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## **Melanism in a Chinese population of *Harmonia axyridis* (Coleoptera: Coccinellidae): a criterion for male investment with pleiotropic effects on behavior and fertility**

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1 **Melanism in a Chinese Population of *Harmonia axyridis***  
2 **(Coleoptera: Coccinellidae): A Criterion for Male**  
3 **Investment with Pleiotropic Effects on Behavior and**  
4 **Fertility**

5

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32 **Running head: Male mating strategies in *Harmonia axyridis***

33 **Abstract** In Beijing, China, females of *Harmonia axyridis* are promiscuous but  
34 prefer typical (*succinea* form) males to melanic ones in the spring generation,  
35 ostensibly due to the thermal disadvantages of melanism during summer. We  
36 used laboratory observations to test whether males invested differentially in  
37 females according to their elytral color, and whether male behavior was  
38 phenotype-dependent. Video-recording was used to monitor no-choice mating  
39 tests between virgin adults in all phenotype combinations and females were  
40 isolated post-copula to observe their egg retention times and reproduction over  
41 five days. Females tended to wait longer before using the sperm of melanic  
42 males, and melanic females delayed longer than succinic females. Melanic  
43 males spent longer in copula with succinic than melanic females and the latter  
44 received fewer bouts of male abdominal shaking that correlate with sperm  
45 transfer, regardless of the phenotype of their mate. Although melanic males  
46 abandoned melanic females faster than did succinic males, they remained in  
47 copula with females of both phenotypes for a longer period after shaking,  
48 suggesting a larger investment in mate guarding by the less-preferred male  
49 phenotype. Although female fecundity did not vary among phenotype  
50 combinations, egg fertility was lower for females mated to melanic males,  
51 suggesting a pleiotropic effect of melanism on male fertility in addition to its  
52 effects on male mating behavior.

53

54 **Keywords**

55

56 male mate choice, mate guarding, pleiotropy, reproductive investment

57

58

59 **Introduction**

60

61 In promiscuous insect mating systems, females mate repeatedly with different  
62 males, setting the stage for sperm competition (Dickinson 1997; Simmons and  
63 Siva-Jothy 1998) that can exert strong selection on male mating behavior and  
64 lead to an evolutionary escalation of both intra- and intersexual conflict  
65 (Alexander et al. 1997). Consequently, males of promiscuous species may  
66 resort to various mating strategies aimed at ensuring or improving their paternity,  
67 including post-coital mate-guarding, the production of mating plugs to impede  
68 female remating (Thornhill and Alcock 1983) and the use of hormones in seminal  
69 fluids to manipulate female reproductive physiology (Eberhard 1996). In  
70 promiscuous mating systems, the potential arises for the availability of sperm (or  
71 associated seminal fluid proteins) to limit male reproductive success more than  
72 mere access to females (Avila et al. 2011). When this occurs, selection may  
73 favor males that invest differentially in females according to their quality,  
74 assuming that males are able to discriminate traits correlated with female fitness  
75 (Edward and Chapman 2011).

76 Many species of Coccinellidae exhibit variable elytral color patterns that  
77 often include melanic forms (e.g., Benham et al. 1974; reviewed in Majerus 1998  
78 and Sloggett and Honek 2012). Melanism influences heat absorption and thus  
79 may affect the fitness of insects under different conditions of temperature  
80 (Muggleton et al. 1975; Clusella Trullas et al. 2007; Martin-Vega and Arturo  
81 2011), hours of sunshine (Brakefield 1984a), and even humidity (Parkash et al.  
82 2009). Dark coloration tends to benefit beetles under conditions of low insolation  
83 and moderate temperature as absorption of more wavelengths of radiation  
84 permits earlier and higher activity levels, thus enhancing foraging activity and  
85 reproductive success (Brakefield 1984b). However, melanism is  
86 disadvantageous at high temperatures because it impedes an insect's ability to  
87 lose excess heat (Stewart and Dixon 1989). Consequently, the frequency of  
88 melanic morphs in beetle populations can vary both geographically (Creed 1966;

