Are attractive male crickets better able to pay the costs of an immune challenge?

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Reproduction and immunity are fitness-related traits that trade-off with each other. Parasite-mediated theories of sexual selection suggest, however, that higher-quality males should suffer smaller costs to reproduction-related traits and behaviours (e.g. sexual display) from an immune challenge because these males possess more resources with which to deal with the challenge. We used *Gryllus texensis* field crickets to test the prediction that attractive males should better maintain the performance of a fitness-related traits (e.g. calling effort) in the face of an immune challenge compared with unattractive males. We found no support for our original predictions. However, that immune activation causes attractive males to significantly increase their calling effort compared with unattractive males suggests that these males might terminally invest in order to compensate for decreased future reproduction.

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35   **Abstract**
36   Reproduction and immunity are fitness-related traits that trade-off with each other. Parasite-
37   mediated theories of sexual selection suggest, however, that higher-quality males should suffer
38   smaller costs to reproduction-related traits and behaviours (e.g. sexual display) from an immune
39   challenge because these males possess more resources with which to deal with the challenge. We
40   used *Gryllus texensis* field crickets to test the prediction that attractive males should better
41   maintain the performance of a fitness-related traits (e.g. calling effort) in the face of an immune
42   challenge compared with unattractive males. We found no support for our original predictions.
43   However, that immune activation causes attractive males to significantly increase their calling
44   effort compared with unattractive males suggests that these males might terminally invest in
45   order to compensate for decreased future reproduction.
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Introduction

Individuals maximize fitness by balancing investment into reproduction with investment into other fitness-related traits, including immunity (Sheldon & Verhulst, 1996; Viney, Riley & Buchanan, 2005; Schmid-Hempel, 2011; Jacobs & Zuk, 2011). Conflict between competing fitness-related traits means that allocation of resources toward immune function by an infected individual will reduce the resources available for other fitness-related traits, such as sexual display, and vice versa (McKean & Nunney, 2001; Jacot, Scheuber & Brinkhof, 2004; McKean & Nunney, 2005; Leman et al., 2009; López, Gabirot & Martin, 2009). Individuals will always face trade-offs when partitioning resources among competing functions (Schmid-Hempel, 2011; Jacobs & Zuk, 2011). Similarly, if individuals vary more in their resource allocation than their variation in resource acquisition (van Noordwijk & de Jong, 1986), a trade-off will emerge among individuals because those that allocate more resources to sexual signals will have fewer resources available for immunity (reviewed by Schmid-Hempel, 2011; Jacobs & Zuk, 2011). For example, Skarstein and Folstad (1996) revealed that male Arctic charr (*Salvelinus alpinus*) with more-colourful sexual ornamentation had weaker immune function.

In contrast, if individuals vary more in their ability to acquire resources than in their allocation of them, then high quality individuals will have more resources (van Noordwijk & de Jong, 1986) and should thus be able to produce an attractive sexual signal while fighting an immune challenge (reviewed by Schmid-Hempel, 2011; Jacobs & Zuk, 2011). This premise is the basis of parasite-mediated theories of sexual selection (Schmid-Hempel, 2011; Jacobs & Zuk, 2011) and has been supported in several empirical studies (Saino & Møller, 1996; López, 1998; Mougeot & Redpath, 2004; Kelly & Jennions, 2009; Schmid-Hempel, 2011; Jacobs & Zuk, 2011). For example, Mougeot et al. (2004) found that the quality of a sexual ornament (redness of beak and eye rings) in male red-legged partridge (*Alectoris rufa*) positively correlated with greater swelling response to PHA.

A third pattern has also commonly be reported in the literature wherein individuals increase their investment in a fitness-related trait after an immune challenge. The 'reproductive compensation hypothesis' posits that immune-challenged individuals adpatively shift more of their resources into the current reproductive event because their residual reproductive value is reduced (Minchella & Loverde, 1981; see also the terminal investment hypothesis: Clutton-Brock, 1984). This hypothesis has been supported empirically in several studies (reviewed by Agnew, Koella & Michalakis, 2000; Reaney & Knell, 2010; see also: McCurdy, Forbes & Boates, 2000; Kivleniece et al., 2010; Krams et al., 2011; Nielsen & Holman, 2012; but see Kolluru, Zuk & Chappell, 2002; Vainikka et al., 2007) with, for example, Polak and Starmer (1998) showing that male *Drosophila nigrospiracula* parasitized with mites court females at a significantly higher rate than unparasitized males. Although we know that an immune-challenge can increase reproductive investment, we know very little about how individual quality mediates this effect.

