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ORIGINAL ARTICLE

Evaluation of the inheritance and dominance of behavioral resistance to imidacloprid in the house fly (*Musca domestica* L.) (Diptera: Muscidae)

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Abstract The house fly, *Musca domestica*, is a cosmopolitan species known for its pestiferous nature and potential to mechanically vector numerous human and animal pathogens. Control of adult house flies often relies on insecticides formulated into food baits. However, due to the overuse of these baits, insecticide resistance has developed to all insecticide classes currently registered for use in the United States. Field populations of house flies have developed resistance to imidacloprid, the most widely used neonicotinoid insecticide for fly control, through both physiological and behavioral resistance mechanisms. In the current study, we conducted a comprehensive analysis of the inheritance and dominance of behavioral resistance to imidacloprid in a lab-selected behaviorally resistant house fly strain. Additionally, we conducted feeding preference assays to assess the feeding responses of genetic cross progeny to imidacloprid. Our results confirmed that behavioral resistance to imidacloprid is inherited as a polygenic trait, though it is inherited differently between male and female flies. We also demonstrated that feeding preference assays can be instrumental in future genetic inheritance studies as they provide direct insight into the behavior of different strains under controlled conditions that reveal, interactions between the organism and the insecticide. The findings of this study carry significant implications for pest management and underscore the need for integrated pest control approaches that consider genetic and ecological factors contributing to resistance.

Key words aversion; behavioral genetics; feeding preference; insecticide; neonicotinoid

Introduction

The house fly (*Musca domestica* L.) (Diptera: Muscidae) is a ubiquitous and synanthropic fly species commonly associated with urban waste storage and concentrated animal feeding operations (CAFOs) (West, 1951; Geden *et al.*, 2021). House flies are considered nuisance pests and pose a significant threat to public health and animal husbandry as they have been implicated in the mechanical transmission of over 200 pathogens (Nayduch & Burrus, 2017; Nayduch *et al.*, 2023), including antimicrobial resistant bacteria (Bertelloni *et al.*, 2023; Nayduch *et al.*, 2023). Failure to control adult flies dispersing from development sites to surrounding communities can result in litigation against CAFOs or urban waste facilities (Meyer, 1993).

Insecticides are frequently relied upon to control adult house flies when populations exceed acceptable abundance or activity levels (Gerry, 2020). Insecticides for fly control are applied as sprays, dusts, pour-ons, or formulated into granular insecticidal baits. Granular insecticidal baits are heavily used due to their low cost, ease of use, and low risk of off-target effects (Keiding, 1975; Chapman *et al.*, 1998; Darbro & Mullens, 2004). Granular insecticidal baits are formulated to contain a toxicant and a phagostimulant to induce fly feeding. However,

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the overreliance on these insecticides has resulted in resistance to all major insecticide classes (reviewed by Geden *et al.*, 2021). Insecticide resistance is a complex and multifactorial phenomenon that can arise through genetic, ecological, and behavioral factors. Insecticide resistance is often characterized as either physiological or behavioral. Physiological resistance to insecticides is associated with well-characterized physiological changes to an organism, such as increased production of toxin-metabolizing enzymes (e.g., GSTs or P450s) and changes to the structure of insecticide target sites (target site insensitivity) (Rinkevich *et al.*, 2006; Ma *et al.*, 2020).

Behavioral resistance is defined as the ability of an insect to modify its behavior in response to insecticide exposure to avoid or reduce exposure to the insecticide (Georghiou, 1972). Behavioral resistance can take many forms, including reduced contact with insecticide-treated surfaces, increased avoidance of insecticide-treated areas, and altered feeding behavior (Fouet *et al.*, 2018; Iqbal & Evans, 2018; Hubbard & Gerry, 2020). Behavioral resistance has been documented in a wide range of insect species, including bed bugs (Agnew & Romero, 2017), cockroaches (Silverman & Selbach, 1998), termites (Iqbal & Evans, 2018), mosquitoes (Gatton *et al.*, 2013), fruit flies (Pluthero *et al.*, 1982), horn flies (Byford *et al.*, 1987), and house flies (Freeman & Pinniger, 1992; Darbro & Mullens, 2004; Gerry & Zhang, 2009; Mullens *et al.*, 2010; Hubbard & Gerry, 2020).

