 0.05, LSD). Mean blank Avert consumptions data was log transformed before ANOVA.

Stability of Resistance. Time–mortality responses to three gel baits, by F_1 and inbred generations F_{2-6} of the reciprocal crosses, are shown in Fig. 3. After six generations, although some reversion was apparent, J♂ × C♀ still showed significant levels of resistance to Avert and Pre-Empt gel baits. The mean corrected mortality of J♂ × C♀ at 7 d in the Avert, Maxforce FC, and Pre-Empt treatments was 44.2 ± 6.8, 92.9 ± 2.1, and 78.7 ± 5.2%, respectively. The F_6 J♂ × C♀ strain was significantly more resistant to Avert, Maxforce FC, and Pre-Empt gel baits than the inbred F_6 generation of the J♀ × C♂ strain (Avert: $F = 24.5$, df = 1, 8; $P = 0.001$; Maxforce FC: $F = 14.5$; df = 1, 8; $P = 0.001$; and Pre-Empt: $F = 11.0$; df = 1, 8; $P = 0.01$). These results suggest that gel bait resistance will very slowly revert toward susceptibility in the absence of selection. In other words, there are apparently very minor or no fitness cost associated with the resistance phenotype.

Discussion

The heritability of behavioral resistance has important implications for managing gel bait resistance in *B. germanica.* In this study, both heterozygous F_1 reciprocal cross strains showed reduced feeding responses to blank Avert and Maxforce FC gel baits (original formulations), indicating the avoidance behavior to food ingredients has a clear genetic basis. These results demonstrate that long periods of exposure to gel baits result in changes in the genetic composition of *B. germanica* populations. With sufficient selection pressure, these changes may lead to control failures in future generations. Rotating baits may not improve the level of control unless substantially different food-based attractants are used, particularly sugar-based attractants.

Additionally, topical assay results show that physiological resistance to fipronil is autosomal, but ranges from incompletely dominant to incompletely recessive, depending on probit mortality level (Fig. 1). The slope of the fipronil dose–response line for the Cincy strain is substantially flatter than the dose–response line of the Jwax strain, supporting that the Cincy strain is qualitatively different (ffrench-Constant and Roush 1990), i.e., the Cincy strain is likely in possession of multiple physiological resistance mechanisms against

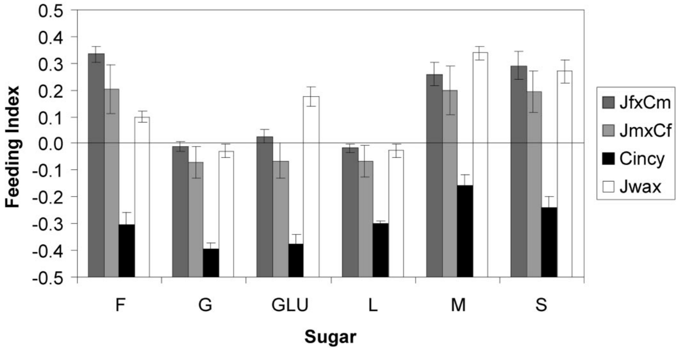

Fig. 2. Differential response of *B. germanica* strains to sugar–agar diets. F, D-fructose; G, D-galactose; GLU, D-glucose; L, D-lactose; M, D-maltose; and S, D-sucrose.

fipronil in more highly tolerant portions of the population. At LD$_{25}$, resistance is incompletely dominant and likely to be caused by enzymatic mechanisms (Parimi et al. 2003). At higher probit mortality levels such as LD$_{75}$ and above, resistance is incompletely recessive (Fig. 1) and likely to be caused by multiple mechanisms. This latter condition is consistent with *Rdl*-like resistance, or target site insensitivity to fipronil at the GABA-gated chloride channel. A similar genetic trend toward incompletely recessive inheritance was reported previously in German cockroaches from Denmark (Kristensen et al. 2005). These cockroaches possess cross-resistance to Dieldrin and fipronil as a result of the *Rdl* mechanism (Hansen et al. 2005).

