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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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Abstract In Beijing, China, females of *Harmonia axyridis* are promiscuous but prefer typical (*succinea* form) males to melanic ones in the spring generation, ostensibly due to the thermal disadvantages of melanism during summer. We used laboratory observations to test whether males invested differentially in females according to their elytral color, and whether male behavior was phenotype-dependent. Video-recording was used to monitor no-choice mating tests between virgin adults in all phenotype combinations and females were isolated post-copula to observe their egg retention times and reproduction over 5 days. Females tended to wait longer before using the sperm of melanic males, and melanic females delayed longer than succinic females. Melanic males spent longer in copula with succinic than melanic females and the latter received fewer bouts of male abdominal shaking that correlate with sperm transfer, regardless of the phenotype of their mate. Although melanic males abandoned melanic females faster than did succinic males, they remained in copula with females of both phenotypes for a longer period after shaking, suggesting a larger investment in mate guarding by the less-preferred male phenotype. Although female fecundity did not vary among phenotype combinations, egg

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fertility was lower for females mated to melanic males, suggesting a pleiotropic effect of melanism on male fertility in addition to its effects on male mating behavior.

Keywords Male mate choice · mate guarding · pleiotropy · reproductive investment

Introduction

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

Many species of Coccinellidae exhibit variable elytral color patterns that often include melanic forms (e.g., Benham et al. 1974; reviewed in Majerus 1998 and Sloggett and Honek 2012). Melanism influences heat absorption and thus may affect the fitness of insects under different conditions of temperature (Muggleton 1975; Clusella Trullas et al. 2007; Martin-Vega and Baz 2011), hours of sunshine (Brakefield 1984a), and even humidity (Parkash et al. 2009). Dark coloration tends to benefit beetles under conditions of low insolation and moderate temperature as absorption of more wavelengths of radiation permits earlier and higher activity levels, thus enhancing foraging activity and reproductive success (Brakefield 1984b). However, melanism is disadvantageous at high temperatures because it impedes an insect's ability to lose excess heat (Stewart and Dixon 1989). Consequently, the frequency of melanic morphs in beetle populations can vary both geographically (Creed 1966; Davies et al. 2007; Sloggett and Honek 2012) and seasonally (Osawa and Nishida 1992; Nedved and Honek 2012).

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

It is generally assumed that coccinellid females are largely (but not entirely) in control of the initiation of copula, whereas males control its duration (Wang et al. 2009; Sloggett and Honek 2012). Duration of copula is not a trivial parameter as coccinellids typically spend a very large fraction of their adult lives in mating pairs (Nedved and Honek 2012). For example, Brakefield (1984a) observed that 23.5–44.0 % of adult *Adalia bipunctata* L. were in copula at any given time in the field, whereas the observations of Haddrill et al. (2008) yielded an estimate of 20 %. Paternity is typically shared by males mating multiply with the same female, and the duration of copula tends to be correlated with probability of paternity (de Jong et al. 1998; Haddrill et al. 2008).

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

Materials and Methods

Insects

Adult *H. axyridis* were collected from an apple orchard in rural Beijing, Changping County (n=402 males and 447 females) during April, 2008 and maintained in the entomology lab of the Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences. The beetles were held in aluminum frame screen cages (75.0×45.0×50.0 cm), 30 pairs of adults per cage and reared for a generation prior to production of the experimental insects. The beetles were fed ad libitum on cowpea aphids, *Aphis craccivora* Koch, reared on *Vicia faba* L. and refreshed every 48 h. The insects were held under constant environmental conditions of 25±1 °C, 16:8 L:D daylength, 60–65 RH and with light supplied by a 3,000 lx LED lamp. The experimental beetles corresponded to a spring generation in which succinic forms are the preferred phenotype (Wang et al. 2009).