Here, we investigate whether sexual attractiveness (i.e. male quality) affects the performance of a sexual signal (calling frequency) after an immune challenge in the Texas field cricket, *Gryllus texensis* (Orthoptera: Gryllidae). Mate choice studies have shown that sexually attractive male crickets can have a particular pheromone profile (Tregenza & Wedell, 1997), larger body size (Simmons, 1986b), call more frequently (Hunt et al., 2004), or have a calling song with, for example, a higher chirp rate, longer intercall duration or louder amplitude (Wagner & Hoback, 1999; Holzer, Jacot & Brinkhof, 2003; Scheuber, Jacot & Brinkhof, 2003; Brooks et al., 2005).

93   Although most mate choice studies tend to examine only a small subset of male traits, it is likely
94   that female crickets simultaneously assess several traits when making their mate choice
95   (Simmons, 1986a; Shackleton, Jennions & Hunt, 2005; Bussiere et al., 2006). The above-listed
96   male sexual signals, particularly those related to calling, are condition-dependent and
97   energetically costly to produce. Consequently, the quality of the signal tends to suffer when
98   males are forced to allocate resources to other fitness-related traits. For example, Jacot et al.
99   (2004) showed that an immune challenge with lipopolysaccharide (LPS) causes a significant
100 reduction in calling rate in male *G. campestris* and Fedorka and Mousseau (2007) observed a
101 post-challenge increase in interpulse interval in the calling song of male *Allonemobius socius*
102 ground crickets.

103 We used controlled laboratory experiments to test the sexual selection hypothesis that sexually
104 attractive males are better able to bear the costs of an immune challenge and thus maintain the
105 performance of one component of sexual signalling, that is calling frequency, during immune
106 activation. We predicted that immune-challenged attractive males would not suffer a significant
107 decrease in calling frequency compared with control males whereas the calling effort of
108 unattractive males would significantly decline after an immune challenge. Alternatively, if
109 sexual signaling trades off with immunity, we would expect that attractive males would suffer a
110 significant drop in signalling relative to controls while unattractive males would not. If immune-
111 challenged males undergo reproductive compensation then attractive and unattractive males
112 should both elevate their sexual signalling relative to controls.
113
114 **Materials and Methods**
115 Experimental crickets were lab-reared descendants of individuals originally caught in Austin, TX
116 (USA) in 2012 and 2013. The laboratory colony of crickets was reared in several large
117 communal plastic bins (73 x 41 x 46 cm) until their penultimate instar at which time they were
118 transferred to large sex-specific communal bins to prevent mating and ensure virginity. Newly
119 eclosed adults were placed in individual 10 cm deli cups. All crickets were housed in an
120 environmentally controlled room (27° C, 12:12 h light:dark cycle, 80% relative humidity) and
121 were supplied with cotton-plugged water vials and dry cat food (Special Kitty Premium Cat
122 Food) *ad libitum*. Crickets were used in experiments 10-14 d post-eclosion to ensure sexual
123 maturity.
124
125 *Quantifying Attractiveness*
126 As in most gryllid species, mating in *G. texensis* follows a highly stereotypical sequence of
127 behaviours. Males will contact a female with his antennae and then male produce a courtship call
128 during which he moves backward toward the female. If the female mounts the male he will
129 attempt to attach an externally positioned spermatophore. Spermatophore transfer is
130 accompanied by rapid and irregular flicking of the male's caudal cerci and takes 5-6 seconds,
131 immediately after which the male unhooks his genitalia. Mating lasts 3 min on average and
132 requires the active cooperation of the female to be successful.

133 Latency to mate is a reliable predictor of male sexual attractiveness and mating success in field
134 cricket species (Simmons, 1987; Bateman, 1998; Shackleton, Jennions & Hunt, 2005; Bussiere et
135 al., 2006). Following Shackleton et al. (2005) we determined male attractiveness by conducting a
136 four-round no-choice tournament that indexed male attractiveness based on the time that elapsed
137 until a female mounted them. Studies have traditionally used a single trait to assess male

138   attractiveness in crickets (Heisler, 1985; Wedell & Tregenza, 1999) but a no-choice tournament
139   is a superior approach because it simultaneously incorporates all relevant factors contributing to
140   short-range male attractiveness (Head et al., 2005; Shackleton, Jennions & Hunt, 2005; Bussiere
141   et al., 2006). Tournaments commenced at the onset of the environmental chamber's dark cycle
142   and were conducted under red light to minimize observer disturbance. In the first round, we
143   placed each of 12 sexually naive males in individual plastic containers (10 cm diameter) with a
144   randomly assigned virgin female from our stock culture and observed them until the female
145   mounted the male. We scored a mounting as successful if (1) the female remained motionless on
146   top of the male for at least 3 seconds and (2) the male commenced spermatophore transfer,
147   characterized by the vibration of his cerci. Pairs were separated prior to spermatophore transfer.
148   Males were ranked 1 to 12 in order of mounting (rank 1 being the fastest) and males that
149   remained unmounted after 60 minutes were given the average of the remaining ranks. This
150   process continued for three more rounds with a new female being assigned to each male in each
151   round. Ranks from the four rounds were summed for each male (four lowest sums were
152   attractive; four highest sums were unattractive). Seven such tournaments were completed
153   yielding 28 attractive males (four lowest-ranked males from each tournament) and 28
154   unattractive males (four highest-ranked males from each tournament).

