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Correlated evolution between male and female primary reproductive characters in seed beetles

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Summary

1. Because males and females of internally inseminating species interact directly during mating, adaptations in one sex in primary reproductive traits may trigger an evolutionary response in the other sex. Divergent postcopulatory sexual selection is considered the main driving force behind the evolution of many male and female reproductive traits, generating unique morphologies and physiologies that can contribute to reproductive isolation and, ultimately, speciation.

2. The focus of most previous studies of the evolution of primary reproductive characters has been male reproductive traits and ejaculate or sperm characteristics. However, in order to more fully understand the evolution of primary reproductive characters it is crucial that we also include female traits.

3. In insects, both the size and the composition of the ejaculate have been shown to influence female reproduction in numerous ways by affecting female remating behaviour, female fecundity and female life span. Here, we employ a phylogenetic comparative approach to assess correlated evolution between primary reproductive characters in males and those in females in a group of seed beetles (Chrysomelidae: Bruchinae). We further explore correlated evolution between ejaculate size and female fitness in these insects.

4. Our analyses revealed positive correlated evolution between three internal female reproductive traits and ejaculate weight as well as correlated evolution between ejaculate weight and female fitness. We discuss the causal factors behind this correlated evolution and suggest that the evolution of larger ejaculates, primarily by postcopulatory sexual selection, causes selection for larger primary sexual traits in females to allow females to more rapidly process ejaculates. This may then feedback on postcopulatory selection in males, reinforcing selection for larger ejaculates.

5. Our results show that the primary reproductive traits of males and females show correlated evolution and suggest that intersexual co-evolution may affect the evolution of female fitness.

Key-words: Bruchinae, Callosobruchus, co-evolution, fitness, genitalia, phylogenetic least squares regression, postcopulatory sexual selection, reproductive traits, sexual selection, sperm competition

Introduction

It has become more and more evident that multiple mating is virtually ubiquitous in nature, implying that sexual selection generally continues after copulation. Divergent postcopulatory sexual selection is the main driving force behind the evolution of many male and female reproductive traits (e.g. Parker 1970, 1998; Eberhard 1985; Arnqvist 1998; Birkhead & Møller 1998; Simmons 2001; Birkhead & Pizzari 2002; Hosken & Stockley 2004). For example, male genitalia are frequently the only trait by which closely related species can be identified (Eberhard 1985; Hosken & Stockley 2004; Kulikov *et al.* 2004; Franco *et al.* 2006). It has been suggested that male-female co-evolution of reproductive traits generates unique morphologies and physiologies that can contribute to reproductive isolation and, ultimately, speciation (Rice 1996; Partridge & Hurst 1998; Arnqvist *et al.* 2000; Gavrilets 2000; Martin & Hosken 2003; Pitnick *et al.* 2003).

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Postcopulatory sexual selection is generated by sperm competition and/or cryptic female choice (Birkhead & Pizzari 2002). Sperm competition occurs after mating, when gametes of two or more males compete over the fertilization of the female ova (Parker 1970), and affects the evolution of male reproductive behaviour, physiology and morphology (see reviews by Birkhead & Møller 1998; Simmons 2001). Cryptic female choice occurs whenever female traits bias post-mating fertilization success in favour of certain males over others (Thornhill 1983; Eberhard 1996). Cryptic female choice traits may evolve to acquire direct or indirect benefits provided by males (Eberhard 1996), to minimize costs imposed by males (Arnqvist & Rowe 2005) or as a result of selection that is unrelated to sexual interactions (Ryan 1990).