Over the course of 2 days, ca. 40 clutches of eggs laid by different females were collected by clipping leaves with egg clusters and transferring them to plastic Petri dishes (9.0 cm diameter), one or two clusters per dish. At eclosion, first instar larvae were transferred to a rearing cage (as above), 50 larvae per cage. Cowpea aphids were provided as food and once larvae pupated they were isolated in plastic Petri dishes (4.5 cm diameter), one per dish. Newly emerged adults (>4 h post-emergence) were sexed according to the colour of the clypeus, which is pale in males and dark in females. The adults for use in experiments were categorized as melanic (elytral background colour black with either two or four large red-orange spots) or succinic (elytra background colour yellow or orange, either immaculate or with variable numbers of small black spots) and maintained as four separate groups in cages (as above): melanic males, melanic females, succinic males, and succinic females. Virgin adults were used in mating experiments when they were 15–20 days old.

Behavioral Observations

To examine whether male mating behaviour varied among colour morph combinations, all four phenotype combinations were observed: 1) melanic male + melanic female ($♂_M + ♀_M$), 2) melanic male + succinic female ($♂_M + ♀_S$), 3) succinic male + melanic female ($♂_S + ♀_M$) and 4) succinic male + succinic female ($♂_S + ♀_S$). Environmental conditions were adjusted to mimic natural late spring conditions in Beijing (day $T=16$ °C, night $T=11$ °C, L:D=14:10, RH=55±65 %).

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

Video recording was initiated once a male mounted a female and was terminated once the pair separated. We observed 20 mating pairs of each phenotypic combination and tallied data for individual components of male copulation behavior from the video recordings as follows; the initial latent period (the period from genital contact until onset of the first shaking bout), the total duration of copula, and the terminal latent period (the period from the last shaking bout until disengagement of genitalia). A 'shaking bout' was defined as a discrete period of rapid, side-to-side oscillations of the male's abdomen while in copula and the number of shaking bouts was tallied for each mating. Following each observation period, the male was removed, cowpea aphids were provisioned, and all egg clusters laid by the female were collected for

5 days. Egg clusters were incubated under the same conditions as adults until eclosion and egg fertility was determined.

Statistical Analysis

The seven responses (initial latent period, duration of copula, terminal latent period, number of shaking bouts, egg retention time, fecundity and fertility) were analyzed as a set by 2-way Multivariate Analysis of Variance (MANOVA) with ‘male’ and ‘female’ phenotypes as fixed factors using the approximate F for Wilks’ Lambda. The partial correlation coefficients with *t*-test statistics were calculated from the MANOVA E-matrix and used to examine correlations between responses. Responses were then analyzed individually by univariate ANOVA to explain differences seen in the MANOVA. Both MANOVA and ANOVA analyses were conducted using the GLM procedure of SAS (version 9.3). Pairwise mean comparisons of the four different phenotype combinations were done when the overall test of phenotype combinations was significant for the MANOVA and seven ANOVA analyses. To control for Type 1 error for the multivariate and univariate analyses, we used a Bonferroni-adjusted alpha level of $0.05/10=0.005$ (where 10 is the total number of tests performed).

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

Results

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

The partial correlations obtained in the MANOVA analysis indicated that there were significant correlations for only two pairs of responses. Duration of copula and number of shaking bouts were positively correlated ($R=0.827$, $P<0.001$) as were terminal latent period and fecundity ($R=0.336$, $P=0.042$). Clearly, a greater number of shaking bouts cannot occur without a corresponding increase in the duration of copula, but correlation of terminal latent period with fecundity is unclear and may be a chance result without biological significance.

