Eur. J. Entomol. **111**(3): 417–420, 2014
doi: 10.14411/eje.2014.037
ISSN 1210-5759 (print), 1802-8829 (online)

Age-related changes in the frequency of harassment-avoidance behaviour of virgin females of the small copper butterfly, *Lycaena phlaeas* (Lepidoptera: Lycaenidae)

Jun-Ya IDE

Department of Education and Creation Engineering, Kurume Institute of Technology, 2228-66 Kamitsu-machi, Kurume, Fukuoka 830-0052, Japan; e-mail: idejy@kurume-it.ac.jp

Key words. Lepidoptera, Lycaenidae, *Lycaena phlaeas*, immature adult, receptivity, sexual conflict, virgin, wing-closing behaviour

Abstract. Mated females of the small copper butterfly *Lycaena phlaeas* avoid harassment by males by closing their wings and concealing themselves when in the proximity of a con-specific butterfly. This wing-closing behaviour is less frequently exhibited by virgin females that are two days old or older (i.e., potentially receptive) than by mated females. During the first 2 days after emergence, females of *L. phlaeas* are sexually immature and unreceptive. To determine whether recently emerged virgin females try to avoid male harassment, age-related changes in the frequency of harassment-avoidance behaviour of virgin females were investigated. On the day of emergence, a high percentage of virgin females exhibited wing-closing behaviour. Over the following 2 days, however, the frequency of this behaviour declined sharply and then reached a constant low level. This observation supports the idea that the harassment-avoidance behaviour exhibited by virgin females of *L. phlaeas* depends on their receptivity.

INTRODUCTION

Sexual conflicts over the timing and frequency of mating are reported for a broad range of animal taxa (Arnqvist & Rowe, 2005). Because the optimal mating frequency is often higher for males than for females, male sexual coercion and corresponding female resistance may have been enhanced through sexually antagonistic coevolution (Clutton-Brock & Parker, 1995). As a means of sexual coercion, male harassment is observed in many animal species (e.g., Sakurai & Kasuya, 2008; Gay et al., 2009).

Females of many species have evolved strategies to avoid male harassment. Often females actively avoid males in time or space (Krupa et al., 1990; Stone, 1995) or aggregating with other females create a dilution effect (Pilastro et al., 2003; Cappozzo et al., 2008). If a female always avoids harassment, she will never mate. Therefore, a female stops avoiding male harassment when her mating receptivity is high but will exhibit harassment-avoidance behaviour when her mating receptivity is low. Among butterflies and quails, virgin females do not avoid males but do exhibit harassment-avoidance behaviour after copulation (Wiklund, 1982; Persaud & Galef, 2004). However, it is not known whether sexually immature females exhibit harassment-avoidance behaviour.

In many animals, adult females are only receptive over specific periods of time and outside these periods, even virgin females do not mate (e.g., Bronson, 1985; Jormalainen, 1998). Therefore, mating attempts with unreceptive females do not result in copulation and are costly for males due to various costs of courtship (South et al., 2009; Wedell, 2010). However, if it is difficult for males to distinguish between receptive and unreceptive females, the males may attempt to mate with unreceptive females to avoid missing an opportunity to mate (Takahashi & Watanabe, 2011). As a result, unreceptive females experience persistent mating attempts by males (Hammers et al., 2009). Another male mating strategy is to guard an unreceptive female until she is receptive (Jormalainen, 1998). In this case, the females suffer an increase in costs such as in the risk of predation or energy costs that are incidental to mate guarding (Jones et al., 2010). Therefore, avoiding male harassment is important, not only for mated females, but also virgin females that are not receptive.

Males of the small copper butterfly *Lycaena phlaeas* (L., 1761) (subspecies *daimio* Seitz, 1909) are persistent in their attempts at mating (Suzuki, 1978). When a male locates a female, he flies closely around her and then walks after her with fluttering wings for an average of 40 s (Ide, 2011). *Lycaena phlaeas* females usually copulate only once during their lives (Watanabe & Nishimura, 2001), indicating low optimal mating frequency and negligible benefits of polyandry. Therefore, such sexual harassment by males is an important and costly problem for the females. It is suggested that female fecundity in the related butterfly species *L. hippothoe* (L.) declines under conditions of male harassment (Turlure & Van Dyck, 2009).

