Sexual selection on a female copulatory device in an insect with nuptial gifts

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Male genitalia are rapidly evolving structures, often driven by sexual conflict and/or sexual selection to increase fertilization success. Although sexual selection on females can be strong in systems where males provide offspring care or feed their mates, sometimes resulting in the evolution of female ornaments to acquire male investments, there are no studies of sexual selection on female genitalia. In New Zealand ground weta, *Hemiandrus pallitarsis*, females possess a genitalic device (the accessory organ) that is necessary for successful copulation and the acquisition of spermatophylax glandular gifts, which are probably important in avoiding starvation during a months-long period when caring for (their lifetime production of) eggs. Here, we test the hypothesis that the accessory organ is a sexually selected device in *Hemiandrus pallitarsis* by measuring the female Bateman gradient and directional sexual selection on the

accessory organ (female mating frequency estimated from microsatellite analysis of offspring and/or stored sperm). As predicted, there was both positive Bateman gradients for females, and evidence of directional sexual selection on accessory organ length. Although organ length did not correlate well with female fecundity (eggs in abdomen), it may increase mating success by indicating maternal condition and thus quality of her offspring care.

Introduction

Genitalia and associated structures (secondary genitalia) are some of the most rapidly evolving animal traits, often exhibiting extreme variation among closely related species (Eberhard 1985; Sloan and Simmons 2019). While genital diversity has been suggested to be a result of species isolation ("lock and key" hypothesis; Mayr 1963; Eberhard 1985; Sloan and Simmons 2019), genital evolution in males is thought to be driven mainly by sexual selection for increased fertilization success (Eberhard 1985; Sloan and Simmons 2019). Because females are typically more selective when mating (Darwin 1871; Trivers 1972), elaborate male genitalia may have evolved in the context of cryptic female choice, where males with the most "attractive" stimulatory genitalia experience the greatest fertilization success (Lloyd 1979; Thornhill 1983; Eberhard 1985; Simmons 2001; Sloan and Simmons 2019). Alternatively, genitalia may function in manipulating female mating behaviour and/or her sperm stores (e.g., grasping devices that coerce copulations or prevent her from remating; Arnqvist and Rowe 2005). Although much of the focus has been on male traits, females are not passive in this process. In response to, for example, male grasping genitalia, females undergo rapid genital co-evolution to reduce potential costs of mating (Arnqvist and Rowe 2005) or to bias fertilization success in favour of high-quality males (Eberhard 1985; Sloan and Simmons 2019). A well-known example is the

47 abdominal structure of female bedbugs (*Cimex lectularius)* that minimize the harm of traumatic
48 insemination (penile piercing of her abdomen) by males (Morrow and Arnqvist 2003).
49 In contrast to these examples of "typical" mating roles, the factors influencing male and
50 female fitness are expected to differ when males make large investments in reproduction, such as
51 paternal care or nuptial gifts. Females receiving these direct benefits are expected to seek
52 additional matings while the potential for frequent mating by males can be limited by investment
53 cost, resulting in male preferences (Trivers 1972; Gwynne 1990; 1991; 2016; Gwynne and
54 Simmons 1990; Simmons 1992; Herridge et al. 2016) for high quality females that increase male
55 reproductive success (Bonduriansky 2001; Herridge et al. 2016). In these systems, especially
56 those where females depend on male investments for offspring production, female competition
57 can cause strong sexual selection on females for traits that are attractive to males (Tobias et al.
58 2012; Herridge et al. 2016; Hare and Simmons 2018). Well-known examples are sex-specific
59 female ornaments in pipefish with paternal care (abdominal color pattern; Flanagan et al. 2014)
60 and in empid dance flies (legs, wings, or abdomens; Cumming 1994; Murray et al 2018) where
61 females rely on male-provided nutrition (insect prey items) to develop their eggs (Downes 1970).
62 Virtually unstudied is direct sexual selection on sex specific female genitalic traits where
63 copulation is associated with acquisition of goods or services from males. Much like males,
64 female genital traits may be expected to evolve either in the context of male mate choice where
65 certain female genital traits have evolved such as in the context of stimulating males to invest or
66 to coerce the male into supplying more food. Suggested examples are (1) the interspecifically
67 varying ridges on female subgenital plates of Australian *Kawanaphila* katydids that grasp the
68 male's genitalia, perhaps allowing longer copulation duration and thus the delivery of a larger
69 spermatophylax gift (Gwynne 2001); and (2) a penis-like female genital modification in two

genera of psocopteran cave insects, *Neotrogla* and *Afrotrogla* where males transfer nutritious semen to females during copulation, thought to be an important source of food (Yoshizawa et al. 2018). The penis-like gynosome, is inserted into the male's genital opening and anchors him during copulation (Yoshizawa et al. 2014), allowing females to prolong the duration of copulation and thus the amount of food-gift received (Yoshizawa et al. 2014; Yoshizawa et al. 2018).

Our study species has female "secondary" genitalia, abdominal devices found in a clade of New Zealand "short-tailed" ground weta (*Hemiandrus*, Anostostomatidae, Orthoptera: Trewick et al. 2020). Similar to *Neotroglea*, male ground weta feed their mates, in this case with a glandular spermatophylax that the mating male adheres to the female's abdomen (separate from the sperm capsule) and is eaten by her after copulation (Gwynne 2004). The female device is an elongated abdominal modification on the 6th sternite, known as an accessory organ (Salmon 1950). In contrast to other species of *Hemiandrus* that, like most crickets and relatives (suborder: Ensifera), abandon their eggs after injecting them into the substrate using long ovipositors (Gwynne 2004; Taylor Smith et al. 2013), "short-tailed" females care for their eggs and newly-hatched offspring (their lifetime reproduction) in an underground chamber for a period of 5-6 months (hence the "short-tailed" reduced ovipositor; Gwynne 2004; Gwynne 2005; Taylor Smith 2015). Care-providing females do not eat so male mating gifts likely represent important nutrition to avoid starvation during the lengthy care period, potentially leading females to compete for mating (feeding) opportunities (Gwynne 2004; Gwynne 2005). Because of this, the accessory organ, positioned close to where the sticky spermatophylax gift is adhered, is expected to be involved in the acquisition of gifts. This structure is not used as a holding device (as suggested in Gwynne 2002) for the gift (Gwynne 2005).