89 Davies et al. 2007; Sloggett and Honek 2012) and seasonally (Osawa and  
90 Nishida 1992; Nedved and Honek 2012).

91 In many populations of *Harmonia axyridis* (Pallas) in China and Japan, the  
92 frequency of melanic morphs fluctuates seasonally in response to seasonal  
93 cycles of female mate preference (e.g., Osawa and Nishida 1992); females of  
94 each generation prefer males that possess the background elytral coloration  
95 (melanic or succinic) most advantageous for conditions to be faced by progeny in  
96 the subsequent generation (Wang et al. 2009). The trait for melanic elytra is  
97 controlled by a single dominant gene in *H. axyridis* (Tan and Li 1934) and  
98 evidence exists to suggest that this allele has pleiotropic effects on mating  
99 behavior. For example, the strength of a female's preference for the seasonally  
100 appropriate male phenotype is influenced by her own elytral color in an adaptive  
101 manner (Wang et al. 2009). In the latter study, succinic females breeding in the  
102 fall generation showed a stronger non-preference for succinic males than did  
103 melanic females because, unlike the latter, the former are unable to produce any  
104 melanic offspring unless they mate with a melanic male. The same study  
105 revealed that female non-preference can be reflected in longer times to copula,  
106 more rejection events, and longer periods of egg retention post-copula when  
107 females are presented with less-preferred males. The mechanisms underlying  
108 the alternations in mate preference between spring and fall generations are not  
109 yet understood, but they appear independent of environmental cues and result in  
110 seasonal shifts in the proportion of melanics from 20% or less in the fall  
111 generation to 40-60% in the spring generation (Wang et al. 2009).

112 It is generally assumed that coccinellid females are largely (but not  
113 entirely) in control of the initiation of copula, whereas males control its duration  
114 (Wang et al. 2009; Sloggett and Honek 2012). Duration of copula is not a trivial  
115 parameter as coccinellids typically spend a very large fraction of their adult lives  
116 in mating pairs (Nedved and Honek, 2012). For example, Brakefield (1984a)  
117 observed that 23.5 – 44.0% of adult *Adalia bipunctata* L. were in copula at any  
118 given time in the field, whereas the observations of Haddrill et al. (2008) yielded  
119 an estimate of 20%. Paternity is typically shared by males mating multiply with

120 the same female, and the duration of copula tends to be correlated with  
121 probability of paternity (de Jong et al. 1998; Haddrill et al. 2008).

122 Copulations by pairs of *H. axyridis* typically last two to three hours (Wang  
123 et al. 2009; Nedved and Honek 2012). The period of copula can be crudely  
124 divided into three sequences of male behavior: (1) the initial latent period (the  
125 period from initial genital contact until the first bout of abdominal shaking), (2) a  
126 series of stereotyped bouts of abdominal shaking by the male, interspersed with  
127 resting intervals, that are assumed to be directly associated with sperm transfer  
128 (Obata 1987; Nedved and Honek 2012) and (3) the terminal latent period (the  
129 period of sustained genital contact from end of the last shaking bout until the pair  
130 separate). In the present experiments, we used continuous video monitoring of  
131 individual pairs to measure the frequency and duration of these male behaviors.  
132 We used the duration of the terminal latent period as a proxy measurement of  
133 male mate-guarding tendency and the duration of copula and number of shaking  
134 bouts as estimates of male reproductive investment per female. We  
135 hypothesized that spring generation *H. axyridis* males would invest more in  
136 succinic females than in melanic and that this preference would be reflected in  
137 differences in the duration of copula and in the frequency of male shaking  
138 behavior. Secondly, we hypothesized that melanism would have pleiotropic  
139 effects on male mating behavior as it does in females, i.e., melanic males reduce  
140 their investment in melanic females more than would succinic males. Thirdly, we  
141 hypothesized that the less-preferred melanic males would remain longer in  
142 copula after sperm transfer was complete, given the risk of their females  
143 remating with a male of the preferred phenotype.

144

## 145 **Materials and Methods**

146

147 Insects

148

149 Adult *H. axyridis* were collected from an apple orchard in rural Beijing, Changping  
150 County (n = 402 males and 447 females) during April, 2008 and maintained in

151 the entomology lab of the Institute of Plant and Environment Protection, Beijing  
152 Academy of Agriculture and Forestry Sciences. The beetles were held in  
153 aluminum frame screen cages (75.0 × 45.0 × 50.0 cm), 30 pairs of adults per  
154 cage and reared for a generation prior to production of the experimental insects.  
155 The beetles were fed *ad libitum* on cowpea aphids, *Aphis craccivora* Koch,  
156 reared on *Vicia faba* L. and refreshed every 48 h. The insects were held under  
157 constant environmental conditions of 25 ± 1 °C, 16:8 L:D daylength, 60-65 RH  
158 and with light supplied by a 3000 lux LED lamp. The experimental beetles  
159 corresponded to a spring generation in which succinic forms are the preferred  
160 phenotype (Wang et al. 2009).