Making themselves invisible to males would help females escape from persistent mating attempts by males. In some satyrine butterflies, females reject mating by playing possum (Shreeve et al., 2006). They feign death with closed wings and let go of the substrate on which they are settled. As a result they drop into the vegetation and become invisible to males. Harassment-avoidance behaviour of females of *L. phlaeas* takes advantage of a similar effect (Ide, 2011). A mated female closes her wings when in the proximity of a conspecific butterfly. When female butterflies close their wings, mating attempts occur significantly

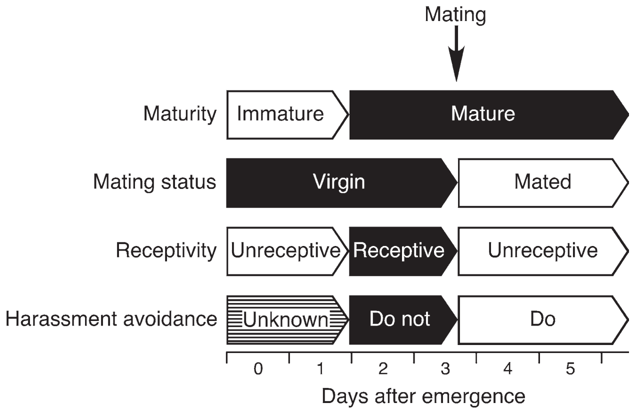

Fig. 1. A schema of the daily changes in maturity, mating status, receptivity and incidence of harassment avoidance behaviour recorded for females of *Lycaena phlaeas*. The case of a female that mated on the fourth day after emergence is shown.

less frequently than when their wings remain open, indicating that the wing-closing behaviour of *L. phlaeas* females functions to deter male mating attempts (Ide, 2011). This is probably because, for male butterflies seeking mates while flying, the visible wing area of a perched individual whose wings are closed is much smaller than that of an individual whose wings are open. Wing-closing behaviour is exhibited less frequently by virgin females that are two days old or older (i.e., potentially receptive) than by mated females, which is believed to increase the probability that a male will court her (Ide, 2011). Females of *L. phlaeas* do not copulate during the first 2 days after emergence, because the eggs in their ovaries are still immature (Watanabe & Nishimura, 2001). It is of interest to determine whether sexually unreceptive virgin females of this age exhibit harassment-avoidance behaviour in the presence of a conspecific male (Fig. 1). A female exhibiting harassment-avoidance behaviour based on her receptivity will close her wings. However, if the harassment-avoidance behaviour is dependent on her mating status (i.e., virgin or mated), she will not close her wings. In this study, I investigated whether the frequency of harassment-avoidance behaviour exhibited by virgin females of *L. phlaeas* changed as they aged.

MATERIAL AND METHODS

Eggs of *L. phlaeas* were collected from females caught in the wild and were reared to adulthood. Male and female adults were housed separately. To determine experimentally whether the reaction of a virgin female to the presence of a male butterfly depends on her age, I moved a dummy male butterfly into the proximity of a female whose age was known. The butterfly dummy consisted of life-size photograph of a male specimen (whose wing span was approximately 30 mm), as in the apparatus described by Douwes (1975). Dorsal and ventral photographs of a male were pasted together, and a rotating axis attached to an electric motor (Model FA-130RA-2270; Mabuchi Motor Co., Ltd., Matsudo, Japan) was inserted between the photographs. The motor was attached to one end of a 1-m rod, and the power switch was attached to the other end, which enabled the dummy to be rotated from a distance. A previous study (Ide, 2011) confirmed that females of *L. phlaeas* reacted similarly to a dummy as to a con-specific individual.

At the start of the experiment, female butterflies were released into a cage and left undisturbed until they opened their wings. The rotating dummy was presented for 10 s, at a height of approximately 5 cm above a female perched with her wings open, and her reaction was recorded. Each individual female was used repeatedly but only once per day. Approximately 70% of copulations occur between 2 and 4 days after emergence (J.-Y. Ide, unpubl. data), hence 0- to 4-day-old virgin females were used for this experiment. A total of 490 females were tested.

Females may close their wings to avoid predators. Recently emerged females are very sensitive to any approaching objects possibly because they are poor at escaping from predators because their wings are still soft. If this is the case, wing-closing behaviour should be more often observed immediately after emergence, even if immature females do not exhibit harassment-avoidance behaviour. To avoid this problem, a dummy of a short tailed blue butterfly *Everes argiades* (Pallas), which often coexists with *L. phlaeas*, was presented to 0- to 4-day-old virgin females as a control. In this treatment, a total of 287 females were tested. If females close their wings to avoid only predators, their wing-closing reaction to the proximity of a dummy of *L. phlaeas* should occur at a similar frequency to their reaction to *E. argiades*, which is harmless to *L. phlaeas*. In contrast, if they close their wings to avoid male harassment, then it should occur more frequently in response to the proximity of a dummy of *L. phlaeas* than of *E. argiades*.