The most widely used granular bait toxicant for fly control is the neonicotinoid imidacloprid (Norris *et al.*, 2023). Neonicotinoids such as imidacloprid bind reversibly to the nicotinic acetylcholine receptor, disrupting normal nerve function, causing paralysis and insect death (Jeschke & Nauen, 2005). Unfortunately, soon after the introduction of imidacloprid-containing granular baits, both physiological (Wen & Scott, 1997; Kaufman *et al.*, 2006) and behavioral resistance to imidacloprid was documented (Gerry & Zhang, 2009; Wasik & Gerry, 2010; Seraydar & Kaufman, 2015).

In some cases, resistance is conferred by a single gene, but more commonly, it is a polygenic trait, with multiple genes contributing to the phenotype. Physiological resistance to imidacloprid is postulated to be caused by an overexpression of a microsomal glutathione S-transferase gene on chromosome 3 and to an unknown trans-regulatory gene on chromosome 4, which results in overexpression of a galactosyltransferase-like gene (Reid *et al.*, 2019). The molecular mechanisms contributing to behavioral resistance to imidacloprid in the house fly are currently unknown, though Hubbard & Gerry (2021) mapped resistance factors to autosomes 1 and 4 which suggests that behavioral resistance is polygenic in nature; however, additional studies are needed to confirm these results. Elucidating the genetic and molecular basis of behavioral resistance to insecticides is essential for the development of effective control strategies and for the management of resistance in pest populations. The goal of the current study was to examine the inheritance and dominance of behavioral resistance to imidacloprid in the house fly as well as to examine if differences in feeding preference could be observed in genetic cross progeny, which might provide a future alternative for screening flies when conducting genetic cross experiments.

Materials and methods

Reference fly colonies

Two house fly strains were used in this study. One had been selected for and exhibited a strong behavioral resistance phenotype to imidacloprid (BRS-1) (Hubbard & Gerry, 2020). The BRS-1 fly strain is maintained by challenging the flies with a choice bioassay every three filial generations (Hubbard & Gerry, 2020). During this challenge bioassay, flies were separated within 8 h of eclosion into same-sex bioassays chambers, allowed to fully mature for 3−5 d, then starved for 14 h before subsequently providing them with a food dish containing sucrose and a second food dish containing sucrose mixed with a very high concentration of imidacloprid (4000 μg imidacloprid per gram of sucrose) where they are allowed to feed for 72 h. Surviving male and female flies are then combined and allowed to mate.

The other fly strain is a known imidacloprid susceptible strain (UCR). The UCR fly strain was collected in 1982 from a dairy farm in Mira Loma, California, and has been maintained without insecticide exposure. All fly strains were maintained in insectary rooms at 27 °C, 14 : 10 L : D, 35% RH, and reared following standard practices (Zahn & Gerry, 2018).

Inheritance and dominance of behavioral resistance to imidacloprid

The F_1 male backcross method (Tsukamoto, 1983; Roush *et al.*, 1986) was used in conjunction with choice and preference assays to evaluate the inheritance and dominance of behavioral resistance to imidacloprid in the highly selected BRS-1 fly strain. Resistant (BRS-1) and susceptible (UCR) flies were sorted by sex within 8 h of eclosion (Murvosh *et al.*, 1964), and reciprocal genetic crosses were set up (BRS-1 ♀ × UCR ♂, BRS-1 ♂× UCR ♀). Flies were allowed to mate for 7 d before

collecting eggs and rearing heterozygous F_1 offspring. F_1 male offspring were then backcrossed to unmated UCR and BRS-1 females to examine the inheritance and dominance of resistance. A visual representation of the F_1 male backcross method can be found as Fig. S1 online. Two complete experimental replicates of all genetic crosses and bioassays were completed (described below).

Examining behavioral resistance and degree of dominance of resistance of genetic cross progeny