Male genitalia in many weta (Anostostomatidae) and some related ensiferans, include genital hooks (falci) on the dorsal side of the 10th segment (Johns 2001) which hook onto the edge of one of the of the female's sternites during copulation (the male is positioned beneath the female: Gwynne 2002). This is likely an ancestral behaviour, used to anchor the female for stability or to coerce her into copulation (D. Gwynne, personal communication). While some weta species have sclerotised cuticle on the sternite edge that receives the male hooks, female short-tailed ground weta are unique in possessing the accessory-organs modifications on the 6th sternite, to which the male genitalia attach (D. Gwynne, personal communication). Females of our study species, *H. pallitarsis,* possess the most elaborate accessory organ (fig. 1), which protrudes from the abdomen. After pairing (female mounted above male), males first make genitalic contact with the accessory organ then move their genitalia back to attach to the female's primary genitalia when they insert the sperm ampulla (Gwynne 2004; Gwynne 2005). Following this, the male detaches from her primary genitalia, but his genitalia remain attached to her abdominal accessory organ while he extends paired phalli that deposit the bilobed spermatophylax gift (Gwynne 2002; Gwynne 2004; Gwynne 2005). The accessory organ is positioned between paired males falci pressed onto its dorsal surface, while his paired epiprocts appear to be inserted ventral to the female organ, and the distal edge of the male's 8th abdominal segment pushes into the region between the female's 5th and 6th sternites (photographic evidence: D. Gwynne unpublished). The female accessory organ has not evolved via sexual conflict to thwart copulation attempts (as in some gerrid water striders: Arnqvist and Rowe 2005) because it is apparently necessary for mating: copulation fails when this device is experimentally removed (Gwynne 2005).

115 Extensive interspecific variation in the accessory organ of short-tailed species (Johns
116 2001; Trewick et al. 2020) suggests they are sexually-selected devices in females, possibly
117 driven by competition for male gifts. This variation is opposite to the usual Darwinian pattern,
118 where male external genitalia vary greatly across closely related species (Gwynne 2005).
119 Interspecific variation in female accessory organs is probably not related to species isolation
120 ("lock and key" hypothesis; Mayr 1963; Eberhard 1985) since there is greater support for sexual
121 selection on complex genitalia in males (Eberhard 1985; Gwynne 2005) and, for ground weta,
122 mating interactions between species are more likely prevented by the species-specific
123 precopulatory drumming behaviour of males and females that precede pairing (Gwynne 2004;
124 Gwynne 2005; Chappell et al. 2012). Furthermore, there is little overlap in ground weta species
125 ranges (Trewick and Bland 2011; Trewick et al. 2020), which makes species mis-pairing
126 unlikely.
127 The accessory organ functions in the transfer of sperm and the gift but may also be under
128 sexual selection to secure mates as envisioned by Eberhard (1990) for males. Examples are the
129 attractive genital structures (e.g., long penes in guppies, *Poecilia reticulata*; Brooks and
130 Caithness 1995, brightly colored penes in some lizards; Bohme 1983) or stimulatory devices
131 (e.g., genital lobes in *Drosophila pachea*; Lefèvre et al. 2021, pneumopophyses in the tsetse fly
132 *Glossina pallidipes;* Briceño et al. 2012, and genital titillators in tettigoniid bushcrickets; Wulff
133 and Lehmann 2016) that increase female receptivity to mating or fertilization. Support for this
134 hypothesis for females in ground weta is that males make close contact with the accessory organ
135 prior to mating and have been observed to reject females after probing her mid-abdomen
136 (Gwynne 2005). As females with longer accessory organs tend to have a greater number of eggs
137 that develop to the nymphal stage (female body size controlled; Gwynne 2005), this trait may

138 help females obtain matings (gifts) by signalling quality to males (as in Andersson 1994;
139 Berglund et al. 1997; Barry 2015). Thus, this structure may have evolved via male choice in the
140 context of female mate/gift acquisition (Gwynne 2005),

141 As the female organ is inserted between two parts of the male genitalia, an alterative
142 hypothesis is that it has evolved to anchor between the male genital falci and epiprocts, and thus
143 assure spermatophylax-gift transfer, in a similar manner to the anchoring female penis of
144 *Neotroglea* bark lice that was suggested by Yoshizawa et al. (2014) as a sexually-selected organ
145 to obtain a nutritious sperm capsule. Gwynne's (2005) finding that accessory organ length
146 correlates with the number of eggs that develop to the nymphal stage is also consistent with this
147 hypothesis, as it may alternatively suggest that *H. pallitarsis* accessory organs result in longer
148 copulation and more nuptial food. Despite this, there is no direct evidence that any female
149 copulation devices such as accessory organs or female penes are ornaments subject to sexual
150 selection. Here, we test this hypothesis by measuring sexual selection on female ground weta *H.*
151 *pallitarsis*. Using estimates of female mating success from microsatellite analysis of stored
152 sperm and measures of lifetime female reproductive success, we determine the degree of sexual
153 selection on females (Bateman gradient) and estimate directional sexual selection on several
154 accessory organ traits. If the accessory organ is sexually-selected in the context of gift
155 acquisition, we predict both directional selection on accessory organ size (length; Gwynne 2005)
156 and that this trait will correlate with female fecundity. Characteristic of sexual selection on a sex
157 (Bateman 1948; Arnold 1994), we also expect there to be a positive relationship between female
158 mating success and reproductive success (Bateman gradient).
159

