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Spermatophore weight and sperm number in the southernmost species of the genus *Poecilimon* Fischer, 1853 (Orthoptera: Phaneropterinae)

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Abstract: Male bushcrickets transfer during copulation edible spermatophores that are consumed by bushcricket females as nutrient sources. They consist of two edible parts; the smaller ampulla that contains the ejaculate and the larger spermatophylax. Both components of the spermatophore and the number of sperm transferred vary greatly in size respectively number between species. This paper examines the relationship between body size and spermatophore components, including sperm number in *Poecilimon* (*Poecilimon*) *syriacus* Brunner von Wattenwyl, 1891 which is known from the southernmost area of the genus distribution. Water content of spermatophylax was found to be about 90% of wet mass. Consistent with many previous studies, the spermatophore components, including dry spermatophylax weight, were positively affected by male body weight, but there was no relationship between body weight and sperm number. A positive relationship between wet spermatophylax weight and ampulla weight supports the ejaculate protection hypothesis. The results of this study suggest that *P. syriacus* makes a large investment in spermatophylax and ampulla relative to its body size in comparison to other species of the genus *Poecilimon*.

Keywords: Spermatophore, Sperm number, Nuptial gift, Bushcricket, *Poecilimon syriacus*, Orthoptera, Turkey.

Introduction

In most of the long-horned bushcrickets, males transfer spermatophores consisting of the spermatophylax and ampulla to females during mating (Vahed, 1998; Lehmann, 2012). After mating, the female consumes the spermatophore starting from the spermatophylax, and during consumption, the sperm in the ampulla moves into the spermatheca of the female (Gwynne, 2001). The consumption of the spermatophylax enables the transfer of sperm into the female spermatheca before the ampulla is being eaten and may delay the female's re-mating with another male. It also increases the involvement of the male in the reproductive success (i.e. paternal investment) (Reinhold, 1999).

Studies show that spermatophore size and the number of sperm in the ampulla vary among species (Vahed and Gilbert, 1996; McCartney et al., 2008). For example, average spermatophore weight in Barbitistini bushcrickets can vary from about 39 mg to 300 mg (Sevgili et al., 2015). Spermatophore contents were studied in around 37 species of the genus *Poecilimon*, especially Barbitistini bushcrickets (McCartney et al., 2008). According to the data obtained, spermatophore size does not show a significant relationship phylogenetically, and at the same time, spermatophore components are largely influenced by diet (Wedell, 1994; Vahed and Gilbert, 1996). Although *Poecilimon* species have a similar diet in general, the size of the spermatophore varies more than in other bushcrickets (McCartney et al., 2008). Similar variability also applies to the sperm number.

The aim of this study is to determine the spermatophore weight and sperm number of *P.* (*s. str.*) *syriacus* Brunner von Wattenwyl, 1891, which represents the southernmost distributed species of the genus *Poecilimon*, and to compare them with body size measurements. A significant part of the previous spermatophore data in this genus was obtained from the species of the Balkans, and there are some data from the species found in North-Western Anatolia and the Aegean region. The presence of *P. syriacus* in a quite different region, which is dominated by semi-arid climatic conditions, will allow comparisons with these closely related species. *Poecilimon syriacus* is found mostly in the driest and hottest region of Turkey: Eastern Anatolia and the Upper Mesopotamian floristic region.

More than half of the species in the genus *Poecilimon* (represented by about 140 species in the Palearctic) show distribution in Anatolia and a significant portion of them are endemic (Çıplak et al., 2002; Eades et al., 2016). Spermatophore characteristics for only 14 species distributed in Anatolia are known, and there is no information about approximately 70 species/subspecies. Because *P. syriacus* is the southernmost species of the genus, it provides a valuable opportunity to collect basic data in contribution to the understanding of the variation among species.