155   *Administering an Immune Challenge*
156   We immune-challenged the attractive and unattractive males the day after the no-choice
157   tournaments by following protocols established for *G. texensis* (Adamo, 1999). Briefly, we cold-
158   anesthetized males by placing them on ice for 10 minutes and then injected them by inserting a
159   pulled-glass microcapillary needle (needles were used only once) along the left pleural region of
160   their abdomen. Microcapillary needles were made in a Flaming/Brown Micropipette Puller
161   (Sutter Instrument Co. model P-97, program 27) with Kwik-Fil's borosilicate glass capillaries.
162   Fourteen attractive males and 14 unattractive males were injected with 5μL of saline (phosphate-
163   buffered saline, Sigma-Aldrich), and 14 attractive males and 14 unattractive males were injected
164   with 100 μg of lipopolysaccharide from *Serratia marcescens* (LPS, Sigma-Aldrich), dissolved in
165   5μL of saline. *S. marcescens* is a soil microbe that is frequently used as an immune challenge
166   model in *G. texensis* because it co-ocurrs with this cricket species in nature and is lethal to it
167   (Adamo, Jensen & Younger, 2001). LPS is a non-pathogenic and non-living elicitor that
168   stimulates several pathways in the immune system of orthopterans (Jacot, Scheuber & Brinkhof,
169   2004; Fedorka & Mousseau, 2007; Leman et al., 2009; Kelly, 2011; 2014) including *G. texensis*
170   (Adamo, 1999).

171   *Quantifying sexual signaling*
172   One hour after males were injected they were put into a 230 mm x 155 mm x 170 mm plastic
173   arena (Exo Terra Faunarium). We began recording calling frequency after 1 h because an
174   immune challenge produces measurable physiological effects in *G. texensis* after 90 minutes
175   (Adamo et al., 2008); this timeframe, therefore, ensured that we captured the full time period.
176   The ventilated section of the lids were removed and replaced with a black mesh screen. The
177   arena contained a paper shelter (made from an Oxford 12.7 x 7.6 cm index card cut into 4 strips),
178   one piece of Special Kitty cat chow, and a water vial affixed with hot glue to a white paper lining
179   the bottom. During each trial a microphone (Dynex USB, DX-USBMIC13) was directed toward
180   each male for 5 seconds every five minutes to assess male calling. For each male, the
181   microphone was held close to the mesh on the top of its arena (within 23 cm of the male). We
182   used QuickTime Player (version 10.2, Apple Inc.) to visualize sounds detected by the

183   microphone and recorded whether or not an individual was calling (0=no, 1=yes) during each of
184   the 60 5 second sample periods. The trials were conducted in a dark room but to assist in
185   appropriately placing the microphone near the focal male, the arenas were illuminated with four
186   CMVision IR200-940 (18 W) infra-red Illuminators and visualized with a Canon Vixia HFG10
187   HD camcorder. Trials were 5 hours in duration and so each male's calling was sampled for a
188   total of 5 min over the course of 5 hours (i.e. sampled for 5 seconds every 5 minutes for 5 hours).
189   Trial duration was within the window of immune-activation for LPS. Although LPS is cleared
190   from insect haemolymph within hours (Kato et al., 1994) it induces a prolonged up-regulation of
191   immune response molecules in orthopterans (i.e. for a period of days to weeks: Jacot et al., 2005;
192   Fedorka & Mousseau, 2007; Kelly, 2011).
193
194   *Morphological Traits*
195   Immediately prior to being placed in a trial, males were weighed on a Denver Instruments TP-64
196   digital balance (to the nearest 0.01 g) and the pronotum length measured using a stereoscope
197   equipped with Leica LAS image analysis software (Leica Microsystems Inc., Buffalo Grove, IL,
198   USA). Pronotum length (the distance from the anterior to posterior edges of the pronotum at the
199   midline) is an excellent proxy for body size in *G. texensis* (Kelly, Tawes & Worthington, 2014).

