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Understanding nuptial gift size in bush-crickets: an analysis of the genus *Poecilimon* (Tettigoniidae: Orthoptera)

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We dedicate this paper to Dagmar von Helversen (1944-2003), who contributed data to this study and devoted many years of her academic career to understanding the nature of Poecilimon.
Anonymous (2004) Bibliographie der wissenschaftlichen Publikationen von Dr. Dr. *h.c.* Dagmar von Helversen (1944-2003). – Articulata 19: 124–126.

Abstract

During mating, male insects of certain species transfer a costly nuptial gift, a large spermatophore, which is eaten by the female as sperm transfer into her. The spermatophore components (the sperm-free spermatophylax and the sperm ampulla) vary greatly in size between species, and have a direct influence on male fitness. Studies of the relationship between spermatophore size variation and male fitness have concentrated on associations between evolutionary changes in spermatophylax size and either ampulla size or sperm number. Two main hypotheses have been put forward to explain the function of the spermatophylax: the ejaculate-protection hypothesis and the paternal investment hypothesis. A strong correlation between the spermatophylax and ampulla or sperm number suggests an ejaculate-protection function because it protects the ampulla from being removed prematurely. However, comparative support comes mainly from disparate bush-cricket species (Tettigoniidae), that vary greatly in relatedness and diet. Furthermore, data are often from animals reared under laboratory conditions. Our study describes the significance of size variation in bush-cricket nuptial gifts, with an analysis from field populations of 33 species within the genus *Poecilimon*. *Poecilimon* share similar diets and the variation in spermatophore size within the genus approximates family-wide variation, so confounding influences from diet and relatedness are, to a certain extent, controlled. Previous support for the ejaculate-protection hypothesis is almost universal, so we expected to find similar results. However, unlike previous studies, there was no relationship between body mass and each of the three spermatophore components when body mass was accounted for, or between spermatophylax mass and sperm number. We also found only a weak relationship between ampulla mass and sperm number, suggesting that caution is needed when using ampulla size to predict sperm number or sperm number to predict ejaculate size. In support of the ejaculate-protection hypothesis we found a positive relationship between spermatophylax size and ampulla mass. While our results support the ejaculate-protection hypothesis, they are not inconsistent with the paternal investment hypothesis.

Key words

mating effort, natural selection, paternal investment, *Poecilimon*, sexual selection, spermatophore function, spermatophore mass

Introduction

The degree to which natural and sexual selection respectively affect mating behavior is largely unknown in evolutionary biology, and few examples delineate the problem more clearly than the maintenance of nuptial gift size in Orthoptera. During mating, male bush-crickets (Tettigoniidae) transfer a variable (in size), yet often substantial, spermatophore to the female (for reviews see Gwynne 1990, 2001; Vahed 1998). When transfer is complete the pair uncouple and the female reaches under her abdomen and starts to consume the spermatophore (Boldyrev 1915). As the ejaculate (sperm and seminal fluid) discharges from the ampulla into the female, she consumes the spermatophylax, a large, sperm-free, gelatinous mass. After that, she consumes the ampulla and remaining ejaculate (Boldyrev 1915, Bowen *et al.* 1984).

Although the function of the ampulla to house the ejaculate is relatively clear, the role the spermatophylax plays in mating is more complicated. Two nonmutually exclusive hypotheses have been suggested for spermatophylax size (for reviews see Vahed 1998, Gwynne 2001). First, the ejaculate-protection hypothesis states that the spermatophylax is sexually selected by preventing the female from removing the ampulla prematurely (Gerhardt 1913, 1914; Boldyrev 1915) and therefore directly increasing a male's assurance in sperm competition in a dose-dependent manner (for reviews see Eberhard 1996, Vahed 1998, Gwynne 2001, Simmons 2001, Arnqvist & Rowe 2005). There may be additional benefits under this hypothesis – consumption of a large spermatophylax may reduce the speed at which a female will remate, thereby indirectly increasing the number of offspring and the number of ova that may be fertilised by the male (Gwynne 1986; Wedell & Arak 1989; Simmons & Gwynne 1991; Wedell 1993a, b; Vahed 2007), or may increase the chance of female survival until oviposition (*e.g.*, Voigt *et al.* 2005, 2006). Males that produce relatively large spermatophores are also more likely to transfer more ejaculate and therefore succeed in sperm competition (for a review see Simmons 2001). A large ejaculate may also induce longer intermating refractory periods in females (Heller & Helversen 1991, Heller & Reinhold 1994, Lehmann & Lehmann 2000a, Vahed 2007), allowing males to father a greater share of eggs laid in the next oviposition (Gwynne 1986; Wedell & Arak 1989; Simmons & Gwynne 1991; Wedell 1993a, b). Under this hypothesis, spermatophylax size should covary with the size of the ampulla (Reinhold & Heller 1993, Wedell 1993a, Heller &

Reinhold 1994) or the number of sperm.

Alternatively, the paternal investment hypothesis suggests that the spermatophylax is under natural selection to provide a positive nutritional effect on the donating male's progeny (Trivers 1972, Gwynne *et al.* 1984). In this case, spermatophylax size should correspond to a relative increase in fitness and/or quantity of offspring (Trivers 1972; Thornhill 1976; Simmons & Parker 1989; Gwynne 1986, 1988, 1990; Wedell 1991; Reinhold 1999) but is not expected to covary with ampulla size or sperm number (for reviews see Vahed 1998, Gwynne 2001).

Both natural and sexual selection functions of the spermatophore have been observed in tettigoniids, and are reflected in considerable interspecific variation in spermatophore size (Gwynne 1983, Wedell 1993a, Vahed & Gilbert 1996). Spermatophore mass ranges from about 2% of total male body mass (relative mass) (*Acripeza reticulata*, Wedell 1993a; *Anonconotus alpinus*, Vahed 2002) to about 40% (*Ephippiger ephippiger*; Busnel & Dumortier 1955), and sperm numbers range between 38,000 (*Phaneroptera nana*, Vahed & Gilbert 1996) and 37.3 million sperm (*P. thessalicus*, McCartney & Heller this issue, p. 227). With respect to spermatophore function it is clear that size variation has significant fitness implications for each sex and species.

Despite the likely benefits to males, producing large spermatophores is expensive, as they represent a loss in future reproductive potential (Simmons 1988a, 1990, 1995a; Heller & von Helversen 1991; Vahed 2007), the costs of which will vary with factors such as local growing conditions and diet (Halliday 1987, Simmons 1988a, Simmons *et al*. 1993).

The variation found in spermatophore size among species may be, at least partly, a consequence of phylogenetic relatedness (Gwynne 1995, Vahed & Gilbert 1996). Nevertheless, in an analysis of 19 bush-cricket genera, Wedell (1993a, 1994a) showed that interspecific differences in spermatophore size, spermatophylax mass and ampulla mass are largely influenced by diet. Controlling for phylogeny in 43 tettigoniid species, Vahed & Gilbert (1996) found that there was also a large residual variation in sperm number and spermatophore size. Vahed & Gilbert (1996) however, did not control for diet, and used laboratory-reared bush-crickets (Vahed 1994) — a condition that may affect sperm number (*e.g.*, Reinhold 1994) and spermatophylax size (*e.g.*, Heller & von Helversen 1991).

Comparisons among species within a genus can be particularly informative because many variables that are shared by congeners are held constant (Ridley 1983, Felsenstein 1985, Harvey 1991, Harvey & Pagel 1991). The aim of this study was to compare spermatophore and body-mass data from field observations within the diverse bush-cricket genus *Poecilimon*. *Poecilimon* species share a similar diet and morphology, and while we recognise that this genus does not represent the full diversity found in bush-crickets, we show here that variation in spermatophore size approximates family-wide variation, so variations in diet and relatedness are, to a certain extent, controlled for. In this paper, we test the ejaculate-protection and paternal-investment hypotheses in *Poecilimon* by examining the correlations between the spermatophore components: spermatophylax mass, ampulla mass and sperm number.

Methods

Poecilimon

Poecilimon Fischer, 1853, (Fig. 8) is a genus of barbistine bush-crickets (Phaneropterinae, Tribe Barbistini) (Orthoptera: Ensifera: Tettigoniidae). There are 128 currently recognized species and subspecies (Otte *et al.* 2005), with about 65 European species, mostly situated in the east Mediterranean (Heller 2004). While the current position of species within the *Poecilimon* clade is under constant review (*e.g.*, Heller 2004, Heller & Lehmann 2004, Heller *et al* 2004, Heller 2006), the status of *Poecilimon* at the genus level is well supported (Ramme 1933, Bey-Bienko 1954, Heller 1984). Since the description of the genus in 1853 there has been no dispute about the homogeneity of this group (see references in Otte 1997). The nomenclature used here follows that of Otte *et al.* (2005), with additional species *P. gerlindae* (Lehmann *et al.* 2006), *P. ege* (Ünal 2005), and *P. ukrainicus* (Bey-Bienko 1951).

The genus *Poecilimon* is quite uniform in terms of behavior and life-history patterns. Notable exceptions include differences in how females consume the spermatophore, and timing of the active mating phase. Most *Poecilimon* species consume the spermatophylax directly from underneath the abdomen, where it remains attached to the ampulla. However, at least one species, *P. erimanthos*, detaches the spermatophylax from the ampulla before consumption. Most species used are nocturnal. Notable exceptions are *P. erimanthos*, *P. mytilenensis*, and *P. werneri*, which are predominantly active during the day. *P. nobilis*, *P. affinis*, and *P. gracilis* seem to be active both night and day (Heller & von Helversen 1993). All species are semelparous, have obligate diapause and most have a univoltine lifecycle. All the *Poecilimon* species employed eat flowers and leaves, so are foliovores when ordered into gross feeding categories, such as those given by Wedell (1994a): 1) omnivorous-predaceous, 2) seed eaters, and 3) foliovores.

Collection.—Previously published and unpublished data were compiled from a range of sources for 33 species (36 taxa, 62 independent observations) of *Poecilimon* to supplement the data we collected ourselves. All were found in Greece, Turkey, Italy, Slovenia or the Ukraine (see Appendix 1 for the location of each population). The data for several species were obtained from the paper by Vahed & Gilbert (1996). Although these authors did not present relative spermatophore, spermatophylax and ampulla mass, we calculated these percentages directly from the table in their paper (see below for calculations of relative mass). The sources for all novel data included here are appended to Table 1; the locations where they were observed are listed in Appendix 1. For 11 species, two (or more) independent measurements from different populations or different years were included (designated by Roman numerals), and two species were sampled at the subspecific level: *P. veluchianus veluchianus*, *P. veluchianus minor*, and *P. jonicus jonicus*, *P. jonicus superbus*, *P. jonicus tessellatus*. In all, 62 taxa-site-year combinations were collated from 36 taxa (Table 1, Appendix 1).