160 **Methods**

161 **Specimen Collection: Bateman Gradient**

162 To determine Bateman gradients for female ground weta *(H. pallitarsis*), females were
163 collected in 2002 (n=10) and 2017 (n=17) from two main sites on the North Island of New
164 Zealand: Kiriwhakapapa trail near Masterton (-40.807627, 175.546532) and private gardens in
165 Palmerston North (-40.413909, 175.662814). Collections took place from mid-February to early
166 March, nearing the end of the ground weta mating season (Gwynne 2004). Live specimens were
167 transported back to our lab in Toronto, Canada to oviposit and obtain measures of lifetime
168 reproductive success. This was a lengthy process that involved placing females in artificial brood
169 chambers formed from potter's clay (2002) or soil (2017) and allowing 2-3 months for them to
170 lay eggs, as well as an additional 5-6 months for eggs to mature. Once eggs began hatching,
171 offspring were counted (including the total number of eggs laid and the number of developed
172 eggs: hatched nymphs or eggs with visible eye spots), and mothers were preserved with their
173 broods at -20°C. We then dissected females to remove the spermatheca, containing the sperm
174 stored after each mating. We isolated the sperm contents from the spermatheca using >70%
175 ethanol, which causes sperm to harden into a pellet (Tripet et al. 2001) and stored them at -20°C.
176 We then imaged females under a dissecting scope fitted with a digital camera and measured
177 pronotum length as an estimate of body size (as in Taylor Smith 2015). Unfortunately, four
178 broods were excluded from the study because females died and rapidly decayed, preventing any
179 further analysis.

180

181 **Specimen Collection: Sexual Selection on Accessory organs**

182 To measure sexual selection on accessory organs, an additional *H. pallitarsis* females (n=58)
183 were collected in the years 2012, 2016, 2017, and 2019 from the same two sites: Kiriwhakapapa

184 and Palmerston North. Following capture, specimens were killed via freezing and placed in
185 strong ethanol (70-95%) to be taken to Toronto, Canada. We then imaged all females under a
186 dissecting scope and measured them using ImageJ software. We measured pronotum length
187 (body size; Taylor Smith 2015) as well as accessory organ length and width (fig. 2). Prior to
188 measurement, all accessory organs were removed and soaked in a 5% Decon 90 solution for 1
189 hour at 40ºC. This was done in order to soften and restore the structures (Banks and Williams
190 1972), as prolonged preservation caused many to become bent and brittle. We then dissected the
191 spermathecae from all females, isolated the sperm contents using strong ethanol (Tripet et al.
192 2001), and stored them at -20°C. During the dissection, we also counted the number of
193 developing ova in the female's abdomen.
194
195 **Locus Characterization & Microsatellite Analysis**
196 Our microsatellite library for *H. pallitarsis* was developed by Genetic Marker Services
197 (Brighton, UK) and consisted of nine candidate loci. Each locus was characterized using samples
198 from both populations, including the females in the current study plus an additional 24 males.
199 After extracting DNA from hind leg tissue of adults, we conducted PCR, each reaction
200 containing 5.75µL sterile filtered water, 1µL 10X PCR reaction buffer, 0.2µL 10mM dNTP
201 mixture, 0.5µL of forward primer (each with fluorescent labels), 0.5µL reverse primer, and 1µL
202 of template DNA. Thermocycling was done using an Eppendorf Mastercycler with the following
203 temperature regime: 2 minutes at 94°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds
204 at 60°C, and 15 seconds at 72°C, then an additional 3 minutes at 72°C. PCR products were sent
205 to The Centre for Applied Genomics in Sick Kids Hospital in Toronto, ON for fragment analysis
206 on an Applied Biosystems 3730xl capillary sequencer. The resulting electropherograms were

207 examined using GeneMarker V1.97, which allowed us to identify fluorescence peaks,
208 representing alleles. For each of the microsatellite loci, we assessed amplification quality and the
209 degree of polymorphism. Using GENEPOP version 4.2 (Raymond and Rousset 1995), we
210 determined if loci deviated from Hardy-Weinberg equilibrium (heterozygote deficiency test), and
211 computed estimates of null allele frequencies (Dempsters EM method) overall and for each
212 population separately. Finally, we assessed the degree of genetic differentiation between our two
213 study populations by determining F_{st} values (GENEPOP version 4.2) for each locus.
214 After identifying three suitable microsatellite loci (wet80, wet81, and wet89), we
215 conducted PCR on samples of stored sperm and offspring using the same protocol as above. We
216 extracted DNA from stored sperm using the Qiagen DNeasy Blood and Tissue Extraction kit.
217 We followed a modified version of the recommended protocol, which involved adding 12μL
218 DTT to each sample to improve lysis of sperm cells and dissolving DNA in a reduced volume
219 (50μL) of elution buffer. We extracted DNA from offspring tissue (nymph or egg) using a
220 Proteinase K based extraction method with multiple ethanol washes. The resulting concentration
221 of DNA was much higher for offspring relative to sperm and so we diluted offspring samples to
222 roughly 50ng/μL.
223
224 **Female mating success**
225 For measures of the Bateman gradient, we used a combination of paternity data and
226 analysis of stored sperm to estimate female mating frequency. Based on offspring genotypes, we
227 first determined the minimum number of sires in each brood using the parentage program
228 GERUD 2.0 (Jones 2005). Any alleles found in female's sperm stores that were not present in
229 the offspring genotypes were assumed to be additional mates and were factored into a female's

230 total mating frequency. We used a method described by Bretman and Tregenza (2005) to
231 estimate the most probable number of mates at each locus, given the additional alleles observed
232 in stored sperm (See Bretman and Tregenza 2005; Herridge 2016 thesis for details). This is
233 considered a less conservative method than allele counting (which assumes heterozygosity)
234 although it is still limited by an inability to compute multi-locus data (Bretman and Tregenza
235 2005; Herridge 2016) and so we used the highest estimate. One female was known to have
236 mated, based on the observed consumption of a spermatophylax gift; however, the male failed to
237 inseminate the female as his genotype did not appear in her sperm stores, nor her offspring.
238 Because acquisition of male-provided materials (in this case the spermatophylax gift) is the key
239 factor affecting female fitness (Clutton-Brock 2007), an additional mate was added to this
240 female's total mate number.