UCR, BRS-1, F_1, F_2, Backcross to BRS-1 (F_1 backcross to BRS-1), and Backcross to UCR (F_1 backcross to UCR) male and female flies were examined for behavioral resistance to imidacloprid. Resistance to imidacloprid was evaluated using standard methods described by Hubbard & Gerry (2020). Briefly, 3 to 5 d-old, mixed-sex adult house flies were starved overnight for 14−18 h by capping off and removing the flies food source, aspirated from a colony cage, and sorted into groups of 25 same-sex individuals on an electronic chill plate (Catalog #1431, BioQuip Products Inc., Compton, CA) (n = 125 total flies per trial) and placed into assay chambers (inverted 947 mL polypropylene deli containers with a removable plastic lid and a bottom modified by adding a fiberglass screen). Flies were provisioned with water and two 37 mL soufflé cups, one containing 1 g of granular sucrose treated with acetone only and the other containing 1 g of granular sucrose formulated with 4000 μg/g technical grade imidacloprid (CAS: 138261-41-3, Chem Service Inc., West Chester, PA). Sucrose treated with imidacloprid was created by dissolving the desired test concentration of imidacloprid per gram of sucrose into acetone. The acetone-imidacloprid solution was then applied to the granular sucrose, mixed thoroughly to ensure even dispersal of the insecticide through the sucrose, and placed in the fume hood for 24 h to allow the acetone to evaporate. An additional set of five assay chambers were set up as a negative control, where flies were provisioned with water and two 37 mL soufflé cup containing 1 g of granular sucrose treated with acetone without imidacloprid as described above. Fly survival was documented and converted to a proportion of flies surviving per assay chamber, then transformed using the arcsine of the square root of the proportion surviving prior to statistical analysis. Survival differences among fly strain and sex were evaluated using one-way ANOVA, with Tukey's post hoc test used to separate means.

Mortality results from the F_1 offspring were also used to calculate a degree of dominance (h) utilizing the single concentration method (Hartl, 1992; Tabashnik, 1994). Dominance (h) is calculated utilizing the following formula:

$$h = \frac{w_{12} - w_{22}}{w_{11} - w_{22}},$$

where w_{11}, w_{12}, and w_{22} are the fitnesses of flies exposed to a choice 4000 μg/g imidacloprid for resistant (BRS-1), heterozygotes (F_1), and susceptible (UCR) flies, respectively. The fitness of resistant flies (BRS-1) is defined as 1. The fitness of susceptible (UCR) flies was calculated as the percent survival of UCR flies divided by the percent survival of resistant (BRS-1) flies. F_1 fitness is calculated as the percent survival of F_1 flies divided by the percent survival of resistant (BRS-1) flies. The values of h range from 0−1, with 0 indicating resistance is inherited in a completely recessive manner, 0.5 indicating resistance is inherited in a codominant or additive manner, and 1 indicating resistance is inherited in a completely dominant manner (Liu & Tabashnik, 1997).

Additionally, mortality results from the choice bioassays for F_1 Backcross to UCR, F_1 Backcross to BRS-1, and F_2 were used to determine the number of genes involved in behavioral resistance to imidacloprid. A chi-square (χ^2) goodness of fit was performed to test the null hypothesis of monogenic resistance following the methods of Sokal & Rohlf (1981).

The expected response mortality was calculated utilizing the formulas:

$$\chi = W_{(F1)} \times 0.50 + W_{(UCR)} \times 0.50,$$

$$\chi = W_{(F1)} \times 0.50 + W_{(BRS\ 1)} \times 0.50,$$

and

$$\chi = W_{(UCR)} \times 0.25 + W_{(F1)} \times 0.50 + W_{(BRS\ 1)} \times 0.25,$$

for F_1 Backcross to UCR, F_1 Backcross to BRS-1, and F_2 respectively (Georghiou, 1969). χ is the expected mortality, and W is the observed mortality of UCR, F_1, and BRS-1 flies tested during choice assays described above.

Concentration-dependent feeding on imidacloprid

To determine if fly lines from the genetic cross experiments exhibited a preference to feed on sucrose or imidacloprid, feeding preference assays were performed with the UCR, BRS-1, F_1, Backcross to UCR, Backcross to BRS-1, and F_2. Three to-five-day-old adult male and female house flies were starved overnight for 14 h,