Determination of male body mass, spermatophore size, and sperm number.—We separated field-caught juveniles (ex-field larvae) and field-caught adults (EL and F respectively, Table 1) into cages of each sex. Field-caught juveniles were separated until at least seven days after their imaginal moult, in order to ensure sexual maturity (Heller & Reinhold 1994). Field-caught adults were separated for at least three days prior to pairing, in order to ensure full receptivity (Heller & von Helversen 1991, Lehmann & Lehmann 2000b). Two exceptions to this were *P. thessalicus* I and *P. v. minor* III (taken from independent mating experiments) where individuals were paired immediately after they were collected. Some data were used from individuals that were reared in the laboratory (for example *P. elegans*, *P. gracilis*, Table 1). While their treatment and the experimental

procedures were otherwise the same as those in the field, they are not included in final interspecific analyses.

For mating, pairs were typically placed in 500-ml containers and observed every 15 min or less until the female bore a spermatophore, which we then carefully removed with forceps for weighing. All weights were measured to the nearest 1 mg. In some cases, the measurements were made in the field from wild matings. Where possible, the spermatophore, spermatophylax and ampulla masses were measured immediately after mating. When this was not possible (for example, *P. laevissimus* IV), male weight loss and female weight gain (with the spermatophore attached) before and after mating were compared (Reinhold & Helversen 1997). If the difference between the male weight loss and female weight gain was larger than 20%, that datum was excluded (following the procedure of Heller & Reinhold 1994). On occasion, either the spermatophylax or the ampulla mass was not measured; in these cases the missing component was calculated as the difference between the full spermatophore mass and the mass from the known component.

Relative spermatophore mass was calculated as the percentage of male body mass for each individual, and then the mean for all individuals taken to calculate a species average. On occasion, the spermatophore mass and male body mass were taken from different males, so the average spermatophore mass was divided by the average male mass to give relative spermatophore mass.

After weighing, the ampulla was cut from the spermatophylax, added to a known quantity of water (between 1 and 5 ml depending on the organ size), and sliced with a scalpel. We further mixed the solution by passing it repeatedly through a syringe until the sperm had been suspended in the water and fully homogenised. A subsample was taken and the sperm counted on a field haemocytometer (Swift: Neubauer improved). Normally three subsamples were taken and the solution remixed before taking each new subsample. If there was a large variation between subsamples or the sperm was not evenly distributed over the slide, the solution was remixed and further subsamples taken. Sperm from a known volume (50 µl - 200 µl) were counted and multiplied by the appropriate dilution factor to give the total number of sperm for the entire ampulla. For *P. mariannae* a Coulter counter was used (for details of the method see Lehmann & Festing 1998). Relative sperm number was calculated as the number of sperm per mg of mean male body mass and expressed as sperm number $\times 10^3$.

Analysis.—Using data from multiple populations or seasons means that some species are over-represented and may inflate the contribution of those taxa in the analyses. However, full data sets with multiple species may give a better understanding of how the environment affects spermatophore size. Therefore, we restricted our use of the full data set to descriptive comparisons, and only performed analyses on reduced data sets that included only one of each taxa. Priority for removal was first given to observation location (*i.e.*, field observations were preferred over lab observations) and then to sample size (Table 1). Unless otherwise stated, statistics with multiple observations removed are presented in text and figures.

P. mytilenensis is unusual as it has a greatly enlarged ampulla and a large variation in sperm number (between 6.3 and 15.8 million sperm, Heller *et al.* 2004). Data for the current paper were from laboratory-reared individuals for this species, although observations from the field show that this variation in size approximates that found in its natural environment. Our intention in this paper was to compare among field-observed animals, avoiding any confounds imposed by lab-reared species. However, in terms of taxonomy, *P. mytilenensis* is quite typical for *Poecilimon* and large variations in spermatophore components are likely to represent realistic variations within the genus. Preliminary analysis that included data from *P. mytilenensis* also indicated that its impact on our understanding of mating systems within *Poecilimon* required further exploration. We therefore duplicated all analyses a second time, with the inclusion of *P. mytilenensis*, in order to directly compare this with variations found in the rest of the genus.

To normalize the data, all variables were $\log_{10}$ transformed prior to analysis unless otherwise stated. Two types of analysis were performed. First, the correlation coefficients between male body mass and each of spermatophore mass, spermatophylax mass, ampulla mass, and sperm number were calculated. Second, the overall effect of male body mass (MBM) was estimated for each parameter using least-squares regressions and the residuals for each population examined, to reveal cases where male investments were over or under expectation based on the overall allometric relationships. All data were analysed using SAS 9.1.3.

Results

Comparisons between Poecilimon and other Tettigoniidae.—The wide range in each spermatophore component within the genus *Poecilimon* approximates that occurring among the Tettigoniidae as a whole (Fig. 1., *Poecilimon* dataset not reduced). However, the smallest relative spermatophore size in *Poecilimon* is around 6.1% (*P. laevissimus* IV, Table 1), while some other tettigoniids have spermatophores that are even smaller than this: *Mecopoda elongata* and *Meconema thalassinum*, for example, have spermatophores that are barely 1% of male body mass, with little or no spermatophylax. *Poecilimon* have relatively large spermatophores (always >5% relative mass) and nearly always have a larger spermatophylax than an ampulla. *Poecilimon mytilenensis* (Fig. 1), however, is an exception with an

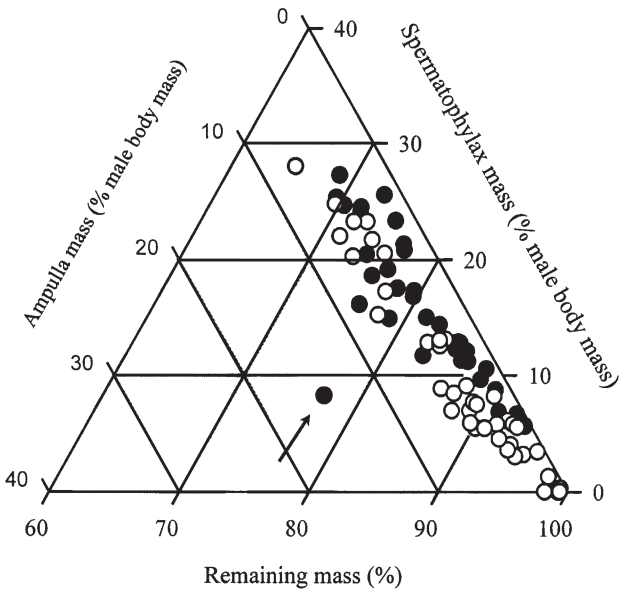

Fig. 1. Male body, spermatophylax and ampulla mass as proportions of combined mass in 29 *Poecilimon* species (solid circles, 31 taxa; n = 37) and 40 other tettigoniid species (open circles, see Vahed & Gilbert 1996 for details), showing that variation in *Poecilimon* approximates family-wide variation. The solid arrow points to *P. mytilenensis*, a species that has a remarkably large ampulla (Heller *et al.* 2004).

Table 1. Mean male body mass and sperm number with relative and actual mean spermatophore, spermatophylax, ampulla masses and sperm number of 33 *Poecilimon* spp. (36 taxa, 62 independent observations) (n = number of individuals). Each species is listed with the describer and with reference to the collectors or source of publication (see key at bottom for reference). Some species with more than one independent observation are distinguished by Roman numerals. Status of observations: field observations (F); exlarvae specimens (EL) that were field-obtained, but allowed to mature in large cages in the location of the natural population; purely lab-reared (L) individuals. Relative sperm number (rel#) = sperm number / male body weight (µg). Dashes (-) indicate a lack of gathered information and on occasion data have been published more than once, so we refer to original publications.