241 To measure directional sexual selection on accessory organs, we again estimated female
242 mating frequency using the probable mate number (Bretman and Tregenza 2005), this time using
243 all the male alleles detected in stored sperm. In cases where an allele was found in sperm stores
244 that was not previously detected in the population, it was assumed to be rare and assigned a
245 frequency equal to the least common allele in the population. We were unable to estimate the
246 mate number for four females due to poor amplification at all three loci.

247

248 **Statistical Analysis: Bateman Gradients**

249 We measured the Bateman gradient for female *H. pallitarsis* using our estimates of
250 mating frequency and the number of offspring in single broods each female cares for before
251 dying (lifetime reproductive success). We calculated standardized Bateman gradients (β'_{ss};
252 Bateman 1948; Arnold 1994) using separate ordinary least squares (OLS) regressions of the

253 relative number of mates and relative number of offspring- both the number of eggs laid, and the
254 number of eggs that developed to the nymphal stage (determined by hatching or visible eye
255 spots). Decomposition of several females prevented us from controlling for body size in our
256 regression model, however, we used the remaining females to test whether the inclusion of
257 pronotum length (an indicator of body size e.g., Taylor Smith 2015) altered our results and
258 whether body size alone explained significant variation in reproductive success. Significance of
259 β'_{ss} was determined using a generalized linear model (GLM) with Poisson error distribution of
260 the unstandardized traits (similar to Gerlach et al. 2012; Martin et al. 2015). We tested whether
261 measures of sexual selection varied by collection year or population by including interaction
262 terms in our regression model.
263
264 **Statistical Analysis: Sexual selection on female accessory organs**
265 We assessed the relationship between phenotype (body size, accessory organ length,
266 accessory organ width) and number of mates in female ground weta using multiple least squares
267 regression. We first z-score standardized all trait values and converted the fitness response
268 variable to relative mating success by dividing by the mean. Low sample size limited the
269 complexity of our regression model; thus, we were unable to include quadratic and correlational
270 terms (Lande and Arnold 1983) in our analysis. Our estimates of sexual selection only included
271 linear selection gradients, indicative of directional selection. Apparent differences in the strength
272 of sexual selection between the two collection sites led us to examine sexual selection in each
273 population separately. We re-standardized the data within each population and again regressed
274 the trait values against female mating success. Additionally, we tested if the accessory organ has

275 the potential to signal female fecundity by measuring the relationship between female phenotype
276 and the number of developing eggs in their abdomen.
277
278 **Results**
279 **Locus Characterization**
280 All microsatellite loci used in the study were polymorphic, with wet80, wet 81, and
281 wet89 all showing more than 20 distinct alleles (table 1). Several of the loci deviated from
282 Hardy-Weinberg Equilibrium (wet18, wet21, wet81, wet83, wet89), however the frequency of
283 null alleles was estimated to be low (< 20%; Dakin and Avise 2004) for both wet81 and wet89
284 (table 1), consistent across populations (table 2). Issues with unreliable or non-specific
285 amplification were present at three loci (wet18, wet14, wet22, and wet27), limiting their use in
286 future studies. Finally, the degree of genetic differentiation (F_{st}) was low between our two study
287 populations, ranging from 0 to 0.06 across all loci tested (table 2).
288
289 **Bateman Gradients**
290 Consistent with our prediction that female ground weta are subject to sexual selection, the
291 standardized Bateman gradient (table 3; Appendix) was positive for both measures of female
292 reproductive success, but only reached significance when using the number of developed eggs.
293 Estimates of β'_{ss} were similar (0.16 for eggs laid; 0.43 for eggs laid and developed) when we
294 controlled for body size using the available pronotum data. Consistent with Gwynne (2005),
295 female body size alone had no significant effect on either measure of reproductive success (R^2 =
296 0.136, p = 0.120 for eggs laid; R^2 = 0.09, p = 0.563 for eggs developed). When using number of
297 eggs to estimate reproductive success, Bateman gradients varied significantly between

298 populations (interaction term from GLM; $p<0.0001$). While mating success apparently had no
299 effect on the number of eggs laid had in the Palmerston North population, this relationship was
300 much stronger in Kiriwhakapa. Notably, estimates of β'_{ss} were significant in Kiriwhakapa
301 regardless of the measure of reproductive success used (eggs laid or eggs developed; table 3;
302 Appendix)
303
304 **Sexual selection on accessory organs**
305 Female mating success ranged from 0-6 mates (mean: 2.3 +/- 1.4 SD) and increased
306 significantly with accessory organ length (table 4). Larger body size and narrower accessory
307 organs were also associated with increased mating success; however, these trends were non-
308 significant. Although there was no significant interaction between phenotype and population
309 (OLS; body size $p=0.112$; accessory organ length $p=0.749$; accessory organ width $p=0.129$), we
310 found that selection appeared to be stronger on all traits in Palmerston North when the two
311 populations were analyzed separately (table 4). Although the magnitude of selection was higher
312 in Palmerston North, the best predictor of female mating success was accessory organ length in
313 both populations, followed by body size and accessory organ width (table 4; Appendix).
314 Interestingly, females with wider accessory organs had lower mating success in the Palmerston
315 North population, but marginally higher mating success in the Kiriwhakapapa population.
316
317 **Accessory organs as a signal of fecundity**
318 None of the measured traits significantly predicted the number of eggs in a female's
319 abdomen although those with wider accessory organs tended to be more fecund (table 5). Again,
320 trends differed between collection sites. In Palmerston North, larger females tended to have

