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Author: Sturm, Robert

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Comparison of sperm number, spermatophore size, and body size in four cricket species

Robert Sturm

Brunnleitenweg 41, A-5061 Elsbethen, Austria. Email: Robert.Sturm@stud.sbg.ac.at

Abstract

This paper examines the relationships between male body size, spermatophore size, and number of sperm per spermatophore, in four cricket species: *Teleogryllus commodus*, *Acheta domesticus*, *Gryllus bimaculatus*, and *Gryllus assimilis*. Within each species, individuals varied considerably in all three characters measured, and generally, spermatophore size, number of sperm, and body size were all correlated; *i.e.*, ampulla diameter and sperm number per spermatophore significantly increased with body mass ($p < 0.001$) according to a linear regression function. Interspecific investigations found considerable differences between species: *G. assimilis* had the largest mean male body mass and length, largest ampullas, and highest numbers of spermatozoa per spermatophore, whilst *A. domesticus* had a small body mass and length, the smallest ampullas, and lowest sperm numbers. Regression analyses of all four cricket species revealed similar results as intraspecific regression computations. Hence, both intra- and interspecifically, larger males produce larger spermatophores containing more sperm, than do smaller males. These results differ from bush crickets (Tettigoniidae), where larger male body size does not necessarily correlate with larger ampullas and more sperm. Possibly male bush crickets have evolved to invest a higher proportion of their resources in the size of the nuptial gift, as opposed to number of spermatozoa.

Key words

body mass, spermatophore, sperm number, ampulla, Orthoptera, Gryllidae

Introduction

Body size, mass, and number of sperm are key components of male fitness (Thornhill & Alcock 1983; Wedell 1997). Body size influences most biological phenomena, making it a determinant of fitness and a target of natural selection (Whitman 2008). Body size can determine male reproductive success, because larger males are often more competitive and can provide more resources and benefits to females than can small males (Leisnham & Jamieson 2004; Fedorka & Mousseau 2002; Whitman 2008; Saleh *et al.* 2013). The number of sperm transferred to the female is also an important component of male fitness (Schaus & Sakaluk 2001), in part, because of male-male sperm competition (Thornhill & Alcock 1983; Simmons 2001).

In the Ensifera (crickets and katydids), males package their sperm into a proteinaceous container called a spermatophore (Lehmann 2012), which is typically composed of three parts: 1) a long thin tube which is threaded into the female spermatheca, 2) an anchor or attachment plate, which is placed into the female genital tract and secures the spermatophore to the female, and 3) a sack-like ampulla which hangs outside the female and holds the sperm. After attaching the spermatophore to the female, the male uncouples from the female. During the next hour, much of the sperm in the dangling, external ampulla is transferred through the spermatophore tube into the female's spermatheca. In many ensiferan species, males also produce a jelly-like spermatophylax (Greek for 'sperm guard'), which surrounds the ampulla. The spermatophylax always lacks sperm and is nutritious for some species (Dewsbury 1982; Mann 1984; Vahed 1994; Reinhold & von Helversen 1997). When present, the spermatophylax is commonly eaten by the female, and thus functions as a nuptial food gift (Voigt *et al.* 2006, 2008). It also serves to delay the female from eating the sperm-filled ampulla, thus allowing time for sperm to pass from the ampulla into the female spermatheca (Sakaluk 1984; Gwynne 1990; Simmons 1990; Simmons & Bailey 1990; Heller & von Helversen 1991). In contrast, most cricket species do not produce a spermatophylax. Instead, males physically guard the females to keep them from eating the sperm-filled ampulla (Alcock 1994; Sturm 2003).

In those insects that do not pass spermatophores, sperm number per ejaculate is often correlated with male body size (Ponlawat & Harrington 2007). But the situation becomes more complex in taxa that produce spermatophores, because larger males tend to produce larger spermatophores (Wedell 1993; Lehmann & Lehmann 2009), and larger spermatophores tend to contain more sperm (Doyle *et al.* 2011). Hence, in the Orthoptera, male body size, spermatophore size, and sperm number should be studied together (Wedell 1997; Schaus & Sakaluk 2001; Brown 2008; McCartney *et al.* 2008). In some crickets, large spermatophores with high sperm numbers are removed by females significantly later than small spermatophores with low sperm numbers (Simmons 1986). Further, males with large spermatophores, containing high numbers of sperm, may attract larger females that are characterized by higher fecundity (Fedorka & Mousseau 2002). Hence, larger spermatophores may have reproductive benefits for males beyond simply having more sperm (Lehmann 2012).

Of course, the number of spermatozoa transferred to the female during a single mating is an important component of male fitness (Simmons 2001). Studies on Orthoptera show considerable interspecific variation in sperm number per spermatophore, adopting values between several thousand and several million: *Gryllodes supplicans*: $1.6\text{-}2.0 \times 10^4$ (Sakaluk 1984), *Teleogryllus commodus*: $0.8\text{-}2.0 \times 10^5$ (Sturm 2003), *Kawanaphila nartee*: 0.2×10^6 (Simmons & Gwynne 1991), *Requena verticalis*: $0.8\text{-}2.0 \times 10^6$ (Gwynne 1986, Simmons *et al.* 1993), *Poecilimon veluchianus*: $6.3\text{-}10.5 \times 10^6$ (Reinhold 1994; Reinhold & Helversen 1997; McCartney *et al.* 2010). This remarkable interspecific variability in sperm number is thought to be the result of differential sexual and natural selection among species. Intraspecifically, sperm number in Orthoptera can correlate with the size, age, health, and nutritional status of the male (Sturm 2011),

