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IMPAIRED ODOR SENSITIVITY IN FIELD-RESISTANT GERMAN COCKROACH

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Abstract Fipronil is a popular toxicant in gel bait formulation for managing German cockroaches because of its fast action, besides other advantages, such as more extended residual activity, safer application, and low environmental impact. However, there are increasing complaints about its ineffectiveness on field-resistant German cockroaches due to the low bait consumption rate. Besides the possibility of physiological resistance and bait aversion behavior contributing to the low efficacy, it is hypothesized that the field German cockroach may have impaired odor sensitivity and thus reduce bait discovery and visitation. A field population that developed high resistance to pyrethroids and fipronil was used to observe their response to fipronil-containing commercial gel bait and compared with laboratory susceptible strain. In addition, next-generation sequencing (NGS) was conducted to identify mutations in the German cockroach genome associated with odor-sensitivity receptors. The result shows that the field-resistant population spent 1498.6 ± 113.74 s visiting the commercial gel bait, significantly longer than in laboratory susceptible strain (24.3 ± 2.1 s). In addition, 80% of male cockroaches were not attracted to the gel bait. We identified two candidate genes of field-resistant populations. Their expression levels were significantly varied compared to laboratory-susceptible populations, suggesting potential functions for these genes in disrupting their olfactory perception.

Key words German cockroach, odor sensitivity, insecticide resistance, odorant receptor

INTRODUCTION

Insecticides with gel bait formulation are popular for managing the German cockroach, *Blattella germanica*. Due to more extended residual activity, safer application, and low environmental impact, gel baits remain highly effective and mostly replace sprays and dust (Appel and Rust, 2021; Gondhalekar et al., 2021; Wada-Katsumata and Schal, 2024). Of the active ingredients used in commercial baits, fipronil is prevalent and has been used for three decades (Kaakeh et al., 1997; Khoobdel, 2022). However, the insecticide resistance caused by the heavy reliance on and frequent use of insecticides has become a prominent challenge in cockroach management (Hu et al., 2020; Scharf and Gondhalekar, 2021; Tisgratog et al., 2023).

In Taiwan, German cockroaches collected from 24 premises reportedly developed high resistance levels towards pyrethroid insecticides (Hu et al., 2020). Metabolic resistance involving detoxifying enzymes such as cytochrome P450 monooxygenases was the most observed mechanism, as the *CYP4G19* gene was reported to overexpress in most pyrethroid-resistant field populations (Hu et al., 2021). Target-site insensitivity associated with *Rdl* mutations was also observed in partial pyrethroid-resistant field populations (Kruaysawat et al., 2024). These resistance mechanisms reduce the efficacy of fipronil in gel baits, leading to failures in cockroach management. Moreover, pest management professionals are increasingly complaining about the ineffectiveness of gel baits on field-resistant German cockroaches due to the low bait consumption rate. Besides the possibility of physiological resistance and bait aversion behavior contributing to the low efficacy, it is hypothesized that the field German cockroach may have impaired odor sensitivity and thus reduce bait discovery and visitation. Boné et al. (2020) reported this reduced odor sensitivity trait by assessing feeding behavior in pyrethroid-resistant and susceptible populations of the German cockroach. However, the relationship between insecticide resistance and reduced odor sensitivity remains unclear.

In this study, we assessed odor sensitivity in 10 field-collected populations of *B. germanica* using a behavioral assay to quantify their odor detection and foraging abilities. Additionally, we compared odorant receptor (*OR*) gene expression levels between insecticide-resistant and susceptible populations to investigate the underlying mechanisms driving differences in odor sensitivity between resistant and susceptible individuals of *B. germanica*.

MATERIALS AND METHODS

Insect collection and rearing 10 field populations of *B. germanica* were collected from infested residences between 2017 and 2021. These populations developed different resistance levels towards several insecticides, especially fipronil and deltamethrin (Table 1) (Kruaysawat et al., 2024). A laboratory strain (EHI) reared in the laboratory without insecticide exposure for more than 40 years was used for comparison. All populations were maintained in round polyethylene containers (24 cm diameter × 32 cm height) under laboratory conditions of 25 ± 1°C, 50 ± 5% RH, and a photoperiod of 12:12 h light: dark. All populations were reared with corrugated cardboard harborage, dog food (RT-Mart chicken flavor dog food, Hsinchu, Taiwan), and water *ad libitum*.