321 somewhat greater fecundity and in Kiriwhakapapa, wider accessory organs were associated with
322 higher fecundity (table 5).
323
324 **Discussion**
325 In *H. pallitarsis* ground weta, sexual competition for male-provided nutrition
326 (spermatophylaxes) prior to an extended maternal care period without food probably creates an
327 opportunity for sexual selection on females when they "forage" for mating gifts. Morphological
328 evidence that females are subject to sexual selection comes from the existence of an apparent
329 female ornament, a secondary-genitalic device known as an accessory organ (Gwynne 2002;
330 Gwynne 2005) that varies greatly between species (Johns 2001; Trewick et al. 2020). Here, we
331 provide empirical evidence that *H. pallitarsis* females are subject to sexual selection, by showing
332 a significant positive relationship between female mating success and lifetime reproductive
333 success (Standardized Bateman gradient; β'_{ss}). Although estimates of the Bateman gradient were
334 not as high as some species where sexually selected females compete for mates, such as bean
335 beetles or pipefish (reviewed in Hare and Simmons 2018; Fromonteil et al. unpublished), they
336 were higher than the non-significant relationship between number of sires and absolute hatching
337 success found for females of another anostostomatid weta, *Hemideina crassidens,* where males
338 are sexually competitive (males fight using very large mandibular weaponry) (β= 0.04; Nason
339 and Kelly 2020). Measures of β'_{ss} for female *H. pallitarsis* ground weta tended to be stronger
340 when using the number of developed eggs to estimate reproductive success rather than just the
341 number of eggs laid. Because these females do not eat for several months while providing care,
342 we suggest that the nutritional benefits from each mating may have greater effects on female
343 fitness via effects on her survival and quality of care rather than on fecundity (total eggs laid). A

344 comparable example is found in birds, where mate-feeding increases hatching success by
345 reducing a mother's energetic constraints and thus improving her nest attentiveness throughout
346 the incubation period (Galván and Sanz 2011).

347 Further evidence of sexual selection on female ground weta comes from our finding that
348 accessory organs are subject to directional selection. There was a significant positive relationship
349 between the length of the accessory organ and female mating success, supporting the hypothesis
350 that this this structure functions as a device that ensures successful mating (there was also a non-
351 significant trend for larger females to mate more frequently). Sexual selection on accessory
352 organs appeared to be greater at the Palmerston North site, as all female traits had a stronger
353 effect on mating success than in Kiriwhakapapa. Despite this, none of these estimates were
354 significant, likely due to the small sample size in the Palmerston North population. Notably,
355 body size appeared to have very little effect on mating success in Kiriwhakapapa and although
356 non-significant, accessory organ width had an opposite (positive) effect on female mating
357 success relative to Palmerston North. Given the apparent lack of genetic differentiation between
358 our study populations (F_{st} low compared to typical values in insects; Ward et al 1992) and their
359 proximity (roughly 100km) relative to the total species range, it is interesting that the strength of
360 sexual selection, and potentially even the traits subject to sexual selection differ. There are
361 morphological differences between populations of *H. pallitarsis* (Chappell et al. 2012), with
362 Chappell et al. (2012) finding that some populations differed in size as well as the drumming
363 pattern performed by males prior to mating. While this variation was not congruent with genetic
364 differences among populations (suggesting that widely separated *H. pallitarsis* populations
365 comprise one interbreeding species; Chappell et al. 2012), it is possible that patterns of selection
366 and mate attraction differ slightly between populations of ground weta in Palmerston North

367 compared to Kiriwhakapapa. Regardless, the accessory organ appears to be involved in acquiring
368 mates and in particular, the length of the device appears to play a role in mating success in both
369 populations.

370 None of the traits measured, including female body size, were related to the number of
371 eggs in a female's abdomen (fecundity). However, this does not mean the accessory organ does
372 not signal attractiveness to males. One reason for the non-significant finding may be that the
373 number of eggs in the abdomen is not the main indicator of female quality for the mating male.
374 Because females oviposit all their eggs and then engage in a long maternal care period, during
375 which the mother does not eat (Gwynne 2004; J. Browne personal observation), choosy males
376 may prioritise female condition and thus the potential quality of care she is able to provide, as
377 this will determine the number of surviving offspring. Some support comes from Gwynne's
378 (2005) study showing a positive relationship between accessory organ length and number of
379 hatched nymphs rather than eggs in the abdomen. Alternatively, these females are able to
380 prolong copulation and thus obtain more nutrients for offspring production.

381 As noted previously, the accessory organ's role in competing successfully for copulations
382 does not exclude a mechanical function for this device (many male genitalia function in sperm
383 transfer as well as stimulating mates; Eberhard 1990). Given the presumed ancestral state where
384 males used genitalic hooks to latch onto a female's unmodified 6th sternite, the accessory organ
385 could have either evolved in the context of mate assessment (in the region that interacts closely
386 with sensitive male genitalia) or as an anchoring device between the male genital falci and
387 epiprocts, that assures full spermatophylax-gift transfer. The association between paired male
388 genitalic structures (falci, paraprocts, and the end of his 8th sternite; D. Gwynne personal
389 communication) almost certainly achieve stability while first the sperm capsule (ampulla) and

390 then the spermatophyax gift is delivered. It is possible, however, that like many male structures
391 (Eberhard 1990), different parts of the accessory organ serve different functions. We note for
392 example that the distal end of the accessory organ has a very prominent bilobed shape that
393 appears to align with the lobes of the males' paired paraprocts (in several *Hemiandrus* species:
394 Gwynne 2002). Because our measures of the accessory organ were simple linear ones, we may
395 not have captured the full complexity of this trait that may be important to successful mating.
396 Future studies should focus on quantifying the shape of this structure and its role in mating.
397 Another limitation of the study is that we were only able to measure directional selection
398 on accessory organs. Due to our limited sample size, we did not compute estimates of non-linear
399 or correlational selection, meaning we may have an incomplete understanding of the type of
400 selection acting on these structures (Lande and Arnold 1983). In particular, it would be valuable
401 to test for stabilizing sexual selection on female accessory organs as preference for
402 intermediately ornamented females has been predicted in some species (typically those with
403 more elaborate and costly traits) due to the energetic costs of investing in these traits (Fitzpatrick
404 1995; Chenoweth et al. 2006; Wheeler et al. 2012). Further, genital structures may be expected
405 to be under stabilizing sexual selection in cases where they have evolved to fit the average male
406 (Eberhard 1998).
407 In conclusion, we show evidence of sexual selection on female ground weta, *H.*
408 *pallitarsis* generally, and also on a secondary genitalic structure known as the accessory organ.
409 Directional selection for longer accessory organs suggests this device is a sexual ornament or a
410 coercive device used to increase mating success, however the strength of selection differed
411 between the two collection sites. Although this structure does not correlate with measures of
412 female fecundity, it may well signal female condition (and thus perhaps her genitalic grasping

ability) or quality of care as important predictors of female reproductive success as females engage in a long and costly maternal care period.

