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Morphology of *Tribolium castaneum* male genitalia and its possible role in sperm competition and cryptic female choice

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ABSTRACT. It is now well recognised that sexual selection proceeds after copula via competition between the sperm of different males, and via female influences on sperm storage and usage. The existence of, and potential for, these selection forces have led to the evolution of a wide range of behavioural, anatomical and physiological adaptations for successful reproduction. Males increase fertilization either by enhancing the success of their own sperm, or by negating or eliminating rival sperm. Ultimately, however, sperm are under the potential control of the female. In the red flour beetle, *Tribolium castaneum*, the last male to mate gains fertilization precedence over previous males that have mated. This phenomenon may be due to the morphology of the female sperm storage organ, which could encourage the temporal stratification of sperm through its narrow tubular structure. In addition, males themselves enhance fertilization precedence by removing rival sperm from the female tract. This study examines the detailed external and internal genital morphology of *T. castaneum* using scanning electron microscopy, and relates form to potential function in intrasexual competition and selection. We show that the aedeagus may articulate in a scoop-like manner to remove sperm, and we observe a retractable brush-like structure and discuss its function. In this beetle, a large and unexplained variation in reproductive success is observed between competing males and we suggest that some of this variance may result from mechanisms of selection and competition that are driven by the complex genitalic structure of *T. castaneum* males.

KEY WORDS: aedeagus, sexual selection, courtship, copula, insecta, coleoptera.

INTRODUCTION

Animal genitalia show remarkable diversity in form and function, and male genitalia in particular have evolved into a bewildering array of forms. Sexual selection may account for the evolution of many of these variant and complex structures because genitalic morphology may play a role in post-copulatory fertilization success (EBERHARD, 1991). When females mate with two or more males sperm competition for fertilization of the ova can occur (PARKER, 1970). Sperm competition is a widespread and influential phenomenon that has been responsible for the evolution of a range of male adaptations at the behavioural, physiological and morphological levels (SMITH, 1984; BIRKHEAD & MØLLER, 1998). For example, the potential for sperm competition has led to the evolution of male genitalia that remove rival sperm from the female reproductive tract (WAAGE, 1979, 1986; HAUBRUGE et al., 1999). It is becoming increasingly recognised that, in addition to post-copulatory sexual selection arising from sperm competition, females may play an active role in sperm competition and sperm selection (EBERHARD, 1991, 1996; PIZZARI & BIRKHEAD, 2000). Accordingly, some of the variance we record between males in sperm competition success may arise through female preference for the sperm of particular males. Since fertilization takes place within their bodies, females may be able to influence a male's reproductive success even after the male has achieved intromission, a phenomenon conceptualized by THORNILL (1983) as 'cryptic female choice'. Females may implement post-copulatory selection either through mechanisms such as active sperm ejection or preferential

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uptake (PIZZARI & BIRKHEAD, 2000), or potentially by more subtle management of ejaculates of different males (EBERHARD, 1991, 1996) such as directional movement of sperm to different spermathecae when multiple storage organs have evolved (OTRONEN, 1997; OTRONEN et al., 1997). *Tribolium castaneum* (Herbst) has a single spermatheca but this consists of multiple tubules (SINHA, 1953), which could be used to accommodate sperm of different males in different spermathecal sites.

Just as there are a number of signals that males have evolved to influence female willingness to mate, males may employ similar signals to influence cryptic female choice. In the red flour beetle *T. castaneum* male fertilization success in sperm competition is influenced by the rate that males rub the female elytra with their tarsi during copula (EDVARDSSON & ARNQVIST, 2000). Leg rubbing may therefore provide honest signals of some aspects of male fitness upon which females subsequently base their sperm selection decisions. In this study, we investigate the detailed morphology of *T. castaneum* male genitalia in relation to intrasexual selection and cryptic female choice. *T. castaneum* provides excellent opportunities for such a study. The outcome of sperm competition between different males in this species is well described and reveals that the last male to mate achieves precedence by fertilizing two-thirds of the ova (SCHLAGER, 1960; WOOL & BERGERSON, 1979; LEWIS & AUSTAD, 1990, 1994; LEWIS & JUTKIEWICZ, 1998; ARNAUD et al., 2001). The mechanism behind this pattern of fertilization precedence may be indirectly driven by the morphology of the female tract since sperm are stored in a tubular spermatheca, which could encourage a last-in first-out mechanism of sperm competition due to the temporal stratification of different ejaculates within the narrow cul-de-sac storage tubules. There is also evidence that male *T. castaneum* have evolved genitalia capable of removing rival sperm from the bursa copulatrix of recently-mated females (HAUBRUGE et al., 1999).

