Differences across taxa in nuptial gift size correlate with differences in sperm number and ejaculate volume in bushcrickets (Orthoptera: Tettigoniidae)

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SUMMARY

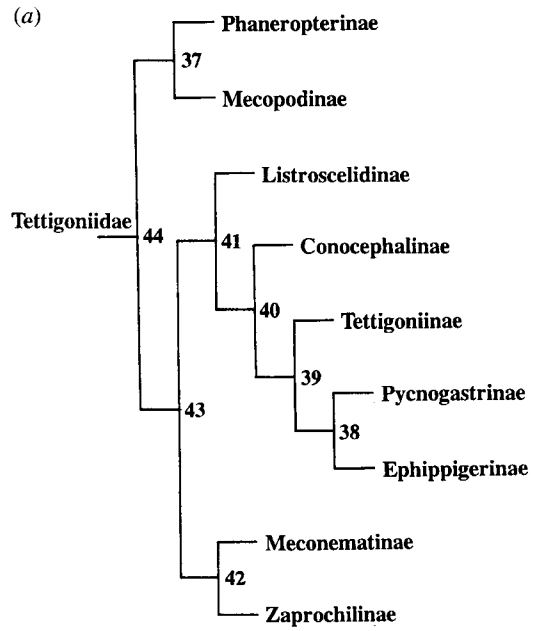
The spermatophores of bushcrickets consist of two parts: an ampulla which contains the sperm and a spermatophylax which the female eats following mating. There are two different, though not mutually exclusive, hypotheses concerning the selective pressures important in the evolutionary enlargement of the spermatophylax in bushcrickets. The paternal investment hypothesis proposes that elaboration of spermatophylax size has proceeded through selection for male nutritional investment in offspring. The ejaculate protection hypothesis, on the other hand, proposes that the evolutionary enlargement of the spermatophylax has proceeded through selection on males to ensure complete ejaculate transfer. The latter hypothesis predicts that evolutionary changes in spermatophylax size should correlate positively with evolutionary changes in ampulla size (i.e. ejaculate volume) and sperm number. Here we present the results of a comparative study designed to test this prediction. Measurements of spermatophylax mass, ampulla mass and male body mass were taken for 43 species of European bushcrickets. Measurements of sperm number were taken for 31 of these species. These data were analysed using the independent comparisons method. As predicted by the ejaculate protection hypothesis, a positive relationship was found, across taxa, between contrasts in spermatophylax mass and contrasts in both ampulla mass and sperm number, controlling for male body weight.

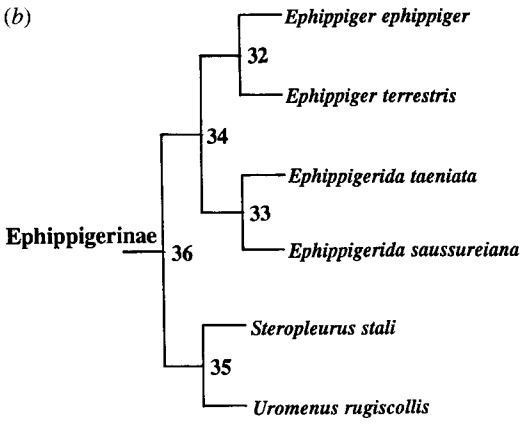
1. INTRODUCTION

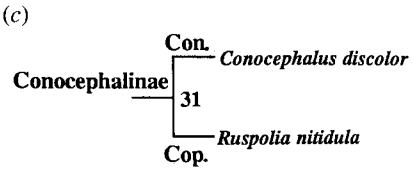
The spermatophores of most species of bushcricket consist of two parts: a sperm-containing ampulla and a large, sperm free mass, the spermatophylax. Following copulation, the female eats the spermatophylax before consuming the ampulla (Boldyrev 1915). The spermatophylax therefore represents a form of nuptial gift (see Thornhill & Alcock 1983; Simmons & Parker 1989 for reviews of nuptial feeding in insects). There is a considerable degree of interspecific variation in the size of the spermatophylax relative to male body weight (Gwynne 1983, 1990*a*; Wedell 1993*a*). At one extreme, the spermatophylax is minute in some species such as *Acripeza reticulata* and only about 2% of male body weight is lost at mating (Wedell 1993*a*). At the other extreme, in certain species such as *Ephippiger ephippiger* the spermatophylax is a very substantial structure which contributes to a loss of up to 40% of male body weight (Busnel & Dumortier 1955).

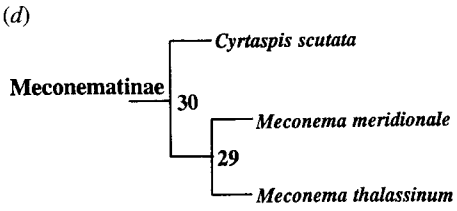
There are two different, though not mutually exclusive, hypotheses to account for the evolution and function of the large spermatophylax in bushcrickets. The ejaculate protection hypothesis proposes that the large spermatophylax has evolved and currently functions to prevent the female from removing the ampulla before sperm transfer is complete (see, for example, Boldyrev 1915; Wedell 1993*a*). The paternal investment hypothesis, on the other hand, proposes that whereas the spermatophylax may have originated as a sperm protection device, its large size is currently maintained by selection for male nutritional investment in offspring (see, for example, Gwynne 1990*a*).