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Tables and Figures

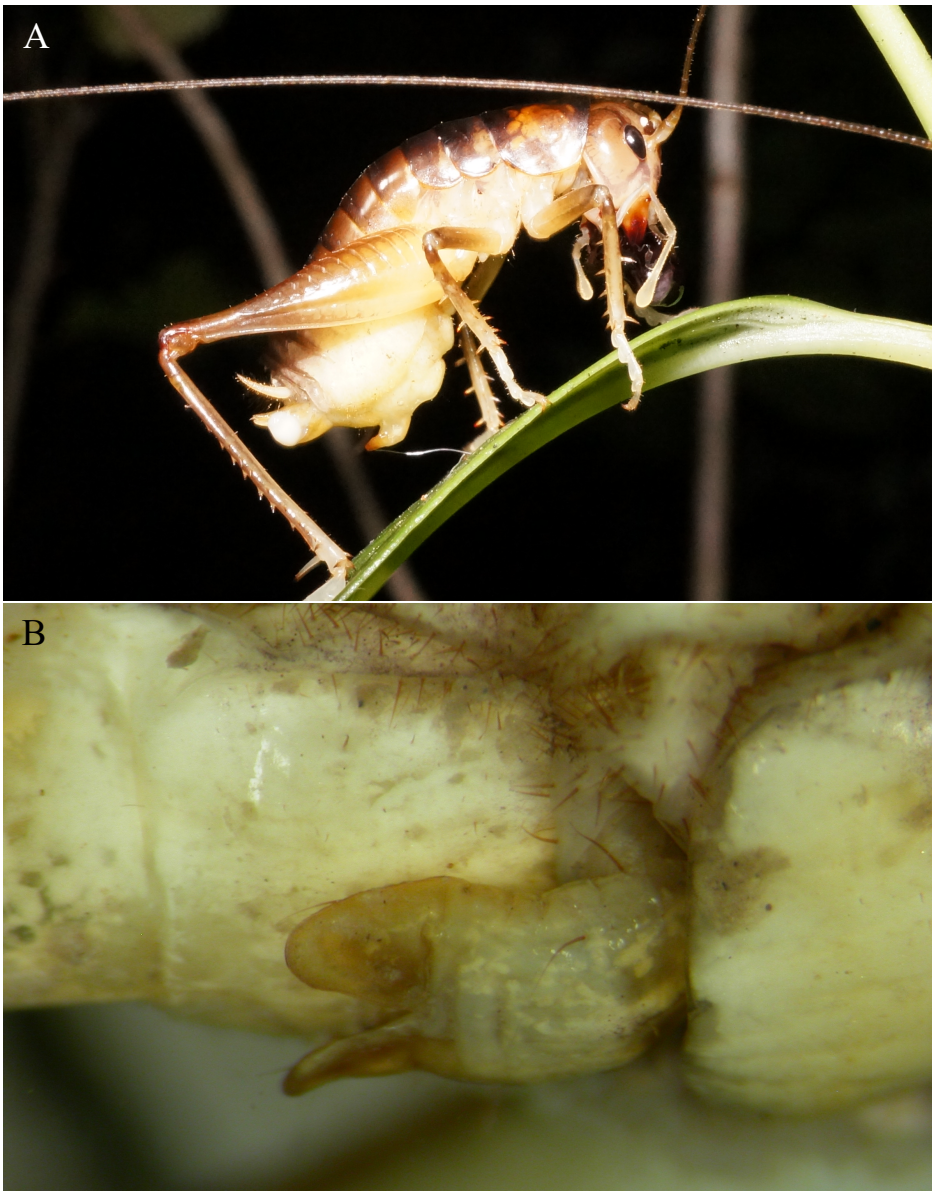

Figure 1. Lateral view of the extended female secondary genitalic accessory organ (6th sternite, located between the hind and middle legs) in A) a newly-mated female *H. pallitarsis* and B) a close-up of the organ. Photos by Darryl Gwynne.

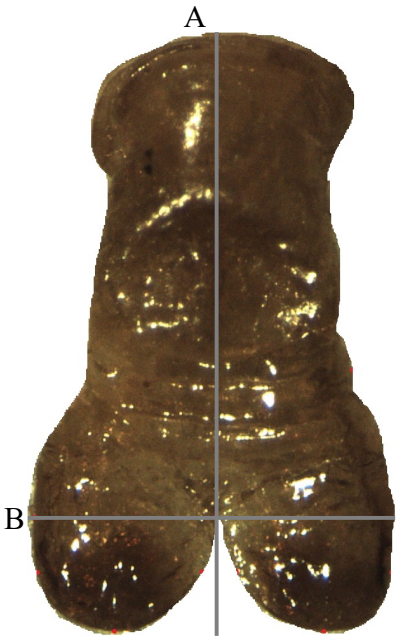

Figure 2. Modified 6th sternite, the accessory organ, on the abdomen of female *H. pallitarsis* ground weta. Image shows the structure removed from the body and the two measurements taken: A) accessory organ length, from the point where the organ attaches to the body to the furthest point on the distal end and B) accessory organ width, left to right on the widest point of the distal end.

