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When males stop having sex: adaptive insect mating tactics during parental care

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The theory of prudent sperm allocation predicts that males should reduce their investment in sperm transfer when their mate's propensity to produce offspring is low. Furthermore, if mating is costly for females, they would benefit from signalling this propensity to males to keep them from attempting to mate. Here we document a remarkable flexibility in insect mating tactic during the period of parental care. Extensive video observations in the biparental burying beetle *Nicrophorus vespilloides* revealed that pairs mate frequently before and during oviposition, temporarily discontinue their sexual activity while young larvae are present, and resume mating when males are close to leaving the brood. An experiment, in which we switched males at different time intervals and determined their P_2 value (i.e. the proportion of progeny sired by the second male), confirmed the lack of mating activity during the first few days of larval care and its resumption later in parental care. Females cease laying eggs when caring for larvae, but females will resume egg laying if none of the larvae from their first clutch reach the carcass. We manipulated females into producing such replacement clutches by withholding their larvae, and we found that in this situation males did not cease to mate, but continued to engage in frequent copulations. Hence, males have the ability to assess a female's propensity for oviposition during the period of parental care. Further experiments demonstrated that males do not use the presence or absence of larvae as a cue to adjust copulation rate, but instead use female-produced cues of reproductive state. Therefore, our study reveals that female beetles express cues that display their reproductive condition and males have evolved the ability to detect these cues and respond with prudent sperm allocation. Our findings further contribute to the developing picture of the sophisticated mating tactics that insects employ.

It has long been established that copulations can entail nontrivial costs, not only to females but also to males (Dewsbury, 1982). The evolutionary consequences are males that are selected to mate or allocate their sperm strategically (Wedell, Gage, & Parker, 2002). Such male prudence can be manifested in male choosiness, or adjustments in mating frequency, mating duration or number of sperm transferred (e.g. Bretman, Fricke, & Chapman, 2009; Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2011; Pilastro, Scaggiante, & Rasotto, 2002; Saether, Fiske, & Kalas, 2001; Thomas & Simmons, 2007) and has been documented in response to a female's genetic quality, mating status, receptivity, reproductive maturity, novelty or the overall risk of sperm competition (see, e.g., references in Bonduriansky, 2001; Edward & Chapman, 2011; Kelly & Jennions, 2011; Parker & Pizzari, 2010; Steiger, Franz, Eggert, & Müller, 2008; Thomas, 2011). Therefore, males that employ strategic mating must have the ability to use cues or signals arising from the female, conspecific males or other sources to evaluate the net benefit of mating. For many invertebrates as well as vertebrate species it is known that the presence of rival males serves as a cue used to determine the risk of sperm competition (Bretman, Westmancoat, Gage, & Chapman, 2011). Male crickets, *Teleogryllus oceanicus*, can directly assess a female's mating status and produce an ejaculate containing sperm of lower viability (proportion of live and dead sperm) when mating with multiply mated females (Thomas & Simmons, 2009). The cue used consists of cuticular compounds left behind on the female by previous mating partners. In the monandrous bee *Andrena nigroaenea*, the females themselves produce specific chemical substances after mating, which render females unattractive to males (Schiestl &

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Ayasse, 2000). Many mammalian males show oestrus testing, sniffing the females' genitalia or urine, and mate only when they are close to ovulation (e.g. Rasmussen & Schulte, 1998; Russell, 1985).

In many taxa with prolonged parental care, caring for young is not compatible with the production of more young. For example, in most mammals, lactation suppresses ovulation until the young are weaned (McNeilly, 2001; Tomogane, Ota, & Yokoyama, 1975). During this infertile time males hardly benefit from copulating, and hence, if mating is costly, should avoid mating with those females. In birds with biparental care, observation of the mating behaviour revealed high mating frequency before or around the time of oviposition, but typically copulation rate dropped drastically afterwards (see, e.g., references in Birkhead, Atkin, & Møller, 1987), supporting the hypothesis of prudent sperm allocation (Wedell et al., 2002) in cases where matings yield no or low benefits.

Although mating strategies in insects have been studied extensively, little is known about mating behaviour during biparental care, probably because parental care in general, and biparental care in particular, is rare in insects (Costa, 2006; Wong, Meunier, & Kölliker, 2013). There is some information on the mating behaviour of biparental insects (Chamorro-Florescano & Favila, 2009; Cook, 1990; Eggert, 1992; Favila, Nolasco, Florescano, & Equihua, 2005; House & Simmons, 2006; House et al., 2008; Müller & Eggert, 1989; Nalepa, 1988; Nalepa & Mullins, 2011; Schuster & Schuster, 1985), but in several of these species, males do not contribute to care after oviposition, and in others, we currently do not know whether mating rate declines once oviposition is complete.

