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1 **Pyrethroid Resistance and Its Inheritance in a Field Population of**
2 ***Hippodamia convergens* (Guérin-Ménéville) (Coleoptera: Coccinellidae)**

3

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21

22 Abstract

23 The convergent lady beetle (CLB), *Hippodamia convergens* (Guérin-Ménéville), a species
24 widely distributed and used in biological control, has exhibited high survival under field and
25 laboratory conditions when treated with field rates of the pyrethroid λ -cyhalothrin, a highly
26 unusual phenomenon for a natural enemy. This work investigated and characterized the
27 phenomenon of pyrethroid resistance in a population of this species collected in Georgia,
28 USA. The mechanism and level of resistance were evaluated by treating parental populations
29 with λ -cyhalothrin \pm piperonyl butoxide (PBO). The inheritance bioassay utilized parental
30 crosses and backcrosses between parental populations to obtain testable progenies. Adult
31 beetles from populations and progenies were topically treated with different doses of λ -
32 cyhalothrin (technical grade) to calculate knockdown (KD) and lethal (LD) doses, and to
33 investigate the dominance based on a single dose and whether resistance is autosomal and
34 monogenic (null hypothesis). Genetic variation in the parental populations was examined by
35 applying a discriminating dose for resistant individuals (0.5 g/L). The data indicate that
36 resistance is due to at least two factors: knockdown resistance and enzymatic detoxification of
37 the insecticide. The knockdown effect is recessive and linked to the X-chromosome.
38 Variability in proportions of individuals within families dying following knockdown indicated
39 genetic variation in the resistant population. Further studies should be done to investigate the
40 role of sex linked inheritance of resistance in the species and interactions of the various
41 mechanisms involved in resistance.

42

43 **KEY WORDS:** Lady beetles; pyrethroid; resistance inheritance; piperonyl butoxide; λ -
44 cyhalothrin

45

46 1. Introduction

47 Effective integration of insecticides and natural enemies has been a goal of integrated
48 pest management (IPM) since the concept was first fully articulated by Stern *et al.* [1],
49 although at the time and in the subsequent decades this integration has seemed highly
50 unlikely. Most organophosphate, carbamate, and pyrethroid insecticides have broad activity
51 spectra, with little selectivity toward natural enemies [2]. Insecticides can affect natural
52 enemies, manifesting as death or alterations in behavior and fitness, via direct intoxication
53 from insecticide application, or indirectly through consumption of contaminated prey or
54 through scarcity of prey or hosts [3, 4].

55 Overcoming this incompatibility is the most difficult aspect of integrating biological
56 control agents and insecticides in IPM strategies. An ideal resolution is to replace all broad
57 spectrum products with insecticides of greater selectivity [5, 6], but this is highly impractical
58 at present. Some efforts have been made to utilize insecticide-resistant natural enemies in
59 IPM, but such resistance in natural enemies is highly unusual relative to that observed in
60 pests.

61 Intensive insecticide use has selected for resistance to multiple classes of insecticides in
62 numerous arthropod species, the vast majority of which are herbivores. Since 1914, when the
63 first instance of resistance was observed in the San Jose scale, *Quadraspidiotus perniciosus*
64 (Comstock) (Hemiptera: Diaspididae), more than 500 pest species resistant to insecticides
65 have been recorded [7]. Insecticide resistance in natural enemies has also been reported, but
66 much less frequently than for pest species. The predatory mite *Neoseiulus* (= *Amblyseius*)
67 *fallacis* (Garman) (Acari: Phytoseiidae) was found to be resistant to azinphosmethyl in the
68 1970s [8]. Subsequently, more cases were observed in predatory mites [9, 10]. Among insect
69 natural enemies, field resistance has been reported for the parasitoid *Anisopteromalus*
70 *calandrae* (Howard) (Hymenoptera: Pteromalidae) to malathion [11], and populations of the

71 lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) have exhibited resistance
72 to carbaryl [12] and organophosphates and pyrethroids [2, 13, 14]. Similarly, Suckling et al.
73 [9] found pyrethroid-resistant predatory mites in apple orchards in New Zealand.

74 Although Coccinellidae have been widely studied and used in biological control for
75 over a century, insecticide resistance has rarely been reported in this group of natural enemies.
76 Lady beetles commonly occur in many ecosystems and are valued for their contributions to
77 biological control of soft-bodied arthropod pests, such as aphids, whiteflies, scales, and mites
78 [6, 15, 16]. Relative to other entomophages, lady beetles tend to be less susceptible to
79 insecticides than other aphidophagous natural enemies, such as lacewings, syrphids,
80 hemipterans, and hymenopteran parasitoids [17]. Studies of different species and populations
81 of lady beetles and insecticides reveal variation in lady beetle susceptibility to insecticides
82 [18, 19, 20, 21, 22, 23, 24], and this variation may be fodder for selection of insecticide
83 resistance in the field. Indeed, *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae)
84 populations in cotton fields were found to be resistant to DDT and several organophosphates
85 by Head *et al.* [25] and Graves *et al.* [26]. More recently, a population of another lady beetle
86 species, *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae), collected from cabbage fields
87 in Brazil was found to be 20-fold resistant to the pyrethroid λ -cyhalothrin relative to other
88 populations [24].

89 The convergent lady beetle (CLB) *Hippodamia convergens* (Guérin-Méneville) is a
90 cosmopolitan species important in numerous agroecosystems [27]. Being widely distributed,
91 populations of CLB are exposed to a wide variety of insecticides across time and space [19,
92 23, 28, 29, 30]. This fact may explain differential survival among lady beetle species of cotton
93 fields in Georgia, USA, when exposed to λ -cyhalothrin, a broad spectrum pyrethroid
94 insecticide frequently used in various crops [23, 28, 30, 31].

95 This study was conducted to investigate pyrethroid resistance (specifically, λ -

96 cyhalothrin) in CLB in Georgia and to determine if the metabolism involved is suppressed by
97 the synergist piperonyl butoxide (PBO). Furthermore, inheritance of the resistance and
98 number of factors involved in the resistance were also examined.

99

100 **2. Material and Methods**

101 This study was carried out at the Biological Control Laboratory of the Tifton Campus of
102 the University of Georgia (Tifton, GA).

103 **2.1. Chemicals.** The insecticide used in the experiments was the pyrethroid λ -cyhalothrin
104 (technical grade 99.5%; Chem Service, West Chester, PA, USA) and the synergist piperonyl
105 butoxide (PBO) at 80% (Endura PB 80 EC-NF, 80% PBO, Endura Fine Chemicals, Bologna,
106 Italy).