Several studies have shown that reproductive traits such as male genitalia and components of the ejaculate evolve more rapidly than most other traits (Arnqvist 1998; Swanson & Vacquier 2002; Eberhard 2004; Hosken & Stockley 2004). However, in order to more fully understand the evolution of primary reproductive characters, we also need to understand the evolution of female traits (Swanson *et al*. 2001; Méndez & Córdoba-Aguilar 2004; Arnqvist 2006). Most studies that have included both male and female reproductive traits have focused on correlated evolution between sperm characteristics and dimensions of the female genital tract (e.g. Presgraves, Baker & Wilkinson 1999; Miller & Pitnick 2002; Minder, Hosken & Ward 2005; Beese, Beier & Baur 2006; Rugman-Jones & Eady 2008; Joly & Schiffer 2010). However, assessments of whether other aspects of the ejaculate show correlated evolution with female reproductive traits have been less investigated (but see Pitnick *et al*. 2003; Pitnick, Wolfner & Suarez 2009; Joly & Schiffer 2010). The ejaculate, typically consisting of a large number of proteins, peptides and additional substances, has been shown to affect female reproduction in several ways (Fowler & Partridge 1989; Poiani 2006). In insects, both the size (Ridley 1988; Simmons 2001) and the composition (Andrés & Arnqvist 2001; Nilsson, Fricke & Arnqvist 2002) of the ejaculate are known to affect female remating propensity. Since males and females of internal inseminating species interact directly during mating, adaptations in one sex in any primary reproductive trait may potentially affect and trigger an evolutionary response in the other sex (Eberhard 1996).

Fig. 1. Mating couple of *Callosobruchus maculatus*. Photo taken by Lena Rönn.

In the current contribution, we employ a comparative approach to explore correlated evolution between male and female primary reproductive characters in seed beetles (Fig. 1). In order to further explore the consequences of such correlated evolution, we also assess evolutionary associations between ejaculate size and female fitness. Furthermore, we discuss possible mechanisms that might cause the observed pattern of intersexual correlated evolution. We use six congeneric *Callosobruchus* ssp. and the Mexican seed beetle *Zabrotes subfasciatus* as a closely related outgroup (Tuda *et al*. 2006). This model system has become an important system for asking questions related to both sexual selection and incipient speciation. It has previously been established that male testis size and female remating rate show correlated evolution in seed beetles (Katvala, Rönn & Arnqvist 2008) and that male genital morphology correlates with the morphology of the female genital tract across species (Rönn, Katvala & Arnqvist 2007). Further, a recent study documented correlated evolution between sperm characteristics and properties of internal female genitalia (Rugman-Jones & Eady 2008). However, whether and how male ejaculate characteristics show correlated evolution with internal female genital traits has not yet been assessed in this model system.

Materials and methods

The species included in this study are closely related, share a common mating system (i.e. both sexes mate multiply) and are ecologically very similar (Fujii *et al*. 1989; Rönn, Katvala & Arnqvist 2006). All species were kept under controlled laboratory conditions at a temperature of 27 °C, under a light : dark cycle of 12 : 12 h and a humidity of 50 ± 10% (see Rönn, Katvala & Arnqvist 2006 regarding rearing and maintenance). Adult beetles require neither food nor water to reproduce successfully (Wightman 1978; Savalli & Fox 1999a). In this study, we included six *Callosobruchus* species: *Callosobruchus maculatus* [PC], *Callosobruchus analis* [RS], *Callosobruchus subinnotatus* [RS], *Callosobruchus phaseoli* [YT], *Callosobruchus chinensis* [RS] and *Callosobruchus rhodesianus* [RS]. Included in our study is also *Z. subfasciatus* [PC] (stocks provided by PC: Peter Credland, University of London; RS: Robert Smith, University of Leicester; YT: Yukihiko Toquenaga, University of Tsukuba). We note here that although the specific affiliation of the *C. analis* population used here is somewhat ambiguous (Y. Toquenaga, pers. comm.), it is genetically distinct from the other *Callosobruchus* clades used (Tuda *et al*. 2006).