Table 1 Results of multivariate tests using Wilks' Lambda, Bonferroni correction for multiple pairwise comparisons, α=0.05/6=0.00833

Comparison	Wilks' Lambda approximate F	df	P
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
(♂$_S$ + ♀$_S$) vs (♂$_S$ + ♀$_M$)	2.20	7,30	0.0623
(♂$_S$ + ♀$_S$) vs (♂$_M$ + ♀$_S$)	5.35	7,30	0.0005
(♂$_S$ + ♀$_S$) vs (♂$_M$ + ♀$_M$)	15.65	7,30	<0.0001
(♂$_S$ + ♀$_M$) vs (♂$_M$ + ♀$_S$)	8.51	7,30	<0.0001
(♂$_S$ + ♀$_M$) vs (♂$_M$ + ♀$_M$)	14.99	7,30	<0.0001
(♂$_M$ + ♀$_S$) vs (♂$_M$ + ♀$_M$)	8.66	7,30	<0.0001

The 2-way ANOVA for initial latent period was significant (F=9.85; df=3,36; P< 0.001) at alpha=0.005, with significant effects of male phenotype (F=28.73; df= 1,36; P<0.001) but not female (F=0.01; df=1,36; P=0.945), and without a significant interaction (F=0.82; df=1,36; P=0.372). Although initial latent periods were relatively short, ranging from 50 to 70 s, melanic males exhibited significantly longer initial latent periods than succinic males, regardless of female phenotype (Fig. 1a). The overall model was also significant for total duration of copula (F=20.40; df= 3,36; P<0.001), with significant effects of both male (F=16.29; df=1,36; P<0.001) and female (F=35.89; df=1,36; P<0.001) phenotype and a significant interaction term (F=9.01; df=1,36; P=0.005). Melanic males spent less time in copula with melanic females than did males in other phenotype combinations, with the latter not significantly different from one another (Fig. 1b). The overall model was also significant for the terminal latent period (F=9.37; df=3,36; P<0.001) with significant effects of male (F=26.43 df=1,36; P<0.001) but not female (F=1.65; df=1,36; P=0.207) phenotype and a nonsignificant interaction term (F=0.04; df=1,36; P= 0.842). Melanic males remained coupled with females for longer periods following completion of shaking behavior than did succinic males (Fig. 1c).

Two way ANOVA of the total number of shaking bouts yielded a significant overall result (F=17.12; df=3,36; P<0.001), with a significant effect of female phenotype (F= 47.19; df=1,36; P<0.001), but not male (F=0.22; df=1,36; P=0.643), and with an interaction that was close to significance (F=3.95; df=1,36; P=0.055). Succinic females received a larger number of shaking bouts than did melanic females, regardless of the phenotype of the male (Fig. 2a). The overall model was also significant for female egg retention time following pair separation (F=16.07; df=3,36; P<0.001), with a significant effect of male phenotype (F=37.24; df=1,36; P<0.001), but not female (F=2.81; df= 1,36; P=0.103), although the interaction term was significant (F=8.16; df=1,36; P= 0.007). Egg retention times were longest for melanic females that mated with melanic males, followed by succinic females that mated with melanic males (Fig. 2b), the latter retaining eggs longer than melanic females mated to succinic males, with ♂$_S$ + ♀$_S$ pairs intermediate between these two (Fig. 2b). There was no effect of phenotype combination