Materials and Methods

The Insect: The bushcricket species investigated in this study, *P. syriacus*, represents the southernmost distribution of the genus *Poecilimon*, and is known from the eastern Mediterranean and southeastern regions of Turkey, Lebanon, Jordan, and Israel (see Sevgili and Çıplak, 2000; Heller et al., 2008). However, a majority of the species records are known from only the southeastern part of Turkey. Phenologically, *P. syriacus* is typical spring species, with a much later appearance than *Isophya sikorai* Ramme, 1951, which is found in similar areas and occupies the same niche for mating period (Uma and Sevgili, 2015). The adult season of *P. syriacus* lasts for around 5-6 weeks at Şanlıurfa Province, which is one of the places in Turkey with highest temperatures (Şensoy et al., 2016). Due to the short length of growing season their development must be fast to rapidly reach adulthood and reproduce (Roff, 1980).

A total of 75 nymphs of *P.* (*s. str*) *syriacus* were collected from Şanlıurfa, Turkey (Tektek Plateau, 600-650 m, 39°12'N, 39°12'E) on 1-2 May 2009 and kept in wooden cages (20 x 30 cm). The nymphs were transferred to the laboratory and were fed mainly with flowers and leaves of *Arthemis hyalina*, *Trifolium stellatum*, *T. speciosum*, *T. tamentasum*, *T. purpureum*, *Trigonella spruneriana* and *Valerinella vesicaria*, in addition to cucumber and apple slices. Bushcrickets were maintained in the laboratory with a photoperiod of 12:12 hr dark:light and temperature of 24-25°C. After emerging, adults were removed and maintained in single sex cages (15 x 30 cm). All individuals were tagged by attaching a small adhesive labels including individual numbers to the dorsal part of pronotum. To avoid over-crowding, each adult cage was stocked with just six individuals.

Mating experiment: Males began to produce calling songs an average of 3 days after the adult molt. Mating trials were initiated when bushcrickets were 10 days old to allow the males to produce sperm and spermatophore properly. Because, it was found that the males did not produce countable sperm in ampulla until 6-10 days (own unpublished data). All the individuals of both sexes were considered virgins at the time of mating. Before mating trials, both sexes were weighed to the nearest 0.1 mg and placed together in small plastic cages (500 ml). When mating did not take place within 1 hr, the mating trial was terminated and a different virgin couple was examined. Individuals who failed a mating trial were not used in further trials due to potential fluctuation of body weight (Uma and Sevgili, 2015). After successful mating, both individuals were immediately weighed again and the transferred spermatophore was gently removed and weighed with the electrobalance (Kern 770) to the nearest 0.1 mg. Then, the spermatophylax and ampulla were separated from each other and weighed. The spermatophylaces were then freeze-dried (Telstar Cryodos) and re-weighed. The ampulla was gently crushed with small forceps in water (0.4 ml). The combination of ejaculate and water was mixed using a fine syringe until the sperm had been homogenised. The sperm number was estimated with a haematocytometer. The sperm found in the four big squares of the Neubauer chamber were counted. The absolute sperm number was calculated as an average of five samples taking the dilution factor into account. A total number of 19 males and 19 females were used for the experiment.

Data analysis: Statistical analyses were performed using R64 (R Development Core; Team 2009). The means with standard errors (SE) of weights for both sexes and spermatophore components were calculated. The normal distribution of the data was evaluated with Shapiro-Wilk's test. Because spermatophylax weight and absolute sperm number were not normally distributed, the first was $\log_{10}$ transformed and sperm number was square root (sqrt) transformed. Relative spermatophore weight was calculated as the percentage of male body weight prior mating for each individual. To determine the factors that may affect spermatophore characteristics, linear mixed-

Table 1. Variable means of spermatophore (mg) and body size (mm) for *Poecilimon* (*s. str.*) *syriacus* (n= 19 females, 19 males; 19 matings) (SE= standard error, Median for non-normally distributed data are given in brackets).