MALE TESTIS SIZE AND EJACULATE WEIGHT

From each species, a number of virgin males (N = 7–9), 24–48 h of age, were collected. The abdomen of decapitated individuals was removed from the thorax and put under a dissecting microscope (Leica® MZ8; Leica Microsystems GmbH, Wetzlar, Germany). The two testes were dissected out and placed on a drop of glycerin on a microscope slide (76 × 26 mm) and covered with a standard coverslip (18 × 18 mm) to flatten the testes. The outline of each testes was then traced twice for each male, using a side mounted camera lucida, and the area of the testes (sum of all four testes for each male) was recorded (using ImageJ 1·36b; Rasband 2006). For each male, we used the average of the two repeated measures. Measures of ejaculate weight were obtained by collecting 20 virgin males from each species.

Table 1. Mean values (SE; *N*) for all traits and species

	Callosobruchus analis	*Callosobruchus rhodesianus*	*Callosobruchus maculatus*	*Callosobruchus subinnotatus*	*Callosobruchus phaseoli*	*Callosobruchus chinensis*	*Zabrotes subfasciatus*
Testes size (mm^2)	1·16 (0·08; 9)	1·16 (0·04; 7)	1·17 (0·09; 9)	2·95 (0·11; 7)	0·66 (0·04; 8)	0·46 (0·03; 8)	0·36 (0·02;7)
Ejaculate weight (μg)	213·4 (11·0; 20)	220·0 (16·0; 20)	192·1 (19·0; 19)	180·9 (16·0; 21)	50·2 (9·0; 21)	34·0 (8·1; 19)	17·2 (7·0; 20)
Male elytra (mm)	1·91 (0·03; 9)	1·88 (0·03; 7)	1·81 (0·04; 9)	2·69 (0·02; 7)	2·10 (0·02; 8)	1·63 (0·02; 8)	1·36 (0·02; 7)
Bursa copulatrix (mm^2)	0·42 (0·02; 6)	0·39 (0·01; 7)	0·46 (0·04; 6)	0·49 (0·02; 7)	0·17 (0·004; 6)	0·07 (0·004; 7)	0·06 (0·002; 8)
Spermatheca (mm^2)	0·0018 (0·00006; 9)	0·0018 (0·0001; 7)	0·0016 (0·0006; 5)	0·0019 (0·000008; 6)	0·0008 (0·0004; 7)	0·0010 (0·00004; 10)	0·0008 (0·00005; 6)
Accessory glands (mm^2)	0·33 (0·03; 6)	0·42 (0·01; 6)	0·39 (0·01; 6)	0·35 (0·02; 6)	0·06 (0·01; 6)	0·04 (0·004; 5)	0·03 (0·002; 7)
Female weight (μg)	0·0047 (0·0002; 20)	0·0047 (0·0002; 19)	0·0052 (0·0002; 19)	0·0078 (0·0003; 21)	0·0050 (0·0002; 22)	0·0048 (0·0001; 18)	0·0036 (0·00009; 20)
Female fitness (no. of eggs)	68·6 (4·5; 15)	67·7 (6·8; 15)	73·7 (4·3; 14)	34·3 (5·3; 15)	42·7 (2·2; 15)	43·7 (3·4; 15)	28·9 (1·4; 15)

These males were mated to virgin females, all individuals being between 24–48 h post-hatching when mated. Matings were performed under the same temperature and humidity as during normal maintenance (see above). Before and after mating, males were weighed twice to the nearest 10^{-5} g, using a Sartorius® ME/SE analytical balance (Sartorius AG, Goettingen, Germany). Mean difference in male weight before and after each mating was used as a measure of ejaculate weight. Male elytra length was obtained using a digitizing tablet (Summsketch III™; Summagraphics Corp., Austin, TX, USA) placed under a dissecting microscope provided with a camera lucida (see above). We note that male elytra length correlates closely with male weight across species ($r = 0{\cdot}97$). We use male elytra length here as a measure of male size to match previous studies (e.g. Wilson & Hill 1989).