sorted into groups of 25 on an electronic chill plate (Catalog #1431, BioQuip Products Inc., Compton, CA), and placed into assay chambers (inverted 947 mL polypropylene deli containers with a removable plastic lid and a bottom modified by adding a fiberglass screen) (Hubbard & Gerry, 2020; Hubbard & Murillo, 2022). Each experimental chamber was provided water and two 37 mL soufflé cups, one containing 1 g sucrose pretreated with a 500 μg/g imidacloprid concentration in acetone (Sigma-Aldrich Chemical Co., St. Louis, Mo) and the other containing 1 g sucrose treated with acetone only ("control") (Hubbard & Gerry, 2020). The test concentration of 500 μg/g was chosen as it was shown in Hubbard & Murillo (2022) to be a concentration to which behaviorally resistant house flies exhibited a strong aversion, similar to that of higher concentrations. For all treatments, the acetone was allowed to volatilize from the sucrose by placement within a fume hood for 24 h prior to each trial. Each sucrose treatment within an experimental chamber was colored either blue or red using food grade coloring solution (McCormick & Co., Inc., Hunt Valley, MD), with colors rotated between treated and untreated sucrose. Flies placed into experimental chambers were allowed to feed for 24 h under dark conditions within the laboratory before being killed by freezing and sorted via abdomen color; red, blue, purple (fed on both treatments), or blank (did not feed). Two technical replicates of experimental assay chambers were paired and run concurrently with food color assigned to the treatment or control food cup alternating among the two paired experimental chambers to account for any possible color effects.

The preference index for each concentration and fly strain was calculated using the following:

$$P_{S/I} = \frac{N_r - N_b}{N_r + N_b + N_p} \text{ or } \frac{N_b - N_r}{N_r + N_b + N_p},$$

where $P_{S/I}$ is the preference of flies to feed on sucrose control over imidacloprid-treated sucrose at the test concentration. N_r, N_b, and N_p, are the number of flies with red, blue, and purple, abdomens, respectively. Only trials that had at least a 50% response rate (feeding on food source) were used to calculate the $P_{S/I}$.

To determine if differences in feeding preference were observed between sex and food color within fly lines from progenitor or genetic cross offspring, two-way ANOVAs were performed. If no differences were observed between sex or food color preference within a fly line, results were pooled.

Reciprocal cross replicates were then compared via *t*-test to determine if differences in feeding preference existed between reciprocal cross fly progeny. If no differences existed, replicates were pooled.

For each progenitor population or genetic cross progeny, one-sample *t*-tests were then performed to detect differences between treatment feeding preference as compared to an expected no preference value ($P_{S/I} = 0$).

Results

Examining behavioral resistance and degree of dominance of resistance of genetic cross progeny

No differences were observed in reciprocal crosses, leading us to pool the data for further analysis (all $t > 2.530$; df = 4; $P \geq 0.0647$).

Behavioral resistance of genetic cross progeny

Significant differences in survival were observed between fly strain ($F = 290.8$; df = 5, 228; $P < 0.0001$) as well as between fly sexes ($F = 23$; df = 1, 228; $P < 0.0001$). Additionally, the interaction between sex and fly strain was significant ($F = 9.339$; df = 5, 228; $P < 0.0001$).

BRS-1 male and female flies exhibited the highest survival (94.00% ± 0.01% ♂, 93.40% ± 0.01% ♀) though they did not differ significantly in survival from F_1 Backcross to BRS-1 progeny (84.55% ± 0.02% ♂, 92.25% ± 0.02% ♀) (Fig. 1). UCR male and female flies exhibited the lowest survival (4.25% ± 0.01% ♂, 3.25% ± 0.01% ♀) though they did not differ significantly in survival from F_1 Backcross to UCR progeny (12.25% ± 0.03% ♂, 10.50% ± 0.02% ♀). F_1 offspring exhibited a moderate level of survival that was significantly higher than UCR fly strains and significantly lower than BRS-1 flies. Significant differences between male and female flies were observed, with females exhibiting higher survival (47.10% ± 0.06% ♂, 64.20% ± 0.04% ♀). Similarly, F_2 flies exhibited a significant difference in survival between sexes, with female flies exhibiting higher survival (23.25% ± 0.03% ♂, 59.50% ± 0.04% ♀). Male F_2 flies exhibited a similar survival to F_1 Backcross to UCR males, while F_2 females exhibited a similar survival to both male and female F_1 flies (Fig. 1).