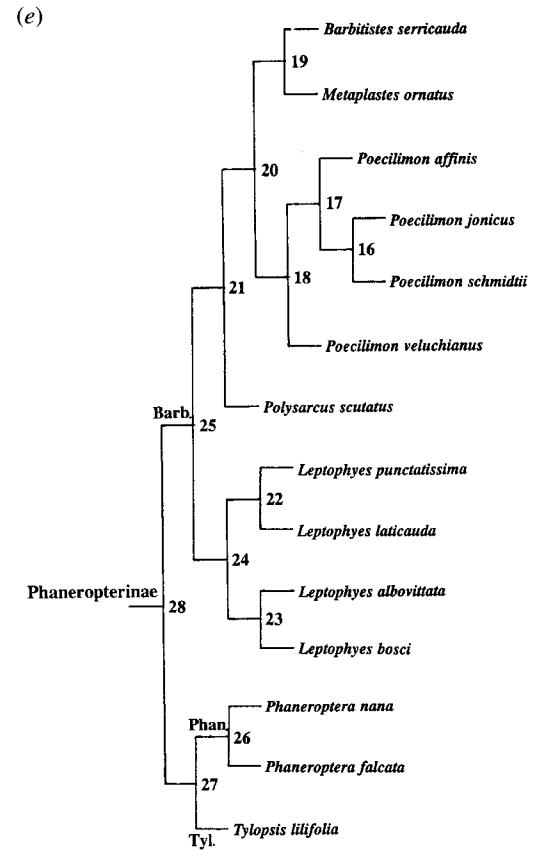
Proteins from the spermatophylax have been traced to developing eggs (Bowen *et al.* 1984; Simmons & Gwynne 1993; Wedell 1993*b*) and have been found to increase the weight and number of eggs in some cases (in *Requena verticalis*, Gwynne 1984, 1988*a*; in *Kawanaphila nartee*, Simmons 1990), but not in others (in *R.verticalis*, Gwynne *et al.* 1984; in *Decticus verrucivorus*, Wedell & Arak 1989; in *Poecilimon veluchianus*, Reinhold & Heller 1993). While these findings support the paternal investment hypothesis, they are not necessarily inconsistent with the ejaculate protection hypothesis because such effects may arise incidentally from the consumption of a food gift that has evolved principally in the context of ensuring sperm transfer (Quinn & Sakaluk 1986). There has been debate over whether the male stands to fertilize the eggs which benefit from his nutritional contribution. In *R.verticalis* and *K.nartee*, this is probably the case (Gwynne 1988*b*; Simmons 1990; Simmons & Gwynne 1993). However, in *P.veluchianus* (Heller & Helversen 1991; Achmann *et al.* 1992; Reinhold &

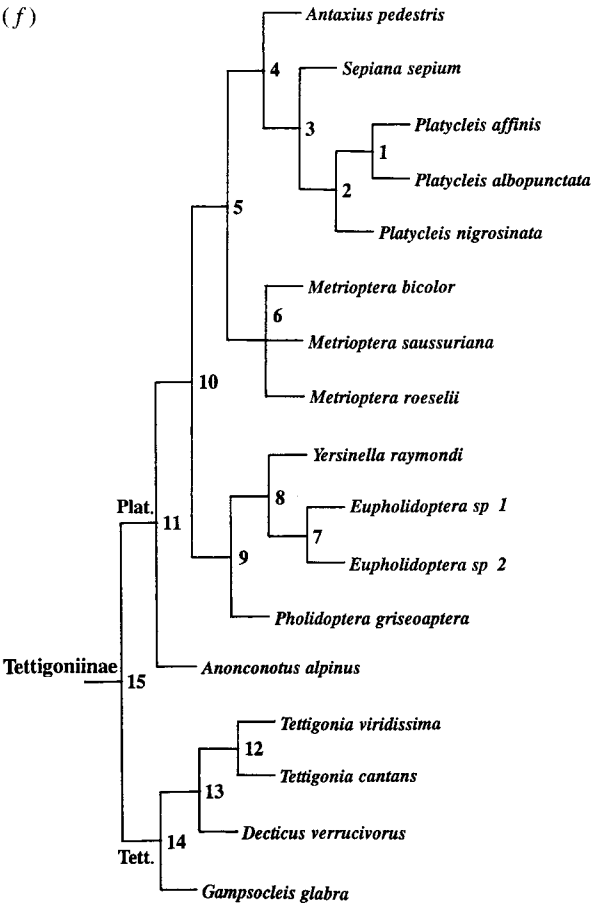

(a)
Phaneropterinae
37
Mecopodinae
Listroscelidinae
Tettigoniidae
44
41
Conocephalinae
40
Tettigoniinae
39
Pycnogastrinae
43
38
Ephippigerinae
Meconematinae
42
Zaprochilinae


(b)
Ephippiger ephippiger
32
Ephippiger terrestris
34
Ephippigerida taeniata
33
Ephippigerinae
Ephippigerida saussureiana
36
Steropleurus stali
35
Uromenus rugiscollis


(c)
Con.
Conocephalus discolor
Conocephalinae
31
Ruspolia nitidula
Cop.