107 **2.2. Sources of *H. convergens* (CLB) populations.** Two populations of *H. convergens* were
108 established and maintained in the laboratory. One population (designated 'Hc-CA'), which
109 originated from field collections in California (Central Valley near Fresno, CA), was
110 purchased in April 2011 from ARBICO Organics (Oro Valley, AZ). The second population
111 (designated 'Hc-GA') was established from beetles collected in crimson clover in Decatur
112 County, Georgia, USA (coordinates 30° 45' 45.34" N and 84° 28' 49.75" W) in April 2011.

113 **2.3. CLB maintenance.** Larvae and adults were reared using eggs of *Ephestia kuehniella*
114 (Zeller) (Lepidoptera: Pyralidae), obtained from Beneficial Insectary Inc. (Redding, CA,
115 USA). Beetles were held in environmentally controlled conditions of $25 \pm 1^\circ\text{C}$, and a
116 photoperiod of 14:10h (L:D) for all rearing and bioassays. The two populations were
117 maintained separately. Adults were kept in cylindrical plastic containers (30cm long, wide and
118 high) containing openings on the sides closed with nylon mesh. Later, individual pairs were
119 held in 500-ml plastic containers with a mesh-covered opening in the lid to allow ventilation,
120 and a piece of paper towel as an oviposition substrate. Eggs were transferred to transparent

121 30-mL plastic cups. Eggs produced by at least 20 adult pairs were used to maintain the
122 colonies and to provide insects for bioassays. Newly eclosed larvae were held individually in
123 30-ml plastic cups and provided *ad libitum* with eggs of *E. kuehniella*.

124 **2.4. Dose-response curves.** Adults of the F₁ generation from both populations (Hc-CA and
125 Hc-GA) were treated with the insecticide λ -cyhalothrin to determine the lethal dose (LD₅₀).
126 Preliminary bioassays were carried out to define doses which resulted in mortality from 0 to
127 100%. Insects were topically treated by applying a 0.5 μ l droplet of the appropriate solution to
128 the venter of the adult abdomen using a Hamilton syringe (25 μ L-volume). Based on
129 preliminary tests six doses for each population (0.001, 0.002, 0.004, 0.006, 0.008, and 0.01 g
130 a.i./L for Hc-CA; and 0.1, 0.3, 0.5, 0.7, 1.0, and 1.3 g a.i./L for Hc-GA) were selected for
131 calculating the dose-mortality curve and the LD₅₀. At least 20 adults (8 to 10 days old) were
132 tested per dose.

133 Treated and control groups were kept in petri dishes (12 cm diameter, and 1.5cm high)
134 lined with filter paper and provided with a 10% honey solution soaked in cotton batting inside
135 the petri dishes. Petri dishes with insects were stored in a climatic chamber at 25 \pm 1°C and
136 photoperiod 14:10h (L:D). Knockdown and mortality were assessed 2 and 24h after
137 insecticide application, respectively. A beetle was considered to be knocked down or dead if it
138 was unable to turn upright and begin to walk after being placed on its dorsum at the respective
139 observation intervals.

140 **2.5. Dose-response curves with the synergist PBO.** The insecticide λ -cyhalothrin (99.5%
141 technical grade) and the synergist PBO were applied in the bioassay diluted in acetone.
142 Previous tests of varying doses of PBO indicated that 10 g a.i. of PBO/L (10 ppm) was the
143 maximum sublethal dose and could be used in the dilutions to be tested. Thus, the synergism
144 ratio using PBO was determined for Hc-GA and Hc-CA populations by treating the insects
145 with λ -cyhalothrin dosage including PBO at 10 g a.i./L. The tested dosages of λ -cyhalothrin

146 alone began with a high dosage of 1 g a.i./L, which was then serially diluted by factors of 10
147 during the preliminary test to obtain the final dosages. The dosages of λ -cyhalothrin + PBO
148 used were: 0.0002, 0.0004, 0.0006, 0.0008, 0.001, and 0.003 g a.i./L for Hc-CA; and 0.005,
149 0.01, 0.03, 0.05, 0.08, 0.10, and 0.5 g a.i./L for Hc-GA. The bioassay was conducted using λ -
150 cyhalothrin + PBO, as well as control treatments using only PBO or acetone.

151 **2.6. Dominance and role of sex linkage in resistance.** The F₁ progeny was tested to evaluate
152 possible sex linkage related to the resistance. Females and males were kept individually in
153 transparent 30-ml plastic cups. Sexes were differentiated based on the shape of the distal
154 margin of the fourth visible abdominal sternite. The posterior margin of the fourth sternite has
155 a concave shape in males while in females it is a straight line. Reciprocal crosses between
156 virgin females (n=30) and males (n=30) from resistant (Hc-GA) and susceptible (Hc-CA)
157 populations were made to obtain F₁ progeny SR (♀ Hc-CA x ♂ Hc-GA) and RS (♀ Hc-GA x
158 ♂ Hc-CA). Free mating choice was allowed by pairing females and males of the two parental
159 populations in plastic containers (30cm long, wide and high). Each F₁ cross progeny (SR and
160 RS) was reared separately to obtain sufficient adults to calculate the LD₅₀.

161 To test for sex linkage, males from both F₁ reciprocal crosses (n=30) (SR and RS) were
162 backcrossed with parental females: BC1 (♀ Hc-GA x ♂ F₁ RS); BC2 (♀ Hc-GA x ♂ F₁ SR);
163 BC3 (♀ H-CA x ♂ F₁ RS); and BC4 (♀ Hc-CA x ♂ F₁SR). The progenies obtained from
164 backcross pairings were reared separately to obtain sufficient adults for each backcross to
165 calculate the LD₅₀ using 6 - 10 λ -cyhalothrin doses.

166 **2.7. Dominance of resistance in *H. convergens* to λ -cyhalothrin based on a single dose.** In
167 this bioassay we used 8-d old adults of the population groups Hc-CA (n = 120), HC-GA (n =
168 120), F₁ RS (n= 120) and F₁ SR (n = 120). Five previously determined doses of λ -cyhalothrin
169 (0.001, 0.01, 0.1, 0.5, and 1.0 g of a.i./L) were administered to adults of the different
170 population groups as previously described. The control group was treated only with acetone

171 (n = 10). The knockdown effect and mortality were assessed 2 and 24h after insecticide
172 application, respectively.

173 **2.8. Genetic variation within susceptible and resistant populations of *H. convergens*.** We

174 tested Hc-CA and Hc-GA for homozygosity of resistance traits in the respective populations.