Degree of dominance (h)

As significant differences in the survival of male and female flies were observed during choice testing (above), the degree of dominance (*h*) was calculated for both male

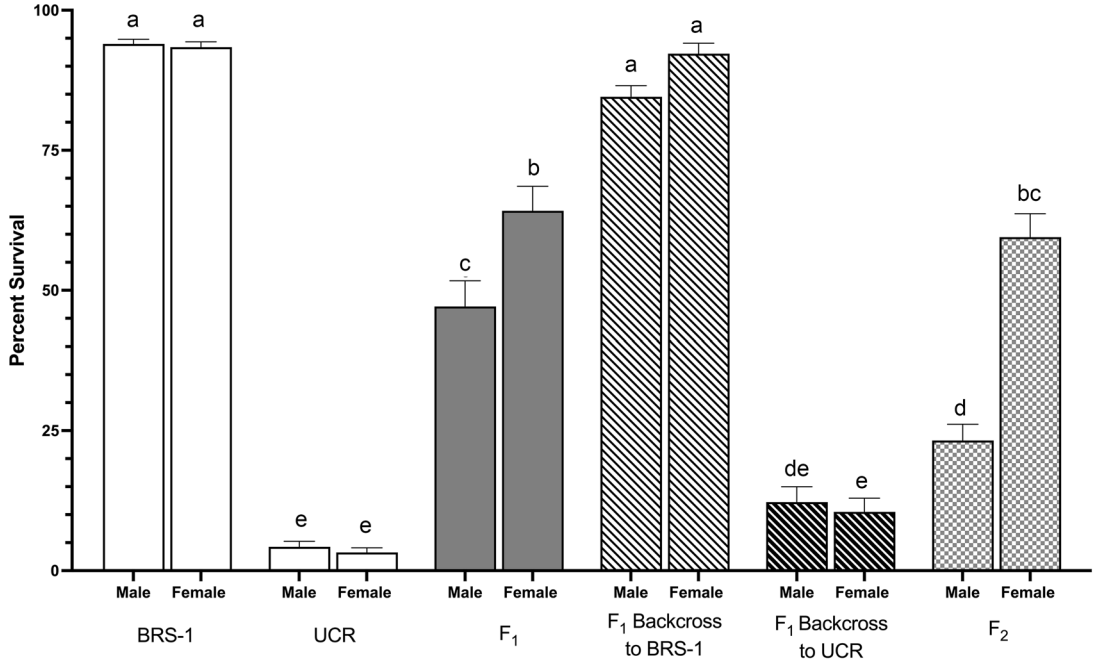

Fig. 1 Mean percent survival ± SE from house fly progenitor (BRS-1 resistant, UCR susceptible) and genetic cross progeny (n = 25/trt) following a 72 h choice feeding assay with flies provided both a food dish containing sucrose alone and a second food dish containing sucrose mixed with a high concentration of imidacloprid (4000 μg/g sucrose). Bars with the same letter indicate that no significant survival differences were observed ($P > 0.05$).

and female flies. Dominance (h) was calculated to be 0.47 (0.43–0.52) for male flies indicating resistance is partially recessive, while dominance (h) was calculated to be 0.67 (0.64–0.72) for female flies indicating resistance is partially dominant (Table 1).

Evaluation of monogenic versus polygenic inheritance of behavioral resistance to imidacloprid

As significant differences in the survival of male and female flies were observed during choice testing, Chi-square goodness-of-fit tests (χ^2) were conducted for both male (♂) and female (♀) flies from F_1 Backcross (BC) to UCR, F_1 Backcross (BC) to BRS-1, and F_2 genetic crosses with monogenic resistance as the null hypothesis. Significant differences ($P < 0.0001$) were detected between observed and expected mortalities in F_1 BC to UCR ♂ $\chi^2(1, N = 500) = 47.58$, $P < 0.0001$, F_1 BC to BRS-1 ♂ $\chi^2(1, N = 500) = 47.51$, $P < 0.0001$, F_2 ♂ $\chi^2(1, N = 500) = 124.3$, $P < 0.0001$, F_1 BC to UCR ♀ $\chi^2(1, N = 500) = 119.6$, $P < 0.0001$, and F_1 BC to BRS-1♀ $\chi^2(1, N = 500) = 53.74$, $P < 0.0001$ indicating that behavioral resistance to imidacloprid involves multiple genetic loci (Table 2). Only the F_2 ♀ exhibited nonsignificant differences between observed and expected mortalities $\chi^2(1, N = 500) = 2.263$, $P = 0.1325$ (Table 2).

Concentration-dependent feeding on imidacloprid

To determine if fly lines from the genetic cross experiments exhibited a preference to feed on sucrose or imidacloprid, feeding preference assays were performed with all the fly strains.