458 **Table 1.** Characterization of microsatellite loci developed for ground weta *H. pallitarsis*

Locus	Primer Sequence (5'-3')	bp	label	N	A	H_E	H_O	f_{null}
wet14	F: TTT TCC AAG TGG GTT ATC GTG R: CGG TAG CTA ATG GTC GGT GT	169-183	HEX	44	6	0.434	0.500	0.025
wet18	F: TGC GAT GTT ACC AAA ACA TCA T R: AGG CGT CCG ATA TCT CCT TT	96-114	HEX	32	9	0.640	0.312*	0.280
wet21	F: TAG TCC ATT ATT CAA TAC CC R: GAG AAT AAG AAG TAG CAA AAT AA	91-127	6-FAM	28	12	0.822	0.357*	0.270
wet22	F: GTA CTG CAT CTT GTC TGA GAG C R: AAG AAG TGT CCA AGG CCA TC	155-235	6-FAM	25	18	0.892	0.840	0.032
wet27	F: TCG CAA GAG TAG GCT ACG TG R: CGT GCG AGT AGG TGT TTG AG	85-97	6-FAM	38	7	0.177	0.184	0.000
wet80	F: TCA ATG AAA TGT TTC AGA CAG ACA R: GCA CAA AGA GCC ATC TGT CA	124-172	HEX	73	22	0.911	0.890	0.013
wet81	F: ACG ACG AGG AAA TGA AAT GT R: GTT GAC AAA ACT CGG GCA GT	158-226	HEX	72	24	0.917	0.736*	0.103
wet83	F: GGT AGA CAA AGC GGT TGA TG R: CAA CAT CCT CCC GTA GTT TT	120-158	6-FAM	56	5	0.650	0.411*	0.156
wet89	F: CCC TTG TTC CGT CCT ACA AG R: CAA AAC CCC ACC ATG ACT TT	180-258	6-FAM	75	21	0.920	0.773*	0.092

459 **bp**= allele size range, **N**= number of individuals tested, **A**= number of alleles detected, $\mathbf{H_E}$ =

460 Expected heterozygosity, $\mathbf{H_O}$ = Observed heterozygosity * indicates significant deviation from

461 Hardy-Weinberg equilibrium, $\mathbf{F_{null}}$ = frequency of null alleles

462 All estimates were obtained from GENEPOP version 4.2 (Raymond and Rousset 1995)

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473 **Table 2.** Characterization of microsatellite loci for ground weta *H. pallitarsis* by population.

	Kiriwhakapapa					Palmerston North					
Locus	**N**	**A**	**H_E**	**H_O**	**f_{null}**	**N**	**A**	**H_E**	**H_O**	**f_{null}**	**F_{st}**
wet14	22	3	0.21	0.23	0.000	22	5	0.58	0.77	0.019	0.16
wet18	17	4	0.53	0.18*	0.230	15	7	0.71	0.47*	0.302	0.06
wet21	13	9	0.80	0.31*	0.303	15	7	0.82	0.40*	0.221	0.01
wet22	7	8	0.87	0.86	0.051	18	13	0.88	0.83	0.000	0.04
wet27	19	5	0.20	0.21	0.000	19	4	0.15	0.16	0.000	0.00
wet80	42	17	0.91	0.93	0.002	31	14	0.85	0.84	0.000	0.06
wet81	36	11	0.89	0.56*	0.172	36	20	0.92	0.92	0.007	0.03
wet83b	31	4	0.66	0.52*	0.084	25	5	0.61	0.28*	0.244	0.036
wet89	44	16	0.90	0.77*	0.085	31	12	0.88	0.77*	0.051	0.05

474 **N=** number of individuals tested**, A**= number of alleles detected, **H_E** = Expected heterozygosity,

475 **H_o** = Observed heterozygosity * indicates significant deviation from Hardy-Weinberg

476 equilibrium**, f_{null} =** estimated frequency of null alleles

477 All calculations were done using GENEPOP version 4.2 (Raymond and Rousset 1995)

478

479 **Table 3.** Standardized Bateman Gradients (β'_{ss}) for female ground weta, *H. pallitarsis*. Estimates

480 are shown overall and in each population.

			Kiriwhakapapa (n=12)		Palmerston North (n=11)	
Reproductive success	**β'_{ss}**	**p-value**	**β'_{ss}**	**p-value**	**β'_{ss}**	**p-value**
Number of eggs laid	0.12	0.072	0.44	<0.001*	-0.10	0.294
Number of developed eggs	0.39	<0.001*	0.49	<0.001*	0.36	0.001*

481 β'_{ss} = Standardized Bateman gradient

482 *Denotes significant p-values, determined using GLM with Poisson error.

483 **Table 4.** Relationship between female phenotype (traits z-score standardized) and mating
484 success (relative number of mates) in ground weta (*H. pallitarsis*). Shown overall and by
485 population.

			Kiriwhakapapa n=35		Palmerston North n=19	
Female traits	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Body size	0.106	0.262	0.037	0.692	0.165	0.449
Accessory organ length	0.195	0.049*	0.103	0.301	0.234	0.318
Accessory organ width	-0.023	0.807	0.031	0.732	-0.079	0.711

486 β= standardized directional selection gradients
487 * Denotes significant p-value determined by OLS regression
488
489 **Table 5.** Relationship between female phenotype (traits z-score standardized) and relative
490 number of eggs in the abdomen. Shown overall and by population.

			Kiriwhakapapa n=37		Palmerston North n=21	
Female traits	**β_R**	**p-value**	**β_R**	**p-value**	**β_R**	**p-value**
Body size	0.001	0.992	-0.077	0.596	0.147	0.610
Accessory organ length	-0.043	0.719	-0.054	0.731	-0.004	0.987
Accessory organ width	0.248	0.057	0.256	0.092	0.006	0.983

491 β_R = standardized regression coefficients
492 * Denotes significant p-value determined by OLS regression
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Appendix: Visualization of directional sexual selection on female *H. pallitarsis*