(d)
Cyrtaspis scutata
Meconematinae
30
Meconema meridionale
29
Meconema thalassinum


(e)
Barbitistes serricauda
19
Metaplastes ornatus
Poecilimon affinis
20
17
Poecilimon jonicus
16
18
Poecilimon schmidtii
21
Poecilimon veluchianus
Polysarcus scutatus
Barb.
25
Leptophyes punctatissima
22
Leptophyes laticauda
24
Phaneropterinae
Leptophyes albovittata
28
23
Leptophyes bosci
Phaneroptera nana
Phan.
26
Phaneroptera falcata
27
Tyl.
Tylopsis lilifolia


(f)
Antaxius pedestris
4
Sepiana sepium
3
Platycleis affinis
1
Platycleis albopunctata
2
5
Platycleis nigrosinata
Metrioptera bicolor
6
Metrioptera saussuriana
10
Metrioptera roeselii
Yersinella raymondi
8
Plat.
Eupholidoptera sp 1
11
7
9
Eupholidoptera sp 2
Pholidoptera griseoaptera
Tettigoniinae
Anonconotus alpinus
15
Tettigonia viridissima
12
Tettigonia cantans
13
Decticus verrucivorus
14
Tett.
Gampsocleis glabra

Heller 1993), *P.affinis* (Heller & Helversen 1991), *Metaplastes ornatus* (Helversen & Helversen 1991), *D.verrucivorus* (Wedell 1993*b*) and *Steropleurus stali* (Vahed 1995) one male's spermatophylax nutrients are likely to benefit primarily eggs fertilized by other males and are therefore unlikely to function as a form of paternal investment.

The large spermatophylax appears to be adapted to ensure complete sperm transfer in two sub-species of *P. veluchianus* (Reinhold & Heller 1993; Heller & Reinhold 1994), in *K.nartee* (Simmons & Gwynne 1991) and in *R.verticalis* (Simmons 1995*a*; but see Gwynne *et al.* 1984 and Gwynne 1986 who argue that the spermatophylax of *R.verticalis* is larger than is necessary to ensure complete sperm transfer). Whereas this provides support for the ejaculate protection hypothesis, it does not exclude the paternal investment hypothesis because, as previously stated, the two hypotheses are not mutually exclusive. As predicted by the ejaculate protection hypothesis, a positive relationship has been found, within species, between the mass of the spermatophylax and the mass of the ampulla (an estimate of ejaculate volume) in the tettigoniids *Decticus verrucivorus* (Wedell & Arak 1989), *Ephippiger ephippiger*, *Metrioptera roeselii* (Wedell 1994*a*) and *Requena verticalis* (Simmons 1995*b*; but see Simmons *et al.* 1993 who found no such relationship) and in the gryllid *Gryllodes sigillatus* (Gage & Barnard 1996; but see Sakaluk & Smith 1988 who found no such relationship). A strong prediction of the ejaculate protection hypothesis is that such a relationship should also be found across species, that is, an evolutionary increase in sperm number and/or ejaculate volume should be associated with an evolutionary increase in the mass of the protective spermatophylax. It should be noted, however, that a positive relationship between nuptial gift size and sperm number across taxa is not necessarily inconsistent with the paternal investment hypothesis. This is because paternal investment is predicted to arise in species in which there is a high confidence of paternity for the investing male (see, for example, Westneat & Sherman 1993) and it is conceivable that the confidence of paternity might be higher in species with larger ejaculates and/or sperm loads (species with larger ejaculates have longer female refractory periods in tettigoniids; Wedell 1993*a*).

In a comparative study of 19 mainly Australian genera of bushcrickets, Wedell (1993*a*, 1994*b*) found a positive correlation between spermatophylax mass and ampulla mass across genera, after controlling for the effects of body mass. However, Wedell (1993*a*, 1994*b*) did not control for the effects of common ancestry or actually measure sperm number. Here we present the results of a comparative study of the relationship between spermatophylax mass, ampulla mass and sperm number in which we controlled both for the effects of male body mass and for the effects of common ancestry.

2. METHODS

In total, 43 species of bushcricket were collected as adults from Spain in August 1990, France in August 1990 and August 1991, Greece in July 1991 and England in September 1990 and 1991. Collecting methods and localities for each species are given in Vahed (1995). Sexes were separated and maintained under laboratory conditions as outlined by Hartley & Dean (1974), detailed in Vahed (1995). Stocks of *Mecopoda elongata*, which originated from Malaysia, were purchased from an entomological dealer.