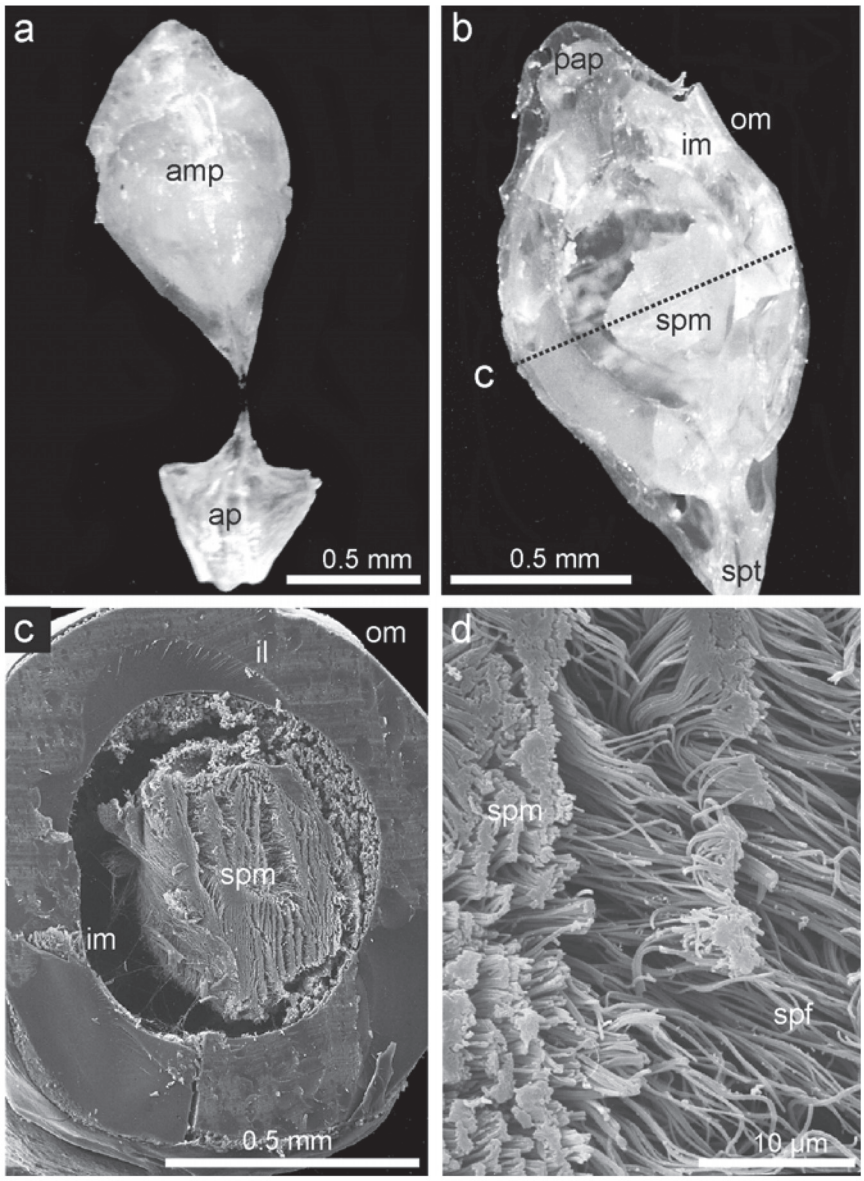

Fig. 1. Cricket spermatophores: a) Fresh spermatophore of *Teleogryllus commodus*, showing the sperm-containing ampulla (amp) and attachment plate (ap), b) main components of the ampulla in *T. commodus* (longitudinal section): apical papilla (pap), outer membrane (om), inner membrane (im), sperm mass (spm), and spermatphore tube (spt), c) electron micrograph of cross section of *T. commodus* ampulla, showing the internal structure (il: inner layer), d) detailed view on the sperm mass residing in ampulla of *T. commodus* (spf: sperm flagella).

and the length of the time between two spermatophore transfers: generally, the longer the refractory time, the higher the number of transferred spermatozoa (Reinhold & Heller 1993; Lehmann & Lehmann 2000, 2009). However, physiological condition itself influences the length of the refractory time (Simmons 1988).

In the study presented here, I examine the intra- and interspecific relationships among male body size, spermatophore size, and spermatozoa number per spermatophore in four cricket species (*T. commodus*, *A. domesticus*, *G. bimaculatus*, and *Gryllus assimilis*). These four species mostly survive in different habitats. These crickets exhibit similar male refractory periods, but differ in both male body size and spermatophore size. My hypothesis is that male body size, spermatophore size, and sperm number per spermatophore are positively correlated in crickets, both intra- and interspecifically.

Material and methods

Breeding and keeping of the crickets.—Four cricket species were used in this study: *Teleogryllus commodus* (Walker 1869), *Acheta domesticus* (Linnaeus 1758), *Gryllus bimaculatus* (DeGeer 1773), and *Gryllus assimilis* (Linnaeus 1758). Three cricket species (*Teleogryllus commodus*, *Gryllus bimaculatus*, *Gryllus assimilis*) were obtained from retailers specialized for feed animals, whereas *Acheta domesticus* was collected as adults in the field in Austria. All were reared and kept under identical conditions (constant 25°C, Light:Dark = 12:12 h, relative humidity ≈ 60%), using an environmental chamber at the former Institute of Zoology, University of Salzburg. Rearing of early, intermediate, and late nymphal stages took place in separate plastic boxes (50 cm × 30 cm × 30 cm), which were filled with dry peat soil (thickness of the layer: 3 cm), food *ad libitum*, and egg cartons serving as shelter for the animals. Immediately after their final molt adult animals of each species were separated by gender and individually kept in five-liter glass vessels filled with crumpled paper. They were provided with food *ad libitum*, consisting of standard laboratory diet (Altromin® 1222), lettuce, and water that was placed into small dishes plugged with cotton wicks (Sturm & Pohlhammer 2000, Sturm 2002).