No significant differences in feeding preference were observed between sex (all $F \leq 3.148$; df = 1, 12; $P \geq 0.1014$), food color (all $F \leq 3.515$; df = 1, 12; $P \geq 0.0854$), or interactions (all $F \leq 0.6519$; df = 1, 12; $P \geq 0.4351$) within fly lines tested, so results for each strain were pooled. Additionally, no significant differences were observed between reciprocal cross feeding preferences (all $t \geq 1.390$; df = 30; $P \geq 0.1746$), so results were pooled for further analysis.

Significant feeding preferences for untreated sucrose over sucrose treated with 500 μg/g imidacloprid were observed in BRS-1 ($t = 29.85$; df = 15; $P < 0.0001$), F_1 ($t = 14.47$; df = 31; $P < 0.0001$), F_1 Backcross to BRS-1 ($t = 17.69$; df = 31; $P < 0.0001$), and F_2 ($t = 3.955$; df = 31; $P = 0.0004$) fly lines (Fig. 2). The UCR

Table 1 Dominance (h) of resistance to imidacloprid in the male and female behaviorally resistant house flies.

Concentration (μg/g)	Strain[†]	% Survival (SE)	Fitness[‡]	h[§]
4000	UCR ♂	4.25 (3.27–5.23)	0.045 (0.035–0.055)	
	BRS-1 ♂	94.4 (93.6–95.2)	1	
	F_1 ♂	47.1 (42.5–51.7)	0.499 (0.454–0.543)	0.475 (0.43–0.52)
4000	UCR ♀	3.25 (2.42–4.08)	0.035 (0.026–0.043)	
	BRS-1 ♀	93.3 (92.3–94.3)	1	
	F_1 ♀	64.2 (59.8–68.6)	0.68 (0.648–0.727)	0.668 (0.64–0.72)

[†] UCR (♂/♀) is the susceptible strain, BRS-1 (♂/♀) is the resistant strain, and F_1 are the hybrid progeny from the reciprocal crosses between UCR and BRS-1.

[‡] Fitness is the survival rate of the adult flies divided by the survival rate of the BRS-1 flies.

[§] h can vary from 0 to 1, with 0 indicating resistance is completely recessive and 1 indicating resistance is completely dominant.

Table 2 The monogenic inheritance to resistance hypothesis was examined utilizing a chi-square (χ^2) goodness-of-fit test. Male and female expected versus observed mortality for each backcross were analyzed separately.

Strain	Expected %	Observed %	Chi-square	df	P-value
F_1 BC to UCR ♂	25.68	12.2	47.58	1	< 0.0001
F_1 BC to BRS-1 ♂	70.55	84.6	47.51	1	< 0.0001
F_2 ♂	48.11	23.2	124.3	1	< 0.0001
F_1 BC to UCR ♀	33.73	10.6	119.6	1	< 0.0001
F_1 BC to BRS-1 ♀	78.80	92.2	53.74	1	< 0.0001
F_2 ♀	56.26	59.6	2263	1	0.1325

The expected response mortality was calculated using the formulas: $\chi = W_{(F1)} \times 0.50 + W_{(UCR)} \times 0.50$, $\chi = W_{(F1)} \times 0.50 + W_{(BRS\text{-}1)} \times 0.50$, and $\chi = W_{(UCR)} \times 0.25 + W_{(F1)} \times 0.50 + W_{(BRS\text{-}1)} \times 0.25$, for F_1 Backcross to UCR, F_1 Backcross to BRS 1, and F_2 respectively. χ is the expected mortality, and W is the observed mortality of UCR, F_1, and BRS-1 flies tested during choice assays described above.

fly strain exhibited a significant preference to feed on imidacloprid-treated sucrose over untreated sucrose ($t = 3.557$; df = 15; $P = 0.0029$), while F_1 Backcross to UCR did not exhibit a feeding preference for sucrose treated with or without 500 μg/g imidacloprid ($t = 1.071$; df = 31; $P = 0.2923$) (Fig. 2).