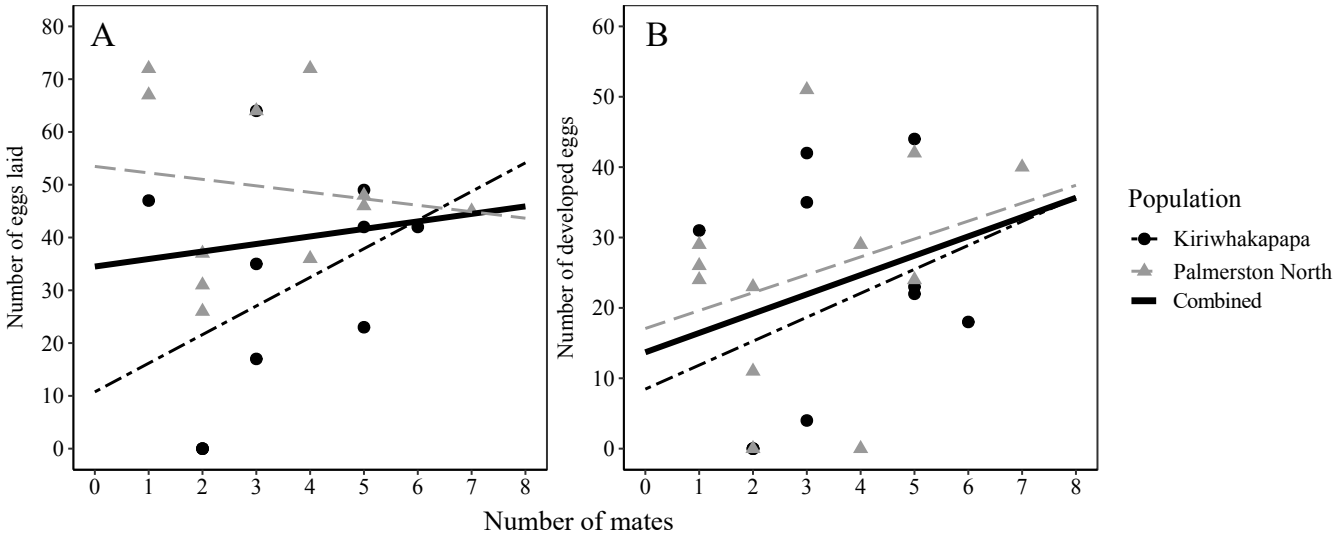

Figure A1 Raw Bateman Gradients for female *H. pallitarsis* showing relationship between number of mates and either A) number of eggs laid or B) number of developed eggs across populations

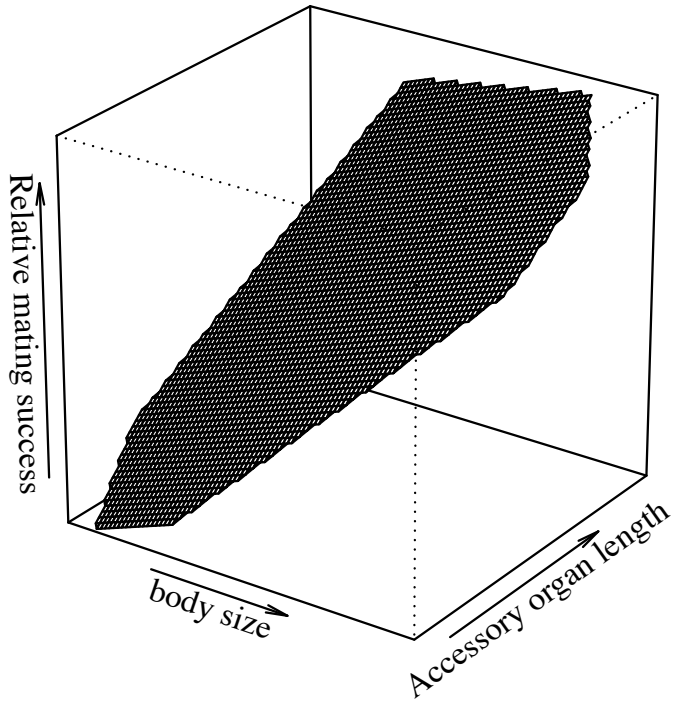

Figure A2 Thin-plate spline visualization of female body size and accessory organ length and their effect on relative mating success in female ground weta (*H. pallitarsis*) collected from Kiriwhakapapa. Relative mating success was estimated from the number of alleles found in a female's stored sperm.

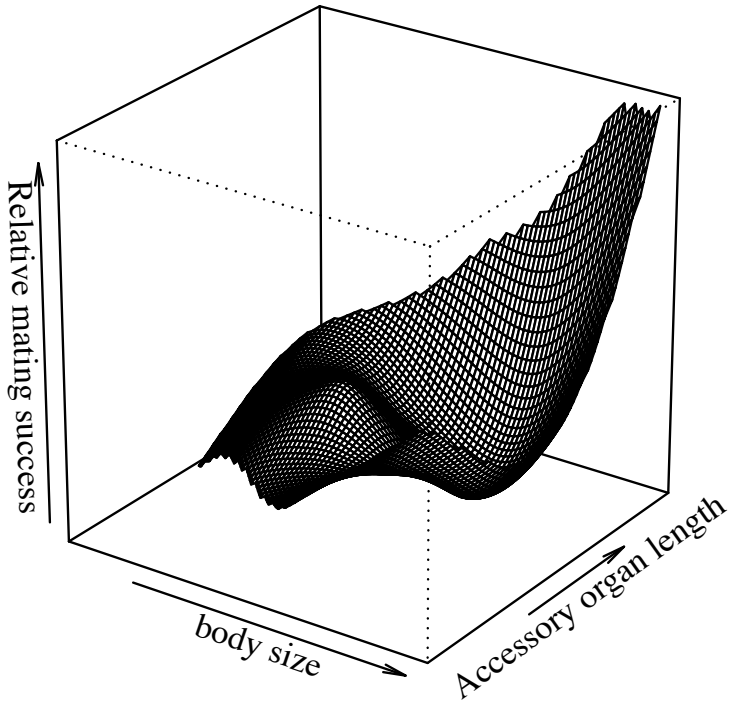

Figure A3 Thin-plate spline visualization of female body size and accessory organ length and their effect on relative mating success in female ground weta (*H. pallitarsis*) collected from Palmerston North. Relative mating success was estimated from the number of alleles found in a female's stored sperm.

B

A

A
B