200   *Statistical Analysis*
201   We examined whether attractive males differed phenotypically from unattractive males by using
202   one-way analysis of variance (ANOVA) to compare pronotum lengths (proxy for body size) and
203   scaled mass indices (proxy for body condition). Body condition was calculated using the scaled
204   mass index (SMI) following Kelly et al. (2014). We forgot to weigh and measure one
205   unattractive and one attractive male prior to the experiment so the sample sizes differ between
206   the morphological (N=54) and calling (N=56) analyses. A generalized linear model was used to
207   test whether the fixed factors male attractiveness (attractive or unattractive), immune status
208   (LPS- or saline-injected), and time since injection affected the number of calls (family=poisson).
209   We pooled calling data into ten 30 minute bins for analysis. All statistical analyses were
210   conducted in *R* 3.0.3. (R Development Core Team, 2014) with data visualized using *ggplot2*
211   (Wickham, 2009). Means are given ± 1 SE and α=0.05.

212
213   **Results**

214   Attractive and unattractive males did not differ significantly in either pronotum length (one-way
215   ANOVA: $F_{1,52}$=0.001 p=0.98) or scaled body mass prior to experimental treatment (one-way
216   ANOVA: $F_{1,52}$=0.361 p=0.55).

217   We found a significant interaction between male attractiveness and immune status on calling
218   frequency (Table 1a; Figure 1). We explored this interaction further by examining the effect of
219   immune status on calling frequency separately within each attractiveness category. These
220   analyses found that attractive males called more frequently on average when injected with LPS
221   (8.36±2.74 calls/5 min, n=14) than with saline (3.28±1.54, n=14) (Table 1b) whereas LPS-
222   injected (6.14±3.02, n=14) and saline-injected (8.64±3.39, n=14) unattractive males did not
223   differ in their calling effort (Table 1c). Males also called more frequently as trials progressed
224   (Table 1). All males called more frequently as the trial progressed, with saline-injected males
225   also generally calling less than immune-challenged males (Table 1).

226   **Discussion**

227   We found that an immune challenge had little statistical effect on the calling effort of
228   unattractive males whereas an immune challenge caused attractive males to *increase* their calling
229   frequency compared with saline-injected controls. This result does not support the sexual
230   selection hypothesis that the calling effort of unattractive males should decline after an immune
231   challenge while that of attractive males would not.

232   In contrast, that immune-challenged attractive males elevated their calling rate relative to
233   controls supports the reproductive compensation hypothesis (see also the terminal invesment
234   hypothesis: Clutton-Brock, 1984): by increasing invesment in current fitness attractive males
235   might compensate for their reduced future fitness. On the other hand, the lack of difference
236   between control and treatment unattractive males does not support the reproductive
237   compensation hypothesis. The observed similarity between saline- and LPS-injected unattractive
238   males is counter-intuitive because immune-challenged unattractive males should have the
239   poorest prospects for survival and, thus, the most to gain from increased investment in
240   reproduction. Our results raise the question as to why attractive, but not unattractive, males were
241   able to increase their calling effort post-challenge? Perhaps attractive males possessed a larger
242   pool of resources from which to draw for use in both signalling and immunity compared with
243   unattractive males. The scaled mass indices did not differ, on average, between the male
244   attractiveness groups and suggests that the males in both groups had similar energetic reserves
245   (i.e. fat load) and water content since these two variables scale positively with SMI (see Kelly,
246   Tawes & Worthington, 2014). Despite the similarity in these two components of scaled mass
247   there might still have been important differences in the resource pools of the two groups. For
248   example, attractive males might have had more of a certain amino acid or other important
249   micronutrient that is critical to immune function. Thus, perhaps immune-challenged unattractive
250   males *are* terminally investing, but since they have a poor resource pool from which to draw, that
251   after allocating some portion of resources to immunity they can only achieve calling rates on par
252   with healthy counterparts (i.e. they cannot increase calling rates relative to control males).
253
254   Alternatively, perhaps unattractive male *G. texensis* invest in sexual signaling at the expense of
255   survival (i.e. immunity) regardless of whether they are sick or healthy. In other words,
256   unattractive males might adopt a strategy that resembles reproductive compensation (and
257   terminal investment) simply given their poor prospects of acquiring mates and whether they
258   recieve an immune-challenge makes little difference to their resource allocation straetgy. This
259   'live fast, die young' hypothesis predicts that there should be little reproductive benefit to
260   holding back resource consumption (e.g. via a reduced signaling rate) if caution yields no mates,
261   and thus, no fitness gains. That unattractive males (both saline- and LPS-injected) in our study
262   called at a rate similar to that of healthy attractive males suggests that unattractive males are
263   investing relatively heavily in signaling. This is likely not a general pattern across taxa as Hunt et
264   al. (2004) found that it is high-quality, attractive male *T. commodus* crickets that invest in calling
265   at the expense of longevity.
266
267   Both alternative explanations would benefit from an examinination of the investment in
268   immunity by healthy and sick individuals with the prediction being that terminally-investing
269   males should have a weaker immune response due to a shift of resources into current
270   reproduction. However, Sadd et al. (2006) showed that immune-challenged males that were

