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Sociosexual environment influences patterns of ejaculate transfer and female kicking in *Callosobruchus maculatus*

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There is growing evidence that many organisms adjust their physiology and behaviour during sexual encounters according to changes in their sociosexual situation. Selection tends to favour plasticity in males that can strategically ejaculate and females that can alter their resistance or remating behaviour adaptively. We investigated plasticity both in the ejaculate transfer patterns of males and in the pericopulatory kicking behaviour of female seed beetles, *Callosobruchus maculatus*. We altered the sociosexual environment of the beetles by (1) manipulating male mating history, (2) preventing females from kicking and (3) altering the risk of sperm competition. Males mating for the second time transferred half the weight of ejaculate of virgin males, but at the same rate. Males mating in the presence of rivals did not alter the weight of ejaculate, as might have been expected; however, the transfer of ejaculate began sooner. By looking at the effect of female kicking on male ejaculate transfer, we found that when females have their hindlegs ablated to prevent kicking, the rate of ejaculate transfer slows, but the weight remains the same. From the female perspective we found that the probability of females kicking was positively related to both copula duration and ejaculate weight, but also to male mating history; females were less likely to kick males that had mated more times previously even after controlling for copula duration and ejaculate weight. Finally, we found that removing a female's hindlegs increased the probability that females (attempted to) kick. Our results show that males are, to some extent, plastic in their ejaculatory responses to the sociosexual environment. More striking, however, and not previously documented, is that female kicking is affected by environmental variables, including male mating history.

Although individual sperm are not generally costly to produce, the complex package of seminal fluid and sperm that make up an ejaculate can be (Dewsbury, 1982; Kelly & Jennions, 2011; Perry, Sirot, & Wigby, 2013). In a recent review, Perry et al. (2013) described how the composition of an ejaculate as a whole (i.e. cells, molecules and third parties) can influence the reproductive success of both sexes. Given this, males that are able to strategically adjust their ejaculates depending on the sociosexual environment should be favoured (Simmons, 2001; review: Wedell, Gage, & Parker, 2002). There is a large body of evidence showing how males make such adaptive adjustments to the quality and quantity of their ejaculates based on a range of environmental circumstances (meta-analysis: Kelly & Jennions, 2011; review: Wedell et al., 2002). One of the most commonly studied sociosexual environments is the risk of sperm competition brought about by the presence of rival males (Parker, 1970). There is ample evidence to support the notion that ejaculate size increases with the risk of sperm competition across a wide range of taxa (see Table 1 in Kelly & Jennions, 2011). However, there are other factors that affect male ejaculate expenditure, in particular sperm depletion caused by male mating history (Preston, Stevenson, Pemberton, & Wilson, 2001).

From a female perspective, the reproductive value of current and future matings may depend on the potential mate's mating history, as females in general have greater fecundity from larger ejaculates (South & Lewis, 2011) and males become sperm depleted with sequential matings (Preston et al., 2001). Female lepidopterans tend to show a greater reproductive output after mating with virgin males (Torres-Vila & Jennions, 2005), and males' spermatophore size is greatly reduced after the first mating (Cook & Wedell, 1996). Hence, in situations in which males strategically allocate ejaculate, or are limited in their ejaculate production, female fitness can be affected by male behaviour.

The importance of dynamic ejaculate allocation is illustrated in the seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). Female *C. maculatus* benefit from receiving larger ejaculates in the form of increased lifetime fecundity (Savalli & Fox, 1999; but see

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Table 1

Models tested to determine the function that best suits both sets of data in order of best fit as judged by the lowest AIC

Model and formula	Experiment 1			Experiment 2		
	AIC	Δ AIC	Parameters	AIC	Δ AIC	Parameters
Three-parameter logistic $f(x)=\frac{A}{1+e\left(\frac{\mathrm{xmid}-x}{\mathrm{scale}}\right)}$	−603.78		**A=0.083** **xmid=3.752** **scale=0.529**	−912.37		**A=0.157** **xmid=4.592** **scale=0.847**
Four-parameter logistic $f(x)=B+\frac{A-B}{1+e\left(\frac{\mathrm{xmid}-x}{\mathrm{scale}}\right)}$	−601.83	−1.95	B=−0.003 **A=0.084** **xmid=3.900** scale=0.861	Did not converge		
Three-parameter asymptotic $f(x)=A+(R_0-A)\cdot e(-e(\mathrm{lrc}\cdot x))$	−598.75	−5.03	**A=0.103** R_0=−0.042 **lrc=−1.415**	−897.99	−14.38	**A=0.323** **R_0=−0.056** **lrc=−2.333**
Two-parameter asymptotic $f(x)=A\cdot(1-e(b\times x))$	−595.00	−8.78	A=0.167 b=0.082	Did not converge		
Michaelis–Menten $f(x)=V\mathrm{m}\cdot\frac{x}{(k+x)}$	−594.78	−9.00	Vm=0.310 k=23.087	Did not converge		
Linear $f(x)=ax+b$	−592.77	−11.01	b=−0.001 **a=0.010**	−894.43	−17.94	**b=−0.029** **a=0.022**