Species/source/collector	Male body mass			Spermatophore mass				Spermatophylax mass				Ampulla mass				Sperm number			
	mg	loc	n	mg	rel %	loc	n	mg	rel %	loc	n	mg	rel %	loc	n	x 10^6	rel #	loc	n
P. aegaeus Werner, 1932[a]	849	EL	10	272	31.4	EL	7	236	27.2	EL	7	34	4.0	EL	7	-	-	-	-
P. affinis I (Frivaldsky, 1867)[b]	1440	F	168	209	15	F	15	-	-	-	-	-	-	-	-	-	-	-	-
P. affinis II (Frivaldsky, 1867)[c]	1572	F	5	230	14.6	F	5	-	-	-	-	-	-	-	-	-	-	-	-
P. affinis III (Frivaldsky, 1867)[d]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21.6	-	L	3
P. affinis IV (Frivaldsky, 1867)[e]	1328	F	4	201	15.1	F	4	170	12.8	EL	4	31	2.3	F	3	4.4	3.3	F	3
P. amissus Brunner von Wattenwyl, 1878[f]	410	EL	8	68	20.5	EL	1	48	11.7	EL	1	20	5.3	EL	1	-	-	-	-
P. anatolicus Ramme, 1933[g]	694	EL	2	149	22.4	EL	2	-	-	-	-	-	-	-	-	-	-	-	-
P. brunneri (Frivaldsky, 1867)[h]	320	F	9	62	20.7	F	1	48	15.0	F	1	14	3.4	F	1	-	-	-	-
P. deplanatus Brunner von Wattenwyl, 1891[i]	449	F	15	41	9.2	F	7	55	12.3	F	2	9	2.0	F	4	-	-	-	-
P. ege Ünal, 2005[f]	568	F	4	168	28.7	F	3	140	24.7	F	3	28	4.9	F	3	11.1	19.5	F	3
P. elegans (Brunner von Wattenwyl, 1878)[j]	272	L	3	56	20.4	L	3	47	17.3	L	3	9	3.2	L	3	1.6	5.9	L	3
P. erimanthos I Willemse & Heller, 1992[k]	650	F	25	47	7.2	F	11	43	6.6	F	13	4	0.6	F	11	0.9	1.4	F	19
P. erimanthos II Willemse & Heller, 1992[l]	583	F	5	80	13.8	EL	8	-	-	-	-	-	-	-	-	1.2	2.1	F	4
P. gerlindae Lehmann Willemse & Heller, 2006[f]	552	F	9	154	29.7	F	9	135	24.5	F	9	19	3.7	F	9	2.4	4.3	F	9
P. gracilis (Fieber, 1853)[d]	530	F	6	102	16.7	EL	6	-	-	-	-	-	-	-	-	3.1	5.8	L	3
P. hamatus I Brunner von Wattenwyl, 1878[f]	517	F	5	121	22.3	F	4	110	21.3	F	4	11	2.1	F	4	0.2	0.4	F	4
P. hamatus II Brunner von Wattenwyl, 1878[f]	466	F	12	67	14.5	F	5	58	12.4	F	3	9	2.0	F	3	-	-	-	-
P. hoelzeli I Harz, 1966[f]	2960	F	3	442	14.6	F	1	381	12.9	F	1	61	2.0	F	1	-	-	-	-
P. hoelzeli II Harz, 1966[d]	2250	F	>10	387	17.2	F	8	-	-	-	-	-	-	-	-	13.4	6.0	F	3
P. ikariensis Willemse, 1982[m]	473	F	5	71	14.5	F	4	56	11.8	F	4	15	3.2	F	4	0.2	0.4	F	4
P. jonicus jonicus I (Kollar, 1853 in Fieber)[f]	352	F	6	52	14.9	F	6	45	12.8	F	5	7	1.9	F	5	0.4	1.1	F	6
P. jonicus jonicus II (Kollar, 1853 in Fieber)[e]	324	F	4	28	8.6	F	4	22	6.8	F	4	6	1.9	F	3	0.2	0.6	F	3
P. jonicus superbus (Fischer, 1853)[f]	306	F	2	57	18.6	F	2	-	-	-	-	-	-	-	-	0.2	0.7	F	1
P. jonicus tessellatus (Fischer, 1853)[n]	721	EL	3	83	11.6	EL	3	69	9.6	EL	3	13	1.9	EL	3	-	-	-	-
P. laevissimus I (Fischer, 1853)[f]	759	EL	1	66	8.7	EL	1	-	-	-	-	-	-	-	-	-	-	-	-
P. laevissimus II (Fischer, 1853)[f]	731	EL	5	85	10.8	EL	3	77	10.5	EL	3	8	1.0	EL	3	1.0	1.14	EL	3
P. laevissimus III (Fischer, 1853)[n]	744	EL	4	73	9.9	EL	4	65	8.7	EL	4	9	1.2	EL	4	-	-	-	-
P. laevissimus IV (Fischer, 1853)[o]	781	F	50	48	6.1	F	9	44	5.6	F	7	4	0.5	F	7	0.7	0.9	F	7
P. macedonicus Ramme, 1926[d]	302	F	12	65	21.8	F	5	-	-	-	-	-	-	-	-	2.0	6.6	F	4
P. mariannae Heller, 1988[p]	583	EL	21	133	22.8	EL	21	109	18.6	F	21	34	5.8	EL	21	2.4	4.1	EL	21
P. marmaraensis Naskrecki, 1991[h]	490	EL	8	104	21.2	EL	7	73	14.9	EL	7	31	6.3	EL	7	-	-	-	-
P. mytilenensis Werner, 1932[q, f]	822	F	4	227	29.3	F	6	114	8.2	F	4	113	14.7	F	5	10.4	12.7	L	3
P. nobilis (Brunner von Wattenwyl, 1878)[f]	1405	F	6	194	13.9	F	6	158	11.3	F	6	36	2.6	F	9	6.6	4.7	F	13
P. obesus (Brunner von Wattenwyl, 1878)[f]	1869	F	5	247	13.4	F	5	209	11.2	F	4	38	2.1	F	4	4.0	2.1	F	10
P. ornatus I (Schmidt, 1849)[r]	2552	F	9	310	11.8	F	7	275	25.5	F	7	35	1.4	F	7	-	-	-	-
P. ornatus II (Schmidt, 1849)[f]	2957	EL	8	268	9.2	EL	14	-	-	-	-	-	-	-	-	-	-	-	-
P. pergamicus Brunner von Wattenwyl, 1891[f]	174	F	5	53	30.4	F	1	44	25.3	F	1	9	5.2	F	1	2.8	16.1	F	1
P. sanctipauli I Brunner von Wattenwyl, 1878[f]	1234	EL	4	308	25	EL	1	-	-	-	-	-	-	-	-	-	-	-	-
P. sanctipauli II Brunner von Wattenwyl, 1878[f]	1355	F	1	337	24.9	F	1	316	23.3	EL	2	21	1.6	F	1	2.6	1.9	F	1
P. schmidtii (Fieber, 1853)[e]	525	F	8	73	13.9	F	6	63	12.1	F	6	9	1.7	F	6	0.9	1.7	F	2
P. thessalicus I Brunner von Wattenwyl, 1891[s]	442	F	48	102	23	F	8	92	20.8	F	8	10	2.2	F	8	3.9	8.8	F	4
P. thessalicus II Brunner von Wattenwyl, 1891[s]	507	F	5	146	29	F	5	122	24.1	F	5	20	3.9	F	5	-	-	-	-
P. thessalicus III Brunner von Wattenwyl, 1891[t]	464	F	20	112	24	F	20	89	19.2	F	20	30	4.3	F	20	14.0	30.2	F	20
P. thessalicus IV Brunner von Wattenwyl, 1891[d]	610	F	3	224	36.7	F	2	-	-	-	-	-	-	-	-	16.5	27.0	F	2
P. turcicus Karabag, 1950[f]	632	EL	3	152	24.1	EL	2	102	16.1	EL	2	50	8.0	EL	2	6.4	10.1	EL	2
P. ukrainicus Bey-Bienko, 1951[f]	274	EL	12	60	21.9	F	7	48	17.5	F	7	12	4.4	F	7	0.4	1.5	F	4
P. unispinosus Brunner von Wattenwyl, 1878[f]	404	F	2	82	20.3	F	2	68	16.8	F	2	14	3.5	F	2	0.9	2.2	F	2
P. v. minor I Heller & Reinhold, 1993[f]	439	F	19	87	20	F	19	-	-	-	-	-	-	-	-	-	-	-	-
P. v. minor II Heller & Reinhold, 1993[u]	400	F	83	74	19.1	F	271	-	-	-	-	-	-	-	-	-	-	-	-
P. v. minor III Heller & Reinhold, 1993[t]	327	F	70	56	17.1	F	19	47	14.4	F	19	9	2.7	F	19	3.4	10.4	F	19
P. v. minor IV Heller & Reinhold, 1993[v]	367	L	15	-	-	-	-	-	-	-	-	-	-	-	-	7.6	20.7	L	18

unusually large ampulla (14.7 % relative mass) and a relatively small spermatophylax (8.2 % relative mass; see Heller *et al.* 2004 for details). The upper limits of spermatophylax size are similar between *Poecilimon* and tettigoniids in general, with *P. thessalicus*, *P. ornatus* and *P. pergamicus*, for example, and *Steropleurus stali*, producing spermatophylaces that represent between 25% to 28% of male body mass (Fig. 1).

There is also a very large range in sperm number within *Poecilimon*, which could not be accounted for simply by body size ($y = 1.11x - 2.73$, $F_{1, 26} = 7.706$, $p = 0.011$, $r^2 = 0.22$; Fig. 2). In most tettigoniids sperm number follows body size quite closely ($y = 1.12x - 3.11$, $F_{1,29} = - 60.45$, $p<0.001$, $r^2 = 0.68$), but in *Poecilimon*, sperm

Table 1. Continued.

Species/source/collector	Male body mass			Spermatophore mass				Spermatophylax mass				Ampulla mass				Sperm number			
	mg	loc	n	mg	rel %	loc	n	mg	rel %	loc	n	mg	rel %	loc	n	x 10^6	rel #	loc	n
P. v. minor V Heller & Reinhold, 1994[v]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.5	-	F	43
P. v. veluchianus I Ramme, 1933[f]	821	F	10	212	26.1	F	10	-	-	-	-	-	-	-	-	-	-	-	-
P. v. veluchianus II Ramme, 1933[c]	661	F	13	150	22.7	F	13	-	-	-	-	-	-	-	-	-	-	-	-
P. v. veluchianus III Ramme, 1933[b]	660	F	107	162	26.4	F	10	-	-	-	-	-	-	-	-	-	-	-	-
P. v. veluchianus IV Ramme, 1933[v]	625	L	29	-	-	-	-	-	-	-	-	-	-	-	-	6.8	10.9	L	36
P. v. veluchianus V Ramme, 1934[v]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.5	-	F	-
P. v. veluchianus VI Ramme, 1933[w]	-	-	-	-	25.4	L	64	-	-	-	-	-	25.4	L	-	6.3	-	L	34
P. v. veluchianus VII Ramme, 1933[e]	710	F	1	182	25.6	F	1	145	20.4	F	1	37	5.3	F	1	10.4	14.6	F	50
P. werneri Ramme, 1933[f]	318	EL	5	47	14.6	EL	5	39	12.3	EL	3	8	2.5	EL	3	0.2	0.6	EL	2
P. zimmeri I Ramme, 1933[l]	711	F	7	150	21.1	F	7	-	-	-	-	-	-	-	-	28.4	39.9	F	5
P. zimmeri II Ramme, 1933[x]	818	EL	91	146	17.8	EL	91	-	-	-	-	-	-	-	-	-	-	-	-

Key:
[a] Lehmann, A. & Lehmann, G. (in press)
[b] Heller & von Helversen (1991)
[c] Heller et al. (1998)
[d] Reinhold, K. (unpub.)
[e] Vahed & Gilbert (1996)
[f] Heller, K.-G. (unpub.)
[g] von Helversen, D. & Heller, K.-G. (unpub.)
[h] Braun, H. (unpub.)
[i] Heller, K.-G., Heller, M. & Volleth, M. (unpub.)
[j] Ingrisch, S. (unpub.)
[k] McCartney, J. & Heller, K.-G. (unpub.)
[l] Reinhold, K. & Heller, K.-G. (unpub.)
[m] Heller, K.-G. & Volleth, M. (unpub.)
[n] Lehmann, G. & Lehmann, A. (unpub.)
[o] McCartney, J. Telscher, K.L. & Heller, K.-G. (unpub.)
[p] Lehmann & Lehmann (2000a)
[q] Heller et al. (2004)
[r] Achmann, R. (unpub.)
[s] McCartney, J., & Telscher, K.L. (unpub.)
[t] McCartney, J., Telscher, K.L., Penny. L. (unpub.)
[u] Heller & Reinhold (1994)
[v] Reinhold (1994)
[w] Reinhold & von Helversen (1997)
[x] Lehmann & Lehmann (2007 and in press)

number ranged between about 200,000 sperm per spermatophore (*P. hamatus*, *P. ikariensis*, *P. jonicus* and *P. werneri*) to about 28 million (*P. zimmeri*), although *P. thessalicus* can reach 37.3 million sperm (McCartney & Heller unpub. data). Within other tettigoniids, sperm number ranges between 38,000 for *Phaneroptera nana*, to about 10 million for *Pycnogaster inermis*. Many species of *Poecilimon* had far more sperm than would be expected for their body size, based on the overall pattern within the tettigoniids (*e.g.*, *P. thessalicus*, *P. zimmeri* and *P. ege*, Table 1), though there are also a few species with unusually low sperm counts for their size (*e.g.*, *P. jonicus* and *P. werneri*).

