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Coevolution of male and female reproductive traits across the Bruchidae (Coleoptera)

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Summary

1. Despite the obvious importance of spermatozoa to individual reproductive success a general explanation of variation in spermatozoan form and function is still lacking. In species with internal fertilization, sperm not only have to interact with the physical and biochemical environment of the female reproductive tract, but frequently face competition from the sperm of rival males. Both sperm competition theory and adaptation to the selective environment of the female reproductive tract have been implicated in the evolution of spermatozoan morphological diversity.
2. Using the comparative method, we examine variation in sperm length in relation to i) sperm competition intensity (as measured by relative testis size) and ii) female reproductive characters, across 15 species of beetle belonging to the family Bruchidae.
3. Stepwise multiple regression within a phylogenetic framework revealed sperm length to be positively correlated with female spermathecal duct length and negatively related to spermathecal volume, but not testes size, indicating that the female reproductive environment rather than sperm competition *per se* exerts selection on sperm length in this taxonomic group.
4. A positive association between testes volume and the volume of the female spermatheca was also evident suggesting correlated evolution of these traits.

5. A number of models of sexual selection could lead to the correlated evolution of male and female reproductive characters, although the underlying mechanisms of cause and effect remain elusive. Divergence between species (and populations) in primary reproductive traits is likely to present a significant barrier to heterospecific fertilisation, and thus contribute to reproductive isolation.

Keywords: Callosobruchus, postcopulatory sexual selection, sperm, spermathecal duct, comparative method.

Introduction

Across insect species, sperm morphology is extremely diverse (Jamieson 1987), suggesting rapid and divergent evolution of this character. Parker (1982) was the first to theorise that post-copulatory sexual selection, in the form of sperm competition, should favour the production of numerous tiny sperm, as observed in the field cricket *Gryllus bimaculatus* (Gage & Morrow 2003). However, positive relationships between sperm length and sperm competition risk have been reported in a variety of taxa. In a comparative study of 25 butterfly species, the length of eupyrene (fertilising), but not apyrene (non-fertilising), sperm was found to increase with the degree of female multiple mating, inferred from spermatophore counts of wild-caught females (Gage 1994). Using testis size as an alternative indirect measure of sperm competition risk, Morrow & Gage (2000) found a similar relationship in moths, but in this group, the length of both sperm types increased with sperm competition risk. Similar positive relationships between sperm length and sperm competition risk have been reported in birds (Briskie, Montgomerie & Birkhead 1997), nematodes (LaMunyon & Ward 1998, 1999), and mammals (Gomendio & Roldan 1991; although this has been refuted by Gage & Freckleton 2003), whilst across species of fish, sperm length was found to be negatively related to the intensity of sperm competition (Stockley *et al.* 1997). Hosken (1997), studying bats found no relationship between sperm size and sperm competition intensity.

Within species, LaMunyon & Ward (2002) found that in the nematode *Caenorhabditis elegans*, the size of sperm from males from artificial lines, genetically induced to have high levels of sperm competition, increased by nearly 20% after 60 generations of selection compared to the sperm of control-line males (LaMunyon & Ward 2002). In this species, larger sperm have been shown to crawl faster and displace smaller sperm (from the spermathecae), thereby taking precedence at fertilisation (LaMunyon & Ward 1998). By way of contrast, in *Drosophila melanogaster*, Pitnick *et al*. (2001) found no evidence that selection for monogamy altered sperm length.

Directional selection via sperm competition alone ignores the reproductive interests of females. Female reproductive morphology is often exceedingly complex and there is growing evidence that processes such as transport of sperm to and from the female sperm storage organs are at least partly under female control (Eberhard 1996), and thus female reproductive characters are likely to exert selection on sperm morphology (Eady 2001). A number of comparative studies have demonstrated correlations between sperm morphology and areas of the female reproductive tract, consistent with female-mediated selection on sperm morphology. In featherwing beetles (Dybas & Dybas 1981), moths (Morrow & Gage 2000) and scathophagid flies (Minder, Hosken & Ward 2005), sperm length has been shown to correlate positively with the length of the ducts leading to the sperm storage organs. Sperm length has also been shown to correlate positively with the size of the female sperm storage site in featherwing beetles (Dybas & Dybas 1981), stalk-

eyed flies (Presgraves, Baker & Wilkinson 1999) and Drosophila (Pitnick, Markow & Spicer 1999). These findings suggest that evolved differences in sperm morphology are adaptations in response to changes in the dimensions of the female reproductive tract, with the value of such adaptations being particularly important during sperm competition (Pitnick *et al.* 1999; Morrow & Gage 2000).