Spermatophores were obtained both from wild-caught individuals and from offspring subsequently reared in the laboratory (details of oviposition media used, treatment of eggs and rearing conditions for nymphs are outlined in Vahed 1995). Males were not used for mating until at least 2 weeks following collection or the final moult, to ensure that spermatophore size and sperm number were unlikely to be reduced because of male age or mating history (see Simmons 1995*b*). For each species, individual stridulating males, and females which showed signs of receptivity (i.e. showing phonotaxis to the male call or exhibiting a response-song, where present), were transferred to black nylon-mesh observation cages (measuring approximately 10 cm^3), one pair per cage. Directly after the end of copulation, the entire spermatophore was removed from the female using watchmakers' forceps. This was weighed on a Cahn-25 electrobalance to an accuracy of 0.01 mg. The ampulla was then separated from the spermatophylax and weighed separately. Ampulla weight was subtracted from the weight of the entire spermatophore to give the spermatophylax weight in each case. The recently mated male in each case was weighed on an electrobalance to an accuracy of 0.1 mg. The weight of the spermatophore produced was added to male body weight to give male pre-mating body weight. In most cases, the ampulla was then placed in a plastic vial in a known volume of physiological locust saline (from 0.05 to 6 ml, depending upon the size of the ampulla). The ampulla was crushed with watchmakers' forceps and its contents were suspended by thorough mixing with watchmakers' forceps for 5 min. This was found to result in an even suspension of sperm. A portion of each sample was then transferred to a haemocytometer (Neubauer, improved), and the number of sperm in the centre grid was counted under a microscope. A total of two sub-samples were counted per sample and a mean value was taken. This value was multiplied by the appropriate dilution factor to give an estimate of the total sperm number in the

Figure 1. Branching diagrams reflecting the possible taxonomic and/or phylogenetic relationships between the different species of bushcricket studied. These diagrams were used in the calculation of the contrasts (see text). (*a*) Probable phylogenetic relationships between the different tettigoniid sub-families studied (from Gorochov (1988)); (*b*) possible taxonomic relationships between the different species of ephippigerine studied; (*c*) taxonomic relationships between the conocephalines studied (Con = tribe Conocephalini; Cop = tribe Copiphorini); (*d*) taxonomic relationships between the different meconematines studied; (*e*) possible taxonomic and/or phylogenetic relationships between the phaneropterines studied (general taxonomy based on Bei-Bienko 1954 and Kevan 1982; relationships between members of the tribe Barbitistini, especially members of the genus *Poecilimon*, based on Heller 1984, 1990) (Barb = Barbitistini; Phan = Phaneropterini; Tyl = Tylopsini); (*f*) phylogenetic relationships between members of the sub-family Tettigoniinae used in this study (from Rentz & Coless 1990) (Tett = Tettigoniini; Plat = Platycleidini).

Table 1. *Mean male weight, ampulla weight, spermatophylax (sp'lax) weight, and sperm number for different bushcricket species*
(Letters indicate source of reference; dashes indicate missing values; *n* = number of different individuals).

