Animal Behaviour 83 (2012) 75–79

Contents lists available at SciVerse ScienceDirect

Animal Behaviour

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

Do longer genital spines in male seed beetles function as better anchors during mating?

Johanna L. Rönn[a,*], Cosima Hotzy[b,1]

[a] Department of Zoology, Ecology, Stockholm University
[b] Evolutionary Biology Centre, Department of Ecology and Evolution, Animal Ecology, University of Uppsala

ARTICLE INFO

Article history:
Received 22 June 2011
Initial acceptance 16 August 2011
Final acceptance 22 September 2011
Available online 8 November 2011
MS. number: 11-00494R

Keywords:
adaptive harm hypothesis
Callosobruchus
fitness
genital spine
male–male competition
mating duration
pleiotropic harm hypothesis
postmating sexual selection
sexual conflict

In a wide variety of taxa, males are equipped with harmful structures on their genitalia such as hooks, barbs or spines. The proximate function of these structures and the evolutionary forces behind their evolution have been discussed and investigated during the last few decades. One model system in which these structures have attracted particular attention is the *Callosobruchus* seed beetle group. The main suggestion for the occurrence of genital spines in this group of species has been that their primary function is to act as an anchor during mating, to aid the male in staying attached to the female. This would prevent females terminating copulation prematurely, or would hinder take-overs by rival males. We used five populations of *Callosobruchus* seed beetles, with differing lengths of the male genital spines, to test whether longer spines provide males with an enhanced attachment during mating. This was tested both with and without male competition in the form of rival males present or not during focal copulations. We found that males from populations with longer spines did not stay in copula for longer than males from populations with shorter spines. In addition, females mating with males with longer genital spines suffered a fitness cost in terms of lower lifetime offspring production. In conclusion, we did not find any support for the hypothesis that the primary function of genital spines in seed beetles is to serve as an anchor.

Male genitalia are among the fastest evolving morphological characters of internal fertilizers and sometimes the only character by which closely related species can be identified (Eberhard 1985; Hosken & Stockley 2004). Most evidence suggests that the driving force behind the extraordinary variation seen in the morphology of male genitalia is postmating sexual selection (Parker 1970; Arnqvist 1998; Birkhead & Møller 1998; Birkhead & Pizzari 2002) through either sperm competition (Parker 1970) or cryptic female choice (Eberhard 1996). Among the various structures and morphologies of the male genitalia, the traits that have been most difficult to explain are those inflicting harm upon females during mating. Playing a central role in male–female coevolution, structures like these have been extensively investigated during the last decade (Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002; Rönn et al. 2007). Structures on the male genitalia, such as spines, hooks, barbs or spikes, can be found in many taxa (von Helversen & von Helversen 1991; Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002) and two main hypotheses to explain the existence of these structures have been suggested. According to one of these, the pleiotropic harm hypothesis (Parker 1979), the harm caused to females during mating is considered to be a negative pleiotropic side-effect of genital structures in males that have a primary function other than harming the females. Structures like these will be selected for as long as the benefit for a male equipped with harmful structures outweighs the costs he suffers by lowering his mate's reproductive output (Parker 1979). According to the second hypothesis, the adaptive harm hypothesis, males may also gain by harming their mates directly (Johnstone & Keller 2000). For example, harming females may directly benefit the male if the harm reduces female remating propensity leading to less sperm competition for the male. It may also increase the female's allocation of resources to current reproduction rather than future reproduction and maintenance (Michaels 1998; Lessells 1999) and males may thus enjoy paternity benefits. Conflicts between the sexes over reproductive decisions, such as the ones described above, are thought to be a key force in the evolution of many reproductive characters (Gavrilets 2000; Martin & Hosken 2003) and evolution of male genitalia that impose physical injury

---

* Correspondence: J. L. Rönn, Department of Zoology, Ecology, Stockholm University, SE-106 91 Stockholm, Sweden.
E-mail address: Johanna.Liljestrand-Ronn@zoologi.su.se (J. L. Rönn).

[1] C. Hotzy is at the Evolutionary Biology Centre, Department of Ecology and Evolution, Animal Ecology, University of Uppsala, Norbyvägen 18D, SE-752 36 Uppsala, Sweden.

0003-3472/$38.00 © 2011 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.anbehav.2011.10.007

on females during mating has been suggested to be a product of sexually antagonistic coevolution (reviewed in Arnqvist & Rowe 2005; Reinhardt et al. 2007).