Despite detailed studies of the mechanisms of sperm competition, an important feature of the *T. castaneum* mating pattern has not been explained, namely that there is significant variation between males in fertilization precedence, and these differences are consistent for individual males (LEWIS & AUSTAD, 1990). Accordingly, there are as yet unrecognised mechanisms influencing fertilization precedence that may proceed within the environment of the female reproductive tract. An obvious trait that could be used to influence female management of sperm is male genitalic morphology, and we therefore conducted a detailed examination of the aedeagal morphology of *T. castaneum* with particular respect to traits that could have evolved to function in intrasexual competition and/or cryptic female choice.

MATERIAL AND METHODS

Beetles were cultured in dark incubators at 30 ± 3°C and 65 ± 5% RH with wheat flour and brewer's yeast (10/1-wt/wt) as rearing medium. Males were sexed as pupae and maintained individually in small vials with rearing medium.

Males were weighed to the nearest 10^{-4} mg on a Cahn electro balance. After anaesthesia and decapitation, genitalia were carefully removed intact. Dissected genitalia were mounted on glass slides (0.75mm diameter) and then critical-point dried through a series of dehydrating alcohol washes before being Au/Pd (60/40) coated.

Morphometric observations. Beetles originating from different geographic areas (Canada, Ivory Coast, Japan, Mozambique, Nigeria, Pakistan, Philippines, UK and USA) were examined to provide a range of body sizes. A total of 14 populations were examined and five males were observed per population. Genitalia for measurement were mounted on their side to present a lateral and perpendicular viewpoint for measurement. Since genitalia can articulate (see Discussion and Fig. 2), total aedeagal length was calculated by summing the lengths of the basal and lateral lobes (between which the articulation occurs, Fig. 2). Measurements were made from micrographs so that two-dimensional images were presented.

External and internal genital morphology. 20 male *T. castaneum* originating from Ivory Coast were used in this study. Genitalia were deliberately mounted in a range of positions so that a three-dimensional image of the aedeagus could be perceived. We also dissected some aedeagi further to observe their internal morphology.

RESULTS

Mean penis length averaged across all populations was 368.8 ± 2.8 µm (mean ± se, n=70). Total aedeagal length was significantly correlated with body weight across 70 males (r=0.351, P=0.003) (Fig. 1). Detailed morphometric measures of aedeagal dimensions showed that residual penis length did not differ significantly between the males of the 14 different populations ($F_{13,56}$= 1.80, P=0.065).

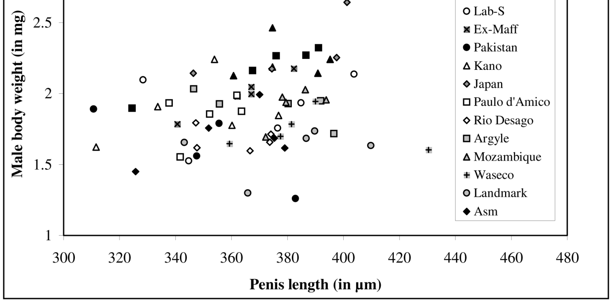

Fig. 1. – Allometric relationship between male body weight (in mg) and penis length (in µm) in *T. castaneum* (r= 0.351, P= 0.003). Data were obtained from 70 males of 14 populations (five males per population).

The detailed external and internal structures of the male genitalia of *T. castaneum* are illustrated in micrographs (Figs 2-5). A total of 20 aedeagi were examined for anatomical purposes. The aedeagus consists of a chitinous organ with a concave scoop lined with sclerotised spines, previously shown to trap and remove rival sperm (Haubruge et al., 1999). The aedeagus appears able to articulate about a central axis (marked on Fig. 2), which may enable the distal part of the aedeagus to swing up and down within the female bursa copulatrix and effect a scooping mechanism.