I used a two-way generalized linear mixed model with a binomial error structure and a logit link function to determine whether the reactions of females of different ages to the two different dummies differed. The dependent variable was whether the female closed her wings when a rotating dummy was presented. The independent variables were the number of days after emergence and the kind of dummy, and the individual was included as a random factor for intercept and slope. The model was run using the “glmer” function in the “lme4” package in R version 2.12.1 (R Development Core Team, 2010).

RESULTS

When presented with the rotating dummy, females either closed their wings or displayed no reaction. The reactions of virgin females of different ages to the dummy of *L. phlaeas* differed significantly (Table 1). On the day of emergence (Day 0), 73% of the virgin females closed their wings, then over the following 2 days (Days 1 and 2) the percentage that closed their wings declined sharply and then remained relatively constant at a low percentage

Table 1. Summary of the results of generalized linear mixed model analysis of the wing-closing reaction of *Lycaena phlaeas* females.

Factor	Estimate	S.E.	*z*	*p*
Intercept	–0.655	0.375	–1.744	0.081
Day	–1.091	0.217	–5.036	<0.0001
Dummy (con-specific)	1.984	0.396	5.006	<0.0001
Day × dummy (con-specific)	–0.099	0.214	–0.461	0.645

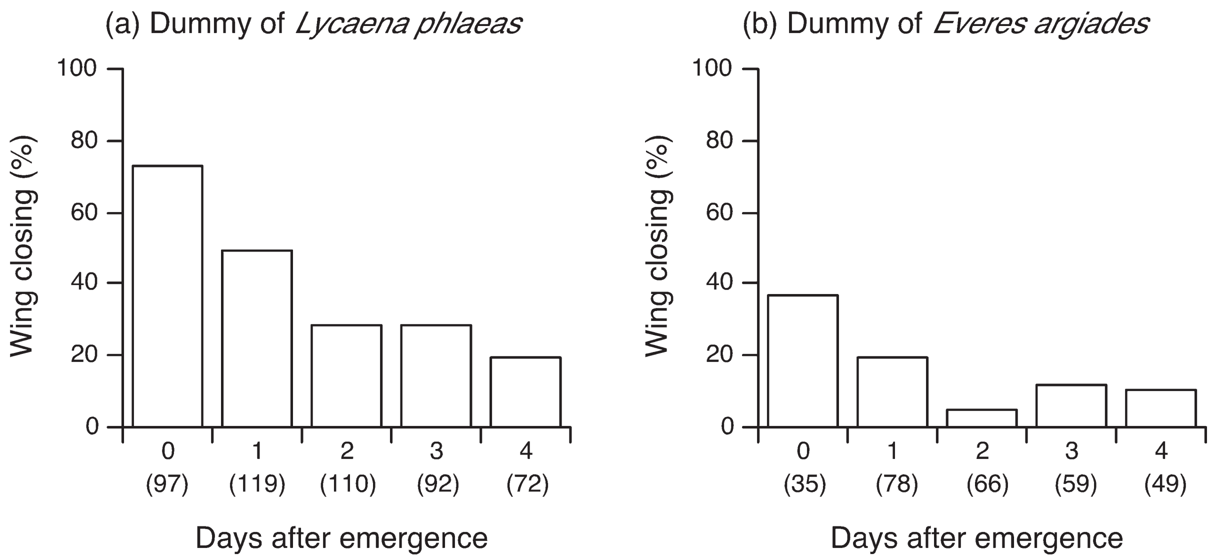

Fig. 2. Changes in the frequency of the wing-closing reaction of virgin females of *Lycaena phlaeas* recorded over a period of 5 days following emergence in response to (a) a dummy of *L. phlaeas* and (b) one of *Everes argiades*. Numbers in parentheses are the sample sizes.

(Fig. 2a). Virgin females also reacted to the dummy of *E. argiades* by closing their wings. The percentage was 37% on the day of emergence, declining to lower levels over the next four days (Fig. 2b). The percentage of females that reacted to the dummy of *E. argiades* by closing their wings was significantly lower than to the dummy of *L. phlaeas* (Table 1).