All models were compared with the best model and sorted by increasing Δ AIC where more negative values indicate a poorer fit. The formula for the function is italicized, and parameters that were significant are in bold font. x = input variable (in this case ejaculate weight), A = right-side asymptote, e = exponential, xmid = value of x at the inflection point, scale = growth rate on x axis , B = left-side asymptote, b = y intercept, R_0 = the response when x is 0, lrc = the natural logarithm of the rate constant, Vm = the maximum value of the response, similar to an asymptote, k = value of x at half maximum, similar to xmid, a = slope of a linear curve. Nonconvergence indicates a poor fit for the data, and so models that did not converge were not considered further.

Eady, 1995) and male *C. maculatus* have particularly large first ejaculates: 5–8% of their body weight (Fox, Hickman, Raleigh, & Mousseau, 1995; Savalli & Fox, 1998). However, producing such a large ejaculate is likely to be costly, and male longevity has been suggested to decline as the volume of ejaculate transferred increases (Paukku & Kotiaho, 2005). Furthermore, males show a dramatic reduction in sperm number after successive matings (Eady, 1995). In terms of the adaptive plasticity of mating behaviours in species like *C. maculatus*, we expect to see males strategically tailoring their ejaculate expenditure, and females altering their behaviour in accordance with male mating status by increasing the rejection rate of males with little sperm or ejaculatory nutrition to transfer.

Female seed beetles appear to resist males by kicking them during mating, a behaviour thought to be an adaptation to reduce the amount of damage caused to the females' reproductive tract by males' sclerotized penile spines (Crudgington & Siva-Jothy, 2000; Gay, Hosken, Eady, Vasudev, & Tregenza, 2011). Savalli and Fox (1998) proposed that females begin kicking when a mating is complete and they get no further benefit from mating, i.e. when the male has finished transferring the ejaculate. From this hypothesis, the prediction arises that strategic ejaculation and patterns of sperm depletion over successive copulations should influence the patterns of female kicking behaviour.

On the one hand, Savalli and Fox's (1998) hypothesis seems intuitive if the kicking phase starts when the female's interests change from continuing to terminating the copulation. On the other hand, however, Edvardsson and Canal (2006) found that ejaculate size continues to increase with copulation duration, i.e. females begin kicking before males have finished transferring their ejaculate. Understanding the behavioural and ejaculatory dynamics of copulation in this species depends on unravelling the relationship between the duration of mating, ejaculate transfer and the onset of female kicking. As males are, at least partially, in control of female kicking behaviour (Wilson & Tomkins, 2014), and male sperm count decreases with additional matings (Eady, 1995), we expect that the more times a male has mated, the slower ejaculate transfer will be and the longer it will take a female to begin kicking. Furthermore, we predict that the probability of a female kicking will increase not only with the weight of the transferred ejaculate, but also with copulation duration.

Sperm competition theory predicts that males experiencing a risk of sperm competition should transfer a larger ejaculate (Parker, 1970). Here, we investigated whether the presence of rival males affects how males transfer their ejaculates and when females begin to kick. If Savalli and Fox's (1998) hypothesis is correct, and females begin kicking when there is no further benefit to copulating, we expect females receiving a larger ejaculate, that is females mating in the presence of rival males (or with virgin males), to have a greater probability of kicking at any given time relative to females mating in isolation (or with nonvirgin males).

In the present study, we carried out a series of experiments that tested Savalli and Fox's (1998) hypothesis against Edvardsson and Canal's (2006) finding that ejaculate weight continues to increase with copula duration by exposing mating pairs to a range of sociosexual environments. To do this (1) we quantified the pattern of ejaculate transfer from males with different mating histories and environments with and without risk of sperm competition, (2) we investigated how female kicking behaviour is affected by male mating history and the risk of sperm competition and (3) we investigated how female kicking behaviour relates to ejaculate transfer.

METHODS

Study Species

Seed beetles, *C. maculatus*, are a global pest of stored legumes, and have been extensively studied in evolution and ecology. Females lay eggs on the seed coat of *Vigna* beans where larvae