Variation within Poecilimon.—Within *Poecilimon* there is a large range in both body mass and spermatophore size. *P. hoelzeli*, for example, is more than fifteen times the weight of *P. pergamicus* (Table 1) and produces an accordingly large spermatophore of up to 454 mg, compared to 18.1 mg in *P. pergamicus*. Within the genus, spermatophore mass is closely correlated with male body mass ($y = 0.7545x + 1.24$, $F_{1,35} = 59.255$, $p = 0.000$, $r^2 = 0.64$, Table 2). Similarly, male body mass is closely correlated with spermatophylax mass ($y = 0.86x - 0.44$, $F_{1,30} = 72.20$, $p<0.001$, $r^2 = 0.71$), and is significantly correlated with ampulla mass ($y = 0.67x - 0.60$, $F_{1,30} = 12.91$, $p = 0.001$, $r^2 = 0.31$; Fig. 3).

Intraspecific variation.—In four taxa, *P. erimanthos*, *P. hamatus*, *P. j. jonicus*, and *P. laevissimus*, spermatophore size varied two-fold within populations among seasons, while the numbers of sperm per spermatophore remained relatively constant (Table 1). Most of this variation is attributable to spermatophylax mass rather than ampulla mass, apart from *P. laevissimus*, where the ampulla mass (actual and relative) also varied two-fold among seasons. *P. affinis* showed only a small range in relative spermatophore size (13 to

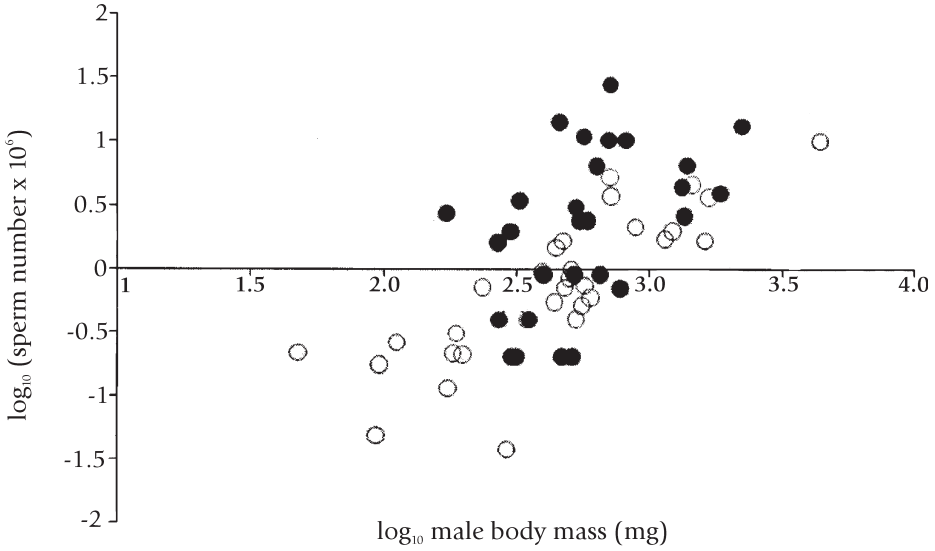

Fig. 2. Male body mass and sperm number within Orthoptera (open circles, Vahed & Gilbert (1996) and *Poecilimon* (solid circles). Male body mass explains little of the variation in sperm number within *Poecilimon*. In contrast, 68% of the sperm number is explained by male body mass in other Orthoptera.

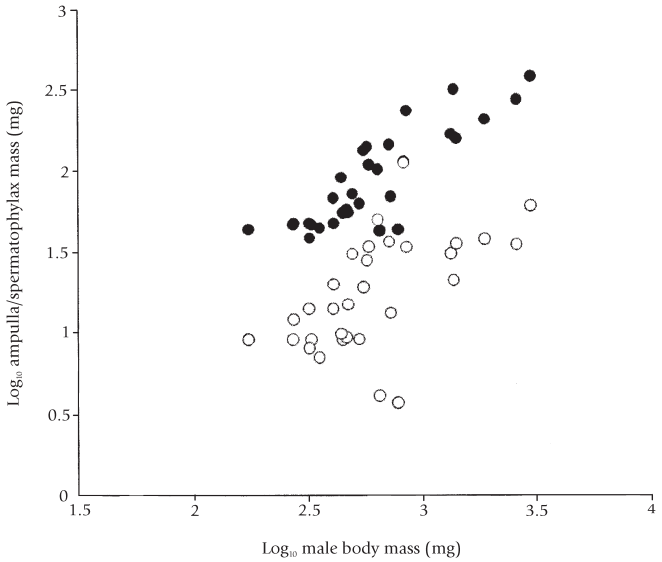

Fig. 3. Spermatophore components are largely dictated by male body mass: the relationships between male body mass and both spermatophylax mass (solid circles) and ampulla mass (open circles) in 31 *Poecilimon* taxa.

15%) among years and populations in field conditions, but there was a remarkable difference in sperm number between field and laboratory-reared individuals (4.4 million sperm and 21.6 million sperm respectively). In *P. thessalicus*, while body size varied between 442 and 610 mg over four seasons, spermatophore mass varied from 102 mg (23% relative mass) to 224 mg (36.7% relative mass), and sperm number showed a four-fold range from 3.9×10^6 to 16.5×10^6 sperm over the same period. The two subspecies of *P. veluchianus* have been sampled repeatedly from both laboratory and field-reared bush-crickets. In *P. v. veluchianus* spermatophore size varied a little from 150 mg to 212 mg (23% to 26% relative mass), but sperm number varied from 6.3 million sperm in laboratory-reared bush-crickets (Reinhold & von Helversen 1997) to 10.5 million sperm in the field (Reinhold 1994). Similarly, the relative spermatophore mass of *P. v. minor* varied from 17% - 20% of body mass but sperm number ranged from 3.4 million to 7.6 million.

Table 2. Regressions between male body mass (MBM), spermatophore mass, spermatophylax mass, ampulla mass and sperm number among 33 species of *Poecilimon* (36 taxa, n=62). *=significant

Hypotheses	F-statistic	p value	r^2-value	df
MBM/spermatophore mass	59.255	<0.001*	0.64	1,35
MBM/spermatophylax mass	72.195	<0.001*	0.71	1,29
MBM/ampulla mass	12.908	0.001*	0.31	1,29
MBM/sperm number	7.406	0.011*	0.22	1,26
MBM/relative spermatophore mass	2.7855	0.104	0.08	1,34
MBM/relative spermatophylax mass	0.0586	0.810	0.00	1,29
MBM/relative ampulla mass	1.4749	0.234	0.05	1,29
MBM/relative sperm number	0.1736	0.680	0.01	1,26
Spermatophylax mass/ampulla mass	16.256	<0.001*	0.36	1,30
" without *P. mytilenensis*	23.789	<0.001*	0.46	1,29
Spermatophylax mass/sperm number	1.4827	0.200	0.06	1,22
" without *P. mytilenensis*	1.7638	0.200	0.08	1,21
Ampulla mass/sperm number	15.705	<0.001*	0.43	1,22
" without *P. mytilenensis*	9.4264	0.006*	0.32	1,21

Spermatophore components.—The previous sections demonstrate that there is a tendency for relative spermatophore size to increase with an increase in body size. However, there is considerable variation among the species in spermatophore investment that is independent of this general pattern. No significant relationship was found between male body mass and relative spermatophylax mass ($y = -0.0004x + 16.22$, $F_{1,29} = 0.06$, $p = 0.81$, $r^2 = 0.002$), relative ampulla mass ($y = -0.0009x + 4.35$, $F_{1,29} = 1.48$, $p = 0.23$, $r^2 = 0.048$), and relative number of sperm ($x10^3$ per 1mg of male body mass, $y = -0.0192x + 86.71$, $F_{1,26} = 0.17$, $p = 0.68$, $r^2 = 0.007$). Allowing for body size reveals that some species invest relatively much more heavily in some spermatophore components than other species (Fig. 4, Table 1). Spermatophore components show considerable variation with some small males producing large spermatophylaces, ampullae or sperm numbers, and some large males producing small spermatophylaces, ampullae or sperm numbers.

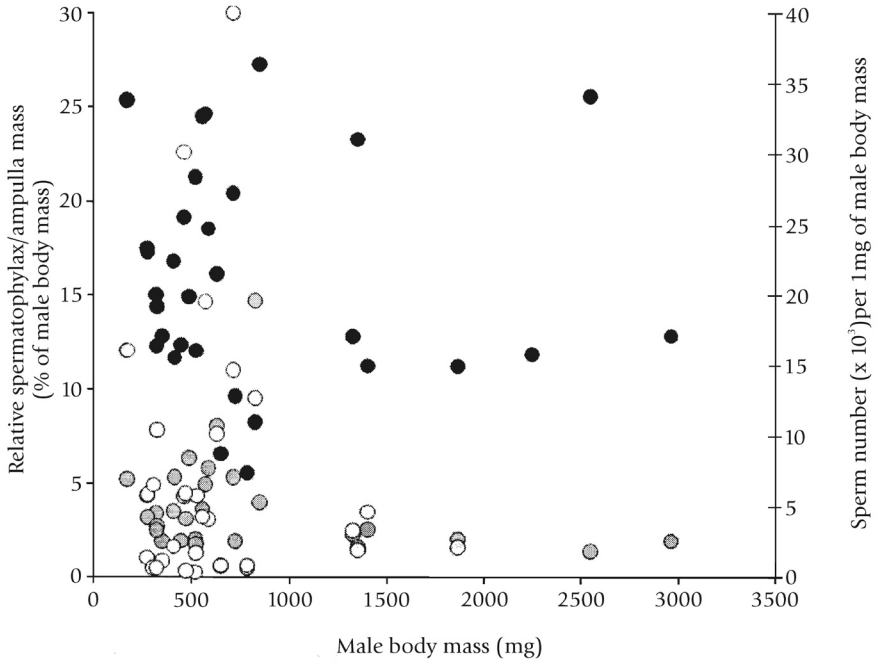

Fig. 4. A large variation in the relative investment in spermatophore components: no relationships between male body mass and relative spermatophylax mass (black/solid circles), relative ampulla mass (grey/solid circles) and relative sperm number (open circles).