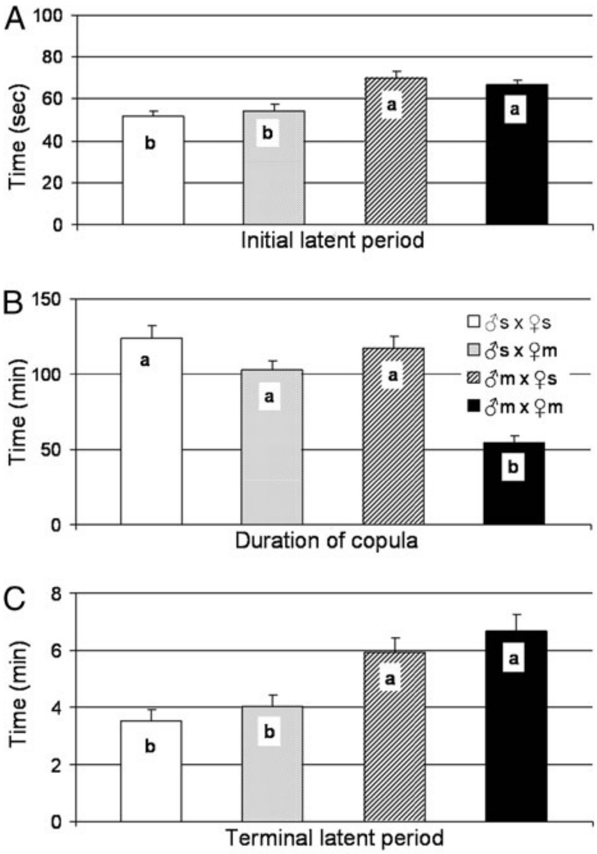

Fig. 1 Mean (+SE) initial latent period (**a**), duration of copula (**b**), and terminal latent period (**c**) for matings between various phenotype combinations of *H. axyridis* in no-choice mating tests (succinic ♂ + succinic ♀, open columns; succinic ♂ + melanic ♀, shaded columns; melanic ♂ + succinic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

on female fecundity over the first 5 days of reproduction (mean=102±3.4 eggs; $F=0.60$; df=3,36; $P=0.622$), but there was a significant effect on fertility ($F=8.99$; df=3,36; $P<0.001$). Male phenotype influenced female fertility ($F=25.80$; df=1,36; $P<0.001$) but female phenotype did not ($F=0.10$; df=1,36; $P=0.751$) and the interaction term was nonsignificant ($F=1.07$; df=1,36; $P=0.307$). Females mating with succinic males hatched a significantly larger proportion of their eggs than did females mating with melanic males (Fig. 2c).

Discussion

The insects in our experiment corresponded to a spring generation in which succinic males are preferred by females in both choice and no-choice situations (Wang et al. 2009), ostensibly because of the fitness disadvantages incurred by melanic forms under the hot conditions of summer (Brakefield and Wilmer 1985). Female

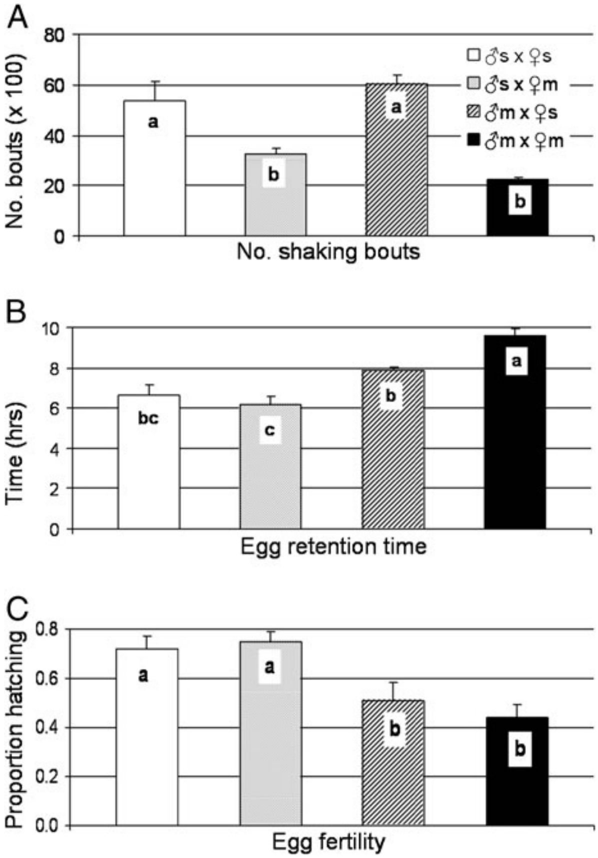

Fig. 2 Mean (+SE) number of male shaking bouts (**a**), female egg retention time (**b**) and egg fertility (**c**) for matings between various phenotype combinations of *H. axyridis* in no-choice mating tests (succinic ♂ + succinic ♀, open columns; succinic ♂ + melanic ♀, shaded columns; melanic ♂ + succinic ♀, hatched columns; melanic ♂ + melanic ♀, solid columns). *Columns bearing the same letters* were not significantly different (LSD test, α>0.05). See text for behavior definitions