FEMALE INTERNAL GENITALIA

From each species, a number of virgin females ($N = 5$–10 females per species), 24–48 h of age, were collected. The abdomen of decapitated individuals was removed from the thorax and put under a dissecting microscope (see above for microscope details). The female bursa copulatrix (the organ that receives the ejaculate) and accessory glands (a secretory organ presumably involved in egg laying and processing of the ejaculate) were dissected out, detached from each other, and placed individually on a drop of glycerin on a microscope slide (76 × 26 mm) and covered with a standard coverslip (18 × 18 mm). To prepare the spermatheca (the sclerotized sperm storage organ), abdomens of females were first macerated for 2 h in 10% KOH-solution and then rinsed in aqueous 50% lactic acid for 24 h. This was done in order to dissolve the fat tissue surrounding the spermatheca which was then placed on a drop of glycerin on a microscope slide (76 × 26 mm) without coverslip. The outline of the bursa copulatrix, accessory glands and spermatheca was then traced and a measurement of the area was recorded using the same procedure as described above for testes size. In all subsequent statistical analyses, female weight was used as a measure of female size (obtained by weighing females twice to the nearest 10^{-5} g, see above). We note here that female weight correlates closely with male weight ($r = 0{\cdot}99$) and male elytra length ($r = 0{\cdot}93$) across species.

FEMALE FITNESS

Female fitness was estimated as female lifetime fecundity. Virgin females ($N = 15$ of each species) were collected and mated to a virgin male (all individuals being between 24–48 h post-hatching when mated). Females were then placed in a petri dish containing 100 beans and two additional virgin males. The two males were then replaced with new virgin males every second day to standardize exposure to males. After females had died, all eggs were counted and used as a measure of lifetime female fitness.

COMPARATIVE METHODS

We estimated the amount of phylogenetic signal present in our data, using PHYSIG.M (Blomberg, Garland & Ives 2003) and a well supported reconstruction of the phylogeny of these taxa based on molecular data (Tuda *et al.* 2006). This analysis revealed a sizeable phylogenetic signal for several traits (see below), suggesting that phylogenetic comparative analyses should be employed. We used

phylogenetic generalized least squares regressions, PGLS (Rohlf 2001), as implemented in REGRESSION.M (Blomberg, Garland & Ives 2003), to assess correlated evolution between male and female reproductive traits. However, we also report the results of phylogenetically uncorrected species level analyses in Appendix 1 (Supporting Information). The basic structure of all inferential models was the same: to control for body size-related covariance across focal traits, we fitted multiple PGLS regression models where a given focal dependent trait was simultaneously related to a second focal trait and body size. Below, we refer to these traits as F1, F2, and FBS or MBS (for female or male body size, respectively).

Results

We first assessed whether species differ in the traits analysed here (Table. 1), using analyses of covariance with species as a factor and body size as a covariate. There was extensive variation across species in ejaculate weight ($F_{6,124} = 52{\cdot}12$, $P < 0{\cdot}001$), size of spermatheca ($F_{6,41} = 45{\cdot}8$, $P < 0{\cdot}001$), size of bursa copulatrix ($F_{6,35} = 151{\cdot}57$, $P < 0{\cdot}001$), size of accessory glands ($F_{6,32} = 190{\cdot}53$, $P < 0{\cdot}001$) and female fitness ($F_{6,92} = 36{\cdot}09$, $P < 0{\cdot}001$).

Several traits showed a sizable phylogenetic signal (ejaculate weight: $K = 2{\cdot}49$ ($P = 0{\cdot}002$); size of testes: $K = 0{\cdot}78$ ($P = 0{\cdot}42$); size of female accessory glands: $K = 2{\cdot}01$ ($P = 0{\cdot}02$); size of bursa copulatrix: $K = 1{\cdot}91$ ($P = 0{\cdot}01$); size of spermatheca: $K = 2{\cdot}04$ ($P = 0{\cdot}01$); female fitness: $K = 1{\cdot}04$ ($P = 0{\cdot}06$)) and we thus report the results of phylogenetically informed analyses below (i.e. PGLS regressions).