The internal morphology of the aedeagus reveals a significant brush-like organ encased within the aedeagal valves (Figs 3 and 4). This brush appears to be retractable and able to move in-and-out of the distal end of the aedeagus.

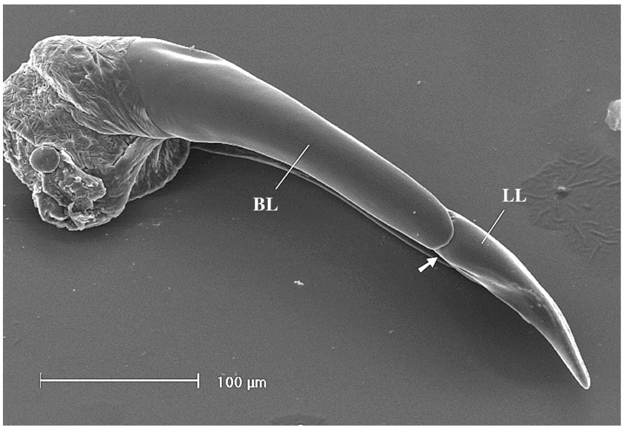

Fig. 2 (upper left). – Lateral view of *T. castaneum* male genitalia showing the two components that have been measured separately (BL: basal lobe and LL: lateral lobe) and are combined to produce a total length measure. The white arrow shows the articulation.

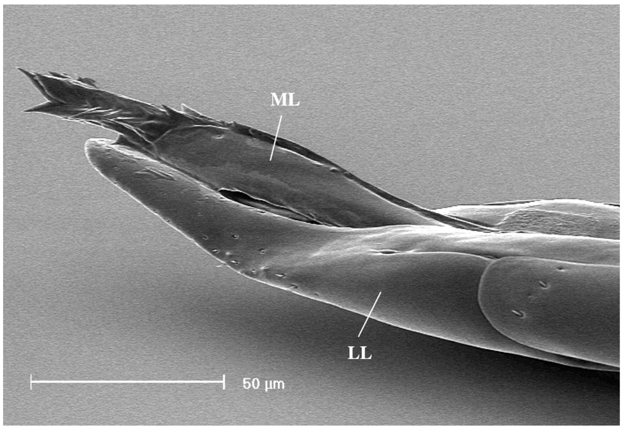

Fig. 3 (upper right). – Side view of *T. castaneum* male genitalia showing the median lobe (ML) expressed. This lobe is comprised of a brush-like structure, which emerges from an aperture in the lateral lobe (LL) (see Fig. 5).

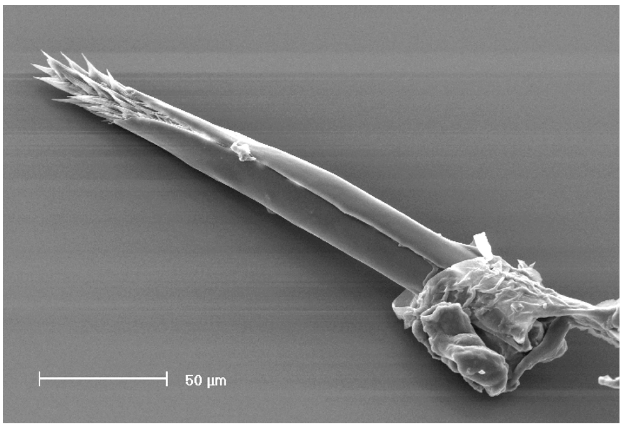

Fig. 4 (lower left). – Detailed view of the internal and retractable brush-like structure within the aedeagus of *T. castaneum*.

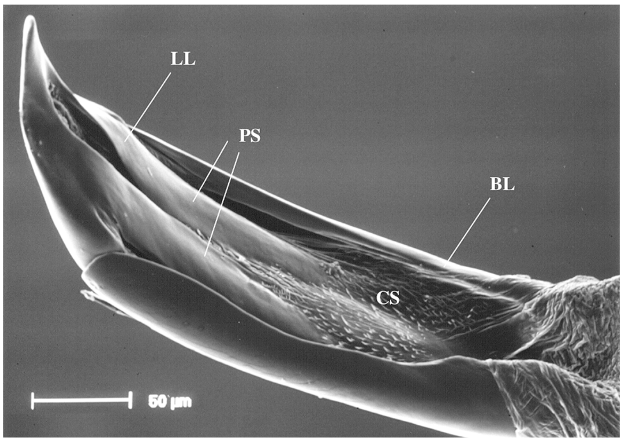

Fig. 5 (lower right). – Ventrolateral view of *T. castaneum* male genitalia with the concave furrow running dorsally. The movement of the lateral lobe (LL) appears to be effected by the sliding of paired struts (PS). An array of chitinous spines (CS) line the proximal half of the basal lobe (BL). The brush-like structure on the median lobe (Fig. 4) emerges from an aperture (see Fig. 3) near the distal tip of the aedeagus.