Discussion

The emergence and spread of insecticide resistance in pest populations poses a significant challenge to sustainable and effective pest control strategies. In the current study, we completed an analysis of the inheritance and dominance of behavioral resistance to imidacloprid in a lab-selected behaviorally resistant house fly strain. Our results confirmed that behavioral resistance to imidacloprid involves multiple genetic loci, which suggests that behavioral resistance is a polygenic trait, as was hypothesized by Hubbard & Gerry (2021). The focus of those experiments was to use the F_1 male backcross method of Tsukamoto (1964) to determine the house fly chromosome(s) that were carrying factors contributing to behavioral resistance. The authors determined that behavioral resistance to imidacloprid in the house fly was linked to factors on autosomes 1 and 4. In the current study, F_1 male and female offspring exhibited moderate levels of survival (47.10% ± 0.06% ♂, 64.20% ± 0.04% ♀) when exposed to choice bioassays. This result is intriguing as female F_1 offspring in the Hubbard & Gerry

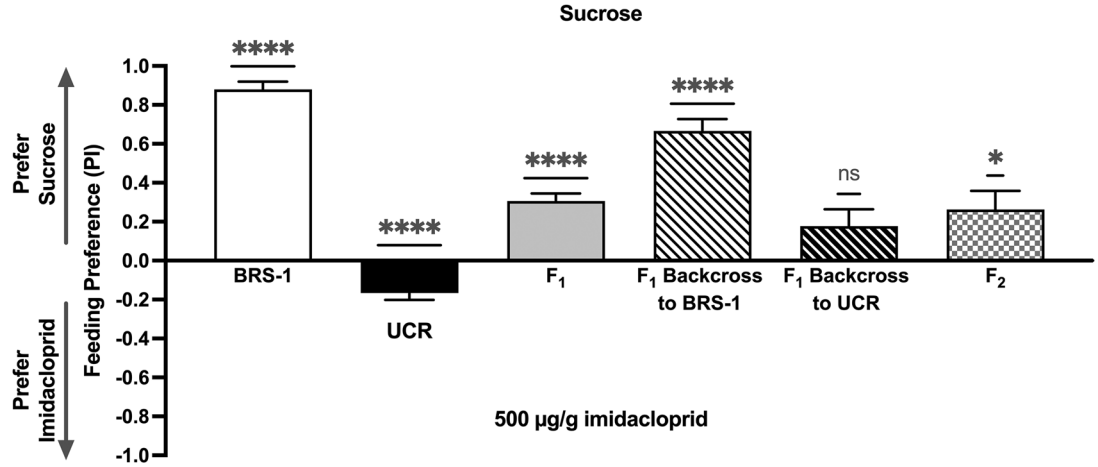

Fig. 2 Fly feeding preference index from house fly progenitor (BRS-1 resistant, UCR susceptible) and genetic cross progeny (n = 25/trt). Flies were provided a choice to feed on either sucrose with or without imidacloprid at 500 μg/g. A $P_{S/I} > 0$ indicates a greater proportion of flies fed on sucrose without imidacloprid, a $P_{S/I} < 0$ indicates a greater proportion of flies fed on sucrose with imidacloprid, and a $P_{S/I} = 0$ indicates that flies fed equally on sucrose with or without imidacloprid. A significant preference for a treatment was determined by a one-sample t-test (ns, not significant, *$P \leq 0.05$, ****$P \leq 0.0001$).

(2021) study exhibited a much lower survival rate (22.7% $\pm$ 3.7%). While both studies indicate that resistance is neither fully dominant nor recessive (Tsukamoto, 1983), as indicated by the survival rate of F_1 offspring being different from both resistant and susceptible flies, the 3 fold difference in survival between studies is surprising.

Interestingly, factors on autosomes 1 and 4 independently elicited the contact-dependent avoidance of imidacloprid documented by Hubbard & Gerry (2020), though a slightly additive interaction (increased survival) between resistance factors on autosomes 1 and 4 was observed when flies were exposed to a choice bioassay (Hubbard & Gerry, 2021). Hubbard & Gerry (2021) hypothesized behavioral resistance to imidacloprid was polygenic in nature, with genes on autosomes 1 and 4 contributing to resistance, though trans-chromosomal regulation of resistance factors or the presence of minor resistance factors on other autosomes could not be ruled out. While the authors successfully determined that resistance factors were located on multiple chromosomes, due to limitations in the study the authors did not examine the degree of dominance of resistance or the survival of backcross progeny.

Our results showed that there were significant differences in survival rates between fly sexes in both F_1 and F_2 genetic cross progeny, with the degree of dominance calculations indicating that male flies inherited behavioral resistance as a partially recessive trait, while female flies inherited behavioral resistance as a partially dominant trait. These results suggest that behavioral resistance may be sex-linked. However, no significant differences in survival were observed in male or female F_1 offspring reciprocal crosses, which should indicate that resistance is not sex-linked. A similar observation was observed by Kavi *et al.* (2014) when investigating the inheritance of physiological resistance to imidacloprid in house flies. This apparent contradiction raises several questions about the possible mechanisms involved and could be attributed to genetic factors outside the X and Y chromosomes influencing the expression of resistance.