Males used for mating (N = 20 per species) were measured for body length (from the front of the head to the end of the abdomen, excluding the antennae and cerci) using mechanical Calipers accurate to 0.02 mm. Fresh, wet body mass was measured with a Satorius® balance (precision: 10^{-4} g). Males were weighed about 30 min before the experiment to avoid any inaccuracies resulting from additional food uptake or excretion.

For the mating process, 5-d old males were placed together with 5-d old virgin females of the same species in respective mating vessels (round glass dishes: di = 30 cm, height = 5 cm). The dishes were empty and were cleaned after each copulation. Only 15 min were allotted for copulation to occur in order to prevent males from any adjustment of their sperm number released into the ampulla in the presence of the female. Immediately after copulation and spermatophore transfer, the sperm-containing capsules were removed from the females by using soft forceps and a stereomicroscope. Separated spermatophores were submerged in insect Ringer's solution (Sturm & Pohlhammer 2000). The ampulla was then measured under the stereomicroscope (Wild®; Fig. 1a, b). There is some evidence that male crickets may modify sperm number in response to both intraspecific competition and female size (Gage & Barnard 1996). Thus, a single male is theoretically able to fill a spermatophore with the highest number of sperm possible or leave it completely empty. In the present study, such factors were controlled as much as possible. For example, during the experiments reported here, all males had similar refractory periods, a single male was always paired with a

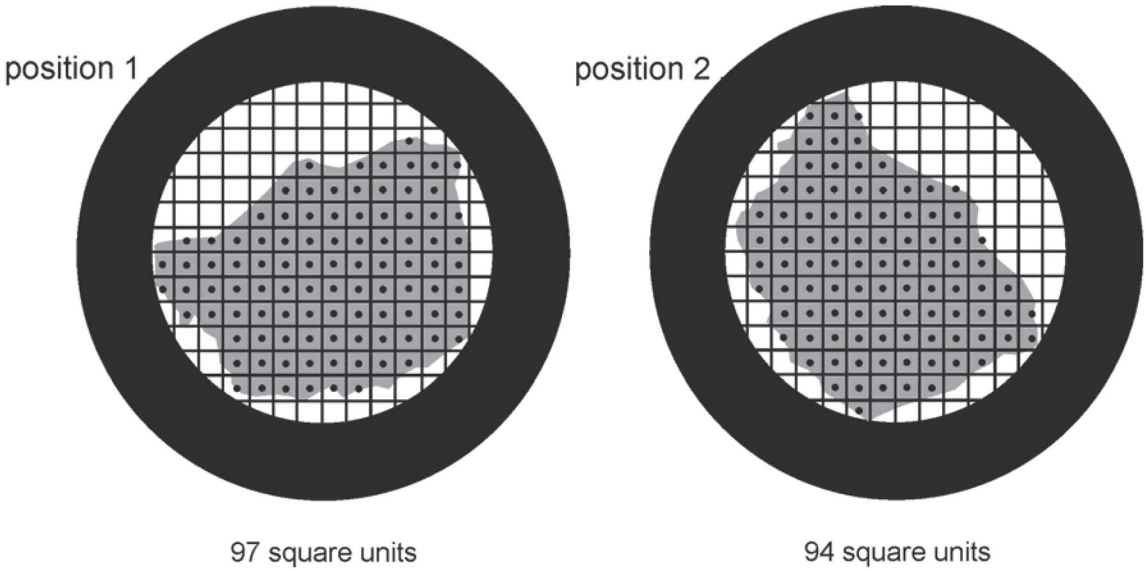

Fig. 2. Determination of the cross-sectional area of the sperm mass (see Fig. 1c) with the help of a simple stereologic counting method. The cross section photographed in the SEM is covered with a grid consisting of a pre-defined number of square units. Those square units that are filled by the sperm mass by more than 50 % are included into the counting and marked by a point. Finally, all counted square units are summed up. The procedure is repeated several times by rotating the photograph below the grid by a pre-defined angle (*e.g.*, compare positions 1 & 2). A mean cross-sectional area is computed.

single female, and male and female sizes were equally matched as much as possible.

Sperm counting.—Sperm numbers per spermatophore were estimated by first fixing isolated capsules in a paraformaldehyde-glutaraldehyde mixture (Karnovsky 1965) for 3 h. Subsequently, they were washed in sodium-cacodylate buffer, dehydrated in series with increasing ethanol content (70% to 96%), and critical-point dried. After the fixation procedure, each oval spermatophore was cut transversely in the middle (thickest) part of the ampulla, using a razor blade and a steriomicroscope. The sperm-containing halves were prepared for electron-microscopy (charged with carbon and sputtered with gold) and subsequently scanned with a Cambridge® 250 SEM at an accelerating voltage of 10-30 kV (Fig. 1c, d).

The resulting SEM micrographs of uniform magnification were analyzed stereographically, as follows (Fig. 2): The cross-sectional area of the sperm mass revealed on the photograph was covered with a grid consisting of a pre-defined number of unit squares. The size of the area was estimated by counting those squares being filled by the mass by more than 50%. Afterwards, the photograph was rotated below the grid by a pre-defined angle and the counting procedure was repeated. Final size of the sperm mass area was computed by simply determining the mean value of the single counting results, *M*. The number of singly held sperm cells within a single square unit, N_{su}, was carefully determined under magnifications (Fig. 1 d). By assuming a homogeneous distribution of sperm within the ampulla the total number of germ cells, N_{tot}, was computed according to the following equation:

$$N_{tot} = N_{su} \cdot M \cdot c \qquad (1)$$

In the equation noted above, *c* represents a correction factor, by which shrinking artifacts and gaps within the sperm mass arising from the fixation process and cutting of the ampulla are considered (Fig. 1c). This factor simply denotes the ratio of the unaffected sectional area of the sperm mass to the whole sectional area of the sperm mass. The factor was individually computed for each spermatophore included into this study. By using this correction factor, I believe that the accuracy of sperm counting is about 90-95%.

