

1 **Functional equivalence of grasping cerci and nuptial food gifts in**  
2 **promoting ejaculate transfer in katydids.**

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32

33 **Abstract**

34 The function of nuptial gifts has generated long standing debate. Nuptial gifts  
35 consumed during ejaculate transfer may allow males to transfer more ejaculate than is  
36 optimal for females. However, gifts may simultaneously represent male investment in  
37 offspring. Evolutionary loss of nuptial gifts can help elucidate pressures driving their  
38 evolution. In most katydids (Orthoptera: Tettigoniidae), males transfer a  
39 spermatophore comprising two parts: the ejaculate-containing ampulla and the  
40 spermatophylax - a gelatinous gift that females eat during ejaculate transfer. Many  
41 species, however, have reduced or no spermatophylaces and many have prolonged  
42 copulation. Across 44 katydid species, we tested whether spermatophylaces and  
43 prolonged copulation following spermatophore transfer are alternative adaptations to  
44 protect the ejaculate. We also tested whether prolonged copulation was associated  
45 with (i) male cercal adaptations, helping prevent female disengagement, and (ii)  
46 female resistance behaviour. As predicted, prolonged copulation following (but not  
47 before) spermatophore transfer was associated with reduced nuptial gifts, differences  
48 in the functional morphology of male cerci and behavioural resistance by females  
49 during copulation. Furthermore, longer copulation following spermatophore transfer  
50 was associated with larger ejaculates, across species with reduced nuptial gifts. Our  
51 results demonstrate that nuptial gifts and the use of grasping cerci to prolong ejaculate  
52 transfer are functionally equivalent.

53

54

**“Gifts are like hooks.”**

55

**Marcus Valerius Martialis (40-103 AD).**

56

57 **Introduction**

58 Nuptial gifts occur in a wide range of animal taxa and take numerous forms, including

59 gifts synthesised or collected by the male and parts of the male’s body (reviewed in

60 Vahed 1998, 2007a; Gwynne 2008; Lewis and South 2012).The evolution and

61 maintenance of nuptial gifts and the extent to which they represent intersexual conflict

62 or co-operation have long been a focus for debate. Nuptial gifts that are consumed by

63 the female during ejaculate transfer could function to prolong ejaculate transfer. By

64 transferring a larger ejaculate, the male could transfer larger doses of allohormones

65 that manipulate female reproduction to favour the male, while imposing direct fitness

66 costs on the female (reviewed in Parker and Simmons 1989; Arnqvist and Nilsson

67 2000; Vahed 2007a). In katydids or bushcrickets (Orthoptera: Ensifera:

68 Tettigoniidae), for example, substances in large ejaculates delay females from re-

69 mating (Wedell 1993; Vahed 2006, 2007b) and may shorten female lifespan (Wedell

70 *et al.* 2008). Nuptial gifts could also be counter to the female’s interests because, by

71 maximising ejaculate transfer, they may circumvent female mating preferences

72 (reviewed in Vahed 2007a). On the other hand, nuptial gifts might additionally

73 function to provide nutrients to the female for use in egg production (the Paternal

74 Investment hypothesis, reviewed in Vahed 1998; Gwynne 2008). Nuptial gifts could

75 thereby benefit both sexes, although the nutritional benefits of nuptial gifts have been

76 questioned (reviewed in Vahed 2007a). The evolutionary loss of nuptial gifts offers a

77 special opportunity to gain insight into these selective pressures, especially via

78 comparative studies (Lewis and South 2012). South *et al.* (2011), for example,

79 concluded that factors associated with the presence or absence of nuptial gifts within  
80 the Lampyridae (fireflies) provided support for the paternal investment hypothesis.  
81  
82 Katydidids are a model clade for studying nuptial gifts (reviewed in Gwynne 2001;  
83 Vahed 2007a; Lehmann 2012). Male katydidids transfer a spermatophore towards the  
84 end of copulation consisting of two parts: an ampulla, containing the ejaculate, and a  
85 spermatophylax, a gelatinous nuptial gift (Fig S1). After copulation, the female  
86 consumes the spermatophylax while the ejaculate is transferred from the ampulla  
87 (Boldyrev 1915). The spermatophylax may prolong ejaculate transfer by delaying the  
88 female from eating the ampulla (reviewed in Vahed 2007a). For example,  
89 spermatophylax size is adjusted within species to occupy females long enough to  
90 ensure complete ejaculate transfer (Sakaluk 1984; Reinhold and Ramm 2013); species  
91 with relatively larger ejaculates and more sperm also produce larger spermatophylaxes  
92 (Wedell 1993; Vahed and Gilbert 1996). Additionally, female katydidids (and Ensifera  
93 generally) routinely eat ampullae before complete sperm transfer, unless prevented by  
94 males (Boldyrev 1915; Alexander and Otte 1967). Large spermatophylaxes might  
95 additionally function as paternal investment: spermatophylax feeding can increase  
96 female reproductive output in some species (reviewed in Gwynne 2001; Vahed  
97 2007a; Lehmann 2012).

98

99 Katydidids represent an ideal opportunity to test for factors associated with the loss of  
100 nuptial gifts (see Lewis and South 2012). Although the spermatophylax appears to be  
101 ancestral in this clade (Gwynne 2001), in a variety of species spermatophylaxes are  
102 reduced or absent. The hypothesis that the spermatophylax functions to protect the  
103 ejaculate predicts that, in taxa where the spermatophylax is evolutionarily lost, its loss

104 will be associated with other methods of ejaculate protection. One such possible  
105 candidate method is prolonged copulation following spermatophore transfer, a  
106 behaviour that occurs in a variety of katydids, whereby the body of the male itself acts  
107 as a barrier to prevent the female from consuming the ampulla before complete  
108 ejaculate transfer (Boldyrev 1915; Vahed 1996, 1997; Wedell 1998).