	sub-families and species	male weight mg	*n*	ampulla weight mg	*n*	sp'lax weight mg	*n*	sperm number $\times 10^4$	*n*
Phaneropterinae									
1	*Phaneroptera nana*	289	7	5.24	7	9.16	7	3.8	5
2	*Phaneroptera falcata*	187	1	10.02	1	16.51	1	31.2	1
3	*Tylopsis lilifolia*	340	6	13.17	6	69.72	6	—	—
4	*Barbitistes serricauda*	721	1	47.5	1	158.6	1	369.0	1
5	*Leptophyes punctatissima*	175	15	1.05	10	5.97	14	11.5	16
6	*Leptophyes laticauda*	478	17	20.28	17	103.65	17	168.76	17
7	*Leptophyes albovittata*	112	2	1.78	2	6.75	2	26.35	2
8	*Leptophyes bosci*	235	4	3.08	4	13.53	4	71.11	4
9	*Poecilimon schmidtii*	525	8	9.17	6	63.39	6	84.5	2
10	*Poecilimon jonicus*	324	4	5.82	3	21.96	4	20.43	3
11	*Poecilimon veluchianus*	710	1	37.0	1	145.0	1	1040.0[a]	50
12	*Poecilimon affinis*	1328	4	30.89	3	170.27	4	438.0	3
13	*Polysarcus scutatus*	1688	4	48.6	2	221.3	2	362.0	1
14	*Metaplastes ornatus*	450	2	—	—	72.0	2	149.0[b]	—
Mecopodinae									
15	*Mecopoda elongata*	3699	2	27.24	2	0.0	2	—	—
Tettigoniinae									
16	*Tettigonia viridissima*	1450	1	78.63	1	250.0	1	454.0	1
17	*Tettigonia cantans*	1204	1	52.6	1	154.4	1	—	—
18	*Gampsocleis glabra*	885	5	36.32	5	61.22	5	216.88	4
19	*Decticus verrucivorus*	1618	3	56.09	3	123.42	3	169.56	3
20	*Platycleis affinis*	576	5	13.78	5	23.05	5	75.14	5
21	*Platycleis albopunctata*	479	3	12.20	3	14.37	3	71.7	2
22	*Platycleis nigrosinata*	409	1	11.56	1	14.56	1	—	—
23	*Metrioptera saussuriana*	509	4	13.05	4	29.33	4	100.45	4
24	*Metrioptera bicolor*	438	3	19.22	3	23.78	3	54.63	3
25	*Metrioptera roeselii*	345	3	15.73	3	20.23	3	40.24	3
26	*Sepiana sepium*	529	2	15.98	2	23.87	2	39.55	2
27	*Yersinella raymondi*	200	2	2.26	3	11.0	1	20.83	2
28	*Anonconotus alpinus*	604	6	4.94	6	7.71	6	59.02	5
29	*Antaxius pedestris*	716	1	25.69	1	89.83	1	532.5	1
30	*Pholidoptera griseoaptera*	498	2	16.34	2	37.09	2	84.6	2
31	*Eupholidoptera sp* 1	1233	1	56.40	1	103.6	1	197.0	1
32	*Eupholidoptera sp* 2	1042	1	34.93	1	135.82	1	—	—
Conocephalinae									
33	*Conocephalus discolor*	150	2	2.38	2	12.20	2	—	—
34	*Ruspolia nitidula*	556	3	2.21	3	1.59	3	51.13	2
Meconematinae									
35	*Cyrtaspis scutata*	182	6	6.72	6	9.86	6	21.3	2
36	*Meconema meridionale*	97	8	1.7	8	0.0	8	17.26	5
37	*Meconema thalassinum*	94	4	0.56	4	0.0	4	4.86	5
Ephippigerinae									
38	*Ephippiger ephippiger*	2313	5	148.97	6	468.76	5	—	—
39	*Ephippiger terrestris*	1091	1	60.80	1	270.0	1	—	—
40	*Ephippigerida taeniata*	4075	17	156.81	17	947.01	17	—	—
41	*Ephippigerida saussureiana*	945	1	46.0	1	219.8	1	—	—
42	*Steropleurus stali*	1296	1	90.9	1	362.5	1	—	—
43	*Uromenus rugiscollis*	1143	9	62.72	9	79.01	9	170.25	4
Pycnogastrinae									
44	*Pycnogaster inermis*	4397	2	308.7	2	669.85	2	1020.0	1
Zaprochilinae									
45	Gen.Nov.22.sp1.	48[c]	—	—	—	ca 10[c,d,h]	—	21.97[e]	—
Listeroscelidinae									
46	*Requena verticalis*	400[f]	—	13.07[g]	—	36.2[g]	—	93.35[g]	—

[a] K. Reinhold, personal communication.
[b] Helversen & Helversen 1991.
[c] Simmons & Bailey 1990.
[d] Gwynne & Bailey 1988.
[e] Simmons & Gwynne 1991.
[f] Gwynne 1990*b*.
[g] Simmons *et al.* 1993.
[h] The weight of the entire spermatophore.

original sample. Where possible, all measurements were done on a number of different individuals of each species and a mean value was taken.

Complete sets of data for male body weight, spermatophylax weight and ampulla weight were obtained for 43 species of bushcricket. Data (for all three variables) for one additional species were taken from the literature, giving data for 44 species representing 27 genera and eight sub-families, though most species were from the sub-families Phaneropterinae and Tettigoniinae. Sperm counts were done on 31 of these species and a further four were taken from the literature, giving data for 35 species representing 23 genera and eight sub-families.

We used the non-directional independent-comparisons method (see Harvey & Pagel 1991; Harvey & Purvis 1991 for details) to examine the relationships between differences across taxa in spermatophylax size and differences in ampulla size and sperm number. This method allows for phylogenetic effects using the principle that differences in a character between two taxa which share an immediate common ancestor should not be confounded by phylogenetic differences (see Felsenstein 1985). The set of differences (contrasts) for character x and for character y provide a way to test whether changes in x and y are correlated (see Harvey & Pagel 1991).

Ideally, this method requires that the true branching phylogeny is known. However, in the absence of such, a taxonomy may be used to represent the branching of species (Harvey & Pagel 1991; Harvey & Purvis 1991). There is currently no detailed phylogeny for the family Tettigoniidae. However, there is a phylogeny at the level of the genus for the sub-family Tettigoniinae (Rentz & Coless 1990) and Gorochov (1988) gives the possible phylogenetic relationships between the sub-families of the Tettigoniidae. We have used these sources together with the taxonomies of the remaining groups to construct the branching diagrams which were used to generate the comparisons (see figure 1 *a–f*).

The overall classification for the sub-family Phaneropterinae (figure 1 *e*) was based on that given by Bei-Bienko (1954) and Kevan (1982), while the relationships between species in the tribe Barbitistini and genus *Poecilimon* were taken from Heller (1984, 1990). Within the sub-family Ephippigerinae (figure 1 *b*), we grouped *Steropleurus stali* and *Uromenus rugiscollis* together because these species are placed within the same genus (*Uromenus*) by Harz (1969). Within the sub-family Tettigoniinae (figure 1*f*), the relationships between the three members of the genus *Platycleis* for which data were obtained was based on the sub-generic groupings given by Harz (1969).