Statistical analysis.—Interspecific differences in male fresh mass, body length, ampulla diameter, and number of sperm per spermatophore were analyzed by ANOVA. To examine possible correlations between body mass and ampulla size or sperm number, least-squares regression analyses were carried out independently for each species as well as for all species together. Constants and intersects of the regression lines were tested for significance using Student's t-test.

Results

Intraspecific comparisons.—For all four cricket species, both ampulla diameter and number of sperm contained in the ampulla increase linearly with body mass (Figs 3, 4). Ampulla diameter (dependent variable) was highly correlated ($p < 0.001$) to male fresh body mass (independent variable) in all four species. Fig. 3 illustrates all four regression lines, based on the equation $y = b_1 x$ (linear homogeneous function). Pearson's correlation coefficients, indicating the accuracy of the regression fit, varied between 0.57 in the case of *A. domesticus* and 0.94 in the case of *T. commodus*, with r(*G. bimaculatus*) = 0.87, and r(*G. assimilis*) = 0.90.

Sperm number per spermatophore (dependent variable) was also highly correlated ($p < 0.001$) to fresh male body mass (independent variable), for all four cricket species. Contrary to the first regression computation for ampulla diameter, noted above, the calculated regression lines for spermatophore number did not cross the origins of the graphs and are thus founded on the equation $y = b_0 + b_1 x$ (linear non-homogeneous function). Goodness of fit (Pearson's correlation coefficients) ranged from 0.72 in the case of *G. bimaculatus* to 0.88 in the case of *T. commodus* (Fig. 4). Intersections between the regression lines and the x-axes of the graphs indicate a theoretical minimal body mass of the males, below which no spermatophore formation takes place.

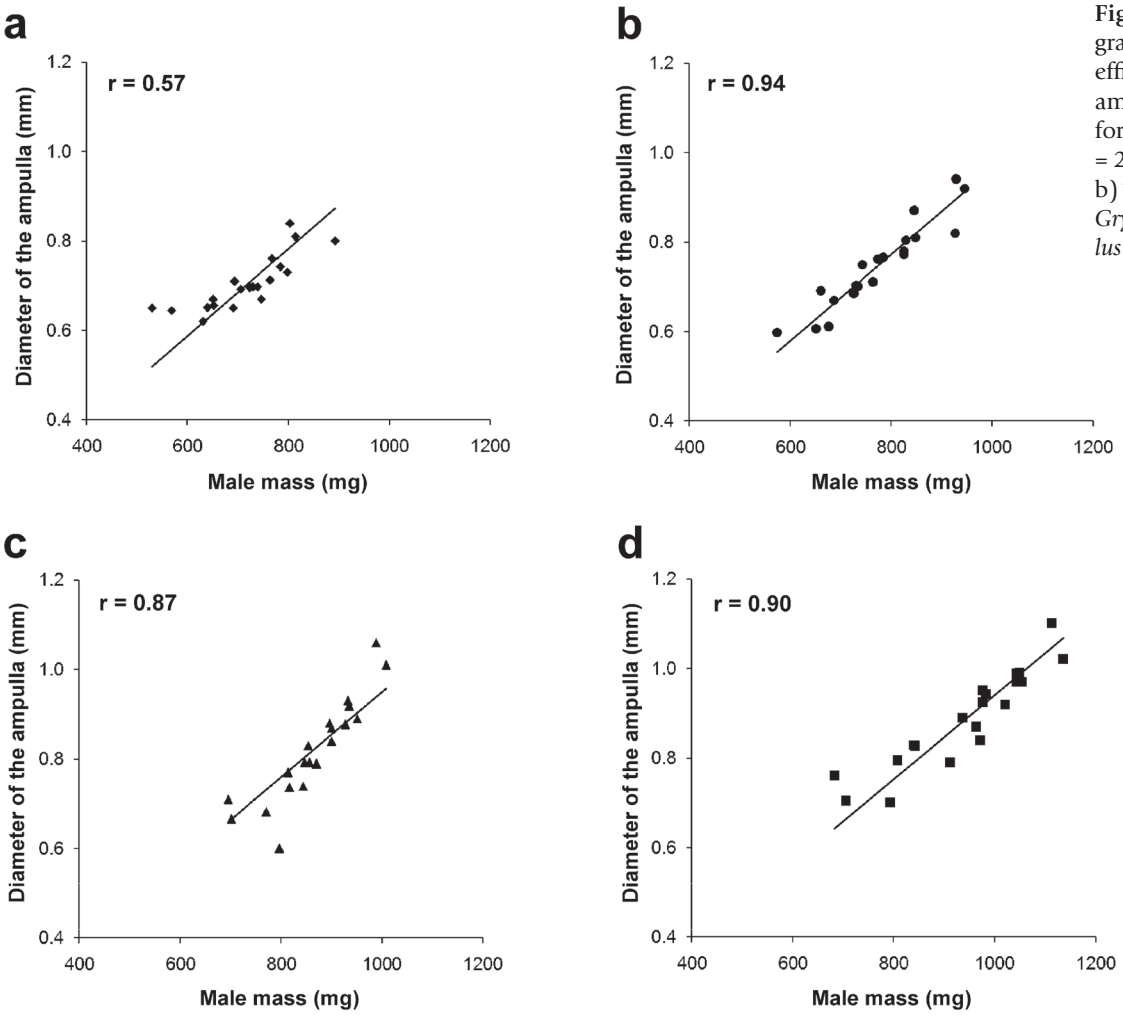

Fig. 3. Linear regression graphs and correlation coefficients (r), of ampulla diameter on male body mass for four species of cricket (N = 20); a) *Acheta domesticus*, b) *Teleogryllus commodus*, c) *Gryllus bimaculatus*, d) *Gryllus assimilis*.