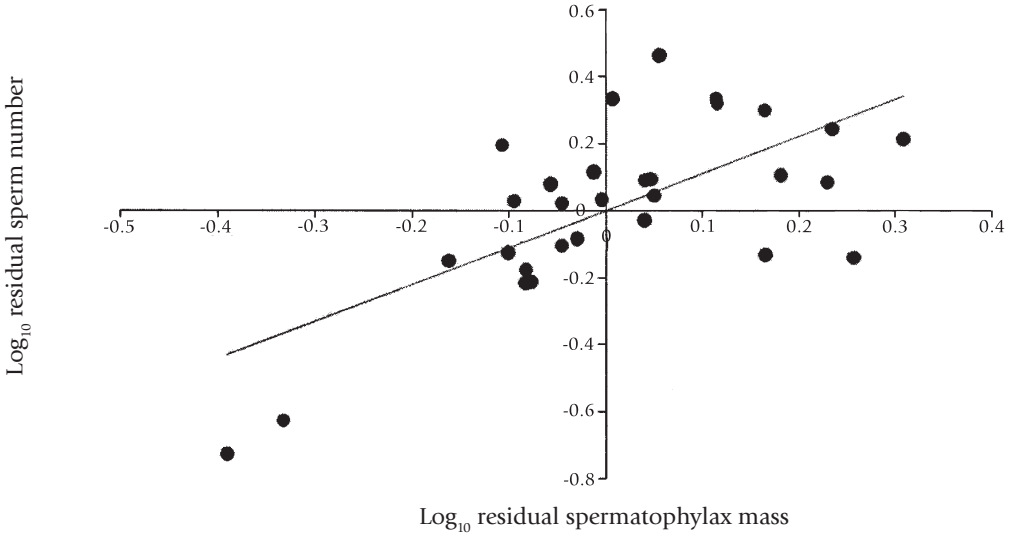

Fig. 5. Positive relationship between residual ampulla mass and residual spermatophylax mass across 31 *Poecilimon* species.

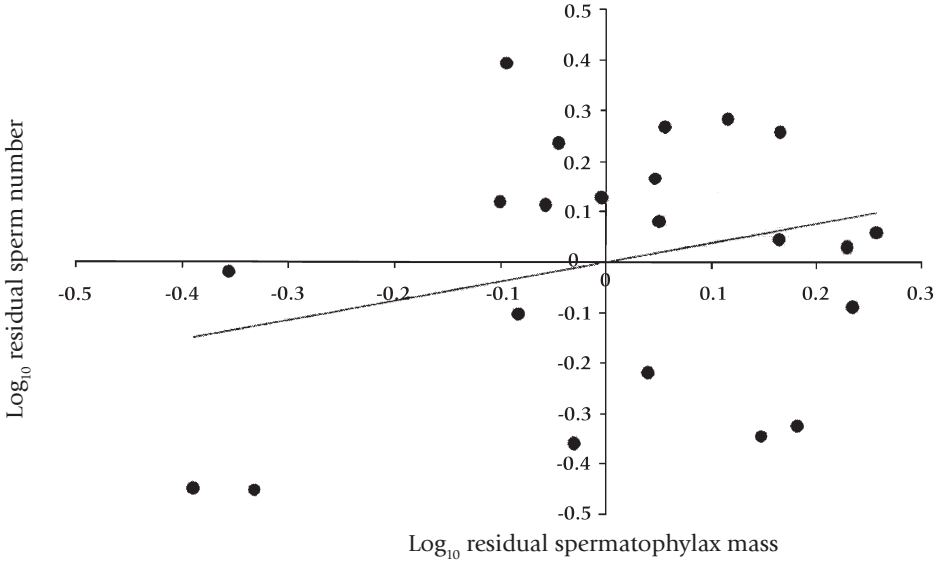

Fig. 6. No relationship exists between residual sperm number and residual spermatophylax mass across 22 species of *Poecilimon*.

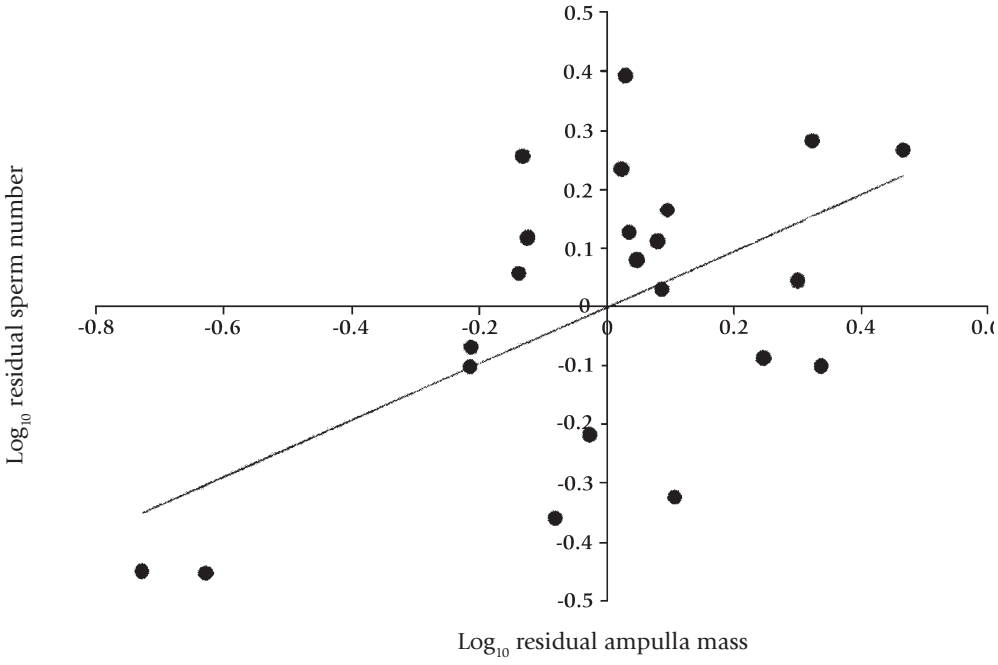

Fig. 7. The relationship between residual sperm number and residual ampulla size across 22 *Poecilimon* species.

We found a significant correlation between the residuals of spermatophylax and ampulla mass ($y = 1.1116x$, $F_{1,30} = 23.79$, $p<0.001$, $r^2 = 0.46$), although a substantial portion of the variance in residuals did not co-vary (Fig. 5). Including data from *P. mytilenensis* predictably decreased the relationship further ($y = 1.10x + 0.023$, $F_{1,31} = 16.26$, $p<0.001$, $r^2 = 0.36$; Table 2). Surprisingly, residual spermatophylax mass did not correlate with residual sperm number across *Poecilimon* species ($y = 0.38x$, $F_{1,21} = 1.76$, $p = 0.2$, $r^2 = 0.08$; Fig. 6), and was largely unaffected by the inclusion of *P. mytilenensis*, ($y = 0.39x$, $F_{1,22} = 1.48$, $p = 0.2$, $r^2 = 0.06$; Table 2).

A significant correlation was found between the residuals of ampulla mass and sperm number ($y = 0.4817x - 0.0032$, $F_{1,21} = 9.426$, $p = 0.006$, $r^2 = 0.32$; Fig. 7, Table 2), although a substantial portion of the variance in residuals, about 68%, could not be explained by the model. Including *P. mytilenensis* in this model strengthened this association so that 57% of the variation could not be accounted for ($y = 0.54x$, $F_{1,22} = 15.71$, $p<0.000$, $r^2 = 0.43$).

Discussion

Spermatophore variation, ejaculate protection and paternal investment.— The positive correlation we found between residual spermatophylax mass and residual ampulla mass is consistent with other research supporting the ejaculate protection hypothesis (Reinhold & Heller 1993; Wedell 1993a, 1994b; Heller & Reinhold 1994; Vahed & Gilbert 1996). Vahed & Gilbert (1996) also found a strong correlation between residual spermatophylax mass and residual ampulla mass within 43 species from nine subfamilies of mostly European bush-crickets. Similarly, Wedell (1993a, 1994b) found a positive correlation between spermatophylax mass and ampulla mass in 19 genera of mostly Australian bush-crickets. While the correlation found between these components within *Poecilimon* was moderate, the relationship was strengthened by the removal of *P. mytilenensis* — a species known to have an inordinately large ampulla, but a modestly sized spermatophylax (Heller *et al.* 2004).

While our findings are consistent with the ejaculate protection hypothesis, they are not inconsistent with the paternal investment hypothesis.

The spermatophylax of *P. veluchianus,* for example, is approximately the size required to allow for an optimum amount of sperm to enter into the female (Reinhold & Heller 1993, Heller & Reinhold 1994), although the spermatophore of the last male to mate will have a positive effect on the dry weight of his own offspring (Reinhold 1999). The paternal investment hypothesis assumes selection acts on the spermatophylax through a direct nutritional benefit to the offspring (Trivers 1972, Gwynne *et al.* 1984). Yet compared to the spermatophylax, the ejaculate may be produced relatively inexpensively (*e.g.*, Bateman 1948, Trivers 1972, but see Dewsbury 1982, Reinhold & Helversen 1997; Wedell *et al.* 2002 provide a review) and is critical to male fertilization success. Ampullae size (ejaculate volume) may still modulate spermatophylax size through influences of ejaculate protection, while the primary factors influencing spermatophylax size itself are paternal investment. Males that primarily invest heavily in spermatophylaces and as a result, provide a significant nutrient investment to their offspring, may also produce greater than normal quantities of sperm in order to 'hedge their bets' and maintain paternity shares in the face of sperm competition (Reinhold & von Helversen 1997, Lehmann & Lehmann 2000b). The ejaculate and/or spermatophylax mass may also have flow-on effects in females by influencing female intermating refractory period (Heller & Helversen 1991, Heller & Reinhold 1994, Lehmann & Lehmann 2000b, Vahed 2007), female lifespan (Brown 1997), the timing of oviposition (Wedell & Arak 1989), and the share of eggs that are laid with the donating males' nutritional investment (Simmons 1990, Vahed 2003).

Under the ejaculate-protection hypothesis, the cost of extra sperm or ejaculate fluid is assumed to be negligible in comparison to the gain in paternity (Simmons 1995b). Evidence showing sperm to be less costly than the production of the spermatophylax has been observed in *P. mariannae*: parasitized males lose their ability to replenish their spermatophylax, but not their sperm (Lehmann & Lehmann 2000b). Similarly, Reinhold & von Helversen (1997) found that spermatophore replenishment rather than sperm number limits intermating interval in male *P. veluchianus*.

In contrast to predictions of the ejaculate-protection hypothesis, we did not observe a relationship between sperm number and spermatophylax size in *Poecilimon*. This runs counter to findings from other studies where a positive relationship existed across taxa (*e.g.*, Wedell 1994b, Vahed & Gilbert 1996). Sperm number has also been found to be independent of spermatophylax mass in an Australian bush-cricket, *R. verticalis* (Simmons *et al.* 1993), and in *P. veluchianus*, (Reinhold & von Helversen 1997). Reinhold & von Helversen (1997) further predicted that this lack of relationship may represent a general trend in bush-crickets. However, sperm number and spermatophylax mass are adjusted in concert in parasitized *P. mariannae* (Lehmann & Lehmann 2000b), so the situation appears to be more complicated in *Poecilimon*.