175 Individual virgin females and males (n=5) were paired for mating and egg production to

176 compose five separate families. Then virgin female and male offspring of Hc-CA, Hc-GA, F₁

177 reciprocal crosses, F₁ RS and SR, and the four backcrosses (BC1 to BC4) were tested with a

178 discriminating dose of 0.5 g a.i of λ -cyhalothrin/L for homozygous resistance ($X^R X^R$ and

179 $X^R y$) following the same procedures used in the previous tests. Each adult pair corresponded

180 to a population family or specified cross progeny. By examining offspring in individual

181 families we could compare observed results with what would be expected for a homozygous

182 population in detail, allowing us to discern individual deviations from homozygosity that

183 could otherwise confound interpretation of results [32, 33]. As a component of this, the sex

184 determination system of *H. convergens* must be considered in evaluating a sex linkage model

185 for inheritance of insecticide resistance. The CLB has been characterized as $2n = 18$

186 autosomal and having homogametic females (XX) and heterogametic (Xy) males [34].

187 Therefore, males will be homozygous for traits acquired from the female on the X

188 chromosome.

189 **2.9. Data analysis.** The number of individuals exhibiting knockdown, death or survival per

190 dose in the resistance inheritance and synergism tests were used to calculate the knockdown

191 dose (KD) and the lethal dose (LD) for each population or progeny with the computer

192 program Polo PC [35], based on Probit analysis [36]. Correction for natural mortality was

193 unnecessary since control survival in all cases was 100%. A χ^2 goodness-of-fit test was used

194 to test for parallelism and equality of the dose-mortality curves between populations. Data

195 from resistance inheritance bioassays were used to obtain the resistance ratio (RR) between

196 resistant and susceptible populations based on the KD and LD calculated for each population,
197 F₁ progenies, and backcrosses. Likewise, the synergism ratio (SR) and the resistance ratio
198 (RR) were calculated for treatments with λ -cyhalothrin only or when the synergist PBO was
199 added. The RR and SR and their respective 95% confidence intervals (CI) were calculated and
200 considered significant when the CI did not include the value 1.0, following the method of
201 Robertson & Preisler [37].

202 Autosomal or sex-linked inheritance of resistance in *H. convergens* to λ -cyhalothrin was
203 tested using the KD and LD determined for F₁ adults from reciprocal crosses between Hc-GA
204 and Hc-CA populations, F₁ RS and F₁ SR progenies. The degree of dominance (*D*) was
205 estimated using the method of Stone [38], which is based on the KD or LD values. The
206 standard error (SE) of the degree of dominance was calculated following the method of
207 Lehmann [39], and interpreted after Preisler *et al.* [40]. The dominance (*h*) was estimated
208 based on a single dose, following Hartl [41].

209 The minimum number of genes controlling resistance was investigated using the
210 method of Lande [42] based on KD₅₀ and LD₅₀ responses. The minimum number of genes
211 driving resistance was calculated separately for F₁ progeny of *H. convergens* and the
212 respective backcrosses.

213 To evaluate genetic variation of parental populations, observed knockdown and
214 mortality were initially corrected for the number of males and females of *H. convergens*
215 tested. Thus, the testable hypothesis for genetic homozygosity is that the proportion of
216 observed knockdown or mortality would be equal to the proportion of expected knockdown or
217 mortality based on the sex-linked inheritance for *H. convergens*, assuming the recessive
218 inheritance of resistance found with the discriminatory dose (0.5 g a.i. of λ -cyhalothrin/L).
219 Thus, using the G-statistic goodness of fit test for heterogeneity [43], homogeneity was tested
220 among families and the hypothesis of absence of genetic variation was tested within and

221 among families. The goodness of fit test was carried out only on the results for F₁ RS and for
222 the backcross BC2 (♀Hc-GA x ♂ F₁ SR). The test was not conducted for families of the
223 susceptible population (Hc-CA), the F₁ SR progeny or their respective backcrosses (BC3 and
224 BC4) because the knockdown and mortality responses observed were as expected for all
225 families (1.00). Furthermore, for the resistant population (Hc-GA) and the backcross BC1 (♀
226 Hc-GA x ♂ F₁ RS), the expected mortality is null (0.00) and, therefore, a *G*-statistic could not
227 be calculated.

228

229 3. Results

230 **3.1. Dose-response curves.** The knockdown results fit the Probit model ($P > 0.05$). In contrast,
231 the dose-mortality curves differed in parallelism and equality ($P < 0.05$); thus the KD_{50s} and
232 KD_{90s} were calculated (Table 1). Based on KD₅₀ and KD₉₀ from evaluations 2h post-treatment
233 the Hc-GA population was over 286 and 461-fold more resistant by knockdown effect to λ-
234 cyhalothrin than Hc-CA adults (Table 1). The LD₅₀ and LD₉₀ of the Hc-CA population were,
235 respectively, 0.004 and 0.816 g a.i. of λ-cyhalothrin/L, compared to 0.015 and 4.595,
236 respectively, for the Hc-CA and Hc-GA populations. Based on these values, the Hc-GA
237 population was over 220 (LD₅₀) and 308.0-fold (LD₉₀) more resistant to λ-cyhalothrin than
238 the Hc-CA population (Table 1).

239 **3.2. Dose-mortality curves with the synergist PBO.** Adults from both populations exhibited
240 similar patterns of response for knockdown and mortality when treated with λ-cyhalothrin
241 plus the synergist PBO, but differed when using λ-cyhalothrin alone (Table 2). The KD₅₀ and
242 LD₅₀, however, were lower than when only λ-cyhalothrin was applied. The KD₅₀ and LD₅₀
243 synergism ratios were 1.62 and 6.94 (KD); and 5.53 and 17.24 (LD) for Hc-CA and Hc-GA
244 populations, respectively. The resistance ratio (RR) of λ-cyhalothrin based on the KD₅₀ or
245 LD₅₀ was reduced approximately 3-4 fold to ~70 for Hc-GA relative to Hc-CA when PBO was

246 added (Table 2). These results further demonstrate that the Hc-GA population is more resistant
247 to λ -cyhalothrin than the Hc-CA population. Furthermore, the LD₉₀ calculated for the Hc-GA
248 population is 10.44 times greater than the highest field rate of λ -cyhalothrin recommended to
249 spray cotton (0.44 g a.i./L).

250 **3.3. Dominance and role of sex linkage in resistance.** The RR for the F₁ RS beetles was
251 greater than that of the F₁ SR beetles when calculated using the KD₅₀, KD₉₀, LD₅₀, and LD₉₀
252 values, suggesting that resistance is X-linked (Table 1). Further the degree of dominance
253 varied from -0.66 to -0.13 based on KD₅₀, and from -0.48 to 0.27 based on KD₉₀ (Table 1).
254 The resistance ratios of the KD₅₀ for BC1 and BC2, both of which were offspring of Hc-GA
255 mothers, were 211.33 and 70.47-fold, respectively, whereas the KD₅₀ resistance ratios for
256 BC3 and BC4, which were offspring of Hc-CA mothers, were 2.81 and 2.91, respectively.
257 These results are consistent with X-linked resistance. Despite the low ratios for BC3 and BC4
258 they were significantly different from the parental Hc-CA population according to the method
259 of Robertson and Preisler [37] (Table 1).