Here, we use the comparative method of analysing phylogenetically independent contrasts (Felsenstein 1985) to investigate whether sperm length is associated with testes size (an accepted indirect measure of sperm competition risk; Morrow & Gage 2000) and/or dimensions of the female reproductive tract, across 15 species of bruchid beetle (Bruchidae). In the Bruchidae, sperm are usually transferred to the female via a spermatophore deposited in the female's bursa copulatrix (*Zabrotes subfaciatus* does not appear to transfer a spermatophore; personal observation). Following copulation, sperm migrate from the spermatophore, through the spermathecal duct, to a chitinised spermatheca where they are stored until they are 'moved' back through the spermathecal duct to the site of fertilisation. Under a coevolutionary scenario, sperm length may exhibit correlated evolution with any one of these regions of the female reproductive tract.

Methods

(a) *Species: collection & culturing*

Collection details for each species (where known) are presented in Table 1. Species were transported to Sunderland as egg/larvae

infested seeds and immediately placed in small aerated plastic boxes and kept in a constant temperature environment at 27°C and 35% r.h., with a 12L:12D photoperiod (host plant seeds of *Kytorhinus sharpianus* were first incubated at 10°C for 4 weeks in order to break larval diapause; Ishihara & Shimada 1995). A few days prior to eclosion seeds were isolated in cell trays and subsequent emergent beetles isolated and identified. Virgins were used wherever possible to control for mating history and only first generation adults were used. All host plant material was destroyed 6 weeks after it was first received at Sunderland. One exception to this procedure was *Bruchus rufimanus*, specimens of which were collected in the field as adults and so their mating history was unknown (although the spermatheca of all three females contained sperm).

(b) *Microdissection and data collection*

Three days post eclosion, beetles were euthanised and dissected in insect saline. Digital images of male and female characters (see below) were captured using a Sony DXC-390P 3CCD colour video camera mounted on an Olympus SZH10 binocular microscope or Zeiss phase contrast microscope (used to measure sperm length), and measured using NIH Image (Scion Corporation). Length of the right elytron was also measured for all beetles, being an accepted correlate of body mass in *Callosobruchus maculatus* (Wilson & Hill 1989). To check this relationship held at the level of family, male beetles were also weighed, either individually or as a 'species batch', within 24 h of eclosion.

Measurement repeatability was estimated in *C. maculatus* via the intra-class correlation coefficient. All female traits (see below) were measured 3x's across 6 females, whilst in males, the repeatability of sperm size was estimated within an individual male by measuring 10 separate sperm 3x's and across males by measuring 10 separate sperm each, from 8 males. The repeatability of testes volume was estimated by measuring testes 3x's across 9 males.

Male traits. The two pairs of spherical testes were dissected out of each male and placed on a 'Thomas Buerker' haemocytometer and flattened, using a coverslip, to a depth of 0.1 mm. The flattened area of each testis was measured and used to calculate total testes volume. Mature sperm were recovered from the spermathecae of females, approximately 4h after a single copulation with a virgin male. Individual spermathecae were dissected free and transferred to cavity slides containing 40 μl insect saline. Sperm were expelled by gently squeezing the spermatheca with watchmaker's forceps in a series of soft pulses. This method ejected sperm through the severed spermathecal duct. The spermatheca was removed and the sperm solution mixed for 2 min using the tips of the forceps. A single drop of solution was transferred to a glass microscope slide and covered with a glass coverslip. The overall length and head length of the first three sperm encountered was measured from each dissection.