271 apparently terminally investing in current reproduction also had significantly higher
272 phenoloxidase activity compared with unchallenged males.
273
274 We also found that time had a significant effect on calling effort: as trials progressed over the
275 course of 5 hours, all males significantly called more often. The lack of a significant treatment by
276 time interaction suggests that males increase their calling effort independent of immune status or
277 sexual attractiveness. Rost and Honneger (1987) also observed that *G. campestris* males in the
278 wild increase their calling during the period from just after sunset to midnight.
279
280 Our study tested the hypothesis that higher-quality males suffer smaller costs to fitness-related
281 traits from an immune challenge because these males possess more resources with which to pay
282 such costs. We found that attractive males significantly increased their calling effort after an
283 immune-challenge while an immune challenge had little effect on signaling in unattractive
284 males. We suggest that our results might be explained by the reproductive compensation
285 hypothesis but more testing is required to unequivocally support this conclusion.
286

287 **References**
288 Adamo SA 1999. Evidence for adaptive changes in egg laying in crickets exposed to bacteria and
289   parasites. *Animal Behaviour* 57:117–124.
290 Adamo SA, Jensen M, Younger M 2001. Changes in lifetime immunocompetence in male and
291   female *Gryllus texensis* (formerly *G. integer*): trade-offs between immunity and
292   reproduction. *Animal Behaviour* 62:417–425.
293 Adamo SA, Roberts JL, Easy RH, Ross NW 2008. Competition between immune function and
294   lipid transport for the protein apolipophorin III leads to stress-induced immunosuppression
295   in crickets. *Journal of Experimental Biology* 211:531–538.
296 Agnew P, Koella JC, Michalakis Y 2000. Host life history responses to parasitism. *Microbes and*
297   *Infection* 2:891–896.
298 Bateman PW 1998. Assortative mating by both sexes in the cricket *Platygryllus primiformis*
299   (Orthoptera: Gryllidae; Gryllinae). *Transactions of the American Entomological Society*.
300 Brooks RC, Hunt J, Blows MW, Smith M, Bussiere LF, Jennions MD 2005. Experimental
301   evidence for multivariate stabilizing sexual selection. 59:871–880.
302 Bussiere LF, Hunt J, Jennions MD, Brooks RC 2006. Sexual conflict and cryptic female choice
303   in the black field cricket, *Teleogryllus commodus*. *Evolution* 60:792–800.
304 Clutton-Brock TH 1984. Reproductive effort and terminal investment in iteroparous animals.
305   *The American Naturalist* 123:212–229.
306 Fedorka KM, Mousseau TA 2007. Immune system activation affects male sexual signal and
307   reproductive potential in crickets. *Behavioral Ecology* 18:231–235.
308 Head ML, Hunt J, Jennions MD, Brooks RC 2005. The indirect benefits of mating with attractive
309   males outweigh the direct costs. *PLoS biology* 3:e33.
310 Heisler IL 1985. Quantitative genetic models of female choice based on "arbitrary" male
311   characters. *Heredity* 55:187–198.
312 Holzer B, Jacot A, Brinkhof MWG 2003. Condition-dependent signaling affects male sexual
313   attractiveness in field crickets, *Gryllus campestris*. *Behavioral Ecology* 14:353–359.
314 Hunt J, Brooks RC, Jennions MD, Smith M, Bentsen C, Bussiere LF 2004. High-quality male
315   field crickets invest heavily in sexual display but die young. *Nature* 432:1024–1027.
316 Jacobs AC, Zuk M 2011. Sex selection and parasites: Do mechanisms matter? In: Demas GE,