Interspecific comparisons.—Most of the morphological and reproductive variables analyzed in this study differed significantly among the four cricket species (Fig. 5). *A. domesticus* was the smallest species of the study (mass: 724 ± 97 mg, length: 19.7 ± 1.9 mm, N = 20), and *G. assimilis* the largest (mass: 935 ± 166 mg, length: 24.1 ± 3.6 mm, N = 20). The remaining two cricket species were intermediate in size: *G. bimaculatus* (mass: 866 ± 115 mg, length: 22.5 ± 2.4, N = 20), and *T. commodus* (mass: 757 ± 103 mg, length: 21.3 ± 2.2 mm, N = 20). Parametric tests found significant differences ($p < 0.05$) of the measured body parameters between all species with three exceptions (Fig. 5a, b): *A. domesticus* did not differ significantly in weight from *T. commodus*. *G. bimaculatus* did not differ significantly in weight from *G. assimilis*, and *T. commodus* did not differ significantly in length from *G. bimaculatus*.

Regarding the diameter of the ampulla, a trend similar to that derived from body measurements was obtained. As summarized in Table 1 and Fig. 5c, d, males of *A. domesticus* produced the smallest ampulla ($D_{ampulla}$ = 0.71 ± 0.07 mm, N = 20), whilst *G. assimilis* produced the largest ($p < 0.05$, $D_{ampulla}$ = 0.89 ± 0.11). Ampullas of *T. commodus* averaged 0.76 ± 0.09 mm, and those of the Mediterranean field cricket *G. bimaculatus* averaged 0.83 ± 0.12 mm in diameter. There was no significant difference in mean diameter values between *A. domesticus vs T. commodus*, *T. commodus vs G. bimaculatus* or *G. bimaculatus vs G. assimilis* (Fig. 5c). However, all other species comparisons in Fig. 5c were significant. The number of sperm contained in the spermatophores ranged from 1.23×10^5 ± 0.65×10^5 (*A. domesticus*) to 2.21×10^5 ± 1.05×10^5 (*G. assimilis*, Table 1), however a significant difference was found only between *A. domesticus* and *G. assimilis* Fig. 5d.

In order to obtain more generalized information of possible correlations between ampulla size and body mass as well as sperm number and body mass, a linear regression analysis of all four cricket species was carried out (Fig. 6). Concerning the correlation between $D_{ampulla}$ and body mass (Fig. 6a), $D_{ampulla}$ increases by 0.001 mm per each additional mg body mass ($p < 0.001$, r = 0.91). The relationship between sperm number and body mass is described by a regression line with the intercept being located at -2.699×10^5 and the constant b_1 adopting a value of 5.55×10^2. Both regression coefficients are characterized by high significance ($p < 0.001$, r = 0.77; Fig. 6b).

Discussion

This study supports the hypotheses that in crickets, male body size, spermatophore size, and number of sperm per spermatophore are positively correlated, both intra- and interspecifically.

Scaling among traits.—More interesting than the simple correlations among these three traits, is the phenomenal increase in ampulla volume and sperm number corresponding to relatively small increases in body size. This holds true for both intraspecific and interspecific relationships. Hence, for *A. domesticus*, male body length (for males who produced spermatophores) varied from 17.4-22.8 mm (a 31% increase from shortest to longest male), but ampulla diameter varied

