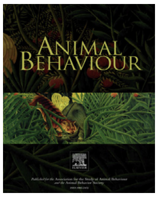
Animal Behaviour 109 (2015) 161–166

Contents lists available at ScienceDirect

Animal Behaviour

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



Male birch catkin bugs vary copula duration to invest more in matings with novel females

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ARTICLE INFO

Article history:
Received 26 May 2015
Initial acceptance 18 June 2015
Final acceptance 27 July 2015
Available online 20 September 2015
MS. number: 15-00449R

Keywords:
Coolidge effect
copula duration
male mate choice
mating behaviour
sexual conflict
sexual selection
sperm allocation

Recent developments in the study of mating behaviour have emphasized the importance of strategic investment of limited reproductive resources. However, in many cases it can be difficult to interpret traits such as copula duration, because they are interacting phenotypes that ultimately depend upon both the male and female mating partner, and the sexes may frequently disagree over the optimal outcome. Here we report the results of experiments designed to establish which sex controls copula duration in the birch catkin bug, *Kleidocerys resedae*, and to test for strategic investment by the controlling sex. First, we found that matings of field-caught individuals were relatively short, but that copula duration increased following a period of sexual isolation, reaching a maximum after 2 days. However, copula duration was again shorter in re-pairings of the same individuals 1 h after their first mating. Because these results could be interpreted as a response to sexual isolation by either sex, we next investigated whether copula duration is under male or female control in this species. Experimental pairings between males and females isolated for 1 h or 48 h in all four possible combinations revealed that copula duration depended strongly on the period of male but not of female sexual isolation, implying that this trait is under male control. Finally, if males mated once were re-paired after 1 h with either the same or a novel (but still recently mated) female, we found that they mated for significantly longer with the latter. Collectively, our results imply that male birch catkin bugs in nature are frequently time-, sperm- or seminal fluid-limited, and that, as predicted by theory, they strategically allocate more of their mating effort and ejaculate reserves to novel females, a form of (cryptic) male mate choice.

There is increasing recognition that males prudently adjust many aspects of their mating behaviour and ejaculate allocation to maximize fitness returns on their reproductive investment (Dewsbury, 1982; Parker, 1983). Indeed, male mate choice is now recognized as an important aspect of sexual selection in many animal taxa (Bonduriansky, 2001; Edward & Chapman, 2011), potentially leading to sperm limitation for females and sexual conflict over mating decisions (Bro-Jørgensen, 2007; Warner, Shapiro, Marcanato, & Petersen, 1995; Wedell, Gage, & Parker, 2002). However, the optimum mating rate is usually expected to be higher for males than for females, leading to frequent sexual conflict (A. J. Bateman, 1948; Parker, 2006). Even where both mating partners readily agree to mate, the risk of sperm competition means that males then often transfer many more sperm or other ejaculate components than the female would prefer to receive, creating a further arena for sexual conflict (Edward, Stockley, & Hosken, 2014).

One well-studied phenomenon in the context of male mating selectivity is the Coolidge effect, originally defined in mammals as the restoration of sexual activity among males that had previously reached sexual satiety when presented with a novel female (reviewed in Dewsbury, 1981b; see also e.g. Dewsbury, 1981a; Pierce, O'Brien, & Dewsbury, 1992; Wilson, Kuehn, & Beach, 1963). More generally, males in other vertebrate taxa have also been found to express a preference for novel females (e.g. fish: Kelley, Graves, & Magurran, 1999; Spence, Reichard, & Smith, 2013; birds: Pizzari, Cornwallis, Løvlie, Jakobsson, & Birkhead, 2003; lizards: Tokarz, 2008). Evidence for invertebrates is more limited, but suggests that the novelty of the mating partner may also be an important factor driving male reproductive investment. For example, in the simultaneously hermaphroditic great pond snail, *Lymnaea stagnalis*, the propensity to mate in the male role is increased in situations in which the snail is presented with a novel

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http://dx.doi.org/10.1016/j.anbehav.2015.08.020
0003-3472/

mating partner (Koene & Ter Maat, 2007). Similarly, when presented with a mating opportunity with a novel female, male burying beetles, *Nicrophorus vespilloides*, have shorter mating latencies, a behaviour that has recently been interpreted as a 'Coolidge effect' (Steiger, Franz, Eggert, & Müller, 2008; see also Tan et al., 2013). By contrast, male decorated field crickets, *Gryllodes sigillatus*, show no evidence of differential investment towards novel females, with respect to either courtship effort or spermatophore size (Gershman & Sakaluk, 2009; see also Haederer, Werminghausen, Michiels, Timmermeyer, & Anthes, 2009; Newcomer, Zeh, & Zeh, 1999 for further invertebrate counterexamples). The extent to which male insects respond to female novelty by strategically adjusting aspects of mating effort thus remains an open question, and so far as we are aware no study has investigated potential responses to novel/familiar females with respect to copula duration. In insects, there is often a positive correlation between copula duration and sperm transfer (e.g. Engqvist & Sauer, 2003; Sakaluk & Eggert, 1996; Simmons & Parker, 1992). Furthermore, long copulations are often costly (Scharf, Peter, & Martin, 2013), making copula duration a likely target for strategic male investment (Kelly & Jennions, 2011; Parker & Pizzari, 2010; Wedell et al., 2002).