DISCUSSION

Virgin females closed their wings when presented with both dummies, but more frequently to the dummy of *L. phlaeas* than *E. argiades*. This indicates that this response is harassment-avoidance behaviour. It was most often exhibited by virgin females on the day of emergence, after which the frequency declined sharply over the course of the following 4 days. Most virgin females of *L. phlaeas* mate within 2 to 4 days of emerging (Suzuki, 1978; Watanabe & Nishimura, 2001). Therefore, the period in which they did not exhibit wing-closing behaviour largely coincided with the period for which they were receptive. This result indicates that the harassment-avoidance behaviour exhibited by virgin females of *L. phlaeas* depends on whether they are receptive or not. The orange pattern on the wings of female *L. phlaeas* are attractive to males (Ide, 2010) and receptive virgin females are thought to increase their probability of attracting males by prominently displaying this orange pattern. Similar behaviour is recorded in the speckled wood butterfly *Pararge aegeria*, whose virgin females behave more conspicuously if they remain unmated for a long period (i.e., their receptivity is high; Bergman et al. 2011).

Young females of *L. phlaeas* do not mate for 2 days after emergence, but males try to court these young and unreceptive females (Suzuki, 1978). Perhaps males cannot discriminate between females of different ages. Another possibility is that it may be advantageous for males to court young females that are likely to be virgins. If this is true, it is likely that freshly emerged females will be courted more frequently than older females. Therefore, not attracting the attention of males may be particularly important for immature females.

The percentage of virgin females of *L. phlaeas* that closed their wings declined on the day following emergence. It was reported that the percentage of females of *L. phlaeas* that mated on the day following emergence was 13.3% (Suzuki, 1978). In another experiment, no virgin females mated on the day following emergence (J.-Y. Ide, unpubl. data), which is inconsistent with the previous observation that some of these females did not show harassment-avoidance behaviour. However, a decline in female reproductive value with age results in their becoming less choosy (Kodric-Brown & Nicoletto, 2001; Moore & Moore, 2001). It is quite likely that young females of *L. phlaeas* are very choosy (i.e., they do not mate easily). If this is true, then the discrepancy in the timing of the loss of harassment-avoidance behaviour and increase in receptivity may be due to some females accepting the most attractive of the males.

In Lepidoptera, female receptivity often declines sharply after mating and females simultaneously display mate-rejection and/or harassment-avoidance behaviour (Wedell, 2005). Males that can induce non-receptivity in their mates can avoid sperm competition and assure paternity (Simmons, 2001). Indeed, male-induced non-receptivity can be accomplished by means of mating plugs or more often by sperm and male-derived accessory gland substances transferred at mating, yet the fitness consequences for females remain ambiguous (Simmons, 2001; Arnqvist & Rowe, 2005; and references therein). Females of *L. phlaeas* start to exhibit wing-closing behaviour on the day of mating (Ide, 2011). It is unknown whether this post-mating change in behaviour in *L. phlaeas* is caused by male-derived factors. However, the fact that virgin females show the same harassment-avoidance behaviour as mated females suggests that it does not always need male-derived stimuli and females always have the potential for such behaviour. Therefore, manipulation by males using substances that are transferred during mating may easily evolve by exploiting an existing behavioural pattern in females. Further comparisons of the mate-rejection or harassment-avoidance behaviour of virgin and mated females might clarify the evolutionary mechanisms involved in the manipulation of female behaviour by males.

ACKNOWLEDGEMENTS. I thank two anonymous reviewers for their helpful comments on the manuscript. This study was partly supported by a JSPS Research Fellowship for Young Scientists (no. 16-380).

REFERENCES

ARNQVIST G. & ROWE L. 2005: *Sexual Conflict*. Princeton University Press, Princeton, 330 pp.

BERGMAN M., GOTTHARD K. & WIKLUND C. 2011: Mate acquisition by females in a butterfly: the effects of mating status and age on female mate-locating behaviour. — *Anim. Behav.* **81**: 225–229.

BRONSON F.H. 1985: Mammalian reproduction: an ecological perspective. — *Biol. Reprod.* **32**: 1–26.

CAPPOZZO H.L., TÚNEZ J.I. & CASSINI M.H. 2008: Sexual harassment and female gregariousness in the South American sea lion, *Otaria flavescens*. — *Naturwissenschaften* **95**: 625–630.

CLUTTON-BROCK T.H. & PARKER G.A. 1995: Sexual coercion in animal societies. — *Anim. Behav.* **49**: 1345–1365.

DOUWES P. 1975: Territorial behaviour in *Heodes virgaureae* L. (Lep., Lycaenidae) with particular reference to visual stimuli. — *Norw. J. Entomol.* **22**: 143–154.

GAY L., EADY P.E., VASUDEV R., HOSKEN D.J. & TREGENZA T. 2009: Costly sexual harassment in a beetle. — *Physiol. Entomol.* **34**: 86–92.