109

110 Prolonged copulation is predicted to select for male ability to grasp and hold the  
111 female via “copulatory structures” (both genital and non-genital grasping and  
112 intromittent organs) to prevent the female from uncoupling (Alexander and Otte  
113 1967). Male copulatory structures diverge more rapidly than other morphological  
114 traits (Eberhard 1985; Rowe and Arnqvist 2012). Sexual selection is currently  
115 regarded as the main hypothesis accounting for this (reviewed in Eberhard 2004,  
116 2006; Arnqvist and Rowe 2005; Simmons 2014), with three main mechanisms: sperm  
117 competition, cryptic female choice and sexually antagonistic co-evolution, which  
118 need not be mutually exclusive (Kokko *et al.* 2003; Simmons 2014). Under the  
119 sexually antagonistic co-evolution hypothesis, male and female copulatory structures  
120 co-evolve in an arms race over mating, each sex being selected to achieve different  
121 optimum mating rates or copulation duration, for example (reviewed in Eberhard  
122 2004, 2006; Arnqvist and Rowe 2005). Although evidence for sexually antagonistic  
123 co-evolution in genital evolution is compelling in some species (reviewed in Eberhard  
124 2004, 2006; Arnqvist and Rowe 2005; Perry and Rowe 2012), its general applicability  
125 has been questioned (Eberhard 2004, 2006, 2010). For example, even where  
126 potentially sexually antagonistic co-evolution is evident, parts of the female contacted  
127 by male copulatory structures mostly lack evidence of counter-adaptations to resist

128 copulation (Eberhard 2004, 2006). Female resistance to male coercion, however, may  
129 be behavioural rather than morphological (Eberhard 2004).

130

131 In katydids, males generally possess two different types of copulatory structures: the  
132 cerci and the titillators (Hartz 1969). Cerci are generally used to clasp the female  
133 during mating (Rentz 1972; Hartley and Warne 1984), while the titillators are  
134 inserted into the female's reproductive tract prior to spermatophore transfer (Vahed *et*  
135 *al.* 2011). Vahed *et al.* (2011) found that the presence of titillators was associated with  
136 prolonged copulation prior to spermatophore transfer in katydids, but the functional  
137 morphology of cerci with respect to copulation duration is as yet unstudied.

138

139 In this paper we test the hypothesis that prolonged copulation following  
140 spermatophore transfer functions in the same way as the spermatophylax, i.e. to  
141 prolong ejaculate transfer by protecting the ampulla of the spermatophore from being  
142 eaten by the female (Boldyrev 1915, Vahed 1996, 1997; Wedell 1998). As a result,  
143 sexual conflict over prolonged copulation should have led to sexually antagonistic co-  
144 evolution between male copulatory structures and female behaviour (Alexander and  
145 Otte 1967; Arnqvist and Rowe 2005). By contrast, prior to spermatophore transfer,  
146 prolonged copulation is less likely to result in sexual conflict (it may represent a  
147 mutual period of mate assessment, Vahed *et al.* 2011). Thus, the following predictions  
148 should be true for copulation duration after, but not before, spermatophore transfer:

149 (1) Prolonged copulation following spermatophore transfer should typically  
150 appear in species in which the spermatophylax is reduced in size, or absent,  
151 and thus should correlate negatively with spermatophylax mass (Vahed 1996,  
152 1997).

153 (2) Because relatively larger ampullae contain more sperm (Vahed and Gilbert  
154 1996) and take longer to transfer their content (Reinhold and Ramm 2013),  
155 copulation duration following spermatophore transfer should correlate  
156 positively with ampulla mass, but only in species in which the spermatophylax  
157 is absent or reduced (ie. is a small percentage of the spermatophore) (Vahed  
158 1996; Wedell 1998).

159 (3) Prolonged copulation following spermatophore transfer should have led  
160 to: a) the evolution of modified morphology and/or use of the male's cerci (to  
161 prevent the female from dis-engaging, Alexander and Otte 1967), which in  
162 turn should have selected for: b) behavioural resistance by the female.

163

## 164 **Materials and methods**

### 165 *The form and use of the male's cerci during copulation*

166 In order to study the morphology of the male's cerci and the parts of the female  
167 contacted by the male's cerci, specimens for the majority of the 44 species in this  
168 study (Tables S1 & S2) were obtained from the field. Collection localities for the  
169 majority of European species were the same as those given in Vahed *et al.* (2011).  
170 Specimens of *Dichopetala* and *Pterophylla beltrani* were collected from near  
171 Victoria, Tamaulipas, Mexico by L. Barrientos-Lozano. Specimens of *Decticita*  
172 *brevicauda* were collected by D. B. Weissman from near Fairfax, Marin County,  
173 California, U.S.A.. Specimens of *Coptaspis* spp., from Bawley Point, New South  
174 Wales, Australia, were supplied by D.C.F. Rentz, specimens of *Docidocercus*  
175 *gigliotosi* from Panama were supplied by H. ter Hofstede, while K-G. Heller supplied

176 specimens of *Poecilimon veluchianus* and *P. affinis* from Florina, Vernon, Greece.  
177 For two species (*Kawanaphila nartee* and *Phasmodes ranatiformis*) the morphology  
178 of the male's cerci was not observed first hand, but was taken from the taxonomic  
179 literature (Rentz 1993). Specimens were preserved in 75% ethanol and were stored at  
180 5°C. A minimum of three males and females of each species were examined under a  
181 dissecting microscope. The right cercus of each male was removed using watch-  
182 maker's forceps. Cerci were then air-dried, gold coated using an Emitech K550X (EM  
183 Technologies Ltd, Ashford, UK) and examined using a scanning electron microscope  
184 (SEM; Leo 1450 VP, Zeiss Ltd, Oxford, UK).