Female traits. The bursa copulatrix with the spermatheca still attached by its associated duct was carefully removed from virgin females. Spermathecal duct length was measured along its entire central axis from

its junction with the spermatheca to where it joins the oviduct. Bruchidaean spermathecae are elongated, cylindrical, tapered and curved (Mukerji & Chatterjee 1951). Assuming the spermatheca to be a coiled cone, we estimated its volume as Pi x mean radius x length. The mean radius was estimated by measuring the longitudinal area of the spermatheca and dividing it by twice the length of the line traced down the central axis of the organ. Dry weight, as a measure of size of the bursa copulatrix (Morrow & Gage 2000), was rejected because it would not take into account differences in the thickness of the bursal walls or the presence of bursal valves and/or various sclerotised structures. Thus, the volume of the bursa copulatrix was estimated in a similar manner to that of the spermatheca, although we appreciate this still fails to take into account the thickness of the bursal wall.

(c) *Comparative and statistical analysis*

Felsenstein's (1985) method of comparing phylogentically independent contrasts was used to test for correlated evolution among characters. The phylogenetic relationships between the species examined in this study (Figure 1) were derived from an extensive systematic analysis of 165 species, generated from the entire Cytochrome Oxidase 1 mitochondrial gene, the D2-D3 expansion segment of 28S nuclear ribosomal DNA, and partial taxonomic sampling (86 species) of an 800bp exon of elongation-factor-1α and approximately 1000bp of 18S nuclear ribosomal DNA (Geoff Morse, unpublished data). The Callosobruchus phylogeny was resolved according to Tuda *et al.* (2006). Morphological data were $\log_{10}$-transformed before analysis to normalise the

distributions, and standardised independent contrasts were calculated from the $\log_{10}$-transformed phenotypic values using the Comparative Analysis by Independent Contrasts (CAIC v. 2.6.8b) program of Purvis & Rambault (1995). Branch lengths were set equal. These standardised independent contrasts were then tested for correlated evolution by fitting least-square (multiple) regressions forced through the origin, and thus, sample sizes represent the number of independent contrasts. Where the evolutionary assumptions of CAIC were violated (i.e. a significant relationship between the absolute contrast value and the nodal mean), weighted regression through the origin was employed (Purvis & Rambault 1995).

Results

Interspecific variation

Across the Bruchidae, substantial variation was found in the parameters measured (Table 2). That these measures represent accurate estimates of mean species level traits can be ascertained from an analysis of trait repeatability carried out on *Callosobruchus maculatus* (Table 3). Comparison of independent contrasts (Table 4) confirmed that across the Bruchidae, male elytron length was a good predictor of male mass, testes size and female elytron length, but not sperm length or sperm head length. Female elytron length was positively related to bursal volume, spermathecal mid-line length and spermathecal volume, but not spermathecal duct length (Table 5). We do not attempt to control for allometric effects by calculating residuals (which may be biased), since

inclusion of body size in multiple regression analysis achieves this (Freckleton 2002).

Correlated evolution of reproductive traits

Correlated evolution between male and female traits was analysed using weighted multiple regression through the origin on independent contrasts in trait variables. In the following analyses the maximal models included female elytron length, bursal volume, spermathecal duct length and spermathecal volume. Backward deletion from the maximal model, based on changes in R^2 revealed sperm length to be unrelated to bursal volume ($\Delta R^2 = 0.019$, $F_{1, 10} = 0.89$, $p = 0.37$). The minimal model revealed sperm length to be positively related to spermathecal duct length ($B = 0.74$, $t = 5.05$, $p < 0.0001$) and female elytra length ($B = 0.48$, $t = 2.69$, $p = 0.02$) and negatively related to spermathecal volume ($B = -87.35$, $t = 4.24$, $p < 0.001$). In a separate multiple regression analysis in which male elytron length was included, sperm length was found to be unrelated to testes size ($\Delta R^2 = 0.21$, $F_{1,11} = 3.82$, $p = 0.078$).

Backward elimination from the maximal model revealed sperm head length to be unrelated to spermathecal volume ($\Delta R^2 = 0.002$, $F_{1,10} = 0.04$, $p = 0.85$) or spermathecal duct length ($\Delta R^2 = 0.09$, $F_{1,11} = 0.21$, $p = 0.66$). Elimination of these two variables revealed the minimal model, in which sperm head length was negatively related to bursal volume ($B = -0.018$, $t = 3.52$, $p < 0.004$) and positively related to female elytron length ($B = 0.012$, $t = 2.81$, $p < 0.016$).