In seed beetles, *Callosobruchus* spp. (Chrysomelidae: Bruchinae), the male genitalia are equipped with sclerotized spines that puncture and cause harm to the inner reproductive tract of females during mating (Crudgington & Siva-Jothy 2000). Females suffer costs because of these injuries (Crudgington & Siva-Jothy 2000; Crudgington 2001; Edvardsson & Tregenza 2005; Rönn et al. 2006, 2007; Hotzy & Arnqvist 2009), and towards the end of copulation females kick males with their hindlegs until the mating is terminated (Qi & Burkholder 1982; Tufton 1993; Eady 1994; Edvardsson & Canal 2006). When females are prevented from kicking, mating duration increases as well as the genital damage suffered by females and therefore it has been suggested that the females are able to minimize the costs of mating caused by the genital spines by simply keeping the matings short (Crudgington 2001; Edvardsson & Tregenza 2005). As a counteradaptation to the harmful genital spines in males, females have evolved a thick layer of connective tissue along their inner reproductive tract and patterns across species show that there is correlated evolution between the harmfulness of the male's genital spines and the amount of connective tissue in the female's reproductive tract (Rönn et al. 2007). It has also been shown that males equipped with longer spines are more successful in sperm competition and that the harm caused to females seems to be a pleiotropic side-effect (Hotzy & Arnqvist 2009). However, by which mechanism the spines provide males with an advantage in sperm competition is still unknown. A suggested function of the spines has been that longer spines work as a more efficient anchor and aid males in prolonging the mating (Edvardsson & Tregenza 2005; Eady et al. 2007; Hotzy & Arnqvist 2009). That the primary function of genital spines, hooks or barbs is to aid males in firmly anchoring to the female during mating has also been suggested in other taxa where these structures occur (Eberhard 1985; Arnqvist 1998; Blanckenhorn et al. 2002; Polak & Rashed 2010; Rodriguez-Marquez & Peretti 2010). Prolonged mating may be beneficial for males for two reasons: (1) it may prevent females from remating and hence work as a form of mate-guarding strategy (Simmons 2001) or (2) it may allow enough time for ejaculate substances to be transferred to the female to reduce her remating propensity or increase displacement of previous ejaculates (Eberhard 1996).

Callosobruchus seed beetles are cosmopolitan pests with the potential to, very rapidly, reach extremely high population densities. Adults are facultative aphagous (i.e. do not need to eat or drink as adults; Wightman 1978; Messina & Slade 1999; Savalli & Fox 1999) and spend their time and energy on mating and laying eggs. Females are thus continuously exposed to harassment by males trying to achieve matings (Rönn et al. 2006; Gay et al. 2009). Not only females but also already mating pairs are harassed by rival males trying to interrupt and take over matings (Gay et al. 2009; J. Rönn). This highly competitive situation, together with the fact that once mating is initiated the sexes are only attached to each other through the male genitalia, has given rise to the hypothesis that the function of spines could be to serve as an anchor. The basis of this hypothesis is that if optimal mating duration differs between males and females, then realized mating duration should be intermediate (owing to male and female adaptations) and males equipped with longer spines should be better able to shift the mating duration towards the male optimum. To test this hypothesis we predicted that longer genital spines would provide the copulating male with benefits both in a situation with male–female conflict over mating duration (i.e. that males equipped with longer spines would be better able to withstand the female's kicking behaviour) and in male–male competition over access to females (i.e. that males with longer spines would be better able to withstand harassment by males). We used five populations of *Callosobruchus maculatus* that differed in the lengths of the male genital spines to test this hypothesis directly. To see whether this also provides males with fitness benefits we assessed the effects of spine length and mating duration on female fitness.

METHODS

Populations and Rearing

We used five populations of *C. maculatus*: South India (hereafter SI), Oman, Zaire, IITA and Oyo (the last two originating from Nigeria), Oyo (also from Nigeria) and Zaire. All populations were reared on cowpea beans, *Vigna unguiculata*, and generation time in the laboratory was approximately 3–4 weeks for all populations. Beetles were maintained under controlled laboratory conditions at 30 °C and 60% relative humidity with a 12:12 h light:dark cycle and populations have been kept under these laboratory conditions for approximately 35 generations. All mating trials were performed at 22 ± 1 °C. The life cycle of these seed beetles starts by females cementing their eggs on the surface of the beans and the larvae then bore themselves into the bean where they go through several larval instars before pupating (Southgate 1979).

The five populations used in this experiment were chosen based on the length of their ventral genital spines, as this has been shown to have an impact on sperm competition success and also as these spines are among the longest spines on the male intromittent organ (Hotzy & Arnqvist 2009). Males originating from the SI population have an intermediate spine length and this population was therefore chosen as a reference population. Of the additional four populations two, Oman and IITA, have males equipped with shorter spines than those in SI and the final two populations, Oyo and Zaire, include males equipped with longer genital spines than those in SI. Previous studies on these populations have shown that, although they are genetically distinct (Dowling et al. 2007), they are fully reproductively compatible, with an egg hatching success of >95% in interpopulation crosses (Hotzy & Arnqvist 2009).

Length of Genital Spines

We measured the length of the ventral genital spines by inflating the male genitalia while males were anaesthetized with CO_2 ($N = 8$–11 males per population). Inflation was done using a micropipette tip connected to an adjustable water-jet vacuum pump. Once fully inflated, the male genitalia were stabilized in water at 100 °C. They were then photographed (lateral view) with a Lumenera Infinity 2-2 digital camera mounted on a Leica MZ8 dissection microscope. Image analysis (ImageJ, http://rsb.info.nih.gov/ij) was used to measure the five longest ventral spines of each male and a mean of these five measures was then used to categorize the population as having either long or short ventral genital spines (results from an ANOVA confirming population differences in spine length: $F_{4,46} = 3.409$, $P = 0.016$). The mean length of ventral spines (standardized length in pixels (SE; N)) for each population was as follows: SI: 47.03 (2.34; 11); Oman: 39.14 (2.68; 8); IITA: 43.65 (2.83; 10); Oyo: 51.50 (2.50; 11); Zaire: 49.42 (2.29; 11).

Experimental Set-up

To quantify the effects of male genital spine length on mating duration and female fitness we used the following mating protocol. Seventy-five virgin females from our reference population were collected and each was randomly assigned to a virgin male from one of the populations, creating the following mating combinations: 15 SI females × 15 SI males, 15 SI females × 15 Oman males, 15 SI females × 15 IITA males, 15 SI females × 15 Oyo males and 15 SI