preference for succinic males is reflected in a higher frequency of melanic male rejections and delayed onset of copula, the effect being strongest in melanic females (Wang et al. 2009). In the present study, female preference for succinic males was evident in egg retention times that reflect cryptic female choice (Eberhard 1997); females tended to wait longer to use the sperm of melanic males, and melanic females tended to wait longer than succinic females (Fig. 2b), consistent with previous observations (Wang et al. 2009). Thus melanism is not only a criterion for female mate choice, but also appears to have pleiotropic effects on the strength of the preference in females. An alternative mechanism could be a linkage disequilibrium among loci controlling different traits, which seems less likely when one considers the observed seasonal fluctuations in multiple traits that would require entire gene complexes to flip back and forth in frequency between generations in order to generate the differences observed between spring and fall populations (Wang et al. 2009).

Bonduransky (2001) reviewed the evidence for male mate choice in insects and noted that selection for male choosiness should be stronger in systems where female fitness increases with number of copulations, as it does in *H. axyridis* (e.g., Quinones Pando et al. 2001). Males mating with melanic females spent less time in copula than did their counterparts with succinic females and there was also an effect of male phenotype; melanic males abandoned melanic females sooner than did succinic males (Fig. 1b). Therefore, possession of the melanic allele strengthens mate preference for the succinic phenotype in males much as it does in females, an adaptive pleiotropy given the dominance of the melanic allele (Tan and Li 1934). This finding differs from previous observations of the same *H. axyridis* population (Wang et al. 2009) in which the duration of copula was relatively invariant among phenotype combinations in no-choice tests, although differences emerged when males were allowed to select females in a choice setting. In the present study, males engaged in fewer shaking bouts with melanic females compared to succinic females regardless of their phenotype (Fig. 1d). Since both duration of copula and bouts of abdominal shaking are correlated with seminal transfer in *H. axyridis* (Obata 1987), these results supported our hypotheses that males would invest differentially in females based on their elytral coloration, and that melanic males would reduce their investment in melanic females more than succinic males.

Even though initial latent periods were likely too short to be of much consequence to male mating strategy, they were significantly longer for melanic males than for succinic males, regardless of female phenotype (Fig. 1a). More significantly, the same pattern was evident for terminal latent periods (Fig. 1c) that were roughly an order of magnitude longer in duration. Thus, melanic males invested more effort in guarding females post-insemination, supporting our third hypothesis. Studies of sperm precedence in *Adalia bipunctata* suggest that the paternity advantage of the second male can be very high in coccinellids (de Jong et al. 1993; Ueno 1994). If the probability of female remating is higher following matings with melanic males, as their longer egg retention times would suggest, an increased investment in mate guarding may be an adaptive strategy for melanic males.

Interestingly, mating with melanic males reduced female fertility by approximately 25 % relative to matings with succinic males, independent of female phenotype (Fig. 2d), suggesting a negative pleiotropic effect of melanism on male fertility. Rhamhalinghan (1998) reported higher fecundity of melanic females compared to typicals in a polymorphic population of *C. septempunctata* but did not report any differences in fertility. The fitness costs of reduced male fertility, in conjunction with exposure of the melanic allele to selection in heterozygotes, may explain why the equilibrium frequency of the succinic phenotype is approximately 2.5 times that of the melanic in this population of *H. axyridis* (Wang et al. 2009). Notably, females mated to melanic males did not reduce the number of eggs they laid relative to those mated with succinic males, suggesting that melanism did not influence any putative allohormonal effects of seminal fluids on female fecundity (Eberhard 1997). Aphidophagous coccinellids exhibit a ‘front-loaded’ distribution of reproductive effort (Dixon and Agarwala 2002; Michaud and Qureshi 2006; Vargas et al. 2012), i.e., daily female fecundity is highest early in life and declines thereafter. Thus, it would seem that mated females simply oviposit at a maximal rate once they initiate oviposition, regardless of the relative quality of their most recent mate.