In the analysis of testes size, male elytron length was included in the maximal model as this covaried with testes volume. Backward elimination revealed testes size to be unrelated to female elytra length ($\Delta R^2 = 0.014$, $F_{1,9} = 4.7$, $p = 0.056$), but positively related to male elytron length (B = 0.82, t = 5.56, p < 0.0001), spermathecal volume (B = 91.1, t = 2.7, p = 0.022), spermathecal duct length (B = 0.602, t = 3.93, p = 0.003) and negatively to bursal volume (B = -0.99, t = 3.86, p = 0.003).

Discussion

A number of studies have shown sperm length to be positively associated with sperm competition intensity (Gage 1994; Morrow & Gage 2000; Briskie, Montgomerie & Birkhead 1997; LaMunyon & Ward 1998, 1999, 2002), a common measure of the latter being relative testis size (e.g., Morrow & Gage 2000; Hosken & Ward 2001). However, the present study, which controlled for any effect of body size on testes size by including both variables in a multiple regression model, found no relationship between sperm length and testes size suggesting that intensity of sperm competition is not a major factor in the evolution of sperm length in the Bruchidae. Instead, our results suggest that in the Bruchidae, sperm length has coevolved with female spermathecal duct length and spermathecal volume respectively.

Rapid coevolution between sperm length and seminal receptacle length has been documented in *Drosophila melanogaster* in which female seminal receptacle length was exposed to artificial selection for either long or short duct length (Miller & Pitnick 2002). In this species,

fertilization success is in part determined by the interaction between sperm length and seminal receptacle length, such that long sperm tend to function better within the reproductive tracts of females with long seminal receptacle lengths. Thus, seminal receptacle length selects for sperm length. A similar scenario might occur in relation to spermathecal duct length and sperm length. Indeed, Werner et al. (2007) have shown that the spermatozoa of *Drusilla canaliculata* (Coleoptera: Staphylinidae) interact with the spermathecal duct to gain progressive motility.

What drives the evolution of spermathecal duct length or indeed any part of the female reproductive tract that alters sperm efficiency at fertilization is open to conjecture. For example, variation in spermathecal duct length may arise as a result of natural selection, sexual selection, genetic drift and/or pleiotropy. Such female biases over paternity may be considered analogous to the sensory biases (or sensory drives, Boughman 2002) observed in precopulatory displays (Ryan *et al.* 1990) and may represent the starting point for a host of mechanisms of sexual selection including Fisherian (Morrow & Gage 2000) and good gene mechanisms (Pitnick *et al.* 1999). Such female biases are also a prerequisite for models of coevolution based on sexual conflict (Rice 1996; Rice & Holland 1997; Pitnick *et al.* 1999). Longer sperm (possibly because of its increased contact area with the walls of the spermathecal duct; Morrow & Gage 2000) may benefit males through its improved ability to displace, or resist displacement by, the sperm of rival males (Dybas & Dybas 1981; Presgraves *et al.* 1999), but such male 'control' may prove costly to females. For instance, females may mate

multiply to gain a mixture of sperm from different males, thereby avoiding the costs of genetic incompatibility (Zeh & Zeh 1997; Treganza & Wedell 2002). Under this scenario, the effectiveness of long sperm may have a detrimental effect on female fitness (since females may be stuck with sperm from a genetically incompatible male). Therefore, females may be counter-selected to increase spermathecal duct length so as to increase access of other ejaculates (Pitnick *et al.* 1999). The result of this conflict is an evolutionary arms race with ever lengthening sperm and spermathecal ducts, resulting in correlated evolution of the two traits. Under any of these mechanisms of sexual selection, extension of sperm beyond some critical length is likely to be opposed by the increased costs of producing longer sperm (Pitnick, Markow & Spicer 1995; Pitnick 1996). This critical length may correspond with the length of the spermathecal duct, since the wall area of the spermathecal duct with which the sperm can contact, and therefore the advantages of increased sperm length, are finite (Morrow & Gage 2000).