161 Over the course of two days, ca. 40 clutches of eggs laid by different  
162 females were collected by clipping leaves with egg clusters and transferring them  
163 to plastic Petri dishes (9.0 cm diameter), one or two clusters per dish. At  
164 eclosion, first instar larvae were transferred to a rearing cage (as above), 50  
165 larvae per cage. Cowpea aphids were provided as food and once larvae  
166 pupated they were isolated in plastic Petri dishes (4.5 cm diameter), one per  
167 dish. Newly emerged adults (> 4 h post-emergence) were sexed according to  
168 the colour of the clypeus, which is pale in males and dark in females (Yu, 2004).  
169 The adults for use in experiments were categorized as melanic (elytral  
170 background colour black with either two or four large red-orange spots) or  
171 succinic (elytra background colour yellow or orange, either immaculate or with  
172 variable numbers of small black spots) and maintained as four separate groups  
173 in cages (as above): melanic males, melanic females, succinic males, and  
174 succinic females. Virgin adults were used in mating experiments when they were  
175 15 - 20 days old.

176

#### 177 Behavioral Observations

178

179 To examine whether male mating behaviour varied among colour morph  
180 combinations, all four phenotype combinations were observed: 1) melanic male +  
181 melanic female ( $\text{♂}_M + \text{♀}_M$ ), 2) melanic male + succinic female ( $\text{♂}_M + \text{♀}_S$ ), 3)

182 succinic male + melanic female ( $\text{♂}_S + \text{♀}_M$ ) and 4) succinic male + succinic female  
183 ( $\text{♂}_S + \text{♀}_S$ ). Environmental conditions were adjusted to mimic natural late spring  
184 conditions in Beijing (day T = 16 °C, night T = 11 °C, L:D = 14:10, RH = 55 ±  
185 65%).

186 For observation, *H. axyridis* adults were paired in plastic Petri dishes (9.0  
187 cm diameter) with *ad libitum* food (> 0.05 g *A. craccivora* / dish) and dishes were  
188 covered with a layer of perforated parafilm. A HD digital video camera (Sony  
189 HDR-FX1E-1080i-Hi vision) fixed on a tripod was then focused on the dish and  
190 video was captured on a computer hard drive for subsequent analysis.

191 Video recording was initiated once a male mounted a female and was  
192 terminated once the pair separated. We observed 20 mating pairs of each  
193 phenotypic combination and tallied data for individual components of male  
194 copulation behavior from the video recordings as follows; the initial latent period  
195 (the period from genital contact until onset of the first shaking bout), the total  
196 duration of copula, and the terminal latent period (the period from the last  
197 shaking bout until disengagement of genitalia). A 'shaking bout' was defined as a  
198 discrete period of rapid, side-to-side oscillations of the male's abdomen while in  
199 copula and the number of shaking bouts was tallied for each mating. Following  
200 each observation period, the male was removed, cowpea aphids were  
201 provisioned, and all egg clusters laid by the female were collected for five days.  
202 Egg clusters were incubated under the same conditions as adults until eclosion  
203 and egg fertility was determined.

204

#### 205 Statistical analysis

206

207 The seven responses (initial latent period, duration of copula, terminal latent  
208 period, number of shaking bouts, egg retention time, fecundity and fertility) were  
209 analyzed as a set by 2-way Multivariate Analysis of Variance (MANOVA) with  
210 'male' and 'female' phenotypes as fixed factors using the approximate F for Wilks'  
211 Lambda. The partial correlation coefficients with t-test statistics were calculated  
212 from the MANOVA E-matrix and used to examine correlations between



213 responses. Responses were then analyzed individually by univariate ANOVA to  
214 explain differences seen in the MANOVA. Both MANOVA and ANOVA analyses  
215 were conducted using the GLM procedure of SAS (version 9.3). Pairwise mean  
216 comparisons of the four different phenotype combinations were done when the  
217 overall test of phenotype combinations was significant for the MANOVA and  
218 seven ANOVA analyses. To control for Type 1 error for the multivariate and  
219 univariate analyses, we used a Bonferroni-adjusted alpha level of  $0.05/10 =$   
220  $0.005$  (where 10 is the total number of tests performed).