Variable	Mean (Median)	SE
Male weight (mg)	442.84	12.25
Female weight (mg)	600.89	13.57
Wet spermatophore weight (mg)	114.32	4.84
(%) spermatophore vs. male body weight	25.85	0.77
Wet spermatophylax weight (mg)	97.21 (94.0)	4.42
(%) spermatophylax vs. male body weight	21.96	0.69
Ampulla weight (mg)	17.11	0.63
(%) ampulla vs. male body weight	3.88	0.14
Dry spermatophylax (mg)	9.16	0.80
(%) dry spermatophylax vs. male body weight	2.04	0.16
Sperm number x10^6	2.07 (1.78)	0.39
Male pronotum length	4.74	0.11
Male wing length	2.02	0.07
Male hind femur length	13.14	0.11
Female pronotum length	4.84	0.16
Female hind femur length	13.87	0.29
Ovipositor	8.20	0.12

effects models were used with restricted maximum likelihood estimation (REML). In this analysis, male identity was considered a random effect and male and female weights (plus spermatophylax and ampulla weights for sperm number) were considered fixed-covariate effects. The correlation between male body size (pronotum, male wing and hind femur lengths) and spermatophore and its contents was not significant, thus these measurements were not included in the analysis.

Results

The average values of the morphological and spermatophore variables belonging to the males and females used in the experiment are given in Table 1. The dry weight of spermatophylax was about 9.36% (SE=±0.71, n=19) of spermatophylax wet weight. There was a positive correlation between female body weight and both hind femur and ovipositor length (Pearson correlation, $P<0.05$, n=18), but not pronotum length. Although there was no correlation between male body weight and both pronotum and wing length, the relationship of body weight and hind femur length was significant (Pearson correlation, $P<0.05$, n=19). The positive relationship between ampulla weight and both wet and dry spermatophylax weight was significant (linear regression, n=19, Adj. $R^2=0.33$, t=3.133, $P=0.006$; Adj. $R_2=0.26$, t= 2.680, $P=0.016$), but the relationship between male weight and sperm number was not (linear regression, n=19, Adj. $R^2=0.13$, t=1.929, $P=0.071$).

Male body weight had a significant positive effect on spermatophore, spermatophylax, and ampulla weight as well as on dry spermatophylax weight (Table 2). While between ampulla weight and sperm number a positive interaction could be found, but this interaction was not observed between spermatophylax and sperm number (Table 2).

Discussion

The results of this study show that the average body weight of male *P.* (*s. str.*) *syriacus* is smaller compared to other species of the genus (see data in McCartney et al., 2008). Heavy males produced larger spermatophores, spermatophylaces and ampullae in *P. syriacus*. Due to the high temperatures and low humidity in the area in which the species is naturally found, the vegetation rapidly dries in spring and the development and maturity period of individuals decreases in length. Body size may be included in the group with proportionally smaller size than the other species of genus. However, proportionally larger spermatophore investment depending on body size contradicts Bergmann's rule in relation to body structure (such as the body cells, eggs) in other ectothermic animals

Table 2. Results of a general linear mixed model (REML estimation, Male ID as random factor) testing the effects of male and female body weights on spermatophore (spermatophylax+ampulla), spermatophylax (logtransformed, wet) and ampulla weights. In addition to these results, there was an interaction between absolute sperm number (SQRT transformed) and ampulla weight, but not wet and dry spermatophylaces.

Variable/Effects	Value	SE	DF	*t value*	*p*
Spermatophore weight					
Intercept	1.654	0.165	16	10.041	**<0.001**
Male weight	0.0008	0.0002	16	3.337	**0.004**
Female weight	0.0000	0.0002	16	0.075	0.941
Spermatophylax weight					
Intercept	1.538	0.177	16	8.644	**<0.001**
Male weight	0.0008	0.0002	16	3.168	**0.006**
Female weight	0.0000	0.0002	16	0.300	0.768
Dryspermatophylax weight					
Intercept	11.495	8.309	16	1.383	0.186
Male weight	0.0382	0.013	16	2.899	**0.010**
Female weight	0.0062	0.012	16	0.516	0.612
Ampulla weight					
Intercept	14.172	6.821	16	2.077	0.054
Male weight	0.0272	0.011	16	2.514	**0.023**
Female weight	0.0151	0.009	16	1.552	0.140
Sperm number (sqrt)					
Intercept	38.612	31.390	15	1.230	0.238
Spermatophylax weight ($\log_{10}$)	28.494	17.703	15	1.609	0.128
Dryspermatophylax	0.202	0.369	15	0.546	0.593
Ampulla weight	1713.088	386.468	15	0.546	**<0.001**