Further studies should investigate this result in both physiological and behavioral imidacloprid resistance. Of particular interest is the potential role of genomic imprinting, where the expression of a gene depends on its parental origin. Although traditionally associated with mammals, some recent studies have suggested that genomic imprinting may also occur in insects (Patten *et al.*, 2014; Pegoraro *et al.*, 2017). Alternatively, the discrepancy in results might be due to a complex interaction among multiple resistance genes, with these interactions varying between the sexes due to differences in gene dosage or other factors.

Another interesting result was that all results except for F_2 ♀ indicated that resistance was inherited as a polygenic trait, as expected mortality was significantly different than observed mortality (Table 2). This result is intriguing and may indicate that epistasis is occurring in female flies, where one gene masks or modifies the expression of another gene. In the F_1 cross, we see observed mortality differing significantly from expected

because one genes effect is masked by another. However, when the F_1 generation is self-crossed to produce the F_2 generation, the masking effect may be resolved due to independent assortment of genes (De Visser *et al.*, 2011).

The results from the feeding preference study provide insight into the feeding preferences and behavioral responses of genetic cross progeny to imidacloprid. Significant preferences for untreated sucrose over imidacloprid-treated sucrose were observed in the behaviorally resistant BRS-1 strain, the F_1 hybrid, the F_1 Backcross to BRS-1, and the F_2 generation. This indicates a clear behavioral aversion to imidacloprid in these groups. In contrast, the UCR susceptible strain showed a significant preference for imidacloprid-treated sucrose over untreated sucrose. This response suggests a lack of recognition or detection of the insecticide by this strain, resulting in flies feeding and consuming a lethal dose of imidacloprid-treated sucrose before feeding on untreated sucrose. The F_1 Backcross to UCR did not show a significant preference for either treated or untreated sucrose, which may also indicate a failure to detect imidacloprid. It is also possible that these flies have an increased physiological resistance profile that allows them to survive feeding on imidacloprid-treated sucrose. We also observed high variation in feeding preference between replicates. This variation increased with each successive genetic cross, and the F_2 and F_1 Backcross to UCR exhibited the greatest variation among replicates. This variation in behavioral response within genetic cross progeny is not detected using traditional binary (alive or dead) no-choice or choice assays, which are what is traditionally conducted when examining the genetic inheritance of a resistance mechanism, which speaks to the importance of conducting additional assays. Feeding preference assays can be instrumental when examining genetic inheritance as they provide direct insight into the behavior of different strains under controlled conditions, revealing interactions between the organism and the insecticide. These assays detect changes in feeding behavior because of resistance and provide additional evidence for evolved avoidance strategies in response to insecticides, thus contributing significantly to the study of evolutionary biology and integrated pest management.

While at a small geographic scale (southern California), we have demonstrated that behavioral resistance is a major factor contributing to imidacloprid-containing fly bait failure for house fly management. It is essential to better understand if behavioral resistance is contributing to the failure of imidacloprid baits at a regional or national level. Monitoring for physiological susceptibility to insecticides has long been conducted (Kaufman *et al.*, 2006; Freeman *et al.*, 2019). To date, no comprehensive surveys have been conducted to determine the presence of behavioral resistance/susceptibility to commonly used insecticides used for fly control.

The study of behavioral resistance to imidacloprid in house flies provides valuable insight into the mechanisms of resistance and the complex nature of this phenomenon. The findings of this study have important implications for pest management and highlight the need for integrated approaches to pest control that consider the genetic and ecological factors that contribute to resistance. Further research is needed to better understand the mechanisms of resistance and to develop more effective management strategies that can slow the development of resistance and ensure sustainable pest control.

In addition to the implications for pest management, the findings of this study also have broader implications for the field of evolutionary biology. The development of resistance to insecticides is an example of rapid evolutionary change in response to strong selection pressure. The remarkable capacity of house flies to rapidly develop resistance to all currently registered insecticide classes, through physiological and behavioral adaptations, positions them as potential model organisms for studying evolutionary change. Understanding the mechanisms of resistance can provide insight into the evolutionary processes that underlie adaptation and the evolution of complex traits.

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Disclosure

The authors declare no competing interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Visual representation of the F_1 male backcross method.