Table 1. Target-site mutation frequency in susceptible and field-collected *B. germanica* according to Kruaysawat et al. (2024)

Population	Target-site mutation rate (R/R, R/S, S/S)	
	kdr (L993F)	*Rdl* (A302S)
EHI	0, 0, 10	0, 0, 10
KS Pizza Mall	1, 6, 3	10, 0, 0
KS Wufu	0, 0, 10	10, 0, 0
KS Zuoying mall	4, 5, 1	10, 0, 0
TC Carrefour	0, 1, 9	3, 5, 2
TC Meat shop	0, 0, 10	3, 6, 1
TC Vimilian	4, 4, 2	10, 0, 0
TP Breeze Mall	1, 6, 3	10, 0, 0
TP DTF Restaurant	0, 6, 4	10, 0, 0
TP Ootoya Daan	5, 3, 2	10, 0, 0
TP Taipei	0, 1, 9	10, 0, 0
TY CPCS	0, 3, 7	10, 0, 0

Asessment of bait odor sensitivity The distinction of odorant sensitivity in field-collected and susceptible populations was determined using the commercial gel bait Jin-Li-Hai Ultra Max (containing 0.05% fipronil) (Kukbo Science Co., Ltd, Cheongju-si, Korea). The experimental area was a 100 cm-length acrylic tube with openings in both ends. Before the experiment, cockroaches were starved and provided with only water for 48 hours. Gel baits were placed on one side of the acrylic tube with a fan to spread the odor of baits. A cockroach was introduced into another end of the acrylic tube and acclimatized at the entrance provision with a stopper. After 10 min, the stopper was lifted, and the time spent by cockroaches reaching and consuming bait was recorded. The time spent is defined as latency, a measure to quantify cockroaches' foraging and food-locating ability. The test was terminated at 30 min, and the cockroaches not responsive to the bait odor were recorded. Thirty male individuals from each population were tested. The experiment was conducted in the dark.

Transcriptome construction, sequencing, and quantification of odorant receptor gene expression In this study, we attempted to examine the associations between mechanisms of insecticide resistance and impaired odor sensitivity with odor receptor-related genes (*OR*). KS Zuoying Mall was selected due to the exceptionally high “no response” percentage observed in the behavioral test. The total RNA of tissue on adult male *B. germanica* was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA) and PureLink RNA Mini Kit (Invitrogen) according to the manufacturer’s protocol. For transcriptome construction of tissue samples, 10 antennae and five heads of males in each population were collected. Each population had three biological replicates. Construction of the cDNA library and Illumina sequencing of the samples were conducted by Tri-I Biotech Inc. Gene mapping and quantification of odorant receptor (OR) genes were conducted with Genome assembly Bger_1.1 (GCA_003018175.1, GenBank) as the reference genome.

Data analysis To assess the odorant sensitivity of field German cockroaches, the mean latency of each population was estimated using Kaplan–Meier analysis in R version 4.2.1. The log-rank test was conducted to examine the significant differences in the mean L between the field-collected population and the susceptible strain (EHI) at $\alpha = 0.05$.

The expression levels of *OR* genes were quantified with fragments per kilobase per million (FPKM) value, which was calculated with the normalization of read counts mapped to the gene of the reference genome. The sequencing depth and gene length of the transcript were also considered in the FPKM calculation. The statistical comparison of FPKM values was calculated using DESeq2 in R version 4.2.1. for generating fold-change values of gene expression levels and confirming significant differences.

RESULTS AND DISCUSSION

Assessment of bait odor sensitivity of 11 populations of *B. germanica* males tested, bait odor sensitivity quantified by mean latency varied across populations (Table 2). All field-collected populations exhibited a significantly greater mean latency value than EHI (Log-rank test, $P < 0.05$, Table 2). 10－80% of males of six field-collected populations showed “no response” in the presence of bait odor. Among these populations, 80% of individuals from KS Zuoying Mall showed “no response” to bait odor.

The results showed that field-collected populations were more insensitive toward bait odors than insecticide-susceptible populations (Table 2). This finding was similar to the results of the feeding behavior assessment conducted by Boné et al. (2022). Pyrethroid-resistant cockroaches took more time to locate food resources than insecticide-susceptible cockroaches. Insecticide-resistant cockroaches were hypothesized to exhibit reduced olfaction sensitivity due to the fitness cost, which appeared with resistance phenotype (Boné et al., 2022). This olfactory-systemic difference with the presence of insecticide resistance had been reported in the peach-potato aphid, *Myzus persicae* (Foster et al., 1999; 2005; 2007). In particular, the insensitivity of aphids toward alarm pheromones might be associated with the presence of detoxification enzymes and *kdr* genotypes in the population.