Burying beetles, long known for their elaborate biparental care (Pukowski, 1933), are an ideal model organism to test the hypothesis of prudent sperm allocation during parental care. Burying beetles reproduce on dead vertebrates, and it is usually a male and a female that bury a carcass and feed and defend their young (Eggert & Müller, 1997; Pukowski, 1933; Scott, 1998). Females lay their eggs, usually between 20 and 60, singly in the soil surrounding the carcass (Müller & Eggert, 1990; Smiseth, Ward, & Moore, 2006). After the first larva has hatched, females normally do not lay any more eggs during the time of larval care (Müller, 1987) and males would thus not immediately benefit from continued mating. Females resume egg laying only when the brood is lost to predators, competitors or microbes and the carcass is still suitable to support a brood (Müller, 1987). In this case, males might benefit from additional matings, because female burying beetles, like many socially monogamous bird species, engage in extrapair copulations (Eggert, 1990; Müller & Eggert, 1989; Pettinger, Steiger, Müller, Sakaluk, & Eggert, 2011).

After having detected a carcass, males and females engage in repeated mating with the same partner (House et al., 2008; Müller & Eggert, 1989; Pettinger et al., 2011). Most females have stored sperm when arriving at a carcass, so the resident male has to compete with other males' ejaculates for the fertilization of the eggs (Müller, Braunisch, Hwang, & Eggert, 2007; Müller & Eggert, 1989). In a laboratory study, a single copulation resulted in only about 11% paternity for the last male to mate, but through the mechanism of repeated mating (on average 70 times in the first 24 h on the carcass) resident males can achieve high paternity even with females that have stored large amounts of sperm (Müller & Eggert, 1989). Several findings demonstrate that male mating behaviour in the biparental burying beetle *Nicrophorus vespilloides* can be flexibly adjusted to specific situations: monogamous pairs mate more frequently in the presence of a carcass than in the absence of one (Müller & Eggert, 1989). When two males competed around a carcass, the dominant male's copulation frequency was positively correlated with that of his subordinate rival, suggesting that dominant males increase mating frequency in response to the increased risk of sperm competition (Pettinger et al., 2011). Subordinate males, whose mating opportunities with resident females are limited, exhibit longer copulation durations than dominants (Sakaluk & Müller, 2008), and male *N. vespilloides* males mate more rapidly when encountering unfamiliar females, which may enable them to inseminate evenly all the females present on the same carcass (Steiger, Franz, et al., 2008). In addition, a recent study found that male age influences mating effort, with older males investing more in copulations than younger ones (Benowitz, Head, Williams, Moore, & Royle, 2013).

Despite all this insight into burying beetle mating tactics, relatively little is known about sexual behaviour during the period of parental care, when females do not oviposit. Fetherston, Scott, and Traniello (1994), for example, observed monogamous pairs of *Nicrophorus orbicollis* caring for larvae, but did not provide any information about mating activity. Therefore, the present study was designed to obtain detailed information about the mating behaviour during an entire breeding attempt, including the period of intensive biparental care. We hypothesized that males on buried carcasses are able to gain information about a female's reproductive state and do refrain from copulations during the period of offspring tending, but mate repeatedly when females are likely to produce eggs, that is in the beginning of the breeding cycle or when the offspring are withheld and females are forced to resume egg laying. We tested this hypothesis using time-lapse video recordings of unmanipulated monogamous pairs and pairs in which females were induced to produce a replacement clutch. An experiment designed to detect sperm transfer during different phases of parental care from the paternity of offspring served to verify the findings of the video observations. Finally, we were interested in the question of how males gain information about whether their breeding partner is or is not about to produce another clutch of eggs, that is which cues they use to adjust mating rate. We tested two nonexclusive possibilities: (1) males use larval cues (i.e. males do not engage in copulations when they are on a carcass and larvae are present); and/or (2) males use female cues (i.e. on a carcass, males only engage in copulations with females that are about to produce an egg clutch). The prerequisite for the second hypothesis is that females express cues that inform about their physiological readiness or unreadiness to lay eggs.

METHODS

Origin and Maintenance of Experimental Animals

Experimental *N. vespilloides* animals were the first- or second-generation offspring of beetles collected from carrion-baited pitfall traps in a deciduous forest near Freiburg, Germany (48° 00′ N, 07° 51′ E). Beetles were maintained in temperature-controlled chambers at 20 °C with a 16:8 h light:dark cycle. Before the experiments, groups of up to five adults of the same sex and family were kept in small plastic containers (10 × 10 cm and 6 cm high) filled with moist peat and fed freshly decapitated mealworms twice a week. At the time of experiments all experimental animals were virgin, between 20 and 40 days old and not related to each other within a specific experiment. After the experiments all beetles were freeze-killed.

Video Analyses of Mating Frequency during Parental Care

Mating frequency of *N. vespilloides* pairs in two different contexts was determined by analysing time-lapse video recordings covering a total of 3360 h of observations. Each pair was recorded for approximately 10 days, from the introduction of the carcass to