The gel bait efficacy experiments using reciprocal mass crosses between Jwax and Cincy showed that the overall resistance was not completely autosomal. Female cockroaches were able to inherit slightly more of the resistance trait(s) than male cockroaches. This was supported by the significantly lower mortality of F$_6$ J♂ × C♀ compared with F$_6$ J♀ × C♂. Although there were not significant differences in blank bait consumption between the reciprocal cross offspring, the experiments revealed consistently less consumption in J♂ × C♀ than J♀ × C♂. This indicates that the feeding behavior is, to a degree, sex-linked. In the sugar diet experiments, F$_1$ J♂ × C♀ had significantly lower responses to sugars compared with F$_1$ J♀ × C♂, further confirming that the behavioral resistance to food ingredients in the baits is not entirely autosomal.

The differing genetic results observed in the current study suggest that glucose aversion (Silverman and Bieman 1993) and gel bait aversion (Wang et al. 2004) are most likely caused by different mechanisms. As suggested by Silverman (2005), bait aversion in the Cincy strain could be caused by either a mutation to a taste receptor (sensory nervous system) or to a downstream signaling pathway in the central nervous system (CNS). (Pridgeon et al. 2002) reported results of physiological investigations on a gel bait averse cockroach strain with multifactorial physiological resistance to pyrethroid insecticides. They found differences in metabolic and respiratory rates between averse and susceptible cockroaches, but it is not clear whether these particular differences are related to aversion, pyrethroid resistance, or both. More importantly, with respect to sugar detection in the averse and nonaverse strains, no electrophysiological differences in sucrose and glucose detection were found to exist for receptors on the maxillary palps (Appel et al. 2005). Because the Cincy strain is averse to not only glucose mono- and disaccharides but also to fructose (Wang et al. 2004, current study), this supports that the mutation(s) responsible for resistance is in the CNS, rather than in a taste receptor (Silverman 2005). If we may assume that a single gel bait aversion phenotype has been selected across wide geographic distances, the results of Appel et al. (2005) further support that aversion is caused by changes in the CNS.

Unlike glucose aversion (Silverman and Bieman 1993; Ross and Silverman 1995a, b), our findings for the Cincy strain indicate that gel bait aversion is incompletely dominant and partially sex-linked. This suggests that aversion may be caused by multiple mutations at multiple genetic loci in the Cincy strain. It remains to be determined whether such mutations cause changes in the CNS. If a *Rdl*-like mechanism of physiological fipronil resistance is present in the CNS of the Cincy strain, it could possibly be linked to the behavioral changes that may be responsible for sugar and gel bait aversion (e.g., drugs that target the GABA system typically exert strong influences on behavior; Bloomquist 2002). Additional research examining sugar perception and insecticide susceptibility in geographically diverse cockroach populations, and among averse and nonaverse strains, is necessary to provide answers to this problem.

Cincy cockroaches exhibited lower fecundity than Jwax cockroaches (Wang et al. 2004). This might be attributed to the sex-linked inheritance of bait aversion. Another possible factor affecting the inheritance (or manifestation of resistance) is the tergal gland secretions and associated mating behavior. Female cockroaches feed on the tergal gland secretions from males just before copulation. Tergal gland secretions in laboratory strain cockroaches contain maltose (Nojima et al. 1999), which stimulates feeding in nonaverse cockroaches (Wang et al. 2004). Does the tergal gland secretion in male Cincy cockroaches have significantly different components than those from the nonaverse strains? Do female Cincy cockroaches re-