(Blanckenhorn and Hellriegel, 2002). Temperature is considered to be one of the most important environmental factor affecting body size. Low food availability, high temperature and short growing season have a limiting effect on body size in general (Davidowitz and Nijhout, 2004; Blanckenhorn and Demont, 2004). The primary range of *P. syriacus* is located in the hottest zone of any of the species in the *Poecilimon* genus.

When compared with the other species of *Poecilimon* studied, males of *P. syriacus* transfer large spermatophores (~ 26% relative mass) (McCartney et al., 2008). Only six previously studied species (*P. aegaeus*, *P. gerlindae*, *P. mytilenensis, P. pergamicus*, *P. thessalicus* and *P. v. veluchianus*) transfer larger spermatophores than *P. syriacus* in terms of body weight and they are much larger than the genus average (17.72% relative mass) (see McCartney et al., 2008; Sevgili et al., 2015). The observed spermatophore size for *P. syriacus* is also higher than that of species of the genera *Isophya* Brunner von Wattenwyl, 1878 and *Phonochorion* Uvarov, 1916, which are closely related to the genus *Poecilimon* (Sevgili et al., 2015). A similar situation also applies to spermatophylax size (~ 22% relative mass). This finding supports the idea that spermatophore investment is quite variable among both the *Poecilimon* species and the other genera of Barbitistini tribe (McCartney et al., 2008). Relative ampulla weight in *P. syriacus* (3.88% relative mass) is lower than in 12 *Poecilimon* species, but when compared with the genus (2.82% relative mass), it is much higher (Sevgili et al., 2015). The average sperm number is much less than the mean of *Poecilimon* species (5.94 x 10^6, see McCartney et al. (2008)). Despite high spermatophore investment, low sperm number suggests different sexual and natural selection pressures among species (Simmons, 2001).

Sperm number was also found to be quite variable within *P. syriacus*, similar to results observed in other species of the genus *Poecilimon* and among other bushcrickets. This supports the idea that sperm number in the ejaculate within the ampulla is characteristic for each individual male (Reinhold and von Helversen, 1997).

The weight of dry spermatophylax was found to be 12.6% of wet spermatophylax weight on average in *P. veluchianus* (Reinhold and von Helversen, 1997) and it is 9.42% in *P. syriacus*. This means that average 90% of the total weight of the spermatophylax consists of water. This finding is quite similar to measurements of dry spermatophylax weight in *Isophya sikorai* (10.12% of spermatophylax wet weight, Uma and Sevgili, 2015). However, the water composition in spermatophylax was found to be 85% for some species belonging to the genera *Barbitistes*, *Ancistrura*, *Metaplastes* and *Poecilimon* (Heller et al., 1998). This suggests that higher percentage of the water composition in spermatophylax may be an adaptation to dry climate to increase female survival and egg production. Although very few data are available, this suggests that a large part of the spermatophylax is composed of water and the females that consume this can eliminate hunger with both the water and the contained food after mating (Voigt et al., 2005). There is also an argument that spermatophore nutrients are rapidly digested and that females need additional plant diets, (Lehmann and Lehmann, 2016). However, it should also be noted that spermatophylax has a very rich nutrient content, including proteins, carbohydrates and lipids (Heller et al., 1998; Pauchet et al., 2015).