221 Normality of residuals was also checked for each response using the  
222 Univariate procedure of SAS v. 9.3 (SAS Institute 2013) with  $\alpha = 0.01$ . Tests  
223 of residual normality indicated no problems for all of the response variables  
224 except for number of shaking bouts (slight upward skew) and egg retention time  
225 (slight downward skew). In addition, the assumption of common variance for the  
226 four male\*female combinations was checked using the Brown-Forsythe test in  
227 GLM for all response variables, with the result that no response variables  
228 displayed nonhomogeneity of variance at an alpha of 0.01 (see Milliken and  
229 Johnson, 2009). Given the sample size, the MANOVA and ANOVA results  
230 should be robust with respect to slight departures from normality.

231

232

## 233 **Results**

234

235 The two-way MANOVA analysis (Table 1) showed significant results for the  
236 overall model, male and female main effects and the male\*female interaction ( $\alpha$   
237  $= 0.005$ ). Pairwise comparisons between the four phenotypes indicated that,  
238 based on the set of seven response variables, all phenotypes were highly  
239 significantly different from each other except for the ( $\sigma_s + \phi_s$ ) vs ( $\sigma_s + \phi_M$ )  
240 comparison ( $P = 0.0623$ ; Table 1). Thus, over all responses, melanic males  
241 differed in their behavior (and its consequences for female reproduction)  
242 according to the phenotype of their mate, whereas succinic males did not.

243

244 **Table 1.** Results of multivariate tests using Wilks' Lambda, Bonferroni correction  
 245 for multiple pairwise comparisons,  $\alpha = 0.05/6 = 0.00833$ .

246

Comparison	Wilks' Lambda Approximate <i>F</i>	df	<i>P</i>
Overall model	7.23	21,86.7	< 0.0001
Male main effect	16.82	7,30	< 0.0001
Female main effect	7.34	7,30	< 0.0001
Interaction	3.53	7,30	0.0070
(♂ <sub>S</sub> + ♀ <sub>S</sub> ) vs (♂ <sub>S</sub> + ♀ <sub>M</sub> )	2.20	7,30	0.0623
(♂ <sub>S</sub> + ♀ <sub>S</sub> ) vs (♂ <sub>M</sub> + ♀ <sub>S</sub> )	5.35	7,30	0.0005
(♂ <sub>S</sub> + ♀ <sub>S</sub> ) vs (♂ <sub>M</sub> + ♀ <sub>M</sub> )	15.65	7,30	< 0.0001
(♂ <sub>S</sub> + ♀ <sub>M</sub> ) vs (♂ <sub>M</sub> + ♀ <sub>S</sub> )	8.51	7,30	< 0.0001
(♂ <sub>S</sub> + ♀ <sub>M</sub> ) vs (♂ <sub>M</sub> + ♀ <sub>M</sub> )	14.99	7,30	< 0.0001
(♂ <sub>M</sub> + ♀ <sub>S</sub> ) vs (♂ <sub>M</sub> + ♀ <sub>M</sub> )	8.66	7,30	< 0.0001

247

248 The partial correlations obtained in the MANOVA analysis indicated that  
 249 there were significant correlations for only two pairs of responses. Duration of  
 250 copula and number of shaking bouts were positively correlated ( $R = 0.827$ ,  $P <$   
 251  $0.001$ ) as were terminal latent period and fecundity ( $R = 0.336$ ,  $P = 0.042$ ).

252 Clearly, a greater number of shaking bouts cannot occur without a corresponding  
 253 increase in the duration of copula, but correlation of terminal latent period with  
 254 fecundity is unclear and may be a chance result without biological significance.