The size of the female bursa copulatrix (F1) showed a positive correlated evolution with the size of female accessory glands (F2: ß = 0·796, $t = 3{\cdot}087$, $P = 0{\cdot}03$; FBS: ß = 34·324, $t = 1{\cdot}44$, $P = 0{\cdot}22$). However, the size of the spermatheca (F1) was not significantly related to either the bursa copulatrix (F2: ß = 0·002, $t = 1{\cdot}646$, $P = 0{\cdot}18$; FBS: ß = 0·067, $t = 0{\cdot}687$, $P = 0{\cdot}53$) or the accessory glands (F2: ß = 0·002, $t = 2{\cdot}243$, $P = 0{\cdot}09$; FBS: ß = 0·111, $t = 1{\cdot}506$, $P = 0{\cdot}21$). Thus, although the female organs that receive and process the male ejaculate (bursa copulatrix and female accessory glands) show correlated evolution, neither correlated significantly with the organ that store received sperm for later use in fertilizing the eggs (the spermatheca).

We have previously shown that the evolution of larger male testis and larger body size are both associated with an increased ejaculate size (Katvala, Rönn & Arnqvist 2008). Here, we are concerned with how these male traits relate to female traits. We found a strong positive correlation between male ejaculate weight (F1) and all female genital traits: spermatheca (F2: ß = 173452·8, $t = 3{\cdot}30$, $P = 0{\cdot}030$; MBS: ß = −58·05, $t = 1{\cdot}339$, $P = 0{\cdot}25$), bursa copulatrix (F2: ß = 441·78, $t = 3{\cdot}92$, $P = 0{\cdot}017$; MBS: ß = −40·12, $t = 1{\cdot}140$, $P = 0{\cdot}318$) and accessory glands (F2: ß = 451·02, $t = 5{\cdot}364$, $P = 0{\cdot}005$; MBS: ß = 1·106, $t = 0{\cdot}045$, $P = 0{\cdot}97$). However, we found no significant correlated evolution between testis size (F1) and any female genital traits: female bursa copulatrix (F2: ß = 1·509, $t = 1{\cdot}132$, $P = 0{\cdot}32$; MBS: ß = 1·676, $t = 4{\cdot}021$, $P = 0{\cdot}016$), spermatheca (F2: ß = 821·31, $t = 1{\cdot}734$, $P = 0{\cdot}16$; MBS: ß = 1·507, $t = 3{\cdot}857$, $P = 0{\cdot}018$) and accessory glands (F2: ß = 1·506, $t = 1{\cdot}174$, $P = 0{\cdot}31$; MBS: ß = 1·818, $t = 4{\cdot}889$, $P = 0{\cdot}008$). Thus, the evolution of a large male ejaculate is associated with increased size of the female organs that process the received ejaculate and store sperm but this pattern of correlated evolution was not found between testis size and female primary sexual traits.

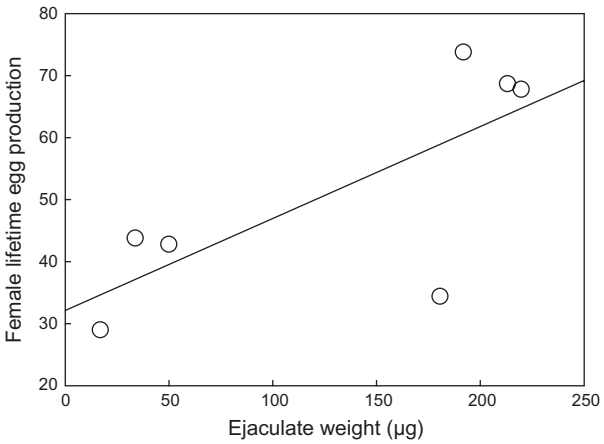

Fig. 2. The relationship between mean female lifetime egg production and mean ejaculate weight across species (species level data).