185

186 In order to observe how the male's cerci were used to contact the female during  
187 copulation, pairs were observed closely during laboratory mating trials in which the  
188 duration of copulation was timed (see below). For six of the species in this study  
189 (Tables S1 & S2), copulation was not observed first hand and details were taken from  
190 the literature (*Docidocercus gigliotosi*, *Coptaspis* spp 2, 5 & 10, *Kawanaphila nartee*  
191 and *Phasmodes ranatiformis*, see Table S1). For a range of species, macro-  
192 photographs of copulating pairs were taken using a digital camera (Nikon D3000,  
193 10.2 MP). For selected species (*Leptophyes punctatissima*, *Dichopetala castanea*, *D.*  
194 *pollicifera*, *Pterophylla beltrani*, *Pholidoptera griseoptera*, *Decticita brevicauda*,  
195 *Conocephalus fuscus*, *Coptaspis* sp. 6), a minimum of three pairs were also preserved  
196 in the copulatory position by placing copulating pairs in a freezer (at either -80 or -  
197 18°C) for 5 min before immersing them in 75 % ethanol. The parts contacted by the  
198 male's cerci were examined under a dissecting microscope. Electron micrographs of  
199 the parts of the female contacted by the males' cerci were taken for *Leptophyes*

200 *punctatissima*, *Conocephalus fuscus* and *Platycleis albopunctata* using methods  
201 described for the cerci, above. For the statistical analysis (see below), we developed a  
202 classification system of the morphology of the male's cerci and the different ways in  
203 which they contact the female during copulation (Table 1).

204

#### 205 ***Copulation duration, male body mass, spermatophylax mass and ampulla mass***

206 Data on the duration of copulation following spermatophore transfer were obtained  
207 for 44 species (all data used in this study are given in Table S1). Novel data for this  
208 variable were obtained for 24 of these species, following methods outlined in Vahed  
209 *et al.* (2011), while data for the remainder of the species were taken from the  
210 literature. Data on the duration of copulation prior to the secretion of the  
211 spermatophore, were obtained for 39 species (Table S1). Data were taken primarily  
212 from Vahed *et al.* (2011), with the addition of data for *Dichopetala* spp. Data on  
213 spermatophylax mass, ampulla mass and male body mass (Table S1) were obtained  
214 from the literature for most species (primarily from Vahed and Gilbert 1996, Vahed  
215 2006 and Vahed 2007b), while novel data for these variables and/or additional  
216 replicates were obtained for nine of the species following methods described in Vahed  
217 and Gilbert (1996) and Vahed *et al.* (2011).

218

#### 219 ***Resistance by females during copulation***

220 For each species, the occurrence of resistance behaviour by the female (consisting of  
221 kicking at the male, rapid locomotion during copulation and/or bending to bite at the  
222 male) during copulation was recorded (Table S1). For five of the 44 species included

223 in this part of the study (*Kawanaphila nartee*, *Phasmodes ranatririformis* and  
224 *Coptaspis* sp 2, 5 and 10), we did not observe copulation first hand, so relied instead  
225 upon accounts of copulation behaviour for these species in the literature (Simmons  
226 and Bailey 1990; Bailey and Lebel 1998; Wedell 1998).

227

### 228 ***The phylogeny***

229 The phylogeny used in the analyses (Fig 1) was derived from the morphological  
230 phylogeny developed by Naskrecki (2000). For the subfamily Tettigoniinae, we used  
231 the morphological phylogeny developed by Rentz and Colless (1990). For the genus  
232 *Poecilimon*, we used the phylogeny developed by Ullrich *et al.* (2010), while for the  
233 genus *Anonconotus*, we used an unpublished molecular phylogeny based on mtDNA  
234 (16S and *cyt b*; R. Szabo, G. Carron, K. Vahed and M. Ritchie, unpublished). There  
235 was no overlap between the source phylogenies, so tree-combining algorithms were  
236 unnecessary and trees were assembled jigsaw fashion. Branch lengths on the complete  
237 phylogeny were not available and so were assigned the arbitrary value of 1.

238

### 239 ***Statistical analyses***

240 We used the program MultiState under a Maximum Likelihood framework  
241 implemented in the program BayesTraits (Pagel and Meade 2006), to reconstruct  
242 ancestral male cercal forms for the whole phylogeny and for each subfamily within it.

243

244 To test for correlated evolutionary transitions between male cercal functional  
245 morphology and female resistance behaviour, we collapsed our classification of male  
246 cercal forms (Table 1) into a binary variable: “Unmodified” (including species with  
247 purely “lock and key”-based systems and those in which the cerci do not engage with  
248 the female: states LK1, LK2, LK3 and N) versus “Modified” (states P, T, MP,  
249 MP/P/LK3 and MP/LK1), and used the program Discrete, again implemented in  
250 BayesTraits under Maximum Likelihood.