While our results confirm the prediction of Reinhold & von Helversen (1997), the validity of the ejaculate-protection hypothesis relies more specifically on the relationship between spermatophore consumption time and sperm discharge time, rather than covariance of spermatophylax mass and sperm number (see for example Reinhold & Heller 1993, McCartney & Heller submitted ms.). An association between spermatophylax consumption time and sperm drainage has been observed in all bush-cricket studies thus far: *R. verticalis* (Gwynne 1984a, 1986, 1997, but see Simmons 1995a, Vahed 1998 for different interpretations), *Decticus verrucivorus* (Wedell & Arak, 1989), *Kawanaphila nartee* (Simmons & Gwynne, 1991), and *Leptophyes laticauda* (Vahed 1994), as well as *Poecilimon hoelzeli* (Achmann 1996), and two subspecies of *Poecilimon veluchianus* (Reinhold & Heller 1993, Heller & Reinhold 1994). However, the spermatophore consumption time and sperm discharge do not correspond in two further *Poecilimon* species (*P. laevissimus* and *P. thessalicus*, McCartney & Heller submitted ms.). This, combined with our detection of a large intraspecific variation in spermatophylax mass and sperm numbers between individuals, populations and years (*e.g.*, *P. thessalicus* and *P. veluchianus*, Table 1) is likely to explain the lack of association we found within the genus.

Under the ejaculate-protection hypothesis, the spermatophylax may be viewed as a sperm-protection device, allowing the transfer of a maximum number of sperm, and being primarily influenced by sperm competition. However, chemicals in the ejaculate itself can increase male fitness by functioning to increase onset of egg-laying, increase total number of eggs laid and to prolong the female intermating period (*e.g.*, Reinhold & von Helversen 1997; Vahed 1998; 2003, 2006, 2007; Arnqvist & Rowe 2005). Our study indicates that discharge of the ejaculate may be more important in terms of spermatophylax function than the discharge of sperm *per se*. While we found a significant association, ampulla mass only explained a small amount of variation in sperm number within *Poecilimon*.

Only one other comparative study seems to have measured the association between ampulla mass and sperm number among

bush-cricket species and no relationship was found (Vahed 2006). This, in combination with our finding that ampulla mass, but not sperm number, correlates with spermatophylax mass, indicates that the spermatophylax, in terms of mating effort, has an ejaculate-protection function, but not a primary sperm-drainage function in *Poecilimon*. Our results lead us to believe that sperm number itself should not be used as an assessment of the ejaculate protection function, nor should ejaculate volume (ampulla size) be used to assess sperm protection or competition (*e.g.*, Wedell 1993a, Wedell 1997) when making interspecific comparisons.

Spermatophore size variation within Poecilimon.—Spermatophore size within the genus *Poecilimon* approximates that found within the entire family Tettigoniidae (*c.f.* Wedell 1993a, Vahed & Gilbert 1996, Wedell 1997, Vahed 2007), indicating that variation in spermatophore size is unlikely due to relatedness or diet alone. This large variation between species is likely to reflect within-species adjustments that male bush-crickets make to specific spermatophore components as a conditional strategy — apparently in order to maximise reproductive output (*e.g.*, *P. affinis*, *P. erimanthos*, *P. hamatus*, *P. jonicus*, *P. laevissimus*, *P. thessalicus*, *P. veluchianus*). We found that all spermatophore components in *Poecilimon* scale approximately with male body mass, but large variations are apparent in relative investment when body mass is taken into consideration.

Preferential investment in spermatophore components suggests that variations in environment and available energy or nutrients are directed to whichever spermatophore component is more effective at increasing reproductive fitness (see for example Voigt *et al.* 2005 and references cited therein). Examples of this have been found in a variety of bush-crickets. Male *Requena verticalis*, for example, increase the number of sperm when mating with older females, or when exposed to a high female sex ratio, effectively increasing their chances of paternity, given the likely increase in sperm competition (Simmons *et al.* 1993, Simmons 1995a). Similarly, *R. verticalis* males disproportionately adjust the ampulla mass over the spermatophylax mass in relation to their remating frequency (Simmons 1995b) or mating potential (Simmons 1995c). Males of another species, *Decticus verrucivorus*, adjust the size of the offered spermatophore depending on whether or not a mate is virgin (Wedell 1992).

Considerable variation in the size of *Poecilimon* spermatophore components was found between and within populations (*e.g.*, *P. erimanthos*, *P. hamatus*, *P. jonicus*, *P. laevissimus*, *P. thessalicus*, *P. veluchianus*). The foundation for this variation is likely the availability of environmental resources (*e.g.*, Hubbell & Johnson 1987, Gwynne & Simmons 1990, Adamo & Hoy 1994) yet, while related, more proximal causes associated with life histories and mating behavior, including population density, operational sex ratio, and sexual size dimorphism, influence the relative pay-offs in spermatophore production (*e.g.*, Gwynne 1981, 1984a, b; Gwynne & Simmons 1990; Heller & von Helversen 1991; Allen 1995; Bateman 1997). There is little published information on intraspecific variation in spermatophore component size among bush-cricket populations, and evidence presented here suggests that further research on *Poecilimon* is needed to help clarify how environmental factors affect male investment in spermatophore components.

Spermatophore differences between field and laboratory-raised individuals.—Importantly, we found large differences between laboratory-reared individuals and those from the field. For example, *P. v. minor* males reared in the laboratory had a larger body mass and over twice as many sperm per spermatophore, compared to those in the field. The converse was true for *P. v. veluchianus*, which had a larger number of sperm in individuals collected in the field. A large range in ampulla mass was also seen in this subspecies (5.3 to 25.4 mg) and previous studies show that spermatophore consumption time also varies greatly between conditions (Reinhold & Heller 1993). Similarly, *P. affinis* differs considerably in sperm number in laboratory and field observations, with nearly five times more sperm in laboratory-reared individuals; however it is difficult to assess whether this reflects environment differences or bias due to small sample size. Laboratory-reared animals it seems, often show extreme variations in spermatophore component size. This may provide important information in some circumstances; however, given the highly variable nature of spermatophore production, we recommend caution when interpreting spermatophore function using laboratory-reared animals, small sample sizes, or means from short-term observations.

Conclusions

Detailed analyses of spermatophore size with respect to phylogeny and diet will be important to developing a more complete understanding of the evolutionary significance of variation in spermatophore size. Spermatophore component size in *Poecilimon* appears to be evolutionarily labile and a general lack of association within *Poecilimon* between relative spermatophore-component size and male body mass, reflects differences related to mating strategy. This, combined with a lack of association between spermatophore component size, indicates that effective ejaculate transfer, not sperm drainage *per se*, is a significant influence in the evolution of spermatophore size. Mating effort and paternal investment are not mutually exclusive and further analysis within *Poecilimon* on the direct association between the amount of sperm that drains into the female and its relationship to spermatophore-consumption time is needed for a full understanding of the relative influences of ejaculate protection and paternal investment on spermatophore size. Given the significance of sperm competition in evolutionary biology, studies within and between closely related species in natural populations are necessary to improve knowledge of the processes that influence the evolution of nuptial feeding in insects.

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References

Achmann R. 1996. Individueller Paarungs und Fortpflanzungserfolg bei der Laubheuschrecke *Poecilimon hoelzeli* : ein molekulargenetischer Ansatz. PhD Thesis. Friedrich Alexander Universität, Institute für Zoology II, Erlangen, Nürnberg, Germany.

Adamo S.A., Hoy R.R. 1994. Mating behaviour of the field cricket *Gryllus bimaculatus* and its dependence on social and environmental cues. Animal Behaviour 47: 857-868.

Allen G.R. 1995. The calling behaviour and spatial distribution of male bushcrickets (*Sciarasaga quadrata*) and their relationship to parasitism by acoustically orienting tachinid flies. Ecological Entomology 20: 303-310.

Arnqvist G., Rowe L. 2005. Sexual Conflict. Monographs in Behavior and Ecology. Princeton University Press, New Jersey.

Bateman A.J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2: 349-368.

Bateman P.W. 1997. Operational sex ratio, female competition and mate choice in the ephippigerine bushcricket *Steropleurus stali* Bolivar. Journal of Orthoptera Research 6: 101-104.

Bey-Bienko, G.Ya. 1954. Orthoptera Vol. II, Sect. 2. Bush-crickets. Subfam. Leaf Bush-crickets (Phaneropterinae). In: Fauna U.S.S.R. – Inst. Zool. Acad. Sci. URSS (NS) 59: 1–385 [Russian; English translation 1965].

Boldyrev B.T. 1915. Contributions à l'étude de la structure des spermatophores et des particularitès de la copulation chez Locustodea et Gryllodea. Horae Societatis Entomologicae Rossicae 6: 1-245.

Bowen B.J., Codd C.G., Gwynne D.T. 1984. The katydid spermatophore (Orthoptera: Tettigoniidae): male investment and its fate in the mated female. Australian Journal of Zoology 32: 23-31.

Brown W.D. 1997. Courtship feeding in tree crickets increases insemination and female reproductive life span. Animal Behaviour 54: 1369-1382.

Busnel R-G., Dumortier B. 1955. Etude du cycle genital du male d' Ephippiger et son rapport avec le comportement acoustique. Bulletin Biologique France Belgique 90: 219-286.

Dewsbury D.A. 1982. Ejaculate cost and male choice. American Naturalist 119: 601-610.

Eberhard W.G. 1996. Female control: Sexual Selection by Cryptic Female Choice. Princeton University Press, New Jersey.

Felsenstein J. 1985. Phylogenies and the comparative method. American Naturalist 125: 1-15.

Gerhardt U. 1913. Copulation und Spermatophoren von Grylliden und Locustiden I. Zoologische Jahrbücher (Syst.) 35: 415-532.

Gerhardt U. 1914. Copulation und Spermatophoren von Grylliden und Locustiden II. Zoologische Jahrbucher (Syst.) 37: 1-64.

Gwynne D.T. 1981. Sexual difference theory: Mormon crickets show role reversal in mate choice. Science 213: 779-780.

Gwynne D.T. 1983. Male nutritional investment and the evolution of sexual differences in the Tettigoniidae and other Orthoptera, pp 337-366. In: Gwynne D.T., Morris G.K. (Eds) Orthopteran Mating Systems. Westview press, Boulder Colorado.

Gwynne D.T. 1984a. Courtship feeding increases female reproductive success in bushcrickets. Nature 307: 361-362.

Gwynne D.T. 1984b. Sexual selection and sexual differences in Mormon crickets (Orthoptera: Tettigoniidae, *Anabrus simplex*). Evolution 38: 1011-1022.

Gwynne D.T. 1986. Courtship feeding in katydids: investment in offspring or in obtaining fertilisations? American Naturalist 128: 342-352.

Gwynne D.T. 1988. Courtship feeding in katydids benefits the mating male's offspring. Behavioural Ecology and Sociobiology 23: 373-377.

Gwynne D.T. 1990. The katydid spermatophore: evolution of a parental investment, pp. 9-27. In: Bailey W.J., Rentz D.C., (Eds) The Tettigoniidae: Biology, Systematics and Evolution. Crawford House, Bathurst.