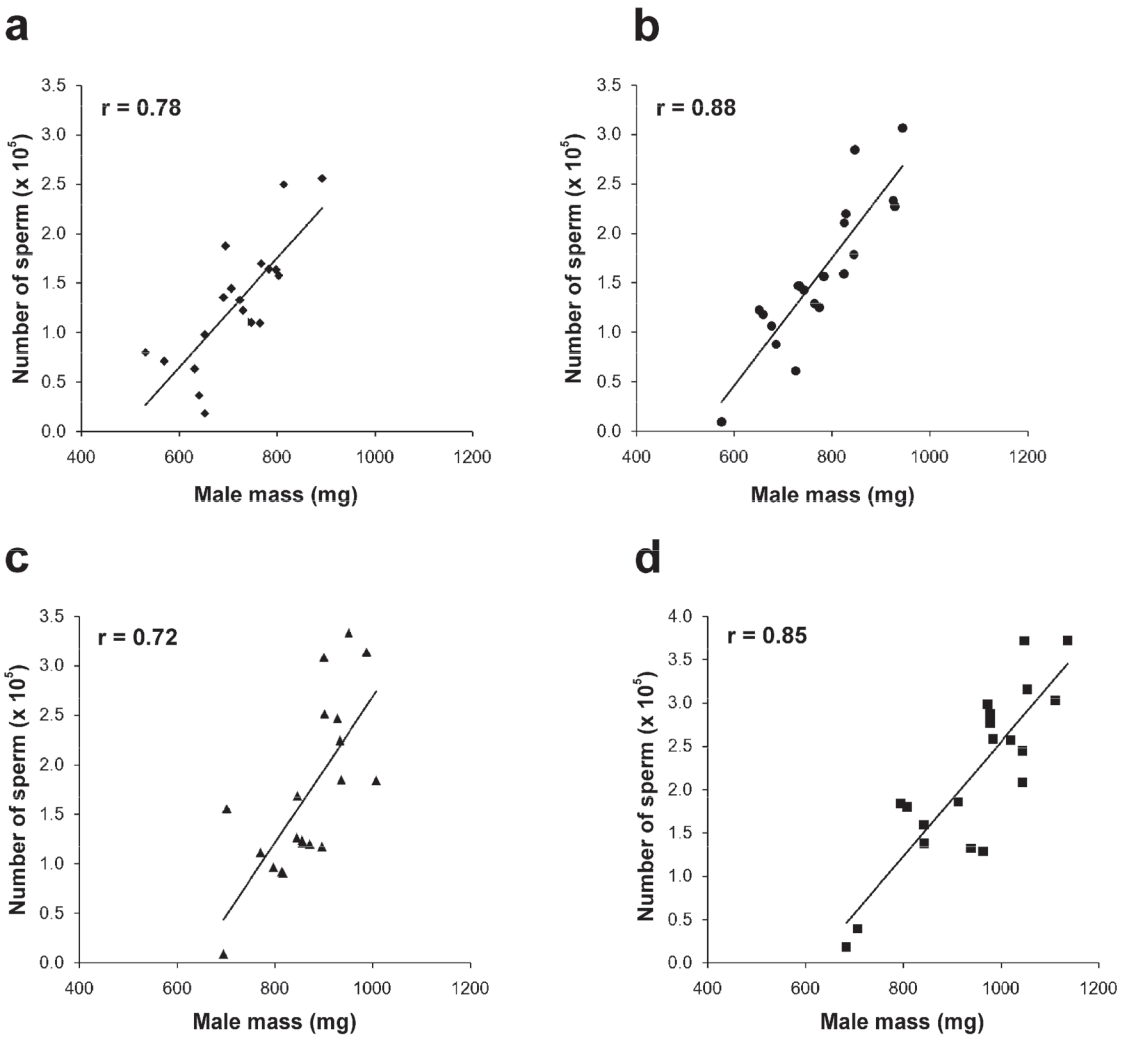

Fig. 4. Linear regression graphs exhibiting the intraspecific dependence of sperm number per spermatophore on body mass of male crickets (N = 20); a) *Acheta domesticus*, b) *Teleogryllus commodus*, c) *Gryllus bimaculatus*, d) *Gryllus assimilis*.

from 0.61 to 0.83 mm (a 36% increase), corresponding to a 150% volume increase. Likewise, for *G. bimaculatus*, male body mass varied by a magnitude of 51% (698-1057 mg), but ampulla diameter varied over a magnitude of 37% (0.69-0.96 mm), corresponding to a volume increase of 169%. In *T. commodus*, body length varied by a magnitude of 30%, while sperm numbers varied by a magnitude of 249% (67,000-234,000 sperm/ampulla). Hence, for this cricket species, an increase in body length by a third nearly triples the number of sperm. This is due, in part, to the scaling relationship between length and volume (Volume µ Length^3) (Whitman 2008). Hence, a doubling of body length produces 8 × the volume for isometric objects. These same relationships linking spermatophore size and sperm number to body size also exist across species (Fig. 6a, b).

Why should sperm numbers increase so rapidly with small changes in body size? One can argue that there is an optimal number of sperm that should be passed to the average female, under average conditions, and that males should evolve to pass that exact amount. And certainly, size-invariant traits exist (Emlen & Nijhout 2000). For example, jumping distance in *Schistocerca gregaria* is relatively invariant across nymphal instars (Bennet-Clark 1990). Two hypotheses compete to explain the strong scaling relationships between male body size and sperm number. The passive scaling hypothesis posits that these relationships are the result of simple physical/growth relationships, and that there has been no evolutionary selection

Table 1. Fresh mass and body length, spermatophore dimensions, and sperm numbers per spermatophore measured for males of four cricket species (mean ± S.D., ranges in brackets, N = 20 per species). Abbreviations: $D_{ampulla}$ = diameter of the ampulla.

Species	Mass (mg)	Length (mm)	$D_{ampulla}$ (mm)	Sperm # (× 10^5)
A. domesticus	724 ± 97 (509-896)	19.7 ± 1.9 (17.4-22.8)	0.71 ± 0.07 (0.61-0.83)	1.23 ± 0.65 (0.24-1.97)
T. commodus	757 ± 103 (567-935)	21.3 ± 2.2 (18.7-24.3)	0.76 ± 0.09 (0.65-0.88)	1.56 ± 0.78 (0.67-2.34)
G. bimaculatus	866 ± 115 (698-1057)	22.5 ± 2.4 (19.8-25.6)	0.83 ± 0.12 (0.69-0.96)	1.76 ± 0.87 (0.79-2.67)
G. assimilis	935 ± 166 (743-1146)	24.6 ± 3.1 (20.5-28.5)	0.89 ± 0.11 (0.75-1.03)	2.21 ± 1.05 (0.98-3.27)