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

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References

Alexander RD, Marshall DC, Cooley JR (1997) Evolutionary perspectives on insect mating. In: Choe JC, Crespi BJ (eds) The evolution of mating systems in insects and arachnids. Cambridge University Press, Cambridge, pp 4–31

Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF (2011) Insect seminal fluid proteins: identification and function. Ann Rev Entomol 56:21–40

Benham BR, Lonsdale D, Muggleton J (1974) Is polymorphism in two-spot ladybird an example of non-industrial melanism? Nature 249:179–180

Bonduransky R (2001) The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev 76:305–339

Brakefield PM (1984a) Ecological studies on the polymorphic ladybird *Adalia bipunctata* in the Netherlands. I. Population biology and geographical variation of melanism. J Anim Ecol 53:761–774

Brakefield PM (1984b) Ecological studies on the polymorphic ladybird *Adalia bipunctata* in the Netherlands. II. Population dynamics, differential timing of reproduction and thermal melanism. J Anim Ecol 53:775–790

Brakefield PM, Wilmer PG (1985) The basis of thermal melanism in the ladybird *Adalia bipunctata*. Differences in reflectance and thermal properties between morphs. Heredity 54:9–14

Clusella Trullas S, van Wyk JH, Spotila JR (2007) Thermal melanism in ectotherms. J Therm Biol 32:235–245

Creed ER (1966) Geographic variation in the two-spot ladybird in England and Wales. Heredity 21:57–72

Davies L, Bouvet S, Vernon P (2007) All-year reproduction and possible thermal melanism in *Amblystogenium pacificum* (Coleoptera: Carabidae) on the sub-Antarctic Ile de la Possession (Iles Crozet). Polar Biol 30:253–260

de Jong PW, Verhoog MD, Brakefield PM (1993) Sperm competition and melanic polymorphism in the 2-spot ladybird, *Adalia bipunctata* (Coleoptera, Coccinellidae). Heredity 70:172–178

de Jong PW, Brakefield PM, Geerinck BP (1998) The effect of female mating history on sperm precedence in the two-spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). Behav Ecol 9:559–565

Dickenson JL (1997) Multiple mating, sperm competition, and cryptic female choice in the leaf beetles (Coleoptera: Chrysomelidae). In: Choe JC, Crespi BJ (eds) The evolution of mating systems in insects and arachnids. Cambridge University Press, Cambridge, pp 164–183

Dixon AFG, Agarwala BK (2002) Triangular fecundity function and ageing in ladybird beetles. Ecol Entomol 27:433–440

Eberhard WG (1996) Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton

Eberhard WG (1997) Sexual selection by cryptic female choice in insects and arachnids. In: Choe JC, Crespi BJ (eds) The evolution of mating systems in insects and arachnids. Cambridge University Press, Cambridge, pp 32–57

Edward DA, Chapman T (2011) The evolution and significance of male mate choice. Trends Ecol Evol 26:647–654

Haddrill PR, Shuker DM, Amos W, Majerus MEN, Mayes S (2008) Female multiple mating in wild and laboratory populations of the two-spot ladybird, *Adalia bipunctata*. Mol Ecol 17:3189–3197

Horth L (2003) Melanic body colour and aggressive mating behaviour are correlated traits in male mosquitofish (*Gambusia holbrooki*). Proc R Soc Lond Ser B 270:1033–1040

Jiang XF, Luo LZ, Zhang L (2007) Relative fitness of near isogenic lines for melanic and typical forms of the oriental armyworm, *Mythimna separata* (Walker). Environ Entomol 36:1296–1301

Ma W, Chen L, Wang M, Li X (2008) Trade-offs between melanisation and life-history traits in *Helicoverpa armiger*a. Ecol Entomol 33:37–44