251

252 To test predictions 1-3 with respect to factors associated with prolonged copulation,  
253 we modelled copulation duration before and after spermatophore transfer using a  
254 Phylogenetic Generalized Least Squares approach (PGLS; Pagel 1999; Martins and  
255 Hansen 1997) with the package “ape” version 3.0-9 (Paradis *et al.* 2004). We included  
256 the predictors (1) ampulla mass as an absolute index of investment by males in the  
257 ejaculate, (2) spermatophylax mass, (3) whether the proportional contribution of the  
258 spermatophylax to the spermatophore exceeded 0.30 (i.e.  
259  $\text{spermatophylax}/[\text{spermatophylax}+\text{ampulla}] > 0.30$ ) as an indicator of evolutionary  
260 reduction of the nuptial gift (breakpoint determined visually based on data; see Fig  
261 S2), (4) presence of male modified cerci, fitted as a binary variable, and (5) male body  
262 mass as a covariate. Prior to analysis, data for pre- and post-transfer copulation  
263 duration and all mass variables were ln-transformed.

264

265 Initial data exploration revealed strong pairwise collinearity among spermatophylax  
266 mass, spermatophylax contribution to spermatophore and the presence of modified  
267 cerci, taking into account body mass as a covariate (PGLS with male body mass as

268 covariate, dropping predictor variable,  $p < 0.0001$  in all cases) whereas none of these  
269 variables was strongly correlated with relative ampulla mass (PGLS as above,  $p > 0.1$   
270 in all cases) except for spermatophylax mass (PGLS as above,  $p < 0.01$ ). Yet, each of  
271 these collinear variables had distinct and specific relevance to our predictions (see  
272 Introduction). As recommended by Zuur *et al.* (2009), to test our predictions in the  
273 light of this collinearity, we did not include collinear predictors in the same analysis.  
274 Instead we first conducted three separate analyses, each with “copulation duration  
275 pre- or post-spermatophore transfer” as the response. In each of these separate  
276 analyses, the full model had four terms: (i) one of the three strongly collinear  
277 predictor variables (spermatophylax mass, whether spermatophylax  $> 30\%$  of  
278 spermatophore, or modified cerci), (ii) ampulla mass, (iii) the interaction of these two  
279 terms, and (iv) male body mass as a covariate. For each analysis we fitted multiple  
280 models including all possible combinations of terms (all models fitted are given in  
281 Table 2). We compared models under an information-theoretic framework, using  
282 corrected Akaike’s Information Criterion (AICc) as a criterion for model selection  
283 (with an AICc difference of 2 as a selection threshold; Burnham and Anderson 2002).  
284 This approach is less sensitive to multicollinearity than alternative methods (Graham  
285 2003) and allows model averaging, a way of providing more meaningful parameter  
286 estimates, and also comparing of non-nested models. We interpreted each of the three  
287 analyses separately with respect to the relevant predictions. Finally, we asked which  
288 of the separately-fitted models was best at explaining copulation duration after  
289 spermatophore transfer, by combining all models from the separate analyses and  
290 comparing all fitted models in a single information-theoretic analysis, again using  
291 AICc as a criterion for model selection.

292

293 **Results**

294 *Evolution of pre- and post spermatophore-transfer copulation duration*

295 *Analyses of pre- spermatophore transfer copulation duration*

296 In all three analyses of pre-spermatophore transfer copulation duration, the top two  
297 models were identical ( $\Delta\text{AICc} < 2$ ; Table 2a); the overall top model was the model  
298 with no terms, i.e. simply an intercept; the next-best model ( $\Delta\text{AICc} = 1.37$  in all cases)  
299 was the model with simply male body mass. Models containing variables relevant to  
300 our predictions always had  $\Delta\text{AICc} > 3$ , and dropping the variable of interest from  
301 these models never resulted in significant reductions in model fit (PGLS,  $\Delta\text{df}=1$ ,  
302  $P > 0.1$  in all cases). When the analyses were combined, the overall top two models  
303 were, identically as above, the intercept alone, followed by male body mass alone.  
304 We conclude that the candidate predictor variables had very limited capacity to  
305 explain variation in pre-transfer copulation duration.

306

307 *Post- transfer copulation duration and spermatophylax mass*

308 There were two best PGLS models of copulation duration with respect to  
309 spermatophylax mass ( $\Delta\text{AICc} < 2$ ; Table 2b). The top model (Akaike weight 0.568)  
310 contained, in addition to male body mass, main effects of spermatophylax mass and  
311 ampulla mass only. Dropping either term from this model significantly reduced model  
312 performance (spermatophylax,  $F_{1,40}=27.5$ ,  $P < 0.0001$ ; ampulla,  $F_{1,40}=8.62$ ,  $P < 0.01$ ).  
313 The second-best model ( $\Delta\text{AICc}=0.66$ , Akaike weight=0.408) additionally contained  
314 their interaction. After model averaging, post-spermatophore transfer copulation  
315 duration was associated negatively with spermatophylax mass, indicating that males  
316 invested less in spermatophylaces where copulation was prolonged after  
317 spermatophore transfer (prediction 1) (Fig 2a). In some species in which copulation

318 following spermatophore transfer was prolonged (e.g. *Dichopetala castanea*, Fig S1,  
319 *D. pollicifera*, *Meconema thalassinum*, *M. meridionale*, *Decticita brevicauda* and  
320 *Pterophylla beltrani*, Fig S3c), the spermatophylax was absent altogether. Post-  
321 spermatophore transfer copulation duration was associated positively with ampulla  
322 mass in the final averaged model, indicating that, across species, males tend to spend  
323 longer in copulation after transferring a larger ampulla (prediction 2), but the  
324 interaction of spermatophylax mass and ampulla mass was not different from zero  
325 (Table 2b).