In *P. syriacus*, the positive effect of the male body weight on the total weights of spermatophore, spermatophylax and ampulla is consistent with other bushcricket species that have been studied (Wedell and Arak, 1989; Heller and Reinhold, 1994; McCartney et al., 2008; Sevgili et al., 2015). Though the cost to produce large spermatophores is high, it is important because large spermatophores are preferred by females in many species, are consumed by the female for a longer time, and therefore reduces the frequency female’s mating with other males (e.g. Heller and Reinhold, 1994; LaMunyon, 1997; Lehmann and Lehmann, 2008a). The strategy of spermatophore allocation may also vary according to age as well as body size of the female being mated and with mating status (Uma and Sevgili, 2015). This indicates that the males with larger bodies may be experience greater reproductive success.

In *P. syriacus*, the positive relationship between both dry and wet spermatophylax weight and ampulla weight supports the ejaculate protection hypothesis, which is consistent with the results of other studies (Heller and Reinhold, 1994; McCartney et al., 2008). The positive effect of the ampulla weight on sperm number indirectly supports this hypothesis as well. On the other hand, no relation was found between either dry or wet spermatophylax weight and sperm number in this study, and this is more in line with findings in other *Poecilimon* species that have been analyzed (McCartney et al., 2008; but see Vahed and Gilbert, 1996; Reinhold and von Helversen, 1997). However, as reported by McCartney et al. (2008), the relationship between spermatophore consumption time and the discharge of the sperm and the covariance between spermatophylax weight and sperm number should be compared in order to understand this situation exactly. Yet, it should be noted that the results of the comparison in these studies are quite contradictory to each other (McCartney et al., 2008). The fact that there is no relationship between the sperm number or body weight with spermatophore weight contradicts findings in many bushcricket species. However, it should be noted that these differences result from environmental factors such as climatic differences, the density of population and food availability, in addition to both proximal and ultimate causes (Lehmann and Lehmann, 2008b; Alcock, 2013).

Relative spermatophylax and ampulla weights are well above the average in comparison to the other species of the genus. The fact that very few of the known 85 *Poecilimon* species in Turkey were studied on this subject is a major gap in terms of understanding possible phylogenetic relationships among species of this genus or its species-groups. In particular, most of the Turkish species of the genus consists of endemic populations distributed in a very narrow area, and this indicates that these species also have different reproductive strategies. In this regard, our next goal will be to study spermatophore characteristics and mating behavior of local endemic populations on a large scale.

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References

Alcock J. 2013. Animal Behavior: An Evolutionary Approach, Tenth edn. Sinauer Associates, 522 p.

Blanckenhorn W.U., Demont M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? Integrative Comparative Biology, 44: 413-424.

Blanckenhorn W.U., Hellriegel B. 2002. Against Bergmann's rule: fly sperm size increases with temperature. Ecology Letters, 5: 7-10.

Çıplak B., Demirsoy A., Yalim B., Sevgili H. 2002. Türkiye'nin Orthoptera (Çekirgeler= Düzkanatlılar) faunası, In: A. Demirsoy (Ed). Genel Zoocoğrafya ve Türkiye Zoocoğrafyası: Hayvan Coğrafyası Meteksan, Ankara, pp. 681-707.

Davidowitz G., Nijhout H.F. 2004. The physiological basis of reaction norms: The interaction among growth rate, the duration of growth and body size. Integrative and

Comparative Biology, 44: 443-449.

Eades D.C., Otte D., Cigliano M.M., Braun H. 2015. Orthoptera Species File, Version 5.0/5.0., retrieved, 28.01.2016.

Eweleit L., Reinhold K. 2014. Body size and elevation: do Bergmann's and Rensch's rule apply in the polytypic bushcricket *Poecilimon veluchianus*?. Ecological Entomology, 39: 133-136.

Gwynne D.T. 2001. Katydids and Bush-Crickets: Reproductive Behavior and Evolution of the Tettigoniidae. Cornell University, USA, 317 p.

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

Heller K.G., Reinhold K. 1994. Mating effort function of the spermatophore in the bush-cricket *Poecilimon veluchianus* (Orthoptera, Phaneropteridae) support from a comparison of the mating-behavior of 2 subspecies. Biological Journal of the Linnean Society, 53: 153-163.