260 The mortality data for the progenies and backcrosses fit a Probit model ($P > 0.05$), except
261 for the mortality of the F₁ RS progeny ($P < 0.05$). There were significant differences between
262 the F₁ progenies (SR and RS) in both the LD₅₀ and LD₉₀ [RR_{50(IC95%)}: 7.44 (4.48-12.35) and
263 TR_{90(IC95%)}: 24.11 (8.56-67.87)], which, taken with the backcross results, strongly suggests a
264 maternal effect or X-linked. The degree of dominance varied from -0.28 to 0.47 for the LD₅₀,
265 from -0.34 to 0.78 for the LD₉₀ (Table 1).

266 **3.4. Dominance of resistance in *H. convergens* to λ -cyhalothrin based on a single dose.**

267 The results indicate recessive dominance in the F₁ progenies tests and variability in the
268 resistance based on single dose results. The resistance was found to be functionally dominant
269 ($h = 1.0$) for the Hc-GA population at the lowest tested dose (0.001) for both reciprocal
270 crosses (RS and SR) (Table 3). For F₁ SR, however, resistance was functionally recessive ($h =$

271 0.0) at doses of 0.1 and 1.0 g a.i. of λ -cyhalothrin/L at 2 and 24h evaluations, respectively;
272 while for F₁ RS it was recessive only at the highest tested dose at knockdown 2h post-
273 treatment (Table 3). Based on mortality evaluated 24h post-treatment the effective dominance
274 ranged from 0.32 to 0.5 for doses greater than 0.1 g a.i. of λ -cyhalothrin/L for F₁ RS (Table
275 3).

276 **3.5. Minimum number of loci.** The number of loci coordinating resistance in *H. convergens*
277 to λ -cyhalothrin was estimated at -4.39 and 0.74 genes for the F₁ RS and F₁ SR progenies, and
278 for their respective backcrosses. On the other hand, when considering the mortality data, the
279 number of genes coordinating resistance is estimated at -1.23 and 3.73 for the F₁ progenies SR
280 and RS, and their backcrosses, respectively.

281 **3.6. Genetic variation within susceptible and resistant populations of *H. convergens*.** The
282 paired females and males from Hc-GA and the F₁ RS progeny resulted in four pairs that
283 produced viable offspring (families), out of the five pairs set up. Thus, only four families were
284 utilized for the BC1 and BC3 backcrosses. The knockdown and mortality results indicated
285 that Hc-GA male parents, used to form the ♀ Hc-GA x ♂ Hc-GA families, were not
286 susceptible to λ -cyhalothrin (i.e. the males of Hc-GA were not X^Sy). The genetic variation in
287 resistance observed in the Hc-GA population is likely related to the proportion of susceptible
288 adults produced by pairings of heterozygous females (X^RX^S) and resistant males (X^Ry)
289 (Tables 4 and 5). Families of the susceptible population (Hc-CA), the progeny of F₁ SR and
290 the backcrosses BC3 and BC4 exhibited responses aligned with the expected frequency of
291 susceptible offspring (1.00) (Tables 4 and 5). Families of F₁ RS were similar to one another in
292 knockdown (P = 0.6611) and mortality (P = 0.0948). Furthermore, the proportion of
293 individuals exhibiting knockdown and mortality was significantly different from the expected
294 proportion in three of the four families (Tables 4 and 5), evidencing genetic variation for
295 knockdown ($\chi^2 = 30.23$, P < 0.0001, df = 4) and mortality ($\chi^2 = 25.35$, P < 0.0001, df = 4).

296 Variation was observed among families of BC2 (♀ Hc-GA x ♂ F₁ SR) for knockdown ($\chi^2 =$
297 26.55, $P < 0.0001$, $df = 5$), but not for mortality ($\chi^2 = 0.55$, $P = 0.9932$, $df = 5$). Variation for
298 the knockdown effect was observed for only two out of five families (Table 4). Regardless of
299 individual family outcome, there was no difference among BC2 families based on knockdown
300 ($P = 0.3277$) or mortality ($P = 0.9942$). For the backcross BC1 (♀ Hc-GA x ♂ F₁ RS), the
301 high variability among families and variation from the expected response confirm the genetic
302 variation of their parental resistant population (Hc-GA).

303

304 **4. Discussion**

305 Resistance in *H. convergens* to λ -cyhalothrin was confirmed in a Georgia population,
306 and it appears to have multiple mechanisms that also may differ in inheritance. Based on
307 knockdown response (KD_{50}), the resistance seems to be autosomally inherited and
308 incompletely recessive, but based on KD_{90} the inheritance also appears to be sex-linked. Sex-
309 linked inheritance of resistance is also indicated based on lethal dose (LD) results calculated
310 for F₁ progenies 24h post-treatment. Several factors might contribute to the variability
311 observed in types of responses, including presence of heterozygotes in the parental population
312 causing unexpected genetic variation in reciprocal crosses (see below) and resulting in dose-
313 mortality curve slopes approaching 1.0 [44]. In addition, we cannot disregard genetic
314 differences of the two studied populations that probably also affect our results.

315 The metabolism of λ -cyhalothrin has at least one resistance mechanism in *H.*
316 *convergens*, as indicated by the action of the synergist PBO in significantly decreasing
317 resistance in the GA population. The estimated KDs and LDs were reduced by adding PBO to
318 λ -cyhalothrin for the resistant population. Recovery from knockdown by 24h post-treatment
319 was reduced by approximately 2/3 with addition of PBO, and a similar reduction was
320 observed in the LD responses (Table 2). However, resistance in the Hc-GA population was

321 not fully suppressed by PBO – resistance in this population was still approximately 70 times
322 that of Hc-CA after PBO was added. Thus, considering that the resistance was not fully
323 inhibited with PBO, further studies are needed to identify the other mechanism(s) present.

324 The hypothesis of sex-linked inheritance should be accepted if the KD and LD
325 calculated for backcrosses BC1 and BC2 are similar to the resistant Hc-GA population and F₁
326 RS, respectively, and if the KDs and LDs of backcrosses BC3 and BC4 are similar to those of
327 the F₁ SR progenies and the susceptible population (Hc-CA), respectively. Only the KDs and
328 LDs of BC2 and BC4 differed from the expected result. However, the limited differences
329 observed also suggest presence of genetic variation [45] or possible natural variation [46]
330 (Table 1). Furthermore, bioassays of single-paired crosses with the discriminating dose of λ -
331 cyhalothrin clearly indicated sex-linked inheritance for both knockdown (KDs) and mortality
332 (LDs) (Table 5). Additionally, the resistance phenotype of males carrying X^R-chromosome
333 yielded responses similar to those of females that were X^RX^R. Finally, estimates of the
334 minimum number of genes responsible for λ -cyhalothrin resistance in *H. convergens* based on
335 KDs and LDs also support sex linkage as the model of inheritance. Sex linkage inheritance
336 patterns tend to inflate phenotypic variances that are critical for estimating the number of
337 genes governing the trait [42]. This inflated variance confounds accurately estimating the
338 number of genes underlying the response, yielding results such as the negative gene estimated
339 values for the F₁ progenies obtained in this study.