326

327 *Post-transfer copulation duration and spermatophylax contribution to the*  
328 *spermatophore*

329 There were three best PGLS models of copulation duration with respect to the  
330 proportional contribution of the spermatophylax to the spermatophore ( $\Delta AICc < 2$ ;  
331 Table 2b). The top model, with Akaike weight of 0.462, was the full model,  
332 containing, in addition to male body mass, the interaction between spermatophylax  
333 contribution to the spermatophore and ampulla mass. In this model, the slope of the  
334 relationship between post-transfer copulation duration and ampulla mass was positive  
335 in species where the spermatophylax comprised less than 30% of the spermatophore,  
336 but was not different from zero in species where this was not the case (prediction 2).  
337 Dropping the interaction from this model resulted in a marginally significant  
338 reduction in explanatory power ( $F_{1,39}=3.59$ ,  $P=0.06$ ). The second-best of the top  
339 models ( $\Delta AICc=0.74$ ; Akaike weight 0.319) contained male body mass and a  
340 negative main effect of the spermatophylax contribution to the spermatophore only,  
341 while the third ( $\Delta AICc=1.49$ ; Akaike weight 0.219) contained a positive main effect  
342 of ampulla mass and a negative main effect of the spermatophylax contribution to the

343 spermatophore, but not their interaction. Combining these models using model  
344 averaging, the interaction was important (i.e. its confidence intervals did not overlap  
345 zero; Table 2b), indicating that the relationship between post-transfer copulation  
346 duration and ampulla mass depended upon whether the spermatophylax was reduced  
347 or absent (prediction 2; Fig 2b).

348

349 *Post- transfer copulation duration and male cercal form*

350 The form and use of the male's cerci during copulation is summarised in Table 1 and  
351 the accompanying figs (Figs. 3, 4 & S3) and is described for each species in Table S2.  
352 In the majority of species, each of the male's cerci has a single tooth which engages  
353 with a sclerotised pit or groove on the female. Some species within each sub-family,  
354 however, depart from these patterns (Fig. 1, Table S2). In such species, the cerci show  
355 a variety of modifications in morphology and in the way in which they contact the  
356 female (Tables 1 & S2, Figs. 4 & S3).

357

358 There were two best models of post-spermatophore transfer copulation duration with  
359 respect to cercal form ( $\Delta AICc < 2$ ; Table 2b). The first, with Akaike weight 0.688,  
360 contained, in addition to male body mass, both main effects of cercal form and  
361 ampulla mass but without their interaction. In this model, longer copulation times  
362 following spermatophore transfer were associated with modified cerci (prediction 3a;  
363 Fig 5) and larger ampullae. In clades where females additionally resisted male  
364 copulation attempts, copulation durations were in fact marginally statistically shorter  
365 after spermatophore transfer than in those species where females did not resist (PGLS,  
366 planned contrast between "modified/non-resisting females" and "modified/resisting  
367 females",  $t = -1.73$ ,  $p = 0.08$ ; Fig 5), indicating that female resistance may be somewhat

368 effective in reducing the duration of copulation after transfer. The second-best model  
369 ( $\Delta\text{AIC}=1.63$ , Akaike weight 0.305) was the full model, containing the interaction of  
370 male cercal form with ampulla mass. In this model, copulation duration following  
371 spermatophore transfer was more strongly positively related to ampulla mass in  
372 species with modified cerci than in those with unmodified cerci. In the averaged  
373 model, only the main effects were different from zero (Table 2b).

374

#### 375 *Combined analysis of post-transfer copulation duration*

376 Comparing all fitted models of post-spermatophore transfer copulation duration, all  
377 the models including “modified cerci” were superior to all other models. Thus, as  
378 above, the two overall best models were (1) “modified cerci” and “ampulla mass” but  
379 not their interaction (Akaike weight 0.672) and (2) “modified cerci” and “ampulla  
380 mass” plus their interaction (Akaike weight 0.326; Table 3b).

381

#### 382 *Evolution of female resistance behaviour*

383 There were no taxa in the dataset in which females resisted copulation by males with  
384 “unmodified” cerci. Thus, female resistance behaviour was, superficially, entirely  
385 contingent upon the presence of modified cerci (prediction 3b). We therefore  
386 amalgamated the two traits into one trait with three extant states, and used MultiState  
387 to model transitions between these three states. This analysis indicated no detectable  
388 transitions between “modified cerci/non-resisting females” and “modified  
389 cerci/resisting females” (Fig S4; note that re-running the model using Discrete did not  
390 produce appreciably different results). This is consistent with a scenario where female  
391 resistance to copulation is ubiquitous in some entire clades where males carry  
392 modified cerci, but is entirely absent from others, as was the case for our data (see Fig

393 1). This lack of variation meant that our phylogeny was not finely resolved enough for  
394 us to ascertain which of the two traits evolved first. Thus, in those clades where  
395 females all resisted copulation (e.g. in the genus *Anonconotus*; see Fig 1), modified  
396 cerci may have evolved to counteract female resistance to copulation, or *vice versa*.  
397 More fine-grained data will be required to resolve this issue, although the fact that  
398 modified cerci occurred independently, whereas female resistance behaviour appeared  
399 to be dependent upon the presence of modified cerci, circumstantially supports the  
400 idea that female resistance follows evolutionary modification of male cerci.