Finally, female lifetime egg production (F1) showed a positive correlated evolution with ejaculate weight (F2: ß = 0·2, $t = 2{\cdot}91$, $P = 0{\cdot}04$; FBS: ß = −8990·28, $t = 2{\cdot}95$, $P = 0{\cdot}04$) (Fig. 2). Thus, heavy ejaculates are positively related to high female fitness across species in this group. However, no relationship could be found between female lifetime egg production (F1) and the testis size (F2: ß = −0·212, $t = 0{\cdot}008$, $P = 0{\cdot}994$; FBS: ß = −7405·51, $t = 0{\cdot}4223$, $P = 0{\cdot}695$).

Discussion

Our analyses revealed correlated evolution between male and female primary reproductive traits in seed beetles. Most importantly, ejaculate weight showed a positive correlation with three internal female genital traits, as well as with a measure of female fitness. We will first discuss possible mechanisms underlying selection on male and female reproductive traits and, secondly, how they may interact to affect male-female co-evolution.

Females of all species studied here mate multiply (Arnqvist, Nilsson & Katvala 2004; Miyatake & Matusmura 2004; Katvala, Rönn & Arnqvist 2008) and there is, thus, sexual selection by both pre-mating male–male competition and post-mating sperm competition. In seed beetles, there is a positive relationship between female remating interval and ejaculate size both within species (Eady 1995; Savalli & Fox 1999b; Takakura 2001) and across species (Katvala, Rönn & Arnqvist 2008). Hence, by transferring a large ejaculate, males delay female remating and a large ejaculate is thus to some extent a defensive sperm competition adaptation (see Simmons 2001). Species included in this study have ejaculate

weights ranging from *c*. 2% to 6% of male body weight (Rönn, Katvala & Arnqvist 2008). Large ejaculates may also benefit females directly (Savalli, Czesak & Fox 2000). Within species, females that receive larger ejaculates or more ejaculate material generally tend to have higher lifetime fecundity (Fox 1993; Savalli & Fox 1999b; Savalli, Czesak & Fox 2000; Rönn, Katvala & Arnqvist 2008). Thus, by transferring large ejaculates, males benefit both in terms of sperm competition success and, potentially, by elevating the fecundity of their mates. Whether sexual selection by sperm competition or natural selection by fecundity selection (i.e. paternal investment) most affects the evolution of ejaculate size in seed beetles is unknown, and disentangling these forms of selection is generally very difficult (Wedell 1993; Vahed 1998). However, ejaculates are clearly not always advantageous to female seed beetles. For example, a single mating prolongs female life span in some species but shortens female life span in others (Rönn, Katvala & Arnqvist 2006) and males of some seed beetle species are known to transfer substances with toxic effects in females (Das *et al*. 1980). Further, larger ejaculates benefit females in some seed beetle species but not in others and ejaculate weight affects different female fitness component in different species (Rönn, Katvala & Arnqvist 2008). To further complicate matters, the effects of large ejaculates depends on environmental conditions and it is thus difficult to predict to what extent fecundity selection generally operates in natural seed beetle populations (Eady & Brown 2000; Edvarssson 2007; Fox & Moya-Laraño 2009).