Whilst the exact mechanisms that produce correlated evolution in traits such as sperm length and spermathecal duct length remain elusive their divergence will almost certainly contribute to reproductive isolation between populations. The prevalence of conspecific sperm precedence supports this idea at a species level (Bella *et al.* 1992; Gregory & Howard 1994; Wade *et al.* 1994; Price 1997; Howard 1999; Rugman-Jones & Eady 2007), whilst patterns of sperm precedence revealed by intraspecific (between population) sperm competition studies (Brown & Eady 2001; Hosken, Blanckenhorn & Garner 2002; Nilsson, Fricke &

Arnqvist 2002, 2003; Rugman-Jones 2003), may also reflect coevolutionary divergence in sperm and female reproductive tract morphology between populations (Eady 2001).

Although testes size was not associated with sperm length, testes size was positively associated with spermathecal volume and spermathecal duct length. A similar relationship exists in moths (Morrow & Gage 2000) and scathophagid flies (Minder *et al.* 2005). Since testis size is commonly associated with male reproductive investment (Simmons 2001), it may be inferred that an evolved increase in spermathecal volume results in selection upon males to produce bigger ejaculates, and/or more sperm (requiring bigger testes) to fill and/or flush previous ejaculates from the spermatheca (Morrow & Gage 2000). At any given time following copulation, the number of sperm in storage (influenced by rates of sperm use and passive sperm loss) may affect female remating decisions and subsequent paternity (Parker 1993; Eady, Rugman-Jones & Brown 2004). In insects, ejaculate size is thought to be a major determinant of female remating (Ridley 1988; Simmons 2001) and several studies have demonstrated a positive relationship between ejaculate size and female remating intervals in bruchids (Eady 1995; Mbata, Shu & Ramaswamy 1997; Savalli & Fox 1999). Since delaying female remating is an important component of male post-copulatory reproductive success (Ridley 1988; Andrés & Arnqvist 2001; Simmons 2001), any evolutionary increase in the size of the female sperm storage organ may result in selection on males to produce more sperm, resulting in the evolution of larger testes.

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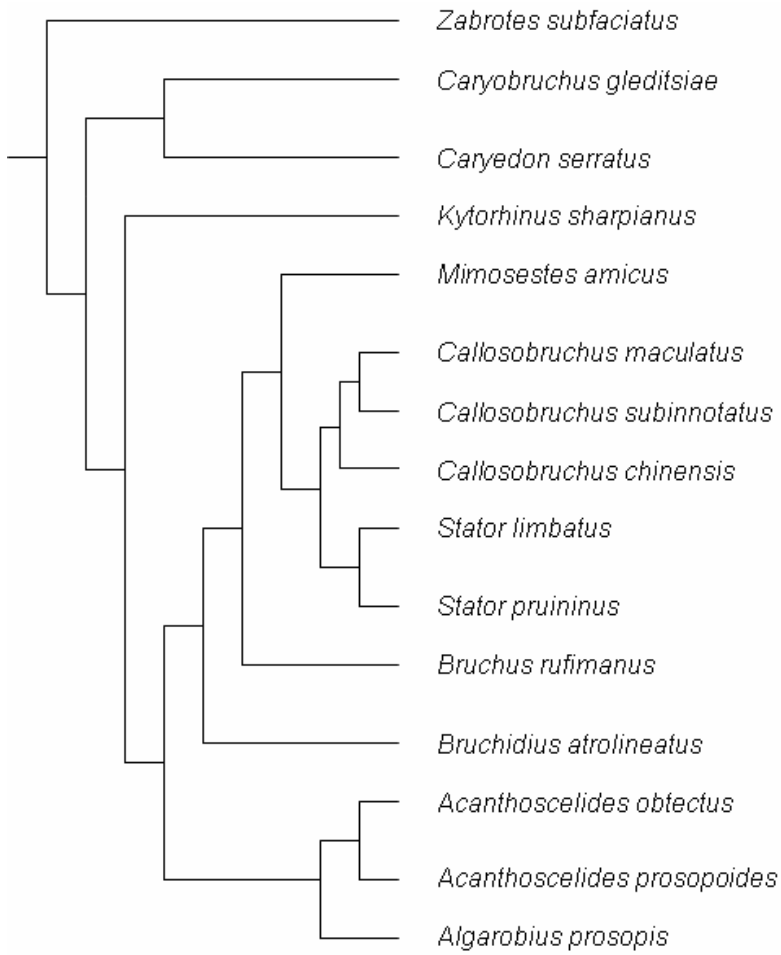
Figure 1. Phylogentic relationships of the study taxa (trimmed from an extensive molecular phylogeny of the Bruchidae; Geoff Morse, unpublished data), with the Callosobruchus phylogeny from Tuda *et al*. (2006). Branch lengths do not imply genetic distance.