401

#### 402 *Ancestral character states of male cerci*

403 Figure 1 shows the phylogeny with the extant states of all traits analysed. We were  
404 statistically unable to resolve which type of cerci was ancestral to katydids as a whole.  
405 In this case outgroup comparison was unhelpful, since in related families within the  
406 Ensifera, such as the Anostomatidae, Stenopelmatidae, Gryllacrididae,  
407 Rhaphidophoridae, and Gryllidae, males carry simple cerci with a sensory function  
408 that are not typically used in mating, and so are uninformative in resolving ancestral  
409 character states (Alexander and Otte 1967; Weissman 2001; Field and Jarman 2001;  
410 Eades *et al.* 2013). Across the whole phylogeny, collapsing cerci into “modified” vs.  
411 “unmodified”, a maximum of 7 origins of modified cerci were evident if unmodified  
412 cerci were treated as ancestral, whereas a maximum of 11 origins of unmodified cerci  
413 were evident if modified cerci were treated as ancestral.

414

415 Within each subfamily, the cercal form ancestral to the Phaneropterinae was most  
416 likely to be “LK3” (Probability, Pr, =0.73) or “MP & P & LK3” (Pr=0.22), for

417 Bradyporinae, “LK1” (Pr=0.93); for Meconematinae, “T” (Pr=0.65), or “N”  
418 (Pr=0.31); for Tettigoniinae, “MP” (Pr=0.51), “LK2” (Pr=0.21) or “MP & LK1”  
419 (Pr=0.19).  
420  
421

422 **Discussion**

423 The present study provides the first comparative evidence that prolonged copulation  
424 during ejaculate transfer and nuptial feeding are functionally analogous (Boldyrev  
425 1915; Vahed 1996, 1997; Wedell 1998). Both predictions 1 and 2 were supported:  
426 prolonged copulation following spermatophore transfer was associated with a loss or  
427 reduction in size of the spermatophylax (both in absolute terms and relative to the rest  
428 of the spermatophore); and larger ejaculates were associated with an increase in the  
429 duration of copulation following spermatophore transfer, but only in species in which  
430 the nuptial gift (spermatophylax) was reduced or absent. Prolonged copulation  
431 following spermatophore transfer, with associated loss or reduction in the size of the  
432 spermatophylax, appears to have evolved independently numerous times. In the  
433 Tettigoniidae, the spermatophylax appears to be the ancestral character state as it  
434 occurs in virtually all subfamilies of katydids studied so far (Gwynne 2001), so it  
435 appears that prolonged copulation has replaced the spermatophylax in function. This  
436 finding supports the hypothesis that the main function of nuptial feeding relates to  
437 enhancing the male's mating or fertilisation success, rather than providing the female  
438 with nutrients for egg production. If nuptial gifts evolved, or currently function, as a  
439 form of paternal investment (reviewed in Gwynne, 2001; Lewis and South 2012), then  
440 there is no reason to expect any association between nuptial gift size and the duration  
441 of copulation following spermatophore transfer.

442

443 The prediction that prolonged copulation following spermatophore transfer would be  
444 associated with a change in the functional morphology of cerci in males (prediction  
445 3a), was supported. In species with brief copulation following spermatophore transfer,  
446 the majority of species in this study, the cercal tooth generally engaged with

447 specialised pits or grooves at the base of the ovipositor or on the female's sub-genital  
448 plate. This mechanism is consistent with inter-sexual co-operation over copulation  
449 rather than conflict. In contrast, prolonged copulation following spermatophore  
450 transfer was associated with three different types of "modified" cerci: those that  
451 contact the female in multiple places, those that encircle the female's abdomen, and  
452 those that pierce the female's abdominal cuticle. Few previous studies have taken  
453 copulation duration into account when seeking to explain inter-specific variation in  
454 the morphology of copulatory structures in males (for insects, see Takami and Sota  
455 2007; Vahed *et al.* 2011; Ronn and Hotzy 2012; for mammals, see Dixson 1995;  
456 Larivière and Ferguson 2002).

457

458 The prediction that prolonged copulation and modified cerci will be associated with  
459 behavioural resistance by the female (prediction 3b), was also supported. Similar  
460 resistance behaviour has been reported in various insect taxa with prolonged or  
461 coercive copulation (reviewed in Arnqvist and Rowe 2005; see also Edvardsson and  
462 Canal 2006; Kuriwada and Kasuya 2009; Mazzi *et al.* 2009). Studies in which  
463 resistance by females during copulation has been prevented have demonstrated that  
464 resistance behaviour can shorten copulations (eg. in *Callosobruchus* beetles and in  
465 *Drosophila montana*; Crudgington and Siva-Jothy 2000; Mazzi 2009). Whether such  
466 behavioural resistance can lead to sexually antagonistic co-evolution has been  
467 questioned: Eberhard (2004, 2006) suggested that species-specific differences in  
468 female resistance behaviour would have to be shown to be effective (i.e. adaptations)  
469 against particular details of male grasping traits. Although our findings cannot satisfy  
470 these strict requirements, we suggest that even general resistance behaviour by the  
471 female, when accompanied by selection on males to prolong ejaculate transfer (i.e. for

472 sperm competition avoidance), can select for copulatory structures in males that are  
473 more effective in maintaining a firm hold of the female.