Only one multiple node occurred in the branching diagram of the taxonomic relationships between the species used in this study, between the three *Metrioptera* species (sub-family Tettigoniinae; figure 1*f*). Each is placed in a separate sub-genus (Harz 1969), making further grouping of the species difficult. We have used the method given by Pagel & Harvey (1989; cited in Harvey & Pagel 1991, p. 157) to generate the contrast in this case.

For each species, the obtained values for male body mass, spermatophylax mass, ampulla mass and sperm number were $\log_{10}$ transformed to meet the assumptions of parametric regressions. Because sperm number data were available for fewer species than ampulla data, two sets of contrasts were calculated for body mass and spermatophylax mass: one set was calculated using species for which there were sperm number data and the other set was calculated using species for which ampulla mass data were available. The confounding effect of body mass was removed by calculating residuals from the linear regressions of a given variable (spermatophylax mass contrasts, ampulla mass contrasts or sperm number contrasts) on contrasts of body mass. Further regression analysis was done on the residual contrasts of spermatophylax mass (dependent variable) against the residual contrasts of ampulla mass, and on the residual contrasts of spermatophylax mass (dependent variable) against the residual contrasts of sperm number to test the prediction that changes in spermatophylax mass should be positively related to changes in ampulla mass and sperm number. In all cases, regressions were forced through the origin, as recommended by Harvey & Pagel (1991). Means are cited $\pm$ standard error.

3. RESULTS

Mean values of male body mass, spermatophylax mass, ampulla mass and sperm number for each species are given in table 1. For the set of contrasts using species for which sperm number data were available, contrasts in sperm number were positively related to contrasts in male body mass ($b = 1.18 \pm 0.21$, $t_{31} = 5.52$, $p \ll 0.001$, $r^2 = 0.49$). The regression coefficient was not significantly different from 1 ($t_{31} = 0.86$, n.s.). For this data set, contrasts in spermatophylax mass were also positively related to contrasts in body mass ($b = 1.3 \pm 0.22$, $t_{31} = 5.9$, $p \ll 0.001$, $r^2 = 0.52$). Again, the regression coefficient for this relationship was not significantly different from 1 ($t_{31} = 1.36$, n.s.). A positive relationship was found between residual contrasts of spermatophylax mass and residual contrasts of sperm number (figure 2; $b = 0.64 \pm 0.14$, $t_{31} = 4.43$, $p < 0.001$, $r^2 = 0.38$). The regression coefficient of this relationship was significantly less than 1 ($t_{31} = 2.57$, $p < 0.05$). Thus, allowing for male body weight, an evolutionary increase of ten times in sperm number would appear to be associated with an increase of 4.4 (anti-log of 0.64) times in spermatophylax mass.

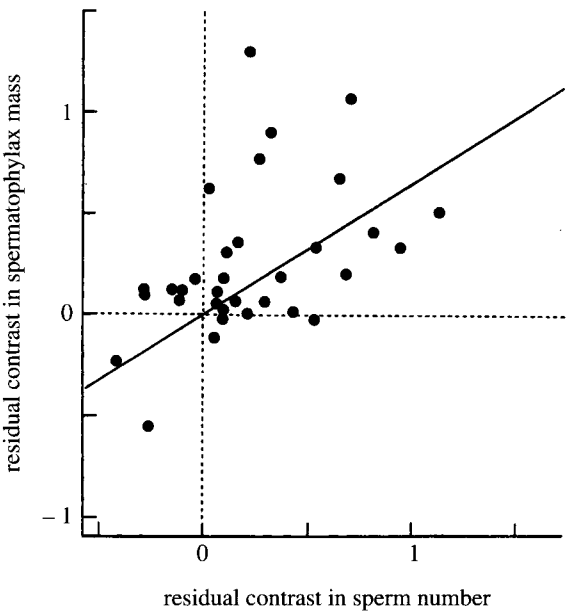


Figure 2. Residual contrasts in spermatophylax mass (residuals from the linear regression of spermatophylax contrasts against body mass contrasts) against residual contrasts in sperm number for the different bushcrickets studied ($b = 0.64 \pm 0.14$, $t_{31} = 4.43$, $p < 0.001$, $r^2 = 0.38$).