317   Nelson RJ eds. *Ecoimmunology*. Ecoimmunology. Oxford, UK: Oxford University Press,
318   468–496.
319   Jacot A, Scheuber H, Brinkhof M 2004. Costs of an induced immune response on sexual display
320   and longevity in field crickets. *Evolution* 58:2280–2286.
321   Jacot A, Scheuber H, Kurtz J, Brinkhof M 2005. Juvenile immune system activation induces a
322   costly upregulation of adult immunity in field crickets *Gryllus campestris*. *Proceedings of*
323   *the Royal Society of London Series B Biological Science* 272:63–69.
324   Kato Y, Motoi Y, Taniai K, Kadonookuda K, Hiramatsu M, Yamakawa M 1994. Clearance of
325   lipopolysaccharide in hemolymph of the silkworm *Bombyx mori:* Its role in the termination
326   of cecropin messenger-RNA induction. *Insect Biochemistry and Molecular Biology* 24:539–
327   545.
328   Kelly CD 2011. Reproductive and physiological costs of repeated immune challenges in female
329   Wellington tree weta (Orthoptera: Anostostomatidae). *Biological Journal of the Linnean*
330   *Society* 104:38–46.
331   Kelly CD 2014. Effect of an immune challenge on the functional performance of male weaponry.
332   *Behavioral Processes* 108:197–203.
333   Kelly CD, Jennions MD 2009. Sexually dimorphic immune response in the harem polygynous
334   Wellington tree weta *Hemideina crassidens*. *Physiological Entomology* 34:174–179.
335   Kelly CD, Tawes BR, Worthington AM 2014. Evaluating indices of body condition in two
336   cricket species. *Ecology and Evolution* 4:4476–4487.
337   Kivleniece I, Krams I, Daukšte J, Krama T, Rantala MJ 2010. Sexual attractiveness of immune-
338   challenged male mealworm beetles suggests terminal investment in reproduction. *Animal*
339   *Behaviour* 80:1015–1021.
340   Kolluru GR, Zuk M, Chappell MA 2002. Reduced reproductive effort in male field crickets
341   infested with parasitoid fly larvae. *Behavioral Ecology* 13:607–614.
342   Krams I, Daukšte J, Kivleniece I, Krama T, Rantala MJ, Ramey G, Šauša L 2011. Female choice
343   reveals terminal investment in male mealworm beetles, *Tenebrio molitor*, after a repeated
344   activation of the immune system. *Journal of Insect Science* 11:56–14.
345   Leman JC, Weddle CB, Gershman SN, Kerr AM, Ower GD, St John JM, Vogel LA, Sakaluk SK
346   2009. Lovesick: immunological costs of mating to male sagebrush crickets. *Journal of*
347   *Evolutionary Biology* 22:163–171.
348   López P, Gabirot M, Martin J 2009. Immune challenge affects sexual coloration of male Iberian
349   wall lizards. *Journal Of Experimental Zoology* 311A:96–104.
350   López S 1998. Acquired resistance affects male sexual display and female choice in guppies.
351   *Proceedings of the Royal Society of London B: Biological Sciences* 265:717–723.
352   McCurdy DG, Forbes MR, Boates JS 2000. Male amphipods increase their mating effort before
353   behavioural manipulation by trematodes. *Canadian Journal of Zoology* 78:606–612.
354   McKean K, Nunney L 2001. Increased sexual activity reduces male immune function in
355   *Drosophila melanogaster. Proceedings of the National Academy of Sciences of the United*
356   *States of America* 98:7904–7909.
357   McKean K, Nunney L 2005. Bateman's principle and immunity: Phenotypically plastic
358   reproductive strategies predict changes in immunological sex differences. *Evolution*
359   59:1510–1517.
360   Minchella DJ, Loverde PT 1981. A cost of increased early reproductive effort in the snail
361   Biomphalaria glabrata. *The American Naturalist*:876–881.
362   Mougeot FO, Redpath SM 2004. Sexual ornamentation relates to immune function in male red