Zabrotes subfaciatus
Caryobruchus gleditsiae
Caryedon serratus
Kytorhinus sharpianus
Mimosestes amicus
Callosobruchus maculatus
Callosobruchus subinnotatus
Callosobruchus chinensis
Stator limbatus
Stator pruininus
Bruchus rufimanus
Bruchidius atrolineatus
Acanthoscelides obtectus
Acanthoscelides prosopoides
Algarobius prosopis

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1 Table 1. Taxa used in the comparative study.

Species	Host seed	Origin	Collector/supplier
Acanthoscelides obtectus	*Phaeseolus vulgaris*	Zimbabwe	Laboratory population supplied by NRI, Chatham, Kent.
Acanthoscelides prosopoides	*Ziziphus obtusifolia*	Yavapai Co., Arizona, USA - 22/07/2001	Collected by C. D. Johnson
Algarobius prosopis	*Prosopis velutina*	Yavapai Co., Arizona, USA - 16/08/2001	Collected by C. D. Johnson
Bruchidius atrolineatus	*Vigna unguiculata*	Burkina Faso	Laboratory population supplied by Peter Credland, Royal Holloway, University of London - February 2002
Bruchus rufimanus	-	Peterborough, UK - 20/06/2001	Collected by Becky Ward, Processors & Growers Research Organisation, UK.
Callosobruchus chinensis	*Vigna angularis*	North Thailand	Laboratory population supplied by Helen Pearson, University of Leicester - June 2001
Callosobruchus maculatus	*Vigna unguiculata*	Niamey, Niger	Laboratory population in culture at Sunderland for 5 years
Callosobruchus subinnotatus	*Voandzeia subterranea*	Tamale, North Ghana, 1998	Laboratory population supplied by Peter Credland - June 2001
Caryedon serratus	*Arachis hypogea*	Senegal - January 2000	Collected by M. Sembéne
Caryobruchus gleditsiae	*Sabal spp.*	Florida, USA - August 2001	Collected by John Kingsolver
Kytorhinus sharpianus	*Sophora flavescens*	Tagawa (Fukuoka Prefecture), Japan - August 2001	Collected by Michihiro Ishihara
Mimosestes amicus	*Prosopis velutina*	Pima Co., Arizona, USA - 22/08/2001	Collected by C. D. Johnson
Stator limbatus	*Acacia greggii*	San Diego Co., California, USA - 25/08/2001	Collected by C. D. Johnson
Stator pruininus	*Acacia constricta*	Yavapai Co., Arizona, USA - 16/08/2001	Collected by C. D. Johnson
Zabrotes subfaciatus	*Phaseolus vulgaris*	Columbia	Laboratory population supplied by Peter Credland - November 1998

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Table 2. Species data: phenotypic values are given as mean *(std. dev.; n)*.