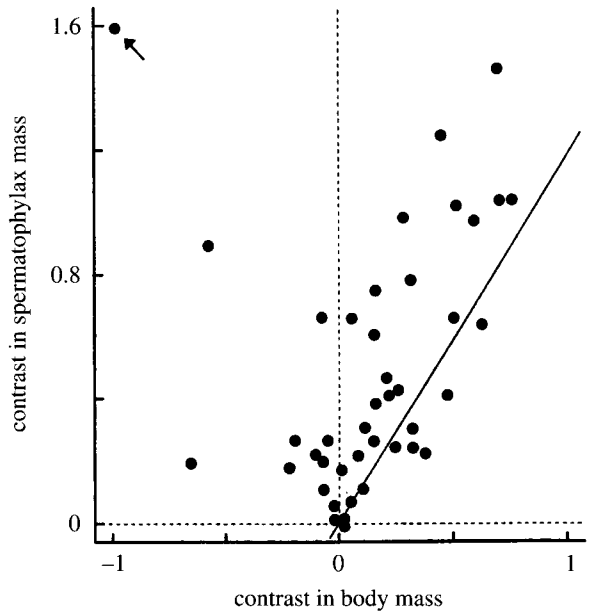


Figure 3. Spermatophylax mass contrasts against body mass contrasts for the species for which ampulla mass data were available ($b = 1.17 \pm 0.2$, $t_{39} = 5.9$, $p \ll 0.001$, $r^2 = 0.47$). The arrow indicates an outlier which was excluded from the calculation of the regression line shown here (with outlier included, $b = 0.72 \pm 0.24$, $t_{40} = 2.98$, $p < 0.01$, $r^2 = 0.18$).

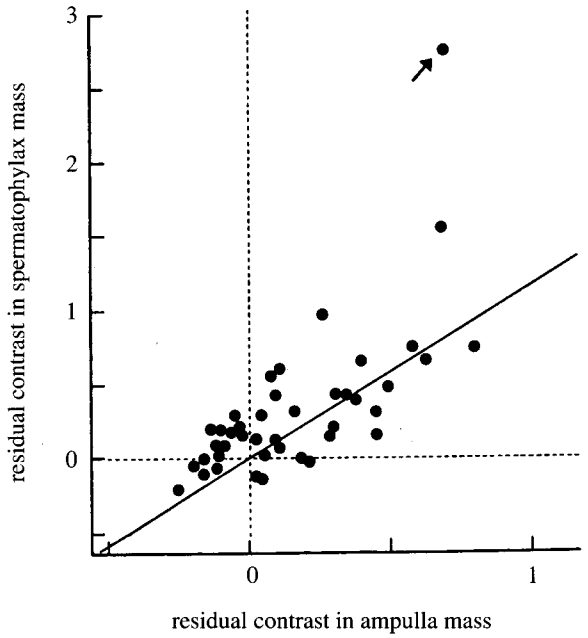


Figure 4. Residual contrasts in spermatophylax mass (residuals from the linear regression of spermatophylax contrasts against body mass contrasts) against residual contrasts of ampulla mass for the bushcrickets studied ($b = 1.16 \pm 0.14$, $t_{39} = 8.2$, $p \ll 0.001$, $r^2 = 0.63$). The arrow indicates an outlier which was excluded both from the calculation of the regression line used to generate the spermatophylax residuals (see figure 3) and from the present regression (with these points included in both regression analyses, $b = 1.49 \pm 0.17$, $t_{40} = 8.72$, $p \ll 0.001$, $r^2 = 0.65$).

For the set of contrasts using species for which ampulla mass data were available, contrasts in ampulla mass were positively related to contrasts in body mass ($b = 1.12 \pm 0.13$, $t_{40} = 8.7$, $p \ll 0.001$, $r^2 = 0.65$), with a regression coefficient which was not significantly different from 1 ($t_{40} = 0.92$, n.s.). The relationship between contrasts in spermatophylax mass and contrasts in body mass for this data set is presented in figure 3. The arrow in figure 3 indicates an apparent outlier. This point represents the contrast between the mean data values for the subfamily Phaneropterinae and the subfamily Mecopodinae (contrast number 37 in figure 1*a*). Data for only a single species of mecopodine were available (*Mecopoda elongata*). This species is comparatively very large and is unusual in that the males do not produce a spermatophylax. By contrast, the phaneropterines for which data were available are relatively small in terms of body size, but produce relatively large spermatophylaxes. Thus the contrast in spermatophylax mass between the two subfamilies was strongly positive, whereas the contrast in body mass was strongly negative. This situation is very different from the apparent trend. Because the anomalous position of this data point appears to have arisen because of the small sample size of mecopodines used, we have done the analysis both with and without this outlier. For this data set, contrasts in spermatophylax mass were positively related to contrasts in body mass (figure 3; with outlier excluded: $b = 1.17 \pm 0.2$, $t_{39} = 5.9$, $p < 0.001$, $r^2 = 0.47$; with outlier included: $b = 0.72 \pm 0.24$, $t_{40} = 2.98$, $p < 0.01$, $r^2 = 0.18$). Whether or not the outlier is included, the regression coefficient is not significantly different from 1 (outlier excluded: $t_{39} = 0.85$, n.s.; outlier included: $t_{40} = 1.17$, n.s.).

A positive relationship was found between residual contrasts of spermatophylax mass and residual contrasts of ampulla mass (figure 4; outlier excluded: $b = 1.16 \pm 0.14$, $t_{39} = 8.2$, $p \ll 0.001$, $r^2 = 0.63$; outlier included: $b = 1.49 \pm 0.17$, $t_{40} = 8.72$, $p \ll 0.001$, $r^2 = 0.65$). With the apparent outlier excluded both from the calculation of the regression line used to generate the residuals (see figure 3) and from the regression of residual contrasts of spermatophylax mass against residual contrasts of ampulla mass, the regression coefficient of this relationship was not significantly different from 1 ($t_{39} = 1.14$, n.s.). However, with the apparent outlier included in both analyses, the regression coefficient of the relationship between residual contrasts of spermatophylax mass and residual contrasts of ampulla mass is significantly greater than 1 ($t_{40} = 2.89$, $p < 0.01$).