255 The 2-way ANOVA for initial latent period was significant ( $F = 9.85$ ;  $df =$   
 256  $3,36$ ;  $P < 0.001$ ) at  $\alpha = 0.005$ , with significant effects of male phenotype ( $F =$   
 257  $28.73$ ;  $df = 1,36$ ;  $P < 0.001$ ) but not female ( $F = 0.01$ ;  $df = 1,36$ ;  $P = 0.945$ ), and  
 258 without a significant interaction ( $F = 0.82$ ;  $df = 1,36$ ;  $P = 0.372$ ). Although initial  
 259 latent periods were relatively short, ranging from 50 -70 seconds, melanic males  
 260 exhibited significantly longer initial latent periods than succinic males, regardless  
 261 of female phenotype (Fig. 1A). The overall model was also significant for total  
 262 duration of copula ( $F = 20.40$ ;  $df = 3,36$ ;  $P < 0.001$ ), with significant effects of  
 263 both male ( $F = 16.29$ ;  $df = 1,36$ ;  $P < 0.001$ ) and female ( $F = 35.89$ ;  $df = 1,36$ ;  $P <$

264 0.001) phenotype and a significant interaction term ( $F = 9.01$ ;  $df = 1,36$ ;  $P =$   
265 0.005). Melanic males spent less time in copula with melanic females than did  
266 males in other phenotype combinations, with the latter not significantly different  
267 from one another (Fig. 1B). The overall model was also significant for the  
268 terminal latent period ( $F = 9.37$ ;  $df = 3,36$ ;  $P < 0.001$ ) with significant effects of  
269 male ( $F = 26.43$   $df = 1,36$ ;  $P < 0.001$ ) but not female ( $F = 1.65$ ;  $df = 1,36$ ;  $P =$   
270 0.207) phenotype and a nonsignificant interaction term ( $F = 0.04$ ;  $df = 1,36$ ;  $P =$   
271 0.842). Melanic males remained coupled with females for longer periods  
272 following completion of shaking behavior than did succinic males (Fig. 1C).

273 Two way ANOVA of the total number of shaking bouts yielded a significant  
274 overall result ( $F = 17.12$ ;  $df = 3,36$ ;  $P < 0.001$ ), with a significant effect of female  
275 phenotype ( $F = 47.19$ ;  $df = 1,36$ ;  $P < 0.001$ ), but not male ( $F = 0.22$ ;  $df = 1,36$ ;  $P$   
276  $= 0.643$ ), and with an interaction that was close to significance ( $F = 3.95$ ;  $df =$   
277  $1,36$ ;  $P = 0.055$ ). Succinic females received a larger number of shaking bouts  
278 than did melanic females, regardless of the phenotype of the male (Fig. 2A). The  
279 overall model was also significant for female egg retention time following pair  
280 separation ( $F = 16.07$ ;  $df = 3,36$ ;  $P < 0.001$ ), with a significant effect of male  
281 phenotype ( $F = 37.24$ ;  $df = 1,36$ ;  $P < 0.001$ ), but not female ( $F = 2.81$ ;  $df = 1,36$ ;  
282  $P = 0.103$ ), although the interaction term was significant ( $F = 8.16$ ;  $df = 1,36$ ;  $P =$   
283  $0.007$ ). Egg retention times were longest for melanic females that mated with  
284 melanic males, followed by succinic females that mated with melanic males (Fig.  
285 2B), the latter retaining eggs longer than melanic females mated to succinic  
286 males, with  $\text{♂}_S + \text{♀}_S$  pairs intermediate between these two (Fig. 2B). There was  
287 no effect of phenotype combination on female fecundity over the first five days of  
288 reproduction (mean =  $102 \pm 3.4$  eggs;  $F = 0.60$ ;  $df = 3,36$ ;  $P = 0.622$ ), but there  
289 was a significant effect on fertility ( $F = 8.99$ ;  $df = 3,36$ ;  $P < 0.001$ ). Male  
290 phenotype influenced female fertility ( $F = 25.80$ ;  $df = 1,36$ ;  $P < 0.001$ ) but female  
291 phenotype did not ( $F = 0.10$ ;  $df = 1,36$ ;  $P = 0.751$ ) and the interaction term was  
292 nonsignificant ( $F = 1.07$ ;  $df = 1,36$ ;  $P = 0.307$ ). Females mating with succinic  
293 males hatched a significantly larger proportion of their eggs than did females  
294 mating with melanic males (Fig. 2C).