Table 2. Mean latency and statistical comparisons of the bait odor sensitivity in 11 populations of *B. germanica* males.

Population	n	Mean latency ± SE (s)	95% CI (s)	χ^2 (df)	[1]*P*
EHI	30	24.3 ± 2.1	18.8 − 28.0	—	—
KS Pizza Mall	30	1220.3 ± 149.75	NA	50.2 (1)	<0.05
KS Wufu	30	55.9 ± 13.8	24.4 − 45.6	8.6 (1)	<0.05
KS Zuoying mall	30	1498.6 ± 113.74	NA	60.7 (1)	<0.05
TC Carrefour	30	53.4 ± 13.74	21.3 − 48.2	7.4 (1)	<0.05
TC Meat shop	30	852.5 ± 127.29	326.7 − NA	66.4 (1)	<0.05
TC Vimilian	30	270.9 ± 101.36	23.4 − 105.7	16.1 (1)	<0.05
TP Breeze Mall	30	129.6 ± 36.31	40.1 − 78.7	31.4 (1)	<0.05
TP DTF Restaurant	30	396.9 ± 101.48	65.0 − 469.9	39.8 (1)	<0.05
TP Ootoya Daan	30	47.5 ± 9.13	21.6 − 45.5	7 (1)	<0.05
TP Taipei	30	621.3 ± 128.02	86.2 − 763.2	54.3 (1)	<0.05
TY CPCS	30	965.4 ± 139.13	238.8 − NA	71.3 (1)	<0.05

Expression levels of odorant receptor (*OR*) genes The profile of odorant receptor (*OR*) genes in two populations of *B. germanica* was constructed with mean FPKM values. *Orco*, *OR36*, *OR49,* and *OR90* exhibited high expression levels in EHI and KS Zuoying Mall (Table 3). Of the 24 *OR* genes analyzed, 14 genes, including odorant co-receptor (Orco), exhibited lower expression levels as indicated by fold change values in KS Zuoying Mall than EHI. However, a significant difference was observed only in *OR37* (Fold change value = -13.27, Wald test, $P < 0.05$) (Table 3). 10 genes exhibited higher expression levels in KS Zuoying Mall compared to EHI, with a significant difference observed only in *OR54* (Fold change value = 18.65, Wald test, $P < 0.05$) (Table 3).

OR37 and *OR54* may have roles in the olfactory process, potentially contributing to the significant differences in sensitivity to bait odors between field-collected and susceptible populations (Table 2). The current finding suggested that the field-collected *B. germanica* with pyrethroid resistance involving cytochrome P450s and kdr genotypes may be associated with impaired odor sensitivity. Further functional analyses of odorant receptors and electrophysiological experiments are needed to validate and support our observations.

Table 3. Statistical comparisons of *OR* gene expression levels in field-collected and susceptible *B. germanica*.

Gene name	Mean FPKM		[1]Fold change	[2]*P*
	EHI	KSZM		
Orco	0.518	0.409	-1.27	0.62
OR7a	0.025	0.026	1.02	0.86
OR9	0.103	0.076	-1.36	0.93
OR34	0.321	0.252	-1.28	0.83
OR36	0.797	0.802	1.01	0.60
OR37	2.215	0.167	-13.27	< 0.05
OR38	0.112	0.065	-1.71	0.69
OR39	0.162	0.075	-2.18	0.53
OR40	0.059	0.102	1.73	0.52
OR48	0.239	0.278	1.17	0.60
OR49	0.718	0.482	-1.49	0.63
OR54	0.012	0.230	18.65	< 0.05
OR56	0.216	0.209	-1.04	0.84
OR57a	0.075	0.050	-1.50	0.82
OR67	0.282	0.333	1.18	0.50
OR68	0.125	0.033	-3.73	0.37
OR72	0.086	0.125	1.46	0.54
OR80	0.177	0.153	-1.15	0.94
OR85	0.042	0.076	1.81	0.50
OR86	0.094	0.135	1.44	0.72
OR90	0.885	0.586	-1.51	0.67
OR96	0.195	0.155	-1.26	0.95
OR102	0.063	0.056	-1.12	0.96

[1]The values of fold change based on FPKM value were calculated by dividing KSZM in EHI, a negative sign was showed when FPKM value of

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