While the evolution of primary reproductive traits in males has been extensively studied in a variety of taxa (e.g. Eberhard 1985; Birkhead & Møller 1998), much less is known about the evolution of female reproductive traits in general (Leonard & Córdoba-Aguilar 2010). We found that ejaculate size shows a positive correlated evolution with female fecundity across seed beetle species, which is in accordance with the fact that ejaculate size often increases female fecundity within seed beetle species (Fox 1993; Savalli & Fox 1999b; Savalli, Czesak & Fox 2000; Rönn, Katvala & Arnqvist 2008). We also found that the evolution of larger ejaculates was associated with the evolution of larger bursas, accessory glands and spermathecas in females. We note that this is line with the recent findings of Rugman-Jones & Eady (2008), who documented a positive correlation between spermathecal volume and testes volume across species, and we suggest that two non-mutually exclusive scenarios may account for this pattern of correlated evolution. First, to the extent that large ejaculates delay female remating beyond their optimal remating interval (Simmons & Gwynne 1991), females may evolve a larger bursa copulatrix and accessory glands as counter adaptations to male manipulation of female remating (Arnqvist & Rowe 2005). This should allow females to more rapidly process the ejaculate and thus also to remate sooner (Wiklund, Karlsson & Leimar 2001). Second, a more rapid processing of the ejaculate is also expected if females are selected to secure water and/or nutrients from the ejaculate. These seed beetle species inhabit dry or semi-dry natural habitats (Southgate 1979; Taylor 1981). One possible function of female accessory glands in insects is to aid in the digestion of ejaculate components (Chapman 1998), at least in some insects (Marchini *et al*. 1993; Hosken & Ward 1999; Hosken, Uhía & Ward 2002) and larger bursa copulatrix and accessory glands might thus allow females to receive larger amounts of ejaculate and/or to metabolize ejaculate material more efficiently. Earlier comparative work has shown that, if anything, the evolution of decreased female remating rate is associated with an increased ejaculate size in seed beetles (Katvala, Rönn & Arnqvist 2008). As pointed out by Vahed (2006), however, both of the above scenarios can predict this pattern of correlated evolution.

To reveal causation from patterns of correlated evolution is generally very difficult (e.g. Martins 2000) and studies of correlated evolution between male and female primary reproductive characters is certainly no exception to this rule (e.g. Pitnick *et al*. 2003; Joly & Schiffer 2010). Previous comparative studies of seed beetles have unveiled correlated evolution, between male genital spines and the robustness of the female reproductive tract (Rönn, Katvala & Arnqvist 2007), between sperm length and spermathecal morphology (Rugman-Jones & Eady 2008) and between female remating behaviour and ejaculate weight (Katvala, Rönn & Arnqvist 2008). Further, experimental studies within species have shown that large ejaculates benefit males in terms of increased fertilization success (Eady 1995; Savalli & Fox 1999b; Takakura 2001). We suggest that the evolution of larger ejaculates, driven by postcopulatory sexual selection, causes selection for larger primary sexual traits in females, to allow females to more rapidly process ejaculate material. The evolution of larger primary sexual traits in females may represent a form of compensatory evolution which does not alter the sperm competition regime. Alternatively, evolutionary changes in the primary sexual traits of females affect postcopulatory sexual selection in males, causing selection for further enlargement of ejaculates. Such a self-reinforcing co-evolutionary process would, in theory, be halted when the elevated costs of ejaculate production reach a point where the reproductive investment becomes similar in the two sexes (Jones 2009). Interestingly enough, in the sex-role reversed seed beetle *Megabruchidius dorsalis* (Takakura 2001), males transfer very large ejaculates and the reproductive investment is similar in magnitude in two sexes (Takakura 2006).

Within the group of seed beetles included in this study, there is substantial variation across species with regards to ejaculate characteristics. Ejaculate weight and allocation of ejaculate over successive matings vary and the effects that the ejaculate allocation has on female reproduction also differs across species (Rönn, Katvala & Arnqvist 2006, 2008). We have shown that the evolution of male ejaculate weight is correlated with evolution of three primary female reproductive traits and we suggest that this may be due to male–female co-evolution. Future studies will need to determine whether these patterns are true also at a higher resolution (e.g. details of ejaculate composition, ejaculate processing rate, female

incorporation of ejaculate material, functional morphology of the female bursa and the accessory glands in both sexes) and, ultimately, to distinguish between alternative causal mechanisms behind correlated evolution in this model system.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Species level analysis.

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