340 The knockdown responses indicate that λ -cyhalothrin resistance in *H. convergens* is
341 inherited as a recessive trait. Thus, the difference in degree of dominance for the sex-linked
342 response is independent of the survival of the heterozygotes in F₁ RS progeny (dominant) and
343 mortality in the F₁ SR progeny (recessive) [47]. The difference is a result of varying mortality
344 patterns between the offspring of the F₁SR reciprocal cross compared to F₁ SR. Male F₁ RS
345 progeny would be resistant (X^Ry), while female progeny would be susceptible (X^RX^S). In

346 contrast, both male ($X^S y$) and female ($X^R X^S$) F_1 SR progeny would be susceptible. In this
347 way, the presence of resistant males in F_1 RS population inflates the KD and LD values,
348 affecting degree of dominance for each reciprocal cross depending on the magnitude of the
349 response for resistant individuals.

350 The mortality data for F_1 RS progeny did not fit the Probit model, indicating that the Hc-
351 GA population was not homozygous for resistance. Assaying for homozygosity revealed
352 presence of $X^R X^S$ females in the Hc-GA population. Despite the heterozygosity in the Hc-GA
353 population, it was not the only influencing factor because the KD for F_1 RS progeny fit the
354 Probit model. Some individuals of the F_1 SR progeny, as well as resistant individuals from Hc-
355 GA, recovered from knockdown (2h) during the 24h post-treatment mortality evaluation in
356 the bioassay of dose-mortality. The results from single-pair families demonstrated that the
357 gene influencing recovery from treatment might be also sex-linked, as males and females of
358 F_1 SR and females of F_1 RS did not recover 24h after treatment. However, the degree of
359 dominance was not conclusive because the discriminatory dose used in the single-pair cross
360 bioassay was sufficiently high to yield functionally recessive inheritance. Thus, a sex linkage
361 model can yield varying results for the resistance mechanisms.

362 Our results indicate that heterozygous Hc-GA females ($X^R X^S$) used in the F_1 RS
363 reciprocal cross can produce susceptible males ($X^S y$). The presence of susceptible males in
364 such a cross would not be anticipated for the offspring of reciprocal crosses (F_1 RS) if the
365 parental populations are homozygous susceptible ($X^S X^S$ and $X^S y$) or resistant ($X^R X^R$ and
366 $X^R y$), based on an “Xyp” sex determination system. Presence of susceptible males might
367 generate unusually low LDs and the conclusion that resistance is autosomally inherited. This
368 occurred with a heterogeneous population of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae)
369 tested for resistance to the CpGV (Baculoviridae), and resistance was originally characterized
370 as autosomally inherited [48]. However, after selection in the laboratory, single-pair

371 experiments with the selected homozygous-resistant *C. pomonella* population revealed that
372 inheritance was sex-linked [33]. Results from single-pair experiments with a heterozygous
373 population of *C. pomonella*, similar to our experiments, supported sex-linked inheritance for
374 resistance [49]. Based on the slopes of the dose-mortality curves calculated for F₁ RS and F₁
375 SR, there is also support for sex-linked heritability of resistance in *H. convergens* similar to *C.*
376 *pomonella* [49].

377 Numerous studies have reported recessive inheritance for pyrethroid resistance in
378 different groups of insects. However, sex-linked inheritance of resistance is not common
379 compared to autosomal inheritance. These results add to the reported cases of sex-linked
380 inheritance of resistance: *Sitophilus oryzae* L. (Col.: Curculionidae) [50], *Culex*
381 *quinquefasciatus* Say [51], *Sitophilus zeamais* Mots. [52], *Spodoptera littoralis* Boisduval
382 (Lepidoptera: Noctuidae) [53], *Helicoverpa armigera* Hübner [54], *Leptinotarsa*
383 *decemlineata* (Say) (Coleoptera: Chrysomelidae) [55], *Grapholita molesta* (Busck)
384 (Lepidoptera: Tortricidae) [56], and *C. pomonella* [33].

385 When λ -cyhalothrin is applied in high doses to resistant *H. convergens*, the effective
386 dominance is best characterized as recessive, but at lower doses it is functionally dominant.
387 This pattern of dominance has been reported in other insects [32, 57, 58, 59, 60, 61, 62].
388 Dominance is not an intrinsic trait of one allele [63], as its expression is dependent on the
389 dose applied [47]. Thus, when a dose is sufficiently high to kill all heterozygotes in the
390 population, the resistance can be functionally recessive, as described by Curtis *et al.* [64]. On
391 the other hand, at low doses in which the heterozygotes survive, resistance would be
392 characterized as functionally dominant. Numerically, we found no functionally recessive
393 response for F₁ RS progeny at high doses of λ -cyhalothrin. This can be explained by
394 inheritance driven by sex linkage due to the presence of X^Ry males.

395 Resistance of *H. convergens* to λ -cyhalothrin was likely selected by historically
396 widespread and intensive insecticide use in Georgia crop systems where the beetles regularly
397 occurred. Using cotton as an example, DDT was widely used during the 1950's to control boll
398 weevil and bollworms in cotton [65]. DDT was replaced with organophosphates (OPs) after
399 DDT resistance was detected in boll weevil [66]. Detection of bollworms resistant to OPs [67]
400 led, in turn, to wide and frequent use of pyrethroid insecticides in Georgia to control this
401 group of pests in the 1980's [68]. The persistence of boll weevil in cotton required repeated
402 applications of broad-spectrum insecticides beginning as early as the appearance of the first
403 flower bud and continuing until close to harvest, producing prolonged negative effects on
404 natural enemy populations [69]. Thus, the historically intensive use of DDT, OPs, and
405 pyrethroids in cotton fields, as well as other surrounding crops frequented by *H. convergens*
406 (e.g., pecans, tobacco, corn), would have applied significant selection pressure to *H.*
407 *convergens* populations for resistance. Even after pesticide use was dramatically reduced by
408 widespread adoption of Bt-transgenic cotton resistant to lepidopteran pests and following
409 eradication of the boll weevil in Georgia [69, 70], pyrethroids and OPs continue to be applied
410 for stink bugs and other pests [71]. The recently reduced application frequency of pyrethroids
411 and OPs to cotton likely reduced the negative effect on *H. convergens* populations and,
412 therefore, permitted resistance-conferring genes to be fixed in the population, affording the
413 stability typical of pyrethroid resistance.

