Animal Behaviour 109 (2015) 235–241

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Aggression, mate guarding and fitness in male fruit flies

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ARTICLE INFO

Article history:
Received 29 May 2015
Initial acceptance 15 July 2015
Final acceptance 6 August 2015
Available online 29 September 2015
MS. number: A15-00462R

Keywords:
aggression
Drosophila melanogaster
fighting
fruit fly
mate guarding
resource defence polygyny
territorial behaviour

Aggression is a central trait affecting fitness, which has been well studied in many animals. As a part of a research programme integrating mechanisms and fitness consequences of aggression, we examined the adaptive functions of antagonistic interactions in fruit flies, *Drosophila melanogaster*, a species in which aggression has been studied primarily in the context of territorial behaviour. In our experiments, males at an attractive food patch were more aggressive towards other males when they were in the presence of their recent mates than when they were in the presence of females mated with other males. Furthermore, while recently mated males accompanied by their mates were more aggressive than virgin males, recently mated males and virgin males showed similar levels of aggression in the presence of females mated with other males. When we allowed focal males to mate inside experimental arenas and then added intruder males, the intruder males spent less time on the food patch, remated with the resident females at lower frequencies and fathered a smaller proportion of offspring when the focals males remained in the arenas than when we removed the focal males. Our results reveal a novel adaptive function of aggression in fruit flies: in addition to fighting to defend attractive food sources that attract prospective mates, males rely on aggression to guard their mates, and such mate guarding enhances their fitness.

Aggression has long been recognized as a primary trait influencing fitness, especially in males, which often fight for territories and prospective mates (Darwin, 1871; Howard, 1920). Fruit flies, *Drosophila melanogaster*, have recently been adopted for a close examination of the genetics and neurobiology of aggression (Alekseyenko et al., 2014; Chen, Lee, Bowens, Huber, & Kravitz, 2002; Dierick & Greenspan, 2006; Zwarts et al., 2011). Because fruit flies are also highly amenable for behavioural, ecological and evolutionary research, we have a unique opportunity for integrating the rapidly accumulating knowledge about the mechanisms that control the varieties of aggressive behaviours with their functional aspects.

Since the first experimental analysis of fruit fly aggression (Dow & Schilcher, 1975), the primary focus in laboratory protocols has been on aggression in the context of territorial behaviour (Certel & Kravitz, 2012; Chen et al., 2002; Dierick & Greenspan, 2006; Hoffmann, 1987b). The limited field work is consistent with the notion that male aggression serves for defending fruits frequented by prospective mates (Markow, 1988). Male aggression, however, can also contribute to other activities such as mate guarding. Male fruit flies defend fruits that are highly suitable for feeding and oviposition. This means that females will most likely remain at their location of mating, because after mating, they increase feeding and then initiate egg laying (Gioti et al., 2012). Remating, however, may be common (Harshman & Clark, 1998) even though recently mated females have lower receptivity than virgin females (Chapman et al., 2003). Because there is a strong last-male sperm precedence in fruit flies (Gromko, Gilbert, & Richmond, 1984; Price, Dyer, & Coyne, 1999), the earlier male to mate will gain little paternity if his recent mate is quick to remate with another male. Thus males can benefit from guarding their mates that remain at the fruit they defend.

Mate guarding has been well studied in many species (Alcock, 1994; Simmons, 2001) and can be expressed in different ways. The most overt way involves cases such as in the dragonfly, *Pachydiplax longipennis*, in which the male remains close to the female after mating and during her oviposition and chases away approaching males (Sherman, 1983). Similarly, in Idaho ground squirrels, *Spermophilus brunneus*, the males stay close to their mates and attack approaching males. Field observations corroborated with genetic tests indeed indicated that males sired the pups

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http://dx.doi.org/10.1016/j.anbehav.2015.08.023
0003-3472/

born to females that they guarded (Sherman, 1989). An alternative form of mate guarding involves the males simply remaining mounted to females after copulation. This probably reduces the guarding males' need for using aggression. For example, male dung flies (*Scatophaga stercoraria*) remain mounted on the females after copulation for the duration of oviposition and thus physically block mounting by other males. Calculations indicate that such mate guarding is advantageous over the alternative strategy of searching for other females (Parker, 1970). In soapberry bugs (*Jadera haematoloma*), copulations can last up to a few days even though sperm transfer takes only a few minutes. The males also remain close to the females during oviposition and remate if other males approach (Carroll, 1991). Another form of mate guarding occurs in a variety of territorial birds. In addition to aggressively defending territories, the males closely follow their mates during their fertile period in order to reduce extrapair copulations (Beasley, 1996; Birkhead, 1979; Chuang-Dobbs, Webster, & Holmes, 2001; Dickinson, 1997; Dickinson & Leonard, 1996). For example, in house wrens, *Troglodytes aedon*, short-term experimental detention of males resulted in higher rates of extrapair copulations and paternity (Brylawski & Whittingham, 2004).