295

296 **Discussion**

297

298 The insects in our experiment corresponded to a spring generation in which  
299 succinic males are preferred by females in both choice and no-choice situations  
300 (Wang et al. 2009), ostensibly because of the fitness disadvantages incurred by  
301 melanic forms under the hot conditions of summer (Brakefield and Wilmer 1987).  
302 Female preference for succinic males is reflected in a higher frequency of  
303 melanic male rejections and delayed onset of copula, the effect being strongest  
304 in melanic females (Wang et al. 2009). In the present study, female preference  
305 for succinic males was evident in egg retention times that reflect cryptic female  
306 choice (Eberhard 1997); females tended to wait longer to use the sperm of  
307 melanic males, and melanic females tended to wait longer than succinic females  
308 (Fig. 2B), consistent with previous observations (Wang et al. 2009). Thus  
309 melanism is not only a criterion for female mate choice, but also appears to have  
310 pleiotropic effects on the strength of the preference in females. An alternative  
311 mechanism could be a linkage disequilibrium among loci controlling different  
312 traits, which seems less likely when one considers the observed seasonal  
313 fluctuations in multiple traits that would require entire gene complexes to flip back  
314 and forth in frequency between generations in order to generate the differences  
315 observed between spring and fall populations (Wang et al. 2009).

316 Bonduransky (2001) reviewed the evidence for male mate choice in  
317 insects and noted that selection for male choosiness should be stronger in  
318 systems where female fitness increases with number of copulations, as it does in  
319 *H. axyridis* (e.g., Quinones Pando et al. 2001). Males mating with melanic  
320 females spent less time in copula than did their counterparts with succinic  
321 females and there was also an effect of male phenotype; melanic males  
322 abandoned melanic females sooner than did succinic males (Fig. 1B).

323 Therefore, possession of the melanic allele strengthens mate preference for the  
324 succinic phenotype in males much as it does in females, an adaptive pleiotropy  
325 given the dominance of the melanic allele (Tan and Li 1934). This finding differs

326 from previous observations of the same *H. axyridis* population (Wang et al. 2009)  
327 in which the duration of copula was relatively invariant among phenotype  
328 combinations in no-choice tests, although differences emerged when males were  
329 allowed to select females in a choice setting. In the present study, males  
330 engaged in fewer shaking bouts with melanic females compared to succinic  
331 females regardless of their phenotype (Fig. 1D). Since both duration of copula  
332 and bouts of abdominal shaking are correlated with seminal transfer in *H.*  
333 *axyridis* (Obata 1987), these results supported our hypotheses that males would  
334 invest differentially in females based on their elytral coloration, and that melanic  
335 males would reduce their investment in melanic females more than succinic  
336 males.

337         Even though initial latent periods were likely too short to be of much  
338 consequence to male mating strategy, they were significantly longer for melanic  
339 males than for succinic males, regardless of female phenotype (Fig. 1A). More  
340 significantly, the same pattern was evident for terminal latent periods (Fig. 1C)  
341 that were roughly an order of magnitude longer in duration. Thus, melanic males  
342 invested more effort in guarding females post-insemination, supporting our third  
343 hypothesis. Studies of sperm precedence in *Adalia bipunctata* suggest that the  
344 paternity advantage of the second male can be very high in coccinellids (de Jong  
345 et al. 1993). If the probability of female remating is higher following matings with  
346 melanic males, as their longer egg retention times would suggest, an increased  
347 investment in mate guarding may be an adaptive strategy for melanic males.

348         Interestingly, mating with melanic males reduced female fertility by  
349 approximately 25% relative to matings with succinic males, independent of  
350 female phenotype (Fig. 2D), suggesting a negative pleiotropic effect of melanism  
351 on male fertility. Rhamhalinghan (1998) reported higher fecundity of melanic  
352 females compared to typicals in a polymorphic population of *C. septempunctata*  
353 but did not report any differences in fertility. The fitness costs of reduced male  
354 fertility, in conjunction with exposure of the melanic allele to selection in  
355 heterozygotes, may explain why the equilibrium frequency of the succinic  
356 phenotype is approximately 2.5 times that of the melanic in this population of *H.*

357 *axyridis* (Wang et al. 2009). Notably, females mated to melanic males did not  
358 reduce the number of eggs they laid relative to those mated with succinic males,  
359 suggesting that melanism did not influence any putative allohormonal effects of  
360 seminal fluids on female fecundity (Eberhard 1997). Aphidophagous coccinellids  
361 exhibit a 'front-loaded' distribution of reproductive effort (Dixon and Agarwala  
362 2002, Michaud and Qureshi 2006; Vargas et al. 2012), i.e., daily female fecundity  
363 is highest early in life and declines thereafter. Thus, it would seem that mated  
364 females simply oviposit at a maximal rate once they initiate oviposition,  
365 regardless of the relative quality of their most recent mate.