414 Unlike the case with autosomally inherited resistance, sex linkage allows males of *H.*
415 *convergens* to exhibit resistance to λ -cyhalothrin even when the allele is present at low levels,
416 because they need only a single resistant allele to confer complete resistance. This capacity
417 may facilitate persistence and rapid spread of the resistant allele(s) in the population.
418 Information on factors that usually influence resistance, such as initial allele frequency in the
419 field population, population size, sex ratio in the field, adaptive costs of resistance, migration,

420 and polyandry in *H. convergens* are needed to better understand evolution of the resistance in
421 this important natural enemy species. However, initial results of resistance selection in Hc-GA
422 under laboratory conditions suggest rapid evolution of resistance can occur, as described for
423 recessive and sex-linked inherited resistance [54]. Variables, such as high frequency of the
424 allele for resistance, heterozygote female $X^R X^S$ being susceptible to λ -cyhalothrin and being
425 killed in the progeny, males requiring only one allele to survive the insecticide application,
426 and the interaction of resistance mechanisms driving the survival of susceptible individuals to
427 the insecticide application, can pace the evolution of resistance in *H. convergens*. Despite the
428 likelihood of multiple genes governing resistance of *H. convergens* to λ -cyhalothrin, the
429 nature of the interactions among these genes was not studied. The interaction among factors
430 governing inheritance of resistance is complex to define [72], but studies focusing on the role
431 of the multiple genes in resistance, the adaptive costs to maintain multiple resistance genes in
432 the absence of insecticide pressure, and the benefits of different resistance mechanisms in the
433 studied species are open avenues for investigation. For instance, we treated adults of Hc-GA
434 and Hc-CA with 10-fold the field rate of the organophosphate dicrotophos and the results
435 showed 100% and 0% survival for these two populations, respectively.

436 In conclusion, the inheritance of λ -cyhalothrin resistance in *H. convergens* is sex-linked
437 and recessive. Likely, the major mechanism of the resistance involves insensitivity of a kdr-
438 type target site, with participation of detoxifying enzymes, which were partially inhibited by
439 PBO leading to greater susceptibility of the resistant population (Hc-GA). These results differ
440 from those obtained for another lady beetle species, *E. connexa*, that exhibits resistance to the
441 λ -cyhalothrin, but in which resistance is autosomally inherited and incompletely dominant,
442 and which was fully inhibited with PBO with high activity of esterase (A.R.S.R. unpublished
443 data). Further, the LD_{50} and LD_{90} for the Hc-GA population (0.816 and 4.595 g) are greater
444 than the highest recommended field rate of λ -cyhalothrin for cotton (44 g of a.i/ha at 100

445 L/ha) Roberts *et al.* [73], indicating the possibility of effectively integrating these predators
 446 with pyrethroid insecticides.

447

448

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450

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Table 1. Knockdown and mortality responses of *Hippodamia convergens* susceptible (Hc-CA) and resistant (Hc-GA) populations, F1 progeny from reciprocal crosses and from backcrosses to λ -cyhalothrin during 2h and 24h evaluation intervals post-treatment, respectively. n, number of tested individuals; df, degrees of freedom; SE, standard error of the slope; CI, confidential intervals at 95% probability; DD, degree of dominance; and χ^2 , Chi-square test.

Population or Progeny ^a	n	df	Slope \pm SE	KD ₅₀ (CI _{95%}) ^b	RR ₅₀ (CI _{95%}) ^c	DD ₅₀ \pm SE	KD ₉₀ (CI _{95%}) ^b	RR ₉₀ (CI _{95%}) ^c	DD ₉₀ \pm SE	χ^2
<i>Knockdown - 2h evaluation</i>										
Hc-CA	191	4	2.39 \pm 0.42	0.001 (0.0004-0.002)	-		0.004 (0.002-0.011)	-		6.76
Hc-GA	221	4	1.73 \pm 0.28	0.297 (0.156-0.439)	286.75 (86.59-949.64)		1.636 (0.955-6.219)	461.16 (133.26-1595.93)		4.76
F ₁ RS	214	5	1.10 \pm 0.20	0.012 (0.005-0.021)	11.91 (5.43-26.11)	-0.13 \pm 0.15	0.182 (0.105-0.474)	51.11 (24.04-108.68)	0.27 \pm 0.17	4.50
F ₁ SR	220	4	1.52 \pm 0.19	0.003 (0.0002-0.007)	2.62 (0.57-12.02)	-0.66 \pm 0.27	0.019 (0.009-0.038)	5.35 (2.81-10.16)	-0.48 \pm 0.11	0.50
BC1	198	6	1.32 \pm 0.19	0.271 (0.162-1.14)	211.33 (111.96-398.90)		2.254 (1.02-15.43)	835.24 (252.59-2761.92)		6.35
BC2	167	4	0.72 \pm 0.20	0.073 (0.026-0.144)	70.47 (31.19-159.24)		4.480 (1.100-396.1)	1259.04 (143.76-11026.3)		6.33
BC3	267	8	2.27 \pm 0.33	0.003 (0.002-0.004)	2.81 (1.71-4.63)		0.011 (0.008-0.017)	3.00 (1.85-4.89)		4.78
BC4	268	8	2.63 \pm 0.40	0.003 (0.002-0.004)	2.91 (1.80-4.71)		0.009 (0.007-0.014)	2.61 (1.64-4.14)		1.78
<i>Mortality - 24h evaluation</i>										
Hc-CA	191	4	2.12 \pm 0.33	LD ₅₀ 0.004 (0.003-0.005)	-		LD ₉₀ 0.015 (0.010-0.028)	-		1.24
Hc-GA	221	4	1.71 \pm 0.32	0.816 (0.631-1.167)	220.03 (76.89-629.65)		4.595 (2.54-15.53)	308.00 (79.62-1191.39)		1.54
F ₁ RS	214	5	1.17 \pm 0.17	0.194 (0.059-1.745)	52.33 (32.30-84.80)	0.47 \pm 0.16	2.423 (0.545-14490)	162.29 (56.64-465.02)	0.78 \pm 0.26	19.63*
F ₁ SR	220	4	2.19 \pm 0.33	0.026 (0.019-0.034)	7.03 (4.89-10.11)	-0.28 \pm 0.09	0.100 (0.072-0.173)	6.73 (3.62-12.52)	-0.34 \pm 0.12	1.46
BC1	198	6	2.03 \pm 0.39	0.804 (0.548-1.441)	216.95 (131.14-358.92)		3.431 (1.793-12.971)	230.03 (85.46-619.16)		1.03
BC2	167	4	1.45 \pm 0.22	0.364 (0.245-0.621)	98.08 (59.26-162.32)		2.754 (1.346-9.637)	184.56 (65.92-516.78)		4.58
BC3	267	8	2.17 \pm 0.25	0.015 (0.012-0.019)	4.07 (2.90-5.71)		0.059 (0.043-0.091)	3.93 (2.19-7.08)		4.78
BC4	268	8	2.24 \pm 0.27	0.011 (0.009-0.014)	3.05 (2.17-4.27)		0.042 (0.031-0.065)	2.83 (1.58-5.08)		4.20