Gwynne D.T. 1995. Variation in bush-cricket nuptial gifts may be due to common ancestry rather than ecology as taxonomy and diet are almost perfectly confounded. Behavioural Ecology 6: 458.

Gwynne D.T. 1997. The evolution of edible 'sperm sacs' and other forms of courtship feeding in crickets, katydids and their kin (Orthoptera: Ensifera), pp 110-129. In: Choe J.C., Crespi B.J. (Eds) Evolution of Mating Systems in Insects and Arachnids. Cambridge University Press, Cambridge.

Gwynne D.T. 2001. Katydids and Bush-crickets: Reproductive behavior and Evolution of the Tettigoniidae. Ithaca, Cornell University Press.

Gwynne D.T., Bowen B.J., Codd C.G. 1984. The function of the katydid spermatophore and its role in fecundity and insemination (Orthoptera: Tettigoniidae). Australian Journal of Zoology 32: 15-22.

Gwynne D.T., Simmons L.W. 1990. Experimental reversal of courtship roles in an insect. Nature 346: 172-174.

Halliday T.R. 1987. Physiological constraints on sexual selection, pp. 247-264. In: Bradbury J.W., Anderson M.B. (Eds) Sexual Selection: Testing the Alternatives. Wiley.

Harvey P.H. 1991. Comparing uncertain relationships: the Swedes in revolt. Trends in Ecology and Evolution 6: 38-39.

Harvey P.H., Pagel M.D. 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford.

Heller K.-G. 1984. Zur Bioakustik und Phylogenie der Gattung *Poecilimon* (Orthoptera: Tettigoniidae, Phaneropteridae). Zoologische Jahrbücher (Syst.) 111: 69-117.

Heller K.-G. 2004. *Poecilimon martinae* n sp. and *P. inflatus* Brunner von Wattenwyl, 1891 (Orthoptera, Tettigonioidea, Phaneropteridae), two bush-cricket species endemic to southwest Anatolia: morphology, bioacoustics and systematics (*Poecilimon*; key to species and subspecies). Articulata 19: 1-17.

Heller K.-G. 2006. Song evolution and speciation in bush-crickets, pp 137-152. In: Drosopoulos S., Claridge M.F. (Eds) Insect Sounds and Communication. Boca Raton.

Heller K-G., Faltin S., Fleischmann P., von Helversen O. 1998. The chemical composition of the spermatophore in some species of phaneropterid bush crickets (Orthoptera: Tettigonioidea). Journal of Insect Physiology 44: 1001-1008.

Heller K-G., von Helversen D. 1991. Operational sex ratio and individual mating frequencies in two bush-cricket species (Orthoptera, Tettigonioidea, *Poecilimon*). Ethology 89: 211-228.

Heller K-G., von Helversen D. 1993. Calling behavior in bush-crickets of the genus *Poecilimon* with differing communication systems (Orthoptera, Tettigonioidea, Phaneropteridae). Journal of Insect Behavior 6: 361-377.

Heller K.-G., Lehmann A. 2004. Taxonomic revision of the European species of the *Poecilimon ampliatus*-group (Orthoptera: Tettigonioidea: Phaneropteridae). Memorie della Societa Entomologica Italiana 82 (2003): 403-422.

Heller K-G., Reinhold K. 1994. Mating effort function of the spermatophore in the bushcricket *Poecilimon veluchianus* (Orthoptera, Phaneropteridae): support from a comparison of the mating behaviour of two subspecies. Biological Journal of the Linnean Society 53: 153-163.

Heller K-G., Willemse F. Sevgili H. 2004. *Poecilimon mytilenensis* Werner, a polytypic phaneropterid bushcricket from the Aegean island of Lesbos (Orthoptera, Tettigonioidea), differing in male mating structures. Journal of Orthoptera Research 13: 221-230.

Hubbell S.P., Johnson L.K. 1987. Environmental variance in lifetime mating success, mate choice and sexual selection. American Naturalist 130: 91-112.

Lehmann G., Festing H. 1998. Alternative Methode zur Bestimmung der Spermienzahl bei Laubheuschrecken. Bioforum 6/98: 358-360.

Lehmann G.U.C., Lehmann A.W. 2000a. Female bushcrickets mated with parasitized males show rapid remating and reduced fecundity (Orthoptera: Phaneropteridae: *Poecilimon mariannae*). Naturwissenschaften 87: 404-407.

Lehmann G.U.C., Lehmann A.W. 2000b. Spermatophore characteristics in bushcrickets vary with parasitism and remating interval. Behavioural Ecology and Sociobiology 47: 393-399.

Lehmann G., Lehmann A. 2008. Bushcricket song as a clue for spermatophore size? Behavioral Ecology and Sociobiology 62: 569-578.

Lehmann A.W., Willemse F., Heller K.-G. 2006. *Poecilimon gerlindae* nov. spec. – a new bushcricket of the *Poecilimon propinquus*-group from Greece (Orthoptera: Phaneropteridae). Articulata 21: 109-119.

Lehmann G., Lehmann A. (in press). Condition-dependent spermatophore size is correlated with male's age in a bushcricket (Orthoptera: Phaneropteridae). Biological Journal of the Linnaean Society.

McCartney J., Heller K-G. (Submitted ms.) Evidence of natural and sexual selection shaping the size of nuptial gifts in a bush-cricket genus (*Poecilimon*; Tettigoniidae, Orthoptera): an analysis of sperm transfer patterns.

Otte D. (1997). Orthoptera Species File 7. Tettigonioidea. 1–373; Philadelphia (Orthopterists' Society at the Academy of Natural Sciences of Philadelphia).

Otte D., Eades D.C., Nasrecki P. 2005. Orthoptera species file online (Version 2.3). 10 Oct. 2005 http://osf2.orthoptera.org

Ramme W. 1933. Revision der Phaneropteriden-Gattung *Poecilimon* Fisch. (Orth. Tettigon.). Mitteilungen aus dem Zoologischen Museum, Berlin 19: 497-575.

Reinhold K. 1994. Inheritance of body and testis size in the bush-cricket *Poecilimon veluchianus* Ramme (Orthoptera, Tettigoniidae) examined by means of subspecies hybrids. Biological Journal of the Linnean Society London 52: 305-316.

Reinhold K. 1999. Paternal investment in *Poecilimon veluchianus* bushcrickets: beneficial effects of nuptial feeding on offspring viability. Behavioural Ecology and Sociobiology 45: 293-299.

Reinhold K., Heller, K-G. 1993. The ultimate function of nuptial feeding in the bushcricket *Poecilimon veluchianus* (Orthoptera: Tettigoniidae: Phaneropterinae). Behavioural Ecology and Sociobiology 32: 55-60.

Reinhold K., von Helversen D. 1997. Sperm number, spermatophore weight and remating in the bushcricket *Poecilimon veluchianus*. Ethology 103: 12-18.

Ridley M. 1983. The Evolution of Organic Diversity. Clarendon Press, Oxford.

Simmons L.W. 1988. Male size, mating potential and lifetime reproductive success in the field cricket, *Gryllus bimaculatus*. Animal Behaviour 36: 372-379.

Simmons L.W. 1990. Nuptial feeding in tettigoniids: male costs and the rates of fecundity increase. Behavioural Ecology and Sociobiology 27: 43-47.
Simmons L.W. 1995a. Courtship feeding in katydids (Orthoptera: Tettigoniidae): investment in offspring and in obtaining fertilizations. American Naturalist 146: 307-315.
Simmons L.W. 1995b. Male bushcrickets tailor spermatophores in relation to their remating intervals. Functional Ecology 9: 881-886.
Simmons L.W. 1995c. Relative parental expenditure, potential reproductive rates, and the control of sexual selection in katydids. American Naturalist 145: 797-808.
Simmons L.W. 2001. Sperm Competition and its Evolutionary Consequences in the Insects. Princeton University Press, Princeton, NJ.
Simmons L.W., Gwynne D.T. 1991. The refractory period of female katydids (Orthoptera: Tettigoniidae): sexual conflict over the remating interval? Behavioural Ecology 2: 276-282.
Simmons, L.W., Parker. G.A. 1989. Nuptial feeding in insects: mating effort versus paternal investment. Ethology 81: 332-343.
Simmons L.W., Craig M., Llorens T., Schinzig M., Hosken D. 1993. Bushcricket spermatophores vary in accord with sperm competition and parental investment theory. Proceedings of the Royal Society London 251: 183-186.
Thornhill R. 1976. Sexual selection and paternal investment in insects. American Naturalist 110: 153-163.
Trivers R.L. 1972. Parental investment and sexual selection. In: Campbell, B. (Ed.) Sexual Selection and the Descent of Man. Aldine, Chicago.
Ünal M. 2005. Phaneropterinae (Orthoptera: Tettigoniidae) from Turkey and the Middle East. Transactions of the American Entomological Society, Philadelphia. 131: 425-448.
Vahed K. 1994. The evolution and function of the spermatophylax in bushcrickets (Orthoptera: Tettigoniidae). PhD Thesis, University of Nottingham.
Vahed K. 1998. The function of nuptial feeding in insects: review of empirical studies. Biological Reviews Cambridge Philosophical Society 73: 43-78.
Vahed K. 2002. Coercive copulation in the alpine bushcricket *Anonconotus alpinus* Yersin (Tettigoniidae: Tettigoniinae: Platycleidini). Ethology 108: 1065-1075.
Vahed K. 2003. Increases in egg production in multiply mated female bushcrickets *Leptophyes punctatissima* are not due to substances in the nuptial gift. Ecological Entomology 28: 124-128.
Vahed K. 2006. Larger ejaculate volumes are associated with a lower degree of polyandry across bushcricket taxa. Proceedings of the Royal Society of London, Series B 273: 2387-2394.
Vahed K. 2007. Comparative evidence for a cost to males of manipulating females in bush-crickets. Behavioral Ecology 18: 499-506.
Vahed K., Gilbert F.S. 1996. Differences across taxa in nuptial gift size correlate with differences in sperm number and ejaculate volume in bushcrickets (Orthoptera: Tettigoniidae). Proceedings of the Royal Society of London, Series B 263: 1257-1265.
Vleck C.M., Brown J.L. 1999. Testosterone and social and reproductive behaviour in *Aphelocoma* jays. Animal Behaviour 58: 943–951.
Voigt C.C., Michener R., Kunz T.H. 2005. The energetics of trading nuptial gifts for copulations in katydids. Physiological and Biochemical Zoology 3: 417-23.
Voigt C.C., Lehmann G.U.C., Michener R.H. Joachimski M.M. 2006. Nuptial feeding is reflected in tissue nitrogen isotope ratios of female katydids. Functional Ecology 20: 656-661.
Wedell N. 1991. Sperm competition selects for nuptial feeding in a bushcricket. Evolution 45: 1975-1978.
Wedell N. 1992. Protandry and mate assessment in the wartbiter *Decticus verrucivorus* (Orthoptera: Tettigoniidae) Behavioral Ecology and Sociobiology 31: 301-308.
Wedell N. 1993a. Spermatophore size in bushcrickets: comparative evidence for nuptial gifts as a sperm protection device. Evolution 47: 1203-1212.
Wedell N. 1993b. Mating effort or paternal investment? Incorporation rate and cost of male donations in the wartbiter. Behavioural Ecology and Sociobiology 32: 239-246.
Wedell N. 1994a. Variation in nuptial gift quality in bush crickets (Orthoptera: Tettigoniidae). Behavioural Ecology 5: 418-425.
Wedell N. 1994b. Dual function of the bushcricket spermatophore. Proceedings of the Royal Society of London B 258: 181-185.
Wedell N. 1997. Ejaculate size in the bushcrickets: the importance of being large. Journal of Evolutionary Biology 10: 315-325.
Wedell N., Arak A. 1989. The wartbiter spermatophore and its effect on female reproductive output (Orthoptera: Tettigoniidae, *Decticus verrucivorus*). Behavioural Ecology and Sociobiology 24: 117-125.
Wedell N., Gage M.J.G., Parker G.A. 2002. Sperm competition, male prudence and sperm-limited females. Trends in Ecology and Evolution 17: 313-320.