366 To the best of our knowledge, the only previous report of melanism  
367 influencing the mating behavior of males was provided by Horth (2003) who  
368 observed that melanic male mosquitofish were more sexually aggressive than  
369 typical silver males. Although melanism commonly affects activity levels in  
370 insects (e.g. Verhoog et al. 1998), it may have no measurable effects on either  
371 fitness or mate selection in some species (e.g., Nahrung and Allen 2005). True  
372 (2003) reviewed the molecular basis of various melanic pleiotropies in insects  
373 and discussed their potential consequences for life history and behavior but  
374 found only one reported effect on reproductive success (de Jong et al. 1998:  
375 melanic males of *A. bipunctata* require longer copulation times). However, Ma et  
376 al. (2008) subsequently examined a spontaneous laboratory example of  
377 dominant autosomal melanism in *Helicoverpa armigera* Hübner and found that  
378 melanism was associated with slower development in all juvenile life stages  
379 (even though it was only expressed in the pupal and adult stages), lower body  
380 weight, lower mating frequency and fecundity, reduced duration of copula, and  
381 assortative mating. In contrast, studies of melanism in *Mythimna separata*  
382 (Walker) revealed opposite results; homozygous melanic moths had higher  
383 juvenile survival, faster development and greater reproductive success than  
384 homozygous typicals (Jiang et al. 2007). Thus, it seems reasonable to expect  
385 that other pleiotropic effects of melanism on insect mating behavior remain to be  
386 discovered.