DISCUSSION AND CONCLUSIONS

Our examinations reveal a detailed picture of the male genitalia of *T. castaneum*. We found an allometric relationship between aedeagal dimension and body size across 70 males. Lewis & Austad (1990) recorded that the relative body size of *T. castaneum* males influenced the degree of second-male fertilization success in sperm competitions. Furthermore, there was a consistent, but unexplained, level of variance between males in the degree of sperm precedence they achieved (Lewis & Austad, 1990). Larger males could achieve an advantage in sperm competition if aedeagal dimensions influence sperm removal ability (Haubruge et al., 1999) and/or ability to stimulate cryptic female choice (Eberhard, 1991).

Microdissections and different orientations suggest that the aedeagus is able to articulate about a central axis. This articulation appears to be effected by the sliding of paired chitinous struts (Fig. 5) along the base of the aedeagus. A forward movement by these struts appears to generate a rise in the distal tip of the aedeagus about the fulcrum at the central axis. Accordingly, this articulation results in a scooping action of the aedeagus that may function to move sperm from the bursa copulatrix back onto the aedeagal spines where the sperm are trapped and removed from the fertilization set at the end of copula (HAUBRUGE et al., 1999). This mechanism may contribute to the fertilization advantage bestowed upon spermatozoa of the last male to mate (SCHLAGER, 1960).

Internal microdissections reveal a retractable brush-like structure within the aedeagus. This brush appears able to slide in-and-out of the aedeagus. This apparatus does not appear adapted to function in sperm transfer as it is non-tubular and could impede the movement of sperm from the male into the female tract. The brush-like apparatus may somehow interfere with previously-deposited rival sperm in the bursa or spermatheca akin to the actions of the secondary genitalia evolved by odonates (WAAGE, 1979). Alternatively, the brush may be used as a sexually-selected signal to which the female is sensitive. More vigorous stimulation of the female's elytra by a mating male's forelimbs encourages sperm selection and increased sperm competition success in *T. castaneum* (EDVARDSSON & ARNQVIST, 2000), and courtship stimulation of females is widespread in insects (EBERHARD, 1994). Similarly, the aedeagal brush may directly stimulate the female reproductive tract. Female insects have evolved sensory structures in the reproductive tract (OBARA et al., 1975 ; SUGAWARA, 1979 ; LUM & ARBOGAST, 1979 ; CORDOBA-AGUILAR, 1999) that are sensitive to the morphology and / or behaviour of male genitalia or ejaculates. For example, in the damselfly, *Calopteryx haemorrhoidalis asturica* (Vander Linden), males stimulate mechano-receptive sensilla of the female genital tract with their aedeagus. This stimulation results in sperm ejection from the spermatheca (CORDOBA-AGUILAR, 1999) and therefore a fertilization advantage for the stimulating male. The multiple-tubule spermatheca of *T. castaneum* provides a mechanistic opportunity for preferential sperm storage and usage, as is claimed for other insects (YUVAL et al., 1996, OTRONEN, 1997 ; OTRONEN et al., 1997). In *Scatophaga stercoraria* (L.), OTRONEN et al. (1997) observed significant interactions between male and female characters on the pattern of sperm storage. Moreover, in the fly *Dryomyza anilis* Fallén, which has multiple sperm storage sites, the single spermatheca is central for male fertilization success, and male-female interactions during copula, such as male leg-tapping on the female, influence the quantity of sperm stored in this spermatheca (OTRONEN, 1997). Similarly, if complex genitalic structures such as retractable aedeagal brushes have evolved as sexually-selected signals, enhanced and direct stimulation of the female reproductive tract during copula could influence sperm storage or usage.

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