474

475 While we interpret behaviour such as biting the male, rapid locomotion during  
476 copulation and kicking the male as reflecting inter-sexual conflict over copulation  
477 duration/ ejaculate transfer, we cannot exclude the possibility that it reflects a means  
478 by which females assess their mates (e.g. Eberhard 1996). If such behaviour is a form  
479 of mate screening, however, it is hard to explain why in the present study this  
480 behaviour only occurred during prolonged copulation *following* spermatophore  
481 transfer and not before spermatophore transfer. Prolonged copulation *prior to*  
482 spermatophore transfer was not associated with either resistance behaviour by the  
483 female or with modified cerci. This could suggest that it is generally not in the  
484 female's interest to break off from copulation *before* receiving the spermatophylax (in  
485 order to gain any nutritional benefits from spermatophylax consumption; reviewed in  
486 Lehmann 2012).

487

488 In species in which males showed “modified” cerci and prolonged copulation in this  
489 study, it is perhaps surprising that females did not appear to show any morphological  
490 adaptations to resist the grasping or piercing male cerci. This may be because  
491 resistance was behavioural rather than morphological. Where females did possess  
492 specialised structures in parts contacted by males, these tended to occur in species in  
493 which copulation following spermatophore transfer was brief and apparently  
494 functioned to *facilitate* copulation (Fig. 3). The tendency for such structures in  
495 females to facilitate rather than to resist copulation is seen in a wide range of

496 arthropod taxa (Eberhard 2004, 2006), although with notable exceptions (reviewed in  
497 Arnqvist and Rowe 2005; Perry and Rowe 2012).

498

499 The present study demonstrates that comparative analyses involving species in which  
500 nuptial gifts have been lost or reduced can provide valuable insights into the selective  
501 pressures underlying gift evolution (South *et al.* 2011; Lewis and South 2012). This  
502 study also underscores the importance of behavioural data in understanding male  
503 copulatory structure evolution. Furthermore, it demonstrates that emphasizing  
504 morphology alone could be misleading: we cannot expect sexually antagonistic co-  
505 evolution always to lead to increases in complexity of male copulatory structures (as  
506 is sometimes implied, e.g. Eberhard 2006); there may be several different  
507 evolutionary pathways by which males can increase grasping efficiency in the face of  
508 resistance by females, not all of which necessarily involve an increase in  
509 morphological complexity.

510

511

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521

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668

669 **Figure and table legends**

670

671 **Figure 1.** Phylogeny used in this study showing extant states for all analysed traits.  
672 Branch lengths have been scaled to make the tree ultrametric and are not  
673 representative of those used in the analysis. For each binary trait, character states are  
674 true (black circles) or false (white circles). “Spx” = spermatophylax; “post-transfer  
675 copulation duration” is the duration for which the male maintains hold on the female  
676 with his cerci following spermatophore transfer. Post-transfer copulation duration has  
677 been scaled so that totally white and black circles represent, respectively, the  
678 minimum and maximum observed in the dataset (see supplementary information for  
679 all raw data). “Male modified cerci” refers to the functional morphology of the cerci  
680 and primarily includes cases which depart from purely “lock and key” based systems  
681 (see text for further details).

682

683 **Figure 2.** (a) Relationship between post-spermatophore transfer copulation duration  
684 and spermatophylax mass. Line shows model-averaged regression  $\pm$ s.e. from PGLS  
685 models of post-spermatophore transfer copulation duration. (b) Relationship between  
686 post-spermatophore transfer copulation duration and ampulla mass in species with  
687 varying spermatophylax contribution to spermatophore (open circles: spermatophylax  
688  $<$  30% of spermatophore; closed circles: spermatophylax  $>$  30% of spermatophore).  
689 Fitted lines are model-averaged regressions from PGLS models of post-  
690 spermatophore transfer copulation duration, and show best-fit lines  $\pm$ s.e. for species  
691 where spermatophylax  $<$  30% of spermatophore (thin line, solid area) and  $>$  30% of  
692 spermatophore (thick line, hatched area).

693

694

695 **Figure 3.** The end of the female’s abdomen in a. *Leptophyes punctatissima*  
696 (Phaneropterinae), b. *Platycoleis albopunctata* (Tettigoniinae), c. *Conocephalus fuscus*  
697 (Conocephalinae) and d. *Steropleurus stalii* (Bradyporinae), showing the structures  
698 that receive male’s the cercal tooth during copulation (p = pit into which the male’s  
699 cercal tooth engages; s= lateral sclerite; l = lamella). The base of the ovipositor (ov) is  
700 visible on the left. See Table 1 & S2 for the accompanying text.

701

702 **Figure 4.** Cerci in male katydids with brief copulation following spermatophore  
703 transfer in comparison with those in which copulation following spermatophore is  
704 prolonged (and coercive, in the case of *Anonconotus pusillus* and *A. baracunensis*)  
705 (see Tables 1 & S2 for the accompanying text). a. *Leptophyes punctatissima*; b.  
706 *Poecilimon affinis*; c. *P. veluchianus*; d. *Dichopetala castanea*; e. *D. pollicifera*  
707 (Phaneropterinae) f. *Docidocercus gigliotosi*, showing the tip of the abdomen (photo  
708 by P. Naskrecki); g. *Pterophylla beltrani*, showing the tip of the abdomen  
709 (Pseudophyllinae). Note the three projections on each cercus (ve = ventral arm; ce =  
710 central tooth; do = dorsal arm); g1. Enlargement of the dorsal arm in *P. beltrani* to  
711 show the sharply pointed hook that grips the female's abdominal cuticle; h. *Yersinella*  
712 *raymondi*; i. *Pholidoptera griseoptera*; j. *Metrioptera roeselii*; k. *Platypleis*  
713 *albopunctata*; l. *Anonconotus pusillus*; m. *A. baracunensis*; n. *Decticita brevicauda*  
714 (Tettigoniinae); o. *Conocephalus fuscus*; p. *Ruspolia nitidula*; q. *Coptaspis* sp. 6  
715 (Conocephalinae); r. *Cyrtaspis scutata*, showing the tip of the abdomen; s. *Meconema*  
716 *thalassinum*, showing the tip of the abdomen; t. *M. meridionale*, showing the tip of  
717 the abdomen (Meconematinae). t1. Enlargement of the tip of a cercus in *M.*  
718 *meridionale*. The arrow indicates the apical tooth (to), which is absent in *M.*  
719 *thalassinum*; u. *Kawanaphila nartee*, showing the tip of the abdomen; *Phasmodes*  
720 *ranatriformis*, showing the tip of the abdomen (adapted from Rentz 1993)  
721 (Zaprochilinae/ Phasmodinae); w. *Ephippigerida taeniata*; x. *Steropleurus stalii*; y.  
722 *Uromenus rugosicollis* (Bradyporinae).