Majerus MEN (1998) Melanism: evolution in action. Oxford University Press, 338 pp

Martin-Vega D, Baz A (2011) Variation in the colour of the necrophagous fly, *Prochyliza nigrimana* (Diptera: Piophilidae): a case of seasonal polymorphism. Eur J Entomol 108:231–234

Michaud JP, Qureshi JA (2006) Reproductive diapause in *Hippodamia convergens* (Coleoptera: Coccinellidae) and its life history consequences. Biol Control 39:193–200

Milliken GA, Johnson DE (2009) Analysis of messy data, volume 1, designed experiments, 2nd edn. CRC Press, Boca Raton

Muggleton J (1975) Melanism in *Adalia bipunctata* (Coleoptera: Coccinellidae) and its relationship to atmospheric pollution. J Appl Ecol 12:451–464

Nahrung HF, Allen GR (2005) Maintenance of colour polymorphism in the leaf beetle *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae: Paropsini). J Nat Hist 39:79–90

Nedved O, Honek A (2012) In: Hodek I, van Emden HF, Honek A (eds) Ecology and behaviour of the Ladybird beetles (Coccinellidae). Wiley-Blackwell, Oxford, pp 54–109

Obata S (1987) Mating behaviour and sperm transfer in the ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Appl Entomol Zool 22:434–442

Osawa N, Nishida T (1992) Seasonal variation in elytra color polymorphism *Harmonia axyridis* (the ladybird beetle): the role of non-random mating. Heredity 69:297–307

Parkash S, Singh S, Ramniwas S (2009) Seasonal changes in humidity level in the tropics impact body color polymorphism and desiccation resistance in *Drosophila jambulina*—evidence for melanism-desiccation hypothesis. J Insect Physiol 55:358–368

Quinones Pando FJ, Chavez Sanchez N, Tarango Rivero SH (2001) Effect of the time of availability of the male in the fecundity of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Fol Entomol Mex 40:47–52

Rhamhalinghan M (1998) The reproductive behaviour of *Coccinella septempunctata* L. var. Confusa Wiedmann (Coleoptera: Coccinellidae). J Adv Zool 19:17–21

SAS Institute (2013) The SAS System for Windows version 9.3. SAS Institute, Cary

Simmons LW, Siva-Jothy MT (1998) Sperm competition in insects: mechanisms and potential for selection. In: Birkhead TR, Moller AP (eds) Sperm competition and sexual selection. Academic, San Diego, pp 341–434

Sloggett JJ, Honek A (2012) Genetic studies. In: Hodek I, van Emden HF, Honek A (eds) Ecology and behaviour of the Ladybird beetles (Coccinellidae). Wiley-Blackwell, Oxford, pp 13–53

Stewart LA, Dixon AFG (1989) Why big species of ladybird beetles are not melanic. Funct Ecol 3:165–177

Tan CC, Li JC (1934) Inheritance of the elytral colour patterns in the lady-bird beetle, *Harmonia axyridis*. Am Nat 68:252–265

Thornhill R, Alcock J (1983) The evolution of insect mating systems. iUniverse.com, Lincoln

True JR (2003) Insect melanism: the molecules matter. Trends Ecol Evol 18:640–647

Ueno H (1994) Intraspecific variation of P2 value in a coccinellid beetle, *Harmonia axyridis*. J Ethol 12:169–174

Vargas G, Michaud JP, Nechols JR (2012) Larval food supply constrains female reproductive schedules in *Hippodamia convergens* (Coleoptera: Coccinellidae). Ann Entomol Soc Am (accepted)

Verhoog MD, Breuker CJ, Brakefield PM (1998) The influence of genes for melanism on the activity of the flour moth, *Ephestia kuehniella*. Anim Behav 56:683–688

Wang S, Michaud JP, Zhang RZ, Zhang F, Liu S (2009) Seasonal cycles of assortative mating and reproductive behaviour in polymorphic populations of *Harmonia axyridis* in China. Ecol Entomol 34:483–491