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^aF₁ RS and F₁ SR stand for reciprocal crosses between ♀ Hc-GA x ♂ Hc-CA and ♀ Hc-CA x ♂ Hc-GA, respectively; BC1, BC2, BC3, and BC4 are the backcrosses of ♀ Hc-GA x ♂ F₁ RS, ♀ Hc-GA x ♂ F₁ SR, ♀ Hc-CA x ♂ F₁ RS; and ♀ Hc-CA x ♂ F₁ SR, respectively. ^bg a.i./L of λ -cyhalothrin at technical grade producing 50 or 90% knockdown effect in the population 2h after treatment. ^cRR, resistance ratio estimated by the relationship of KDs or LDs between resistant and susceptible populations following the method of Robertson and Preisler [37]. *P-value (<0.05)

722 Table 2. Knockdown (2h) and mortality (24h) responses of *Hippodamia convergens* (Hc) populations from California (CA) and Georgia
 723 (GA) to λ -cyhalothrin (99.5% technical grade) only or with 10 ppm of piperonyl butoxide (PBO) added to the solution. n. number of tested
 724 adults; df = degree of freedom; SE = standard error for the slope; LDs = lethal doses in g of a.i./L; CI = 95% confidence intervals; and χ^2 = chi-
 725 square test.

Population/ Progeny	n	df	Slope \pm SE	LD ₅₀ (CI _{95%}) ^a	SR ₅₀ (CI _{95%}) ^b	RR ₅₀ (CI _{95%}) ^c	LD ₉₀ (CI _{95%}) ^a	SR ₉₀ (CI _{95%}) ^b	RR ₉₀ (CI _{95%}) ^c	χ^2
<i>Knockdown - 2h evaluation with λ-cyhalothrin</i>										
Hc-CA	191	4	2.39 \pm 0.42	0.001 (0.0004-0.002)	-	-	0.004 (0.002-0.011)	-	-	6.76
Hc-GA	221	4	1.73 \pm 0.28	0.297 (0.156-0.439)	-	286.75 (86.59-949.64)	1.636 (0.955-6.219)	-	461.16 (133.26-1595.93)	4.76
<i>Knockdown - 2h evaluation with λ-cyhalothrin + PBO</i>										
Hc-CA	278	4	2.64 \pm 0.33	0.0006 (0.0005-0.0008)	1.62 (1.07-2.45)	-	0.002 (0.001-0.004)	1.82 (1.16-2.86)	-	3.87
Hc-GA	182	5	1.45 \pm 0.23	0.043 (0.030-0.061)	6.94 (4.40-10.93)	67.05 (45.70-98.37)	0.327 (0.186-0.881)	5.00 (2.08-12.02)	167.81 (75.53-372.82)	0.69
<i>Mortality - 24h evaluation with λ-cyhalothrin</i>										
Hc-CA	191	4	2.12 \pm 0.33	0.004 (0.003-0.005)	-	-	0.015 (0.010-0.028)	-	-	1.24
Hc-GA	221	4	1.71 \pm 0.32	0.816 (0.631-1.167)	-	220.03 (76.89-629.65)	4.595 (2.54-15.53)	-	308.00 (79.62-1191.39)	1.54
<i>Mortality - 24h evaluation with λ-cyhalothrin + PBO</i>										
Hc-CA	278	4	3.30 \pm 0.42	0.0007 (0.0006-0.0008)	5.53 (4.23-7.22)	-	0.002 (0.001-0.003)	9.10 (5.34-15.49)	-	4.38
Hc-GA	182	5	1.57 \pm 0.24	0.047 (0.034-0.067)	17.24 (11.24-26.70)	70.55 (49.49-100.57)	0.309 (0.182-0.762)	14.84 (5.19-42.39)	188.81 (91.57-389.27)	3.43

726 ^ag a.i./L of λ -cyhalothrin at technical grade producing 50 or 90% knockdown or mortality effect in the population 2 and 24h after treatment, respectively.

727 ^bSR, synergism ratio based on the relationship of LD₅₀ or LD₉₀ calculated from populations treated with λ -cyhalothrin and λ -cyhalothrin + PBO following the method of
 728 Robertson and Preisler [37].

729 ^cRR, resistance ratio based on the relationships of LD₅₀ or LD₉₀ calculated from populations treated with λ -cyhalothrin and λ -cyhalothrin synergized with PBO following the
 730 method of Robertson and Preisler [37].

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733 Table 3. Dominance (h) of resistance in *Hippodamia convergens* adults based on knockdown and mortality
 734 responses evaluated 2h and 24h periods after treatment with different doses (g a.i. of λ -cyhalothrin) for susceptible
 735 (Hc-CA), resistant (Hc-GA), and F1 reciprocal crosses F1 SR (♀ Hc-CA x ♂ Hc-GA), and F1 RS (♀ Hc-GA x ♂ Hc-
 736 CA).
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Doses	Population/ Progeny	n	Knockdown (%)	h^a	Population/ Progeny	n	Mortality (%)	h^a
0.001	Hc-CA	24	33.33		Hc-CA	24	16.67	
	Hc-GA	24	0.00		Hc-GA	24	0.00	
	F ₁ SR	24	0.00	1.00	F ₁ SR	24	0.00	1.00
	F ₁ RS	24	0.00	1.00	F ₁ RS	24	0.00	1.00
0.01	Hc-CA	24	100.00		Hc-CA	24	91.67	
	Hc-GA	24	0.00		Hc-GA	24	0.00	
	F ₁ SR	24	83.33	0.17	F ₁ SR	24	16.67	0.82
	F ₁ RS	24	41.67	0.58	F ₁ RS	24	0.00	1.00
0.1	Hc-CA	24	100.00		Hc-CA	24	100.00	
	Hc-GA	24	33.33		Hc-GA	24	8.33	
	F ₁ SR	24	100.00	0.00	F ₁ SR	24	79.17	0.23
	F ₁ RS	24	75.00	0.38	F ₁ RS	24	54.17	0.50
0.5	Hc-CA	24	100.00		Hc-CA	24	100.00	
	Hc-GA	24	79.17		Hc-GA	24	20.83	
	F ₁ SR	24	100.00	0.00	F ₁ SR	24	95.83	0.05
	F ₁ RS	24	95.83	0.20	F ₁ RS	24	75.00	0.32
1.0	Hc-CA	24	100.00		Hc-CA	24	100.00	
	Hc-GA	24	95.83		Hc-GA	24	33.33	
	F ₁ SR	24	100.00	0.00	F ₁ SR	24	100.00	0.00
	F ₁ RS	24	100.00	0.00	F ₁ RS	24	70.83	0.44

738 ^a h varies between 0 and 1 (0 = survival is recessive and 1 = survival is dominant).