An inherent problem in studying mating interactions is that the traits that are normally measured, such as mating latency, copula duration or sperm transfer, are interacting phenotypes, i.e. the trait value observed potentially depends upon both the male and the female mating partner, and one cannot simply assume that the outcome reflects the optimal trait value of the focal individual (Edward, Poissant, Wilson, & Chapman, 2014; Hall, Lailvaux, & Brooks, 2013; Krebs, 1991; Moore, Brodie, & Wolf, 1997). In the case of copula duration, there is evidence among insects for high degrees of both male (e.g. dung flies, *Scathophaga stercoraria*: Parker, 1970; Parker & Simmons, 1994) and female (e.g. hangingflies, *Harpobittacus nigriceps*: Thornhill, 1983) control, as well as evidence for mutual control (e.g. fruit flies, *Drosophila melanogaster*: Edward, Poissant, et al., 2014). For any particular species, it is therefore important to understand both which sex is able to control different aspects of mating interactions, and how the controlling sex chooses to invest in mating behaviour or ejaculate transfer across successive mating opportunities that differ in their likely fitness returns. In this study, we investigated these questions, i.e. whether males or females control mating duration and whether males invest more in matings with a novel female, in the birch catkin bug, *Kleidocerys resedae* (Heteroptera; Lygaeidae).

METHODS

Kleidocerys resedae is a widespread, univoltine insect that can often be found in large numbers in early summer, on developing birch catkins, into which the females also lay their eggs. Almost whenever they are observed in the field, a large proportion of individual bugs can be found as mating pairs, indicating that males and females frequently mate and invest a large proportion of the available time in mating behaviour. We collected males and females of *K. resedae* from catkins of birch trees, *Betula pendula*, in the vicinity of the Institute of Evolutionary Biology and Ecology, University of Bonn, Germany during the second half of May. For each of the three experiments described below, bugs were collected fresh from the field and held between mating trials in 60 mm diameter petri dishes at room temperature (approximately 20–21 °C), under ambient light and provided with fresh catkins every second day. To prevent the catkins from drying out, petri dishes were sprayed before use with tap water.

Experiment 1: Sexual Isolation and Copula Duration

To measure the effect of sexual isolation on copula duration, we randomly assigned male and female bugs to one of five treatments, keeping individuals isolated for either 0, 1, 2, 3 or 4 days in their own petri dish. After the appropriate period, we transferred individual males on their own catkin to a petri dish containing a randomly selected female from the same treatment also on her own catkin, and made sure the two catkins were touching each other. In the 'no sexual isolation' treatment (0 days isolated), to control for handling effects we also first collected males and females from the field and separated them into individual petri dishes, putting randomly selected males and females together about 1 h later. Usually about six to 10 petri dishes with paired bugs were observed simultaneously to determine copula duration. To ensure that no start or end of a mating was overlooked, each dish was checked at least every 5 min, and we recorded whether the individuals were mating or not. Individual pairs that were in contact but not yet mating, as well as those that had started mating some time ago, were observed more often. To estimate copula duration, we used the mean of the following two estimates: the temporal interval between the first observation in which the pair were seen mating and the first observation after a mating, and the interval between the last observation before the start of mating and the last observation during mating. The pairs were separated after the mating into their individual dishes and paired again about 1 h later to estimate copula duration for a second mating.

Experiment 2: Control over Copula Duration

Because the results of experiment 1 (see below) could be interpreted as either a male or a female response to sexual isolation, we next investigated which sex controls copula duration in *K. resedae*. To determine whether the period of male or female sexual isolation, or the interaction of the two, influences copula duration, we first isolated a new set of field-collected individuals for 2 days in individual petri dishes. Thereafter, approximately half of the individuals were allowed to mate once to produce a pool of recently mated individuals. About 1 h after these matings, the recently mated individuals (designated '1 h' sexual isolation) and the remaining isolated individuals that had not just mated (designated '48 h' sexual isolation) were randomly assigned to male and female pairs (but avoiding the situation in which the same individuals that had just mated together were paired together again). With this design, approximately half of the individuals belonging to one sex and treatment were paired with members of the opposite sex belonging to the same treatment, and half with members of the opposite sex belonging to the opposite treatment, resulting in a balanced 2 × 2 design with respect to the two treatments (isolated for 48 h versus 1 h) for both sexes. The paired individuals were observed and copula duration was estimated as described for experiment 1.

Experiment 3: Male Response to Female Novelty

To determine whether mating with a novel or the same partner influences copula duration, we first collected and isolated several new individuals for 2 or 3 days in petri dishes with birch catkins. Thereafter we transferred individual males with their catkins to individual female dishes and allowed the bugs to mate. After mating, the individuals were separated again. About 1 h later, two pairs of individuals that had mated at approximately the same time were identified, and both randomly assigned to either a 'same' or 'different' treatment. In the same treatment, males were again put