387

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391

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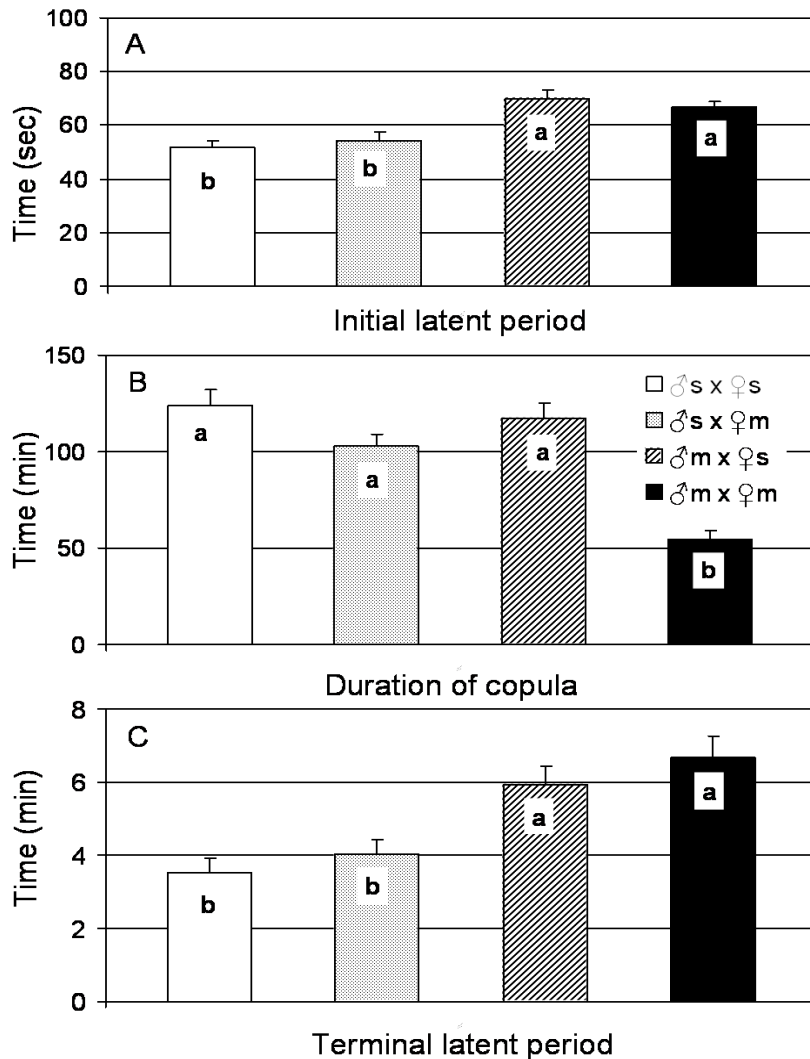
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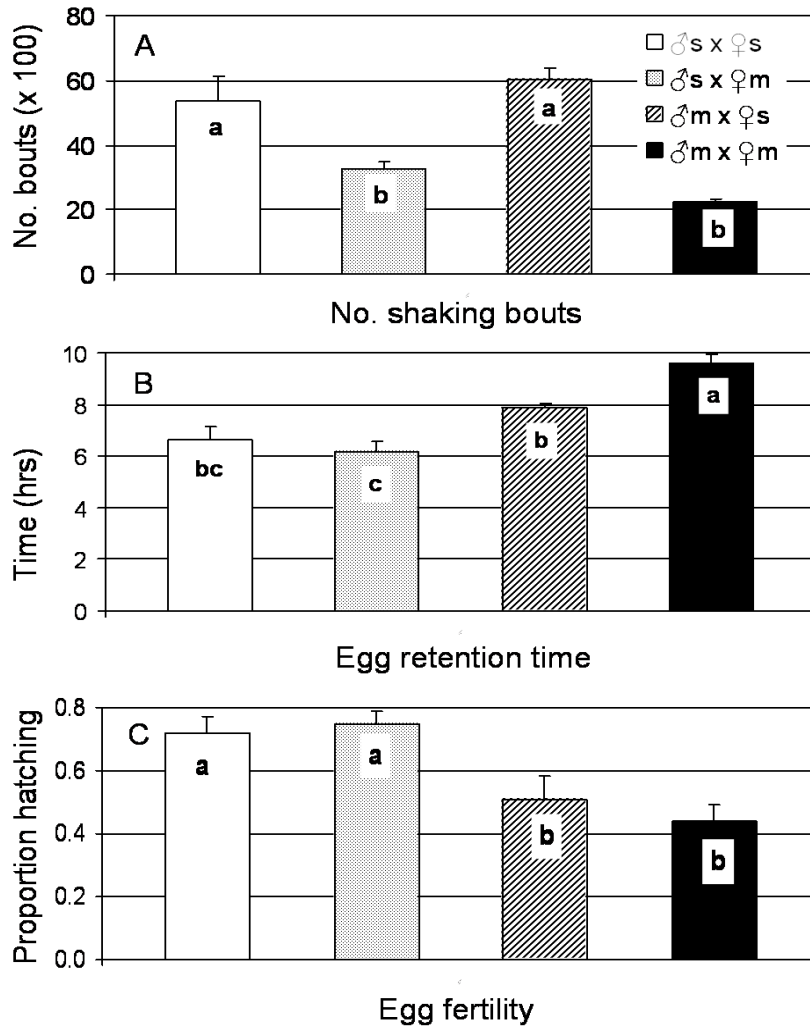
516 Figure 1



517

518 **Figure 1.** Mean (+ SE) initial latent period (A), duration of copula (B), and  
 519 terminal latent period (C) for matings between various phenotype combinations  
 520 of *H. axyridis* in no-choice mating tests (succinic ♂ + succinic ♀, open columns;  
 521 succinic ♂ + melanic ♀, shaded columns; melanic ♂ + succinic ♀, hatched  
 522 columns; melanic ♂ + melanic ♀, solid columns). Columns bearing the same  
 523 letters were not significantly different (LSD test,  $\alpha > 0.05$ ). See text for behavior  
 524 definitions.

525



527

528 **Figure 2.** Mean (+ SE) number of male shaking bouts (A), female egg retention  
 529 time (B) and egg fertility (C) for matings between various phenotype  
 530 combinations of *H. axyridis* in no-choice mating tests (succinic ♂ + succinic ♀,  
 531 open columns; succinic ♂ + melanic ♀, shaded columns; melanic ♂ + succinic ♀,  
 532 hatched columns; melanic ♂ + melanic ♀, solid columns). Columns bearing the  
 533 same letters were not significantly different (LSD test,  $\alpha > 0.05$ ). See text for  
 534 behavior definitions.

535