739 Table 4. Knockdown response (2h evaluation post-treatment) of resistant adults $X^R X^R$
 740 and $X^R y$ of *Hippodamia convergens* treated with a discriminatory dose (0.5 g a.i. of λ -
 741 cyhalothrin/L). Observed and expected proportions of knockdown are presented according to
 742 the progeny genotype and the null hypothesis: parental susceptible and homozygous resistant
 743 as function of inheritance of resistance linked to the X^R -chromosome with 1040 tested adults.
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Population/ Progeny ^a	Sex linkage		Expected proportion	F/n ^c	Observed proportion (SE)	χ^2	P
	Offspring genotype	Adults ^b					
	σ	ϕ	Adults ^b	F/n ^c	Adults ^b		
Hc-GA	$X^R y$	$X^R X^R$	0.00	A/20	0.67 (0.05)	NC ^d	NC
			0.00	B/30	0.37 (0.03)	NC	NC
			0.00	C/30	0.15 (0.06)	NC	NC
			0.00	D/40	0.48 (0.12)	NC	NC
Hc-CA	$X^S y$	$X^S X^S$	1.00	(A-E)/150	1.00 (0.00)	0.00	1.00
F1 RS	$X^R y$	$X^R X^S$	0.50	A/30	0.75 (0.00)	7.50	0.01*
			0.50	B/30	0.65 (0.06)	2.70	0.10
			0.50	C/30	0.77 (0.07)	8.53	<0.00*
			0.50	D/30	0.80 (0.01)	11.5	<0.00*
F1 SR	$X^S y$	$X^R X^S$	1.00	(A-E)/150	1.00 (0.00)	0.00	1.00
BC1	$X^R y$	$X^R X^R$	0.00	A/30	0.00 (0.00)	NC ⁴	NC
			0.00	B/30	0.18 (0.08)	NC	NC
			0.00	C/30	0.05 (0.03)	NC	NC
			0.00	D/30	0.53 (0.02)	NC	NC
BC2	$X^R y$	$X^R X^S$	0.50	A/30	0.63 (0.06)	1.88	0.16
			0.50	B/30	0.64 (0.02)	2.41	0.12
			0.50	C/30	0.63 (0.06)	1.88	0.16
			0.50	D/30	0.71 (0.12)	5.21	0.02*
			0.50	E/30	0.86 (0.04)	15.2	<0.00*
BC3	$X^S y$	$X^R X^S$	1.00	(A-D)/110	1.00 (0.00)	0.00	1.00
BC4	$X^S y$	$X^S X^S$	1.00	(A-E)/150	1.00 (0.00)	0.00	1.00

745 ^aSusceptible (Hc-CA) and resistant (Hc-GA) populations; F1 RS, cross of ϕ Hc-GA x σ Hc-
 746 CA, and F1 SR cross of ϕ Hc-CA x σ Hc-GA. The backcrosses BC1 (ϕ Hc-GA x σ F1 RS),
 747 BC2 (ϕ Hc-GA x σ F1 SR), BC3 (ϕ Hc-CA x σ F1 RS), and BC4 (ϕ Hc-CA x σ F1 SR).

748 ^bProportion of adults (mean pooled for males and females).

749 ^cF stands for families, and n stands for number of insects tested per family for each
 750 population, progeny, and backcrosses.

751 ^dNC stands for qui-square and p-values not determined; while *stands for significant
 752 deviation from the null hypotheses.

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757 Table 5. Mortality response 24h post-treatment of resistant adults $X^R X^R$ and $X^R y$ of
 758 *Hippodamia convergens* treated with a discriminatory dose (0.5 g a.i. of λ -cyhalothrin/L).
 759 Observed and expected proportions of mortality are presented according to the progeny
 760 genotype considering the null hypothesis: parental susceptible and homozygote resistant as
 761 function of inheritance of resistance linked to the X^R -chromosome with 1040 tested adults.

Population/ Progeny ^a	Sex linkage		Expected proportion	F/n ^c	Observed proportion (SE)	χ^2	P
	Offspring genotype						
	σ	♀	Adults ^b	Adults ^b			
Hc-GA	$X^R y$	$X^R X^R$	0.00	A/20	0.54 (0.01)	NC ^d	NC
			0.00	B/30	0.37 (0.03)	NC	NC
			0.00	C/30	0.00 (0.00)	NC	NC
			0.00	D/40	0.40 (0.15)	NC	NC
Hc-CA	$X^S y$	$X^S X^S$	1.00	(A-E)/150	1.00 (0.00)	0.00	1.00
F1 RS	$X^R y$	$X^R X^S$	0.50	A/30	0.75 (0.00)	7.50	0.01*
			0.50	B/30	0.50 (0.00)	0.00	1.00
			0.50	C/30	0.77 (0.09)	8.53	<0.00*
			0.50	D/30	0.78 (0.01)	9.31	<0.00*
F1 SR	$X^S y$	$X^R X^S$	1.00	(A-E)/150	1.00	0.00	1.00
BC1	$X^R y$	$X^R X^R$	0.00	A/30	0.00 (0.00)	NC	NC
			0.00	B/30	0.03 (0.03)	NC	NC
			0.00	C/30	0.00 (0.00)	NC	NC
			0.00	D/30	0.50 (0.00)	NC	NC
BC2	$X^R y$	$X^R X^S$	0.50	A/30	0.50 (0.00)	0.00	1.00
			0.50	B/30	0.53 (0.03)	0.13	0.72
			0.50	C/30	0.54 (0.04)	0.21	0.65
			0.50	D/30	0.50 (0.00)	0.00	1.00
			0.50	E/30	0.54 (0.04)	0.21	0.65
BC3	$X^S y$	$X^R X^S$	1.00	(A-D)/110	1.00	0.00	1.00
BC4	$X^S y$	$X^S X^S$	1.00	(A-E)/150	1.00	0.00	1.00

762 ^aSusceptible (Hc-CA) and resistant (Hc-GA) populations; F1 RS, cross of ♀ Hc-GA x σ Hc-CA, and
 763 F1 SR cross of ♀ Hc-CA x σ Hc-GA. The backcrosses BC1 (♀ Hc-GA x σ F1 RS), BC2 (♀ Hc-
 764 GA x σ F1 SR), BC3 (♀ Hc-CA x σ F1 RS), and BC4 (♀ Hc-CA x σ F1 SR).

765 ^bProportion of adults (pooled for males and females).

766 ^cF stands for families, and n stands for number of insects tested per family for each
 767 population, progeny, and backcrosses.

768 ^dNC stands for qui-square and p-values not determined; while *stands for significant
 769 deviation from the null hypotheses.