HAMMERS M., SÁNCHEZ-GUILLÉN R.A. & VAN GOSSUM H. 2009: Differences in mating propensity between immature female color morphs in the damselfly *Ischnura elegans* (Insecta: Odonata). — *J. Insect Behav.* **22**: 324–337.

IDE J.-Y. 2010: Weather factors affecting the male mate-locating tactics of the small copper butterfly (Lepidoptera: Lycaenidae). — *Eur. J. Entomol.* **107**: 369–376.

IDE J.-Y. 2011: Avoiding male harassment: wing-closing reactions to flying individuals by female small copper butterflies. *Ethology* — **117**: 630–637.

JONES T.M., ELGAR M.A. & ARNQVIST G. 2010: Extreme cost of male riding behaviour for juvenile females of the Zeus bug. — *Anim. Behav.* **79**: 11–16.

JORMALAINEN V. 1998: Precopulatory guarding crustaceans: male competitive strategy and intersexual conflict. — *Quart. Rev. Biol.* **73**: 275–304.

KODRIC-BROWN A. & NICOLETTO P.F. 2001: Age and experience affect female choice in the guppy (*Poecilia reticulata*). — *Am. Nat.* **157**: 316–323.

KRUPA J.J., LEOPOLD W.R. & SIH A. 1990: Avoidance of male giant water striders by females. — *Behaviour* **115**: 247–253.

MOORE P.J. & MOORE A.J. 2001: Reproductive aging and mating: The ticking of the biological clock in female cockroaches. — *Proc. Natl. Acad. Sci. USA* **98**: 9171–9176.

PERSAUD K.N. & GALEF B.G. JR. 2004: Fertilized female quail avoid conspecific males: female tactics when potential benefits of new sexual encounters are reduced. — *Anim. Behav.* **68**: 1411–1416.

PILASTRO A., BENETTON S. & BISAZZA A. 2003: Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*. — *Anim. Behav.* **65**: 1161–1167.

R DEVELOPMENT CORE TEAM 2010: *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, URL http://www.R-project.org/.

SAKURAI G. & KASUYA E. 2008: The costs of harassment in the adzuki bean beetle. — *Anim. Behav.* **75**: 1367–1373.

SHREEVE T.G., DENNIS R.L.H. & WAKEHAM-DAWSON A. 2006: Phylogenetic, habitat, and behavioural aspects of possum behaviour in European Lepidoptera. — *J. Res. Lepid.* **39**: 80–85.

SIMMONS L. 2001: *Sperm Competition and Its Evolutionary Consequences in Insects*. Princeton University Press, Princeton, 456 pp.

SOUTH S.H., STEINER D. & ARNQVIST G. 2009: Male mating costs in a polygynous mosquito with ornaments expressed in both sexes. — *Proc. R. Soc. (B)* **276**: 3671–3678.

STONE G.N. 1995: Female foraging responses to sexual harassment in the solitary bee *Anthophora plumipes*. — *Anim. Behav.* **50**: 405–412.

SUZUKI Y. 1978: Mate-avoiding behaviour in females of the small copper, *Lycaena phlaeas daimio* Seitz (Lepidoptera: Lycaenidae). — *Tyô Ga* **29**: 129–138 [in Japanese with English abstr.].

TAKAHASHI Y. & WATANABE M. 2011: Male mate choice based on ontogenetic colour changes of females in the damselfly *Ischnura senegalensis*. — *J. Ethol.* **29**: 293–299.

TURLURE C. & VAN DYCK H. 2009: On the consequences of aggressive male mate-locating behaviour and micro-climate for female host plant use in the butterfly *Lycaena hippothoe*. — *Behav. Ecol. Sociobiol.* **63**: 1581–1591.

WATANABE M. & NISHIMURA M. 2001: Reproductive output and egg maturation in relation to mate-avoidance in monandrous females of the small copper, *Lycaena phlaeas* (Lycaenidae). — *J. Lepid. Soc.* **54**: 83–87.

WEDELL N. 2005: Female receptivity in butterflies and moths. — *J. Exp. Biol.* — **208**: 3433–3440.

WEDELL N. 2010: Variation in male courtship costs in butterflies. *Behav. Ecol. Sociobiol.* — **64**: 1385–1391.

WIKLUND C. 1982: Behavioural shift from courtship solicitation to mate avoidance in female ringlet butterflies (*Aphantopus hyperanthus*) after copulation. — *Anim. Behav.* **30**: 790–793.

Received August 24, 2013; revised and accepted January 15, 2014
Prepublished online April 23, 2014