Heller K.-G., Sevgili H., Reinhold K. 2008. A re-assessment of the *Poecilimon syriacus* group (Orthoptera Tettigonioidea, Phaneropteridae) based on bioacoustics, morphology and molecular data. Insect Systematics and Evolution, 39: 361-379.

Lamunyon C.W. 1997. Increased fecundity, as a function of multiple mating, in an arctiid moth, *Utetheisa ornatrix*. Ecological Entomology, 22: 69-73.

Lehmann G.U.C. 2012. Weighing costs and benefits of mating in bushcrickets (Insecta: Orthoptera: Tettigoniidae), with an emphasis on nuptial gifts, protandry and mate density. Frontier Zoology, 9.

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

Lehmann G.U.C., Lehmann A.W. 2008b. Variation in body size among populations of the bushcriket *Poecilimon thessalicus* (Orthoptera: Phaneropteridae): an ecological adaptation?. Journal of Orthoptera Research, 17: 165-169.

Lehmann G.U.C., Lehmann A.W. 2016. Material benefit of mating: the bushcricket spermatophylax as a fast uptake nuptial gift. Animal Behavior, 112: 267-271.

McCartney J., Heller K.-G., Potter M.A., Robertson A.W., Telscher K., Lehmann G., Lehmann A.W., Von Helversen D., Reinhold K., Achmann R. 2008: Understanding the size of nuptial gifts in bush-crickets: an analysis of the genus *Poecilimon* (Tettigoniidae: Orthoptera). Journal of Orthoptera Research, 17: 231-242.

Pauchet Y., Wielsch N., Wilkinson P.A., Sakaluk S.K., Svatos A., Ffrench-Constant R.H., Hunt J., Heckel D.G. 2015. What's in the gift? Towards a molecular dissection of nuptial feeding in a cricket. PLoS ONE, 10: e0140191.

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

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

Roff D. 1980. Optimizing development time in a seasonal environment: the ‘ups and downs’ of clinal variation. Oecologia, 45: 202-208.

Şensoy S., Demircan M., Ulupınar Y. 2016. Climate of Turkey. MGM Web site. Turkish State Meteorological Service. http://www.mgm.gov.tr/files/en-us/climateofturkey.pdf

Sevgili H., Çıplak B. 2000. The Orthoptera of Şanlıurfa province from the Mesopotamian part of Turkey. Italian Journal of Zoology, 67: 229-240.

Sevgili H., Önal H., Yigit A. 2015. Mating behavior and spermatophore characteristics in two closely related bushcricket species of the genus *Phonochorion* (Orthoptera: Phaneropterinae). Journal of Insect Behavior, 28: 369-386.

Simmons L.W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton, NY, 456 pp.

Team R.D.C. 2009. R: A Language and Environment for Statistical Computing. R foundation for statistical Computing, Vienna, Austria.

Uma R., Sevgili H. 2015. Spermatophore allocation strategy over successive matings in the bushcricket *Isophya sikorai* (Orthoptera Phaneropterinae). Ethology, Ecology and Evolution, 27: 129-147.

Vahed K. 1998. The function of nuptial feeding in insects: review of empirical studies. Biological Reviews of the Cambridge Philosophical Society, 73: 43-78.

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 B-Biological Sciences, 263: 1257-1265.

Voigt C.C., Michener R., Kunz T.H. 2005. The energetics of trading nuptial gifts for copulations in katydids. Physiological and Biochemical Zoology, 78: 417-423.

Wedell N. 1994. Variation in nuptial gift quality in bush-crickets (Orthoptera, Tettigoniidae). Behavioral Ecology, 5: 418-425.

Wedell N., Arak A. 1989. The wartbiter spermatophore and its effect on female reproductive output (Orthoptera, Tettigoniidae, *Decticus verrucivorus*). Behavioral Ecology and Sociobiology, 24: 117-125.