4. DISCUSSION

As predicted by the ejaculate protection hypothesis, a positive relationship was found between differences between taxa in spermatophylax size and differences in both ampulla size and sperm number, with male body weight and phylogeny controlled for. The results of this study therefore support the ejaculate protection hypothesis, though it should be noted that they could also be interpreted as being consistent with the paternal investment hypothesis (see § 1).

It is of course possible that genes for spermatophylax size, ampulla size and sperm number are linked. If this were the case then an increase in spermatophylax size through selection for paternal investment would automatically lead to an increase in ampulla size and

sperm number. However, this would only occur if the production of a larger amount of sperm had a negligible cost. Otherwise, individuals carrying genes for spermatophylax size and sperm number which were not linked would be at a selective advantage and the linkage might be expected to break down over evolutionary time. The production of large amounts of sperm does appear to have a significant cost (see Dewsbury 1982). Although selection should favour rapid replenishment of sperm, empirical evidence suggests that males may be limited in their capacity to produce sperm (Dewsbury 1982). For example, in the bushcricket *Leptophyes laticauda*, the number of sperm produced is markedly lower both in recently mated males and in recently adult males. The number of sperm produced steadily increases, over a period of at least 30 d, both with time elapsed since the last mating and with male age at mating in this species (Vahed 1995), suggesting that sperm production is costly.

It is interesting that all differences in characters in this study appear to be in direct proportion (i.e. $b = 1$) except for the relationship between differences in relative sperm number and differences in relative spermatophylax mass (dependent variable), the regression coefficient of which is significantly less than unity. This might be because evolutionary changes in spermatophylax eating time (= time available for sperm transfer) may not be directly proportional to changes in spermatophylax mass.

The benefit to a male bushcricket of producing a larger ejaculate and/or more sperm (and hence a larger spermatophylax) probably include an increase in the chance of fertilizing a greater proportion of the eggs of a given female in the event of sperm competition (see Wedell 1991) and the induction of a longer refractory period in the female (see Gwynne 1986; Wedell & Arak 1989; Simmons & Gwynne 1991). Wedell (1993*a*) provides evidence to support the hypothesis that selection on males to induce longer refractory periods in their mates may have been important in the evolution of larger volumes of ejaculate and larger spermatophylaxes in Tettigoniids. In a comparative study, she found a positive relationship between the duration of the female refractory period and both ampulla mass (an estimate of ejaculate volume) and spermatophylax mass, across genera. Another benefit to a male bushcricket of transferring a greater quantity of sperm and/or ejaculate is that this may result in a hastening of the onset and an increase in the rate of oviposition following mating (see Wedell & Arak 1989), thereby increasing the probability that a female will lay eggs before mating with another male.

Given these benefits of producing a larger ejaculate and/or more sperm, male bushcrickets harbouring genes to produce both a larger ejaculate and/or more sperm and a larger spermatophylax to ensure its transfer (larger spermatophylaxes take longer for a female to eat and therefore result in an increase in the time available for ejaculate transfer, Sakaluk 1985; Wedell & Arak 1989) might generally be expected to be at a selective advantage. In the face of sperm competition, selection on sperm number and hence spermatophylax size might proceed as an intraspecific arms race, with males continually being selected to produce larger sperm loads and larger spermatophylaxes than rival males. However, one cost of producing a larger ejaculate and larger spermatophylax would be an increase in the recovery period required between matings (see Dewsbury 1982; Simmons 1990, 1995*b*; Heller & Helversen 1991; Hayashi 1993). Genes for the production of a larger sperm load and/or volume of ejaculate and a larger spermatophylax would only be expected to spread, therefore, if the benefit to a male of fertilizing a greater proportion of a given female's eggs in the event of sperm competition outweighed the cost of a reduction in the number of females a male could inseminate in his lifetime.

Comparative studies of a variety of taxa, including insects (butterflies, Svard & Wicklund 1989; Gage 1994) have demonstrated that in species in which the degree of polyandry (hence the intensity of sperm competition) is greater, males produce relatively more sperm and/or larger ejaculates, or at least have relatively larger testes (reviewed in Simmons *et al.* 1993). Recently even intraspecific studies of a variety of taxa including a fruitfly (Gage 1991), a tenebrionid beetle (Gage & Baker 1991), a noctuid moth (He & Tsubaki 1992) and two species of gryllid cricket (Gage & Barnard 1996) have demonstrated a similar phenomenon (reviewed in Gage & Barnard 1996). Therefore one factor that could favour the evolution of a larger ejaculate and/or sperm load and hence a larger spermatophylax in bushcrickets might be an increase in the intensity of sperm competition.

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