723  
724 **Figure 5.** Pre-spermatophore transfer copulation duration plotted against post-  
725 spermatophore transfer copulation duration in species with unmodified (white circles)  
726 and modified (black circles) cerci, and species with modified cerci where females  
727 resist mating (ringed circles).

728 **Table 1.** Classification of the functional morphology of cerci of male katydids used  
729 in the analysis (see also Table S2).

730

731 **Table 2.** Tables of coefficients and AIC selection criteria for PGLS models of (a) pre-  
732 and (b) post-spermatophore transfer copulation duration. In each case three separate  
733 analyses were carried out with respect to each of three collinear predictor variables  
734 (see text for details). Key: K, number of parameters;  $w_i$ , Akaike weight; INT,  
735 Intercept; M, male body mass; AMP, ampulla mass; SPX, spermatophylax mass;  
736 PSPX, proportional contribution of spermatophylax to spermatophore (binary; greater  
737 or less than 30%); MOD, modified cerci (binary, yes or no). X:Y denotes the  
738 interaction of term X and term Y.

739

740

741 **Supplementary online material legends.**

742

743 **Figure S1.** a.) Female *Ephippiger diurnus* carrying the spermatophore (photo by S.  
744 Dourlot). Note the large spermatophylax (am = ampulla; spx = spermatophylax); b.)  
745 Female *Dichopetala castanea* carrying a spermatophore (am = ampulla). Note the  
746 lack of a spermatophylax in this species.

747

748 **Figure S2.** Frequency distribution of the proportional contribution of the  
749 spermatophylax to the spermatophore across species.

750

751 **Figure S3.** Examples of copulating pairs of tettigoniid species in which copulation is  
752 prolonged following spermatophore transfer. See Table S2 for the accompanying text.  
753 The male is upside-down on the left (ce = the male's cercus; am = ampulla of the  
754 spermatophore). a. *Anonconotus baracunensis* (Tettigoniinae) (modified from a  
755 photo by C. Roesti). Note that the cerci (Fig 4m) grip the sides of the female's  
756 abdomen. The insert shows melanised scarring (sc) on the sides of the female's  
757 abdomen cause by the apical teeth of the male's cerci; b. *Uromenus rugosicollis*  
758 (Bradyporinae) (photo by G. Carron). The insert shows melanised scarring (sc) on  
759 the ventral surface of the female's abdomen from puncture wounds caused by the  
760 sharp teeth of the male's cerci (Fig 4y); c. *Pterophylla beltrani* (Pseudophyllinae)  
761 (photo by L. Barrientos-Lozano) (the cerci of this species are shown in Fig 4g); d.  
762 *Dichopetala pollicifera* (Phaneropterinae) (photo by L. Barrientos-Lozano). The cerci  
763 (Fig 4e) grip the sides of the female's abdomen, causing it to indent; e. *Meconema*  
764 *meridionale* (Meconematinae) (modified from a photo by B. Baur). Note that the  
765 male's cerci (which have been darkened digitally to make them visible, see also Fig  
766 4t) enclose the end of the female's abdomen and cross over one another on the other  
767 side.

768

769 **Figure S4.** Reconstructed evolutionary transitions between cercal states (Unmodified,  
770 U, versus modified, M) and female resistance (no resistance, NR, versus resistance,  
771 R) using the program MultiState. Transition rate parameters represent the relative  
772 probability of a given evolutionary transition along a branch of the phylogeny (Pagel  
773 and Meade 2006). Arrow weights are scaled according to transition rates. Dashed  
774 arrows indicate transition rates that were not different from zero, i.e. which did not

775 reduce the model's explanatory power when restricted to zero. Greyed-out state  
776 combinations did not occur on the phylogeny.

777

778 **Table S1.** Raw data used in the analyses.

779

780 **Table S2.** The form and use of the male's cerci in tettigoniid males in which  
781 copulation following spermatophore transfer is brief in comparison with species in  
782 which copulation following spermatophore transfer is prolonged. "Code" is the  
783 classification of the functional morphology of the cerci used in the analysis (see also  
784 Table 1). For the purposes of this table, species with "prolonged copulation following  
785 spermatophore transfer" include those in which the mean duration of copulation  
786 following spermatophore transfer (see Table S1) is greater than 15 min and/or those in  
787 which ejaculate transfer is likely to occur largely during copulation (because the  
788 female typically eats the ejaculate-containing ampulla within 5 min. following the end  
789 of copulation).

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