363   grouse *Lagopus lagopus scoticus*. *Journal of Avian Biology* 35:425–433.
364 Nielsen ML, Holman L 2012. Terminal investment in multiple sexual signals: immune-
365   challenged males produce more attractive pheromones. *Functional Ecology* 26:20–28.
366 Polak M, Starmer WT 1998. Parasite–induced risk of mortality elevates reproductive effort in
367   male Drosophila. *Proceedings of the Royal Society of London B: Biological Sciences*
368   265:2197–2201.
369 Reaney LT, Knell RJ 2010. Immune activation but not male quality affects female current
370   reproductive investment in a dung beetle. *Behavioral Ecology* 21:1367–1372.
371 Rost R, Honegger HW 1987. The timing of premating and mating behavior in a field population
372   of the cricket *Gryllus campestris* L. *Behavioral Ecology and Sociobiology* 21:279–289.
373 Sadd BM, Holman L, Armitage H, Lock F, Marland R, Siva-Jothy MT 2006. Modulation of
374   sexual signalling by immune challenged male mealworm beetles (*Tenebrio molitor*, L.):
375   evidence for terminal investment and dishonesty. *Journal of Evolutionary Biology* 19:321–
376   325.
377 Saino N, Møller AP 1996. Sexual ornamentation and immunocompetence in the barn swallow.
378   *Behavioral Ecology* 7:227–232.
379 Scheuber H, Jacot A, Brinkhof MWG 2003. Condition dependence of a multicomponent sexual
380   signal in the field cricket *Gryllus campestris*. *Animal Behaviour* 65:721–727.
381 Schmid-Hempel P 2011. *Evolutionary Parasitology*. Oxford, UK: Oxford University Press.
382 Shackleton M, Jennions MD, Hunt J 2005. Fighting success and attractiveness as predictors of
383   male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of
384   no-choice tests. *Behavioural Ecology and Sociobiology* 58:1–8.
385 Sheldon B, Verhulst S 1996. Ecological immunology: costly parasite defences and trade-offs in
386   evolutionary ecology. *Trends in Ecology & Evolution* 11:317–321.
387 Simmons LW 1986a. Female choice in the field cricket *Gryllus bimaculatus* (De Geer). *Animal*
388   *Behaviour* 34:1463–1470.
389 Simmons LW 1986b. Inter-male competition and mating success in the field cricket, *Gryllus*
390   *bimaculatus* (de Geer). *Animal Behaviour* 34:567–579.
391 Simmons LW 1987. Sperm competition as a mechanism of female choice in the field cricket,
392   Gryllus bimaculatus. *Behavioural Ecology and Sociobiology* 21:197–202.
393 Skarstein F, Folstad I 1996. Sexual dichromatism and the immunocompetence handicap: an
394   observational approach using Arctic charr. *Oikos* 76:359.
395 R Development Core Team 2014. R: A language and environment for statistical computing.
396   Version 3.0.3. R Foundation for Statistical Computing, Vienna, Austria.
397 Tregenza T, Wedell N 1997. Definitive evidence for cuticular pheromones in a cricket. *Animal*
398   *Behaviour* 54:979–984.
399 Vainikka A, Rantala MJ, Seppälä O, Suhonen J 2007. Do male mealworm beetles, *Tenebrio*
400   *molitor*, sustain the honesty of pheromone signals under immune challenge? *Acta Ethologica*
401   10:63–72.
402 van Noordwijk A, de Jong G 1986. Acquisition and allocation of resources: Their influence on
403   variation in life-history tactics. *The American Naturalist* 128:137–142.
404 Viney ME, Riley EM, Buchanan KL 2005. Optimal immune responses: immunocompetence
405   revisited. *Trends in Ecology & Evolution* 20:665–669.
406 Wagner WE, Hoback W 1999. Nutritional effects on male calling behaviour in the variable field
407   cricket. *Animal Behaviour* 57:89–95.
408 Wedell N, Tregenza T 1999. Successful fathers sire successful sons. *Evolution* 53:620.

409   Wickham H 2009. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer.

411 **Figure caption**
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413 **Figure 1.** Mean (±SE) number of calls made by male *G. texensis* crickets during each of the ten
414 30 min observation periods. Regression lines for each factorial combination were fit using a
415 mixed model (poisson family of errors) with male ID entered as a random effect. LPS-A = LPS-
416 injected attractive males (n=14); Saline-A = saline-injected attractive males (n=14); LPS-U =
417 LPS-injected unattractive males (n=14); Saline-U = saline-injected unattractive males (n=14).

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Calling bouts for attractive and unattractive males that were injected with either saline or LPS