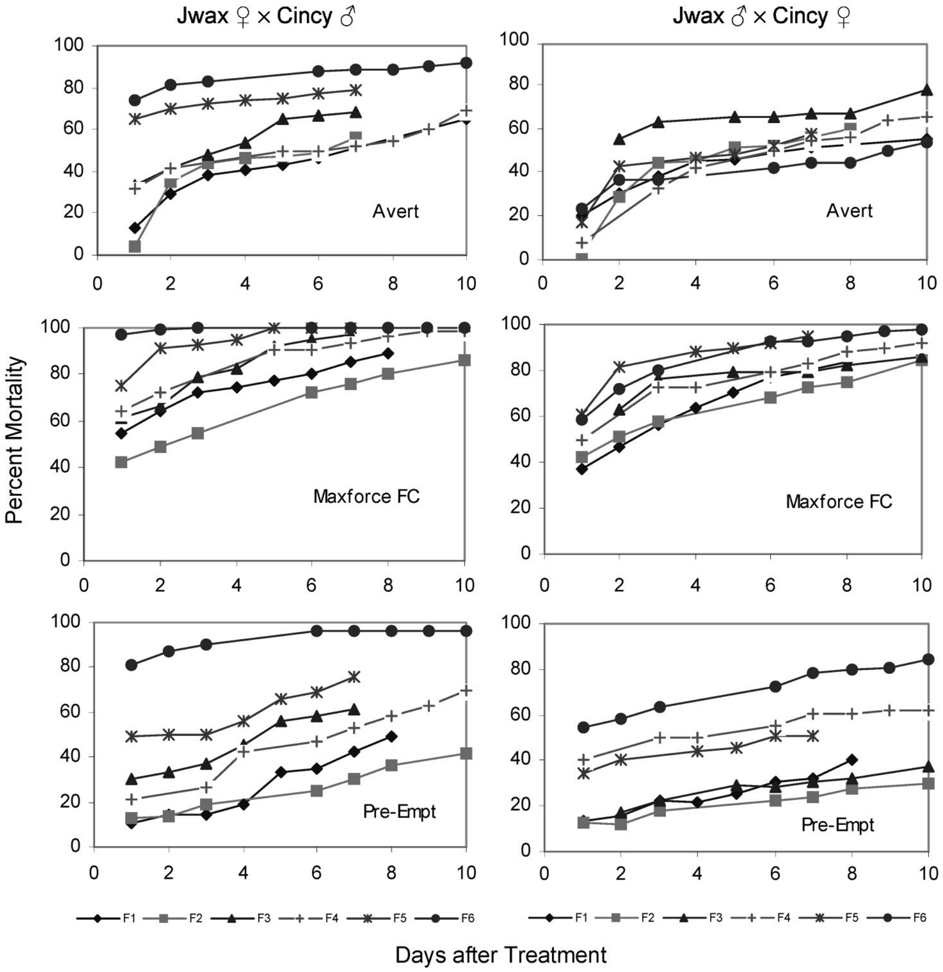

Fig. 3. Stability of resistance to Avert, Maxforce FC, and Pre-Empt gel baits in the heterozygous C♀ × J♂ and J♀ × C♂ strains of *B. germanica.*

spond differently to tergal gland secretions compared with nonaverse strains? These are important questions that need to be addressed to fully understand cockroach bait aversion.

Stability experiments with Avert and Pre-Empt in the J♂ × C♀ indicated that the behavioral resistance trait is incompletely dominant and remains for several generations in the absence of selection. Of particular concern are the fairly stable resistance levels that were apparent after six generations, as seen in the J♂ × C♀. Interestingly, data presented by Appel et al. (2005) suggest that averse cockroaches can derive greater metabolic energy from lipid and protein than from carbohydrates. These data suggest that gel bait–sugar aversion does not have strong fitness costs. Therefore, once aversion is selected to high levels in cockroach populations, it is likely to remain so for long periods, even after gel bait use has ceased.

Because of the convenience, safety, and effectiveness characteristics, gel baits will continue to be widely used in *B. germanica* management in the future. As a result, more instances of behavioral resistance are likely to emerge. Even with the use of improved bait formulations (Morrison et al. 2004), cockroaches will develop resistance to gel bait formulations after repeated exposure. The broad resistance to all gel baits and stability of the resistance that we have observed are particularly alarming. Our conclusions support that, as the proportion of gel bait-resistant individuals increases in natural populations, gel baits will rapidly lose their efficacy. Therefore, improved bait matrices, rotational schemes, and integrated pest management principles should be proactively developed and practiced to mitigate the widespread selection of behavioral resistance in *B. germanica.*

Acknowledgments

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