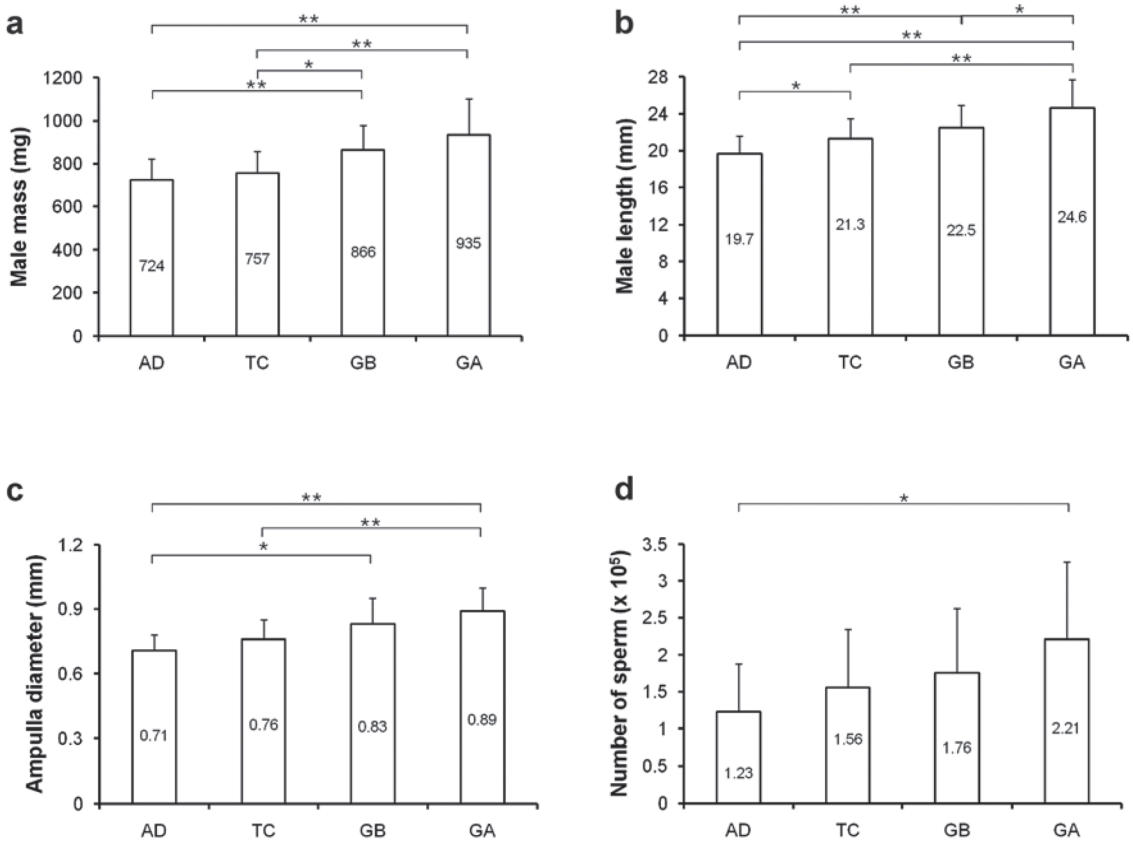

Fig. 5. Interspecific comparisons of four traits among four cricket species: a) body mass, b) body length, c) ampulla diameter, d) sperm number per spermatophore. Abbreviations: TC = *Teleogryllus commodus*, AD = *Acheta domesticus*, GB = *Gryllus bimaculatus*, GA = *Gryllus assimilis*; * = $p < 0.05$, ** = $p < 0.001$ (F-values derived from the ANOVA uniformly indicate normal distribution or close-to-normal distribution.).

for larger spermatophores and more sperm in larger males. The adaptive scaling hypothesis posits that these relationships are the result of adaptive evolution for males to maximize sperm numbers, perhaps because of sperm competition. This hypothesis assumes that each individual male attempts to maximize his reproductive output (sperm numbers), that sperm and spermatophores are costly, that individuals differ in health and nutritional status, and that there are tradeoffs between reproductive output and somatic condition (Stearns 1992; Simmons 2001). As such, individuals that experienced optimal nymphal conditions generally eclose as large adults in good condition, and can afford to allocate a greater proportion of nutritional resources to reproduction (*i.e.*, large spermatophores containing many sperm). In contrast, stressed nymphs eclose at a smaller body size, and cannot afford a large reproductive effort, and hence produce small spermatophores containing fewer sperm. This idea is supported by the fact that in this study, some exceptionally small individuals passed spermatophores that lacked sperm (Fig. 4a, c, d). The fields of scaling, sexual selection, and resource allocation are complex (Stearns 1992; Emlen & Nijhout 2000; Simmons 2001), and answering the interesting question of why larger crickets transfer more sperm, awaits further research.

Intraspecific relationships.—Each cricket species in this study showed a positive correlation between ampulla diameter and body mass as well as between sperm number and body mass. This supports the conclusion that larger and heavier males of *A. domesticus*, *T. commodus*, *G. bimaculatus*, and *G. assimilis* produce larger spermatophores containing higher numbers of sperm. This relationship has been documented for few other crickets (Wedell 1993; McCartney *et al.* 2008). Correlations between sperm number per spermatophore and body size were previously noted for *T. commodus* (Sturm 2011), for the black-horned tree cricket (Brown 2008), and for bushcrickets (Wedell 1997). However, McCartney *et al.* (2008) noted that using ampulla mass (size) to predict the amount of ejaculated or transferred spermatozoa could be problematic due to high natural fluctuations in sperm number. McCartney's warning is strengthened by the present study, where sperm numbers fluctuated widely around the mean values (Table 1).