Figure 1. Mean (±SE) number of calls made by male *G. texensis* crickets during each of the ten 30 min observation periods. Regression lines for each factorial combination were fit using a mixed model (poisson family of errors) with male ID entered as a random effect. LPS-A = LPS-injected attractive males (n=14); Saline-A = saline-injected attractive males (n=14); LPS-U = LPS-injected unattractive males (n=14); Saline-U = saline-injected unattractive males (n=14).
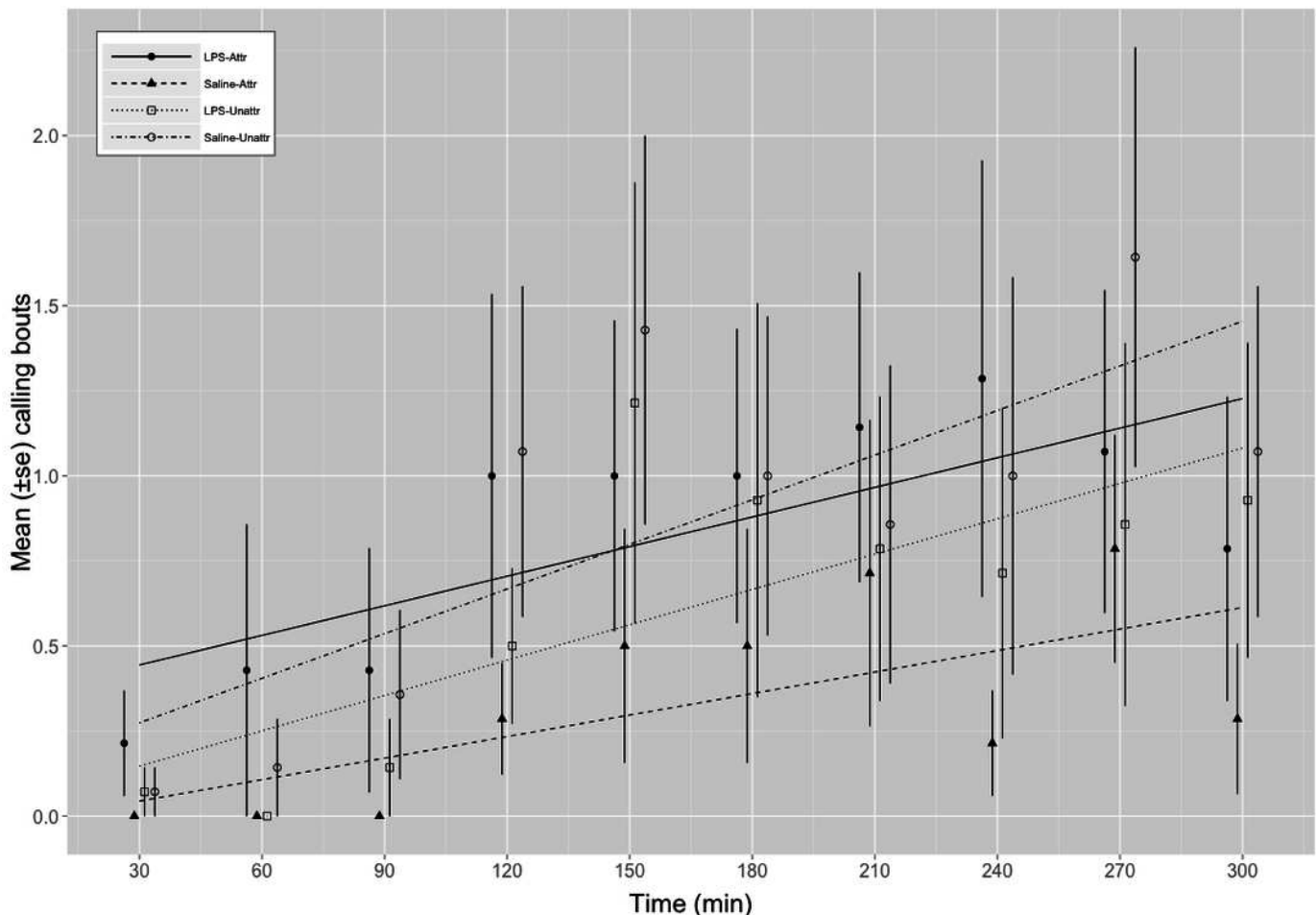
Table 1(on next page)

Results for statistical models examining effect of experimental treatment on and male sexual attractiveness on calling effort.

Table 1. Results from models (see text) with Z tests for estimated parameters. Values in bold are statistically significant at alpha = 0.05.

Table 1. Results from models (see text) with Z tests for estimated parameters. Values in bold are statistically significant at alpha = 0.05.

Response	Predictor	β	SE	Z	P
a) Calling (N=56)	Intercept	-1.911	0.652	-2.931	0.003
	Attractiveness(Un)	-1.855	0.984	-1.886	0.059
	Immune status(Sa)	**-2.056**	**1.012**	**-2.033**	**0.042**
	Time	**0.004**	**0.001**	**3.205**	**0.001**
	Immune status(Sa):Attractiveness(Un)	**3.224**	**1.425**	**2.263**	**0.024**
	Immune status(Sa):Time	0.003	0.002	1.516	0.130
	Attractiveness(Un):Time	0.002	0.002	1.374	0.170
	Immune status(Sa):Attractiveness(Un):Time	-0.004	0.003	-1.419	0.156
b) Attractive males (N=28)	Intercept	-1.773	0.563	-3.15	0.001
	Immune status(Sa)	**-1.942**	**0.881**	**-2.204**	**0.027**
	Time	**0.003**	**0.001**	**3.205**	**0.001**
	Immune status(Sa):Time	0.003	0.002	1.516	0.129
c) Unattractive males (N=28)	Intercept	-4.074	0.952	-4.278	1.88×10^{-05}
	Immune status(Sa)	1.298	1.152	1.127	0.260
	Time	**0.005**	**0.001**	**4.392**	$\mathbf{1.12 \times 10^{-05}}$
	Immune status(Sa):Time	-0.000	0.001	-0.378	0.705

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