A recent study focusing on the mechanisms of aggression (Yuan, Song, Yang, Jan, & Jan, 2014) hinted at the possibility of mate guarding in fruit flies. Because the natural history of fruit flies described above implies that mate guarding may be beneficial under some realistic field settings, we conducted a set of experiments to critically test the role of aggression in mate guarding. Overall, our goal was to expand the scope of research on aggression in fruit flies in order to place it in a broader ecological perspective. This can help us understand both the mechanisms and fitness consequences of aggression in many animals. Specifically, we predicted (1) that males with their recent mates would be more aggressive than control males, (2) that aggression in the context of mate guarding would decrease female remating frequency with other males and (3) that aggression in the context of mate guarding would increase the paternity of mate guards.

GENERAL METHODS

We used descendants of wild-caught *D. melanogaster* collected in several southern Ontario localities in August 2014. We housed the flies in population cages containing several hundred flies per cage. We kept the cages in an environmental chamber at 25 °C and 60% relative humidity with a 12:12 h light:dark cycle, with the lights turning on at 1000 hours. We reared the experimental flies at a low density of about 300 eggs per 240 ml bottle containing 50 ml of standard fly medium made of water, sucrose, cornmeal, yeast, agar and methyl paraben.

We sexed flies within 4 h of eclosion to ensure virginity. We used gentle aspiration to sex and transfer the males into individual food vials, and CO_2 to sex and place females in groups of 20 per food vial. Each 40 ml vial contained 5 ml of the standard fly medium, and the females' vials also contained a dash of live yeast. At the time of testing all flies were 4 days old. We used small amounts of pink fluorescent powder to mark males to allow us to distinguish between males when two males shared an arena. Male colouring was counterbalanced with male treatment.

We conducted all tests in cylindrical arenas (3 cm in diameter, 2.5 cm high) made of Plexiglas. To deter flies from climbing on the arenas' walls and ceilings, we coated the walls with Insect-a-Slip (Fluon; BioQuip, Gardena, CA, U.S.A.) and the ceilings with Surfasil (Sigma Aldrich, Oakville, ON, Canada). The floor of each arena had a piece of moist filter paper, and each arena contained a circular food patch (1.3 cm in diameter, 1.5 mm high) covered with a live-yeast suspension. We recorded all trials using webcams (Logitech HD Pro C920 and iPad Air). Then, observers blind to fly treatment scored the videos using Noldus software (Noldus Information Technology, Wageningen, The Netherlands). We used generalized linear models (GLMs) when there were independent measures, and we used generalized estimating equations (GEEs) when there were repeated measures (SPSS, IBM Corp., 2011). Unless noted otherwise, the models assumed gamma distributions with log linked functions.

AGGRESSION IN THE CONTEXT OF MATE GUARDING

Methods

We began by examining whether males were more aggressive towards other males in the presence of their recent mates than were control males. We used a protocol modified from Yuan et al. (2014). In the mate-guarding treatment ($N = 30$ arenas), we placed two focal males in an arena with two virgin females and allowed them to mate (Fig. 1a). After both males had finished mating, we waited 10 min and then began video recording the arenas for 30 min. In the nonmates treatment ($N = 30$ arenas), we placed one female and one male in each of two vials and allowed them to mate. Following mating, we discarded the males, placed the two mated females and two virgin focal males in an arena, let them acclimate for 10 min and then began video recording for 30 min (Fig. 1a). From these videos, observers who were blind to the male treatment recorded the total duration of aggression, which included all occurrences of lunging, wing threat, high-level fencing, holding, boxing, tussling and charging (Chen et al., 2002;

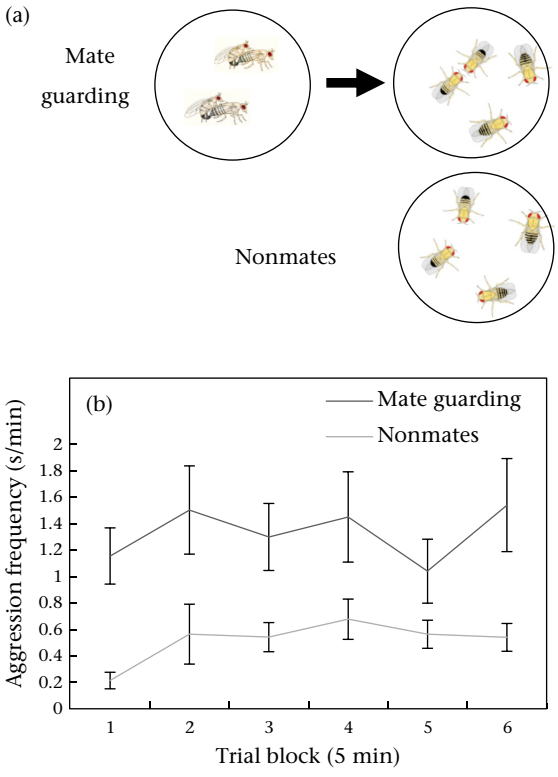

Figure 1. (a) The mate-guarding treatment involved two males in the presence of their recent mates whereas the nonmates treatment had two males together with females recently mated to other males (note that males are distinguished from females by their smaller size and the black tip of their abdomen). (b) Mean ± SE aggression frequency (s/min) per 5 min block per arena in the mate-guarding and nonmates treatments ($N = 60$ arenas).