species	elytron length (mm)	testes volume (mm^3)	sperm length (μm)	sperm-head length (μm)	mass (mg)‡
a) male traits					
Acanthoscelides obtectus	2.07 *(0.08; 4)*	0.06 *(0.00; 4)*	102.08 *(3.78; 3)*	18.50 *(0.47; 3)*	4.90 *(- ; 5)*
Acanthoscelides prosopoides	1.91 *(0.09; 5)*	0.21 *(0.02; 5)*	178.98 *(10.16; 4)*	18.16 *(0.73;4)*	4.71 *(- ; 5)*
Algarobius prosopis	2.44 *(0.03; 2)*	0.56 *(0.03; 2)*	209.20*(10.17; 4)*	17.80 *(1.31;4)*	6.07 *(- ; 15)*
Bruchidius atrolineatus	1.71 *(0.13; 3)*	0.05 *(0.00: 3)*	114.05 *(6.4; 3)*	15.71 *(0.51; 3)*	3.42 *(- ; 10)*
Bruchus rufimanus	2.67 *(0.13; 5)*	0.10 *(0.01; 5)*	98.88 *(4.82; 3)*	12.90 *(0.85; 3)*	9.42 *(- ; 5)*
Callosobruchus chinensis	1.81 *(0.04; 3)*	0.05 *(0.00; 3)*	94.19 *(0.28; 3)*	11.97 *(0.54; 3)*	4.30 *(- ; 5)*
Callosobruchus maculatus	1.96 *(0.08; 5)*	0.19 *(0.02; 3)*	176.67 *(4.7; 3)*	18.7 *(0.1; 3)*	4.75 *(- ; 4)*
Callosobruchus subinnotatus	2.79 *(0.06; 3)*	0.61 *(0.05; 3)*	235.44 *(5.29; 3)*	20.74 *(0.43; 3)*	10.17 *(- ; 10)*
Caryedon serratus	4.20 *(0.05; 2)*	1.21 *(0.04; 2)*	253.84 *(15.05; 3)*	12.14 *(1.69; 3)*	18.03 *(0.82; 4)*
Caryobruchus gleditsae	4.54 *(0.22; 3)*	1.39 *(0.28; 3)*	438.62 *(2.08; 4)*	21.74 *(1.29; 4)*	22.7 *(1.90; 3)*
Kytorhinus sharpianus	2.13 *(0.01; 2)*	0.02 *(0.00; 2)*	71.85 *(1.03; 3)*	15.6 *(0.24; 3)*	5.70 *(- ; 2)*
Mimosestes amicus	2.62 *(0.13; 4)*	0.26 *(0.01; 4)*	411.78 *(16.32; 4)*	15.52 *(0.84; 4)*	9.27 *(1.63; 3)*
Stator limbatus	1.78 *(0.01; 2)*	0.16 *(0.01; 2)*	155.17 *(0.62; 3)*	13.95 *(0.73; 3)*	4.50 *(- ; 10)*
Stator pruininus	1.61 *(0.07; 3)*	0.11 *(0.04; 3)*	92.08 *(1.54; 4)*	11.61 *(0.59; 4)*	4.23 *(0.45; 3)*
Zabrotes subfaciatus	1.42 *(0.01; 3)*	0.06 *(0.01; 3)*	721.07 *(13.9; 2)*	16.42 *(0.08; 2)*	2.16 *(- ; 5)*