The findings presented here relate to the ecology, intraspecific competition, sexual selection, fitness, and behavior of the four cricket species. First is that large body size is known to have both advantages and disadvantages (Weissman *et al.* 2008; Whitman 2008). In many insect species, larger individuals are more powerful, have lower mass-specific metabolic rates (but see Fielding & Defoliart 2008), and more favorable surface-to-volume ratios, which provide numerous physiological benefits (Whitman 2008). Larger males are typically more competitive, and can hold larger or better territories, and win male-male contests (Thornhill & Alcock 1983; Arnott & Elwood 2009). Larger male orthoptera usually produce louder calls (Judge *et al.* 2008; Morris 2008; Römer *et al.* 2008), and often attract more females than small males (Brown 1999; Lehmann 2007; Lehmann & Lehmann 2007; Champagnon & Cueva del Castillo 2008). And, as this paper has shown, larger males often provide larger spermatophores with more sperm. Studies show that female Orthoptera that mate with larger males, can have higher fecundity (Gwynne *et al.* 1984; Honěk 1993; Brown 1997; Fedorka & Mousseau 2002), or produce larger offspring (Bretman *et al.* 2006; Kosal & Niedzlek-Feaver 1997, 2007; Saleh *et al.* 2013). In contrast, larger individuals may require more food, be more conspicuous and less agile, and suffer higher predation rates (Whitman & Vincent 2008). Hence, there are numerous interacting benefits and detriments of large size to males, involving physiological, ecological, and reproductive aspects, and all impact ultimate fitness (Whitman 2008).

The considerable male body size variability within each species,

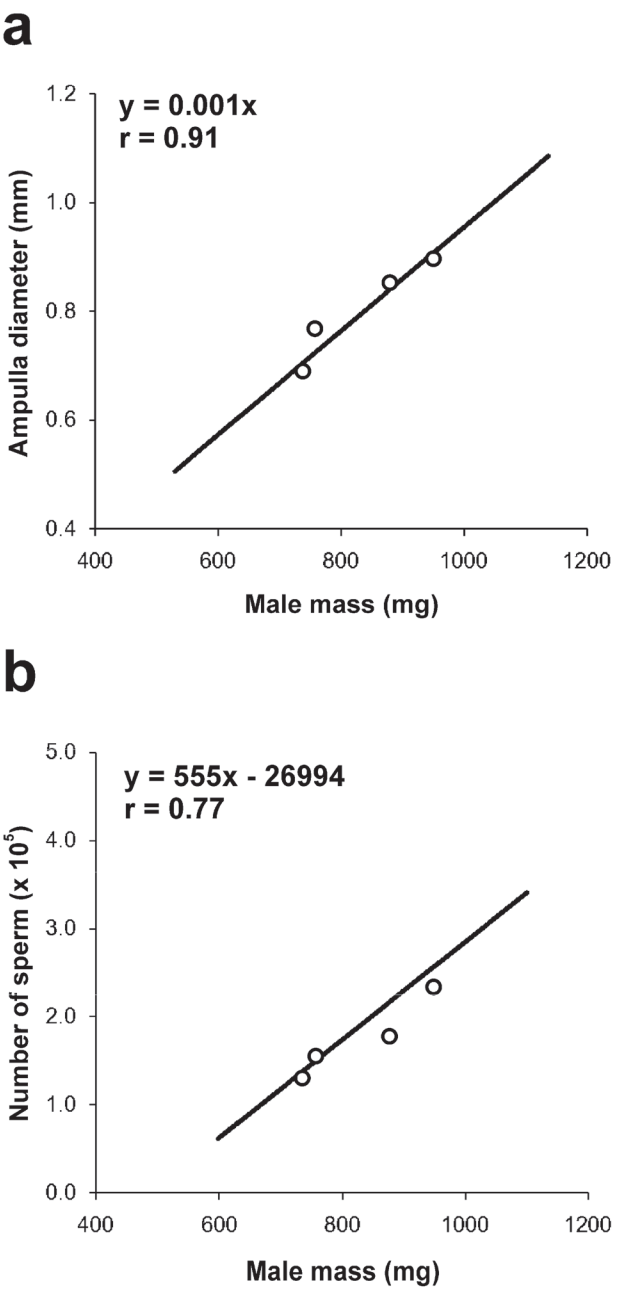

Fig. 6. Interspecific linear regression analyses showing the dependence of ampulla diameter on male's body mass (a; N = 80) and the dependence of sperm number per spermatophore on male's body mass (b; N = 80). Open circles indicate respective mean values of the four species summarized in Table 1.

might also cause intraspecific mating-size incompatibility between males and females (Weissman *et al.* 2008), or foster assortative mating, whereby males and females with similar body sizes tend to mate (Yuexin *et al.* 2013). Differences in size among males may also influence individual behaviors. Small males who are unable to compete against larger, more powerful males, may adopt alternative strategies for gaining access to mating opportunities, such as satellite or sneaky male tactics (Cade 1981; Thornhill & Alcock 1983,). In sum, male body size, spermatophore size, and sperm numbers, and population variation in these traits, have important consequences for males.

Interspecific relationships.—The four cricket species studied tended to differ in mean male body size and mass, ampulla diameter, and number of spermatozoa, although with considerable overlap among species. Under laboratory conditions *G. assimilis* produced the largest males with largest spermatophores and highest sperm numbers, whilst *A domesticus*, had the smallest males and spermatophores, and the lowest sperm numbers.

As George Bartholomew (1981) noted, it is only a slight overstatement to say that the most important attribute of an animal both physiologically and ecologically is its size. Body size influences nearly every aspect of an organism, and as such, is presumed to be a strong target of evolution, resulting in local adaptation of body size (Whitman 2008). Presumably, both natural selection and sexual selection, including both male-male and male-female sexual selection have influenced male body size evolution differently in each of these four cricket species, which, as previously stated, generally survive in different habitats.

The consequences of the interspecific differences in body size, spermatophore size, and sperm number are not known. However, generally, larger species maintain larger territories and/or lower population densities than small species (Bonner 2006). Over evolutionary time, large species tend to go extinct at higher rates than small species (Kingsolver & Pfennig 2007). In contrast, smaller species are thought to be more resistant to extinction, and diversify faster, because they produce more generations per unit time and have higher population densities (LaBarbera 1989).

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