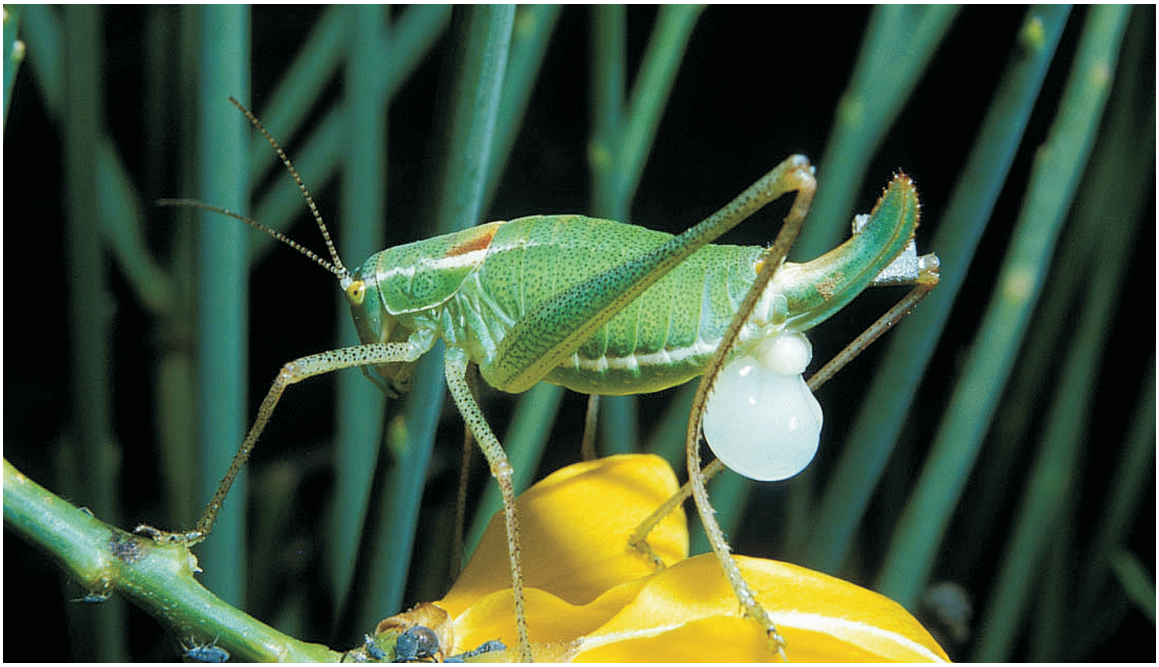

Fig. 8. *Poecilimon veluchianus minor* with attached spermatophore. From a population at Makrakomi, mainland Greece, near the village of Tsouka, 1998. Photo by J. McCartney. See Plate III.

Appendix 1. Table showing the location where each *Poecilimon* species was observed. (The site locations for each species taken from the literature are listed at the bottom of Table 1).

P. aegaeus, GREECE: Island of Andros in the Cyclades, (37°83' N, 24°93'E), 29 iv 1996
P. affinis III, GREECE: Near the village Pisodherion, Florina, (40°46'N, 21°16'E) (date unknown)
P. amissus, GREECE: Island of Lesvos. Mytilini, near Vrissa (39°02'N, 26°11'E), 23 v 1993
P. anatolicus, GREECE: Drama, Kato Vrondou north-east of Serrai (41°16'N, 23°44'E), 1 vi 1983
P. brunneri, GREECE: Evros, 1 km east of Peplos (before the Turkish border) (40°57'N, 26°17'E), 1-31 v 1996
P. deplanatus, GREECE: Island of Karpathos, near Lefkos (35°35'N, 27°4'E), 15-20 v 2005
P. elegans, ITALY: Istrien, near Triest (45°39'N, 13°46'E), 1-31 viii 1992
P. erimanthos I, GREECE: Peloponnes, N. Elia, Erimanthos valley, east of the Koumani village (37°48'N, 21°47'E), 1997
P. erimanthos II, GREECE: Peloponnes, N. Elia, Erimanthos valley, east of the Koumani village (37°48'N, 21°47'E), vi 1990
P. gracilis, GREECE: Near the village Pisodherion, North Florina, (40°46'N, 21°16'E) (date unknown)
P. hamatus I, GREECE: Island of Samos; (37°44'N, 26°46'E), 1998
P. hamatus II, GREECE: Island of Rhodes; (36°11'N, 28°03'E), 2005
P. hoelzeli I, GREECE: Karditsa, between Loutropigi and Mesochori (39°05'N, 22°03'E), 19 v 1989
P. hoelzeli II, GREECE: Karditsa, near Makrirahi, (39°06'N, 22°07'E), vi 1990
P. ikariensis, GREECE: Aegaean Islands, N. Samos, Ikaria: 3 km northwest Ag. Kyrikos (37°37'N, 26°16'E), 22 v 1998
P. jonicus jonicus I, GREECE: Thesprotia, Kallithea, 25 km east of Igoumenitsa (39°33'N, 20°27'E), 4 vi 1992
P. jonicus superbus, ITALY: L'Aquila, Gran Sasso: 10 km west of Fonte Cerreto (42°27'N, 13°25'E), 1300 m, 1-3 ix 1996
P. jonicus tessellatus, GREECE: Peloponnes: N Ano Diakoptó, Haikos gorge (37°83'N, 22°93'E), 27 iv 1996
P. laevissimus I, GREECE: Lakonia, Mistras (37°4'N, 22°22'E), 1-30iv 1983
P. laevissimus II, GREECE: Ilia Peloponnes, Erimanthos -Tal 6 km east of Koumanis (37°48'N, 21°47'E), 24 v 1992 and GREECE: Aitolia-Akarnania, Astakos (38°32'N, 21°4'E), 25 v 1992
P. laevissimus III, GREECE: Peloponnes: Ithómi near the ancient Messenian ruins (37°15'N, 21° 94'E), and near a monastery in the Mistras of Lakonía (37°4'N, 22°22'E), 5-6 v 1996
P. laevissimus IV, GREECE: Peloponnes, N. Elia, Erimanthos valley, east of the Koumani village (37°48'N, 21°47'E), 1997
P. macedonicus, GREECE: Mt. Chortiatis east of Thessaloniki above the town of Panorama (1990) (40°34'N, 23°06'E), 1990
P. marmaraensis TURKEY: Kirklareli, 10 km west of Lüleburgaz (intersection after Saricaali) (41°25'N, 27°15'E), 1-31 v 1996
P. nobilis, GREECE: Peloponnes, N. Elia, Erimanthos valley, east of the Koumani village (37°48'N, 21°47'E), v/vi 1992
P. obesus, GREECE: Aitolia-Akarnania, Bambini, north from Astakos (38°40'N, 21°8'E), 25 v 1992 and GREECE: Aitolia-Akarnania, Acheloos-Münd., Koutsilaris (38°21'N, 21°10'E), 200 m, 25 v 1992
P. ornatus I, ITALY: Medeazza; northern Italy (45°47'N, 13°36'E), 1996
P. ornatus II, SLOVENIA: Loibl-Pass (46°26'N, 14°15'E), 1995
P. pergamicus, GREECE: Island of Lesbos. Mytilini, Moria (Aqueduct) (39°07'N, 26°30'E), 28 v 1993
P. gerlindae, GREECE: Domokos, N. Fthiotis (39°06'N, 22°18'E), 8-17 vi 1992
P. sanctipauli I, GREECE: Island of Rhodos (28°03'E, 36°11'N), 31 v 1996
P. sanctipauli II, GREECE: Island of Samos (37°44'N, 26°46'E), 31 v 1996
P. ege, GREECE: Island of Samos (different localities) (37°44'N, 26°46'E), 31 v 1996
P. thessalicus I, GREECE: Pieria, north west of the village of Elatochori (40°19'N, 22°15'E), 1997
P. thessalicus II, GREECE: Pieria, north west of the village of Elatochori (40°19'N, 22°15'E), 1997
P. thessalicus III, GREECE: Pieria, north west of the village of Elatochori (40°19'N, 22°15'E), 1998
P. thessalicus IV, GREECE: Mt.Ossa, north east of Thessaloniki (40°49'N, 23°08'E), 1990
P. turcicus, GREECE: Island of Lesbos; Mytilini, near Larissos (Kolpos Geras), (39°07'N, 26°26'E), 28 v 1993
P. ukrainicus, UKRAINE: Kiev and Cherkaska Oblast, Kanev Forest Reserve, and surrounding area (49°44'N, 31°30'E), 18-23 vi 1996
P. unispinosus, GREECE: Island of Chios (different localities) (38°22'N, 26°08'E), v 1995
P. v. minor I, GREECE: Nomos Fthiotis, Makrakomi, near the village of Tsouka (38°57'N, 22°05'E), 1995
P. v. minor III, GREECE: Nomos Fthiotis, Makrakomi, near the village of Tsouka (38°57'N, 22°05'E), 1998
P. v. veluchianus I, GREECE: Nomos Fthiotis, 3 km north of the village of Vitoli, near the village of Makrakomi (38°58'N, 22°01'E), 1995
P. werneri, GREECE: Near the city of Astakos, in the area of Aitolia-Akarnania (38°32'N, 21°4'E), 25 v 1992
P. zimmeri I, GREECE: Fokis, near the town of Kalascopi, South of Mt Oiti (38°42'N, 22°19'E), 900 m, v 1990
P. zimmeri II, GREECE: Near the Delphi ancient temple in the area of Fokis (38°28'N, 22°29'E), 2002