species	Elytron length (mm)	spermatheca volume (x 10^{-3} mm^3)	bursa copulatrix volume (mm^3)	spermathecal duct length (μm)	spermatheca mid-line length (mm)
b) female traits					
Acanthoscelides obtectus	2.13 *(0.08; 4)*	0.77 *(0.03; 3)*	0.03 *(0.00; 2)*	190.76 *(17.08; 3)*	0.29 *(0.01; 3)*
Acanthoscelides prosopoides	1.64 *(0.17; 4)*	1.67 *(0.28; 4)*	0.14 *(0.03; 4)*	257.87 *(19.58; 4)*	0.34 *(0.03; 4)*
Algarobius prosopis	2.33 *(0.21: 2)*	1.90 *(0.09; 2)*	0.19 *(0.02; 2)*	489.06 *(23.26; 2)*	*0.33 (0.00; 2)*
Bruchidius atrolineatus	1.76 *(0.09; 5)*	0.72 *(0.12; 3)*	0.02 *(0.00; 2)*	172.88 *(13.00; 3)*	0.26 *(0.02: 3)*
Bruchus rufimanus	2.64 *(0.13; 3)*	2.08 *(0.06; 3)*	0.40 *(0.13; 3)*	125.23 *(11.53; 3)*	0.37 *(0.00; 3)*
Callosobruchus chinensis	1.90 *(0.06; 9)*	0.40 *(0.04; 3)*	0.02 *(0.00; 5)*	159.19 *(21.79; 4)*	0.23 *(0.01; 3)*
Callosobruchus maculatus	2.19 *(0.15; 8)*	1.25 *(0.20; 3)*	0.12 *(0.04; 5)*	189.12 *(21.23; 4)*	0.35 *(0.01; 3)*
Callosobruchus subinnotatus	2.55 *(0.19; 8)*	1.98 *(0.10; 4)*	0.15 *(0.08; 6)*	208.18 *(7.29; 3)*	0.32 *(0.01; 3)*
Caryedon serratus	4.46 *(0.22; 7)*	5.14 *(1.01; 2)*	0.17 *(0.08; 4)*	458.61 *(10.70; 4)*	0.59 *(0.06; 4)*
Caryobruchus gleditsae	4.44 *(0.19; 3)*	7.87 *(0.77; 3)*	0.76 *(0.09; 3)*	819.99 *(5.02; 3)*	0.65 *(0.03; 3)*
Kytorhinus sharpianus	1.95 *(0.15; 2)*	1.57 *(0.18; 3)*	0.02 *(0.00; 3)*	106.42 *(7.55; 2)*	0.28 *(0.02; 3)*
Mimosestes amicus	2.54 *(0.25; 5)*	1.21 *(0.40; 4)*	0.27 *(0.03; 4)*	467.90 *(19.62; 3)*	0.33 *(0.01; 4)*
Stator limbatus	1.72 *(0.05; 2)*	0.85 *(0.20; 2)*	0.13 *(0.04; 2)*	423.25 *(20.50; 2)*	0.30 *(0.01; 2)*
Stator pruininus	1.56 *(0.06; 5)*	0.67 *(0.17; 4)*	0.09 *(0.01; 5)*	356.73 *(32.90; 4)*	0.25 *(0.01; 4)*
Zabrotes subfaciatus	1.75 *(0.05; 3)*	0.61 *(0.03; 2)*	0.01 *(0.00; 2)*	781.63 *(86.85; 3)*	0.28 *(0.00; 2)*

‡ Standard deviations were not available for the mass of those males weighed as a batch (see text).

Table 3. Intra-class correlation coefficients of reproductive trait measures from *C. maculatus*.

Trait	Intra-class correlation coefficient (ri)
Testes volume	ri = 0.70, $F_{8,18} = 7.85$, $p < 0.0001$
Sperm length within single male	ri = 0.88, $F_{9,20} = 22.4$, $p < 0.0001$
Sperm length across males	ri = 0.54, $F_{7,72} = 12.6$, $p < 0.0001$
Sperm head length within single male	ri = 0.72, $F_{9,20} = 8.8$, $p < 0.0001$
Sperm head length across males	ri = 0.14, $F_{7,72} = 2.6$, $p = 0.018$
Spermatheca mid-line	ri = 0.73, $F_{5,12} = 9.1$, $p < 0.001$
Spermathecal duct	ri = 0.95, $F_{5,12} = 62.9$, $p < 0.0001$
Bursa volume	ri = 0.93, $F_{5,12} = 38.1$, $p < 0.0001$

Table 4. Relationship between male elytron length and male reproductive traits and female elytron length. * indicates weighted regression, due to CAIC assumption violation.

Trait	**Statistics**
Male mass	$R^2 = 0.92$, $F_{1,13} = 159.6$, $p < 0.0001$
Sperm length*	$R^2 = 0.002$, $F_{1,13} = 0.021$, $p = 0.88$
Sperm head length	$R^2 = 0.072$, $F_{1,13} = 1.004$, $p = 0.36$
Testes volume	$R^2 = 0.71$, $F_{1,13} = 34.8$, $p < 0.0001$
Female elytron	$R^2 = 0.90$, $F_{1,13} = 127.2$, $p < 0.0001$

Table 5. Relationship between female elytron length and female reproductive traits. * indicates weighted regression, due to CAIC assumption violation.

Trait	**Statistics**
Bursal volume*	$R^2 = 0.20$, $F_{1,13} = 4.53$, $p = 0.053$
Spermathecal mid-line	$R^2 = 0.69$, $F_{1,13} = 29.1$, $p < 0.0001$
Spermathecal volume*	$R^2 = 0.53$, $F_{1,13} = 16.6$, $p < 0.001$
Spermathecal duct length*	$R^2 = 0.06$, $F_{1,13} = 0.20$, $p = 0.65$