Sexual selection on female copulatory devices in an insect with nuptial gifts

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Genitalia are rapidly evolving structures, often driven by sexual conflict and/or sexual selection (especially in males) for coercive, attractive, or stimulatory traits that increase fertilization success. In systems where males make large investments in offspring (i.e., paternal care or nuptial gifts) females may be subject to strong sexual selection, sometimes resulting in the evolution of female ornaments or genital adaptations that increase acquisition of mates and/or male goods and services. In *Hemiandrus*, short-tailed (reduced ovipositor) ground weta, where males feed their mates spermatophylax gifts, females possess a secondary genitalic device (the accessory organ) that is necessary for successful copulation. Females may compete for access to these gifts in order to avoid starvation because following the mating season, they enter a months-long maternal care period without eating. Thus, the accessory organ may be a sexually selected device involved in the acquisition of spermatophylax meals. Here, we test this hypothesis in female ground weta *Hemiandrus pallitarsis* by determining whether an increase in mating frequency (estimated from microsatellite analysis of stored sperm) increases lifetime reproductive success (the Bateman gradient). Additionally, we test if the accessory organ is under directional sexual selection by measuring its relationship with female mating success. As predicted, there was both positive Bateman gradients for females, suggestive of sexual selection, 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 has been suggested that genital diversity is a result of species isolation ("lock and key" hypothesis; Mayr 1963; Eberhard 1985; Sloan and Simmons 2019), genital evolution in males is mainly thought to be driven 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 manipulate female mating behaviour and/or her sperm stores (e.g. grasping devices that coerce copulations or prevent her remating; Arnqvist and Rowe 2005), or 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). 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 bias fertilization success in favour of the highest quality males (Eberhard 1985; Sloan and Simmons 2019). A well-known example are the abdominal structures of female bedbugs (*Cimex lectularius)* that minimize the harm of traumatic insemination (piercing of her abdomen) by males (Morrow and Arnqvist 2003).

In contrast to these "typical" mating roles, the factors influencing male and female fitness are expected to differ when males make large investments in reproduction, such as paternal care or nuptial gifts. Females receiving these direct benefits are highly motivated to mate, but the potential for frequent mating by males can be limited by investment cost, resulting in male

preferences (Trivers 1972; Gwynne 1990; 1991; 2016; Gwynne and Simmons 1990; Simmons 1992; Herridge et al. 2016) for high quality females that increase male reproductive success (Bonduriansky 2001; Herridge et al. 2016). In these systems, especially those where females depend on male investments for offspring production, female competition can cause strong sexual selection on females for traits that are attractive to males (Tobias et al. 2012; Herridge et al. 2016; Hare and Simmons 2018). Well-known examples are sex-specific female ornaments in pipefish with paternal care (abdominal color pattern; Flanagan et al. 2014) and in empid dance flies (legs, wings, or abdomens; Cumming 1994; Murray et al 2018) where females rely on male-provided nutrition (insect prey items) to develop their eggs (Downes 1970).

Less well studied is direct sexual selection on sex specific female genitalic traits in insects where copulation is associated with acquisition of nuptial gifts from males. Much like males, female genital traits may be expected to evolve either in the context of male mate choice where certain female genital traits have evolved such as in the context of stimulating males to invest or to coerce the male into supplying more food. Suggested examples are (1) the interspecifically varying ridges on female subgenital plates of Australian *Kawanaphila* katydids that grasp the male's genitalia, perhaps allowing longer copulation duration and thus the delivery of a larger 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, a device 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 soil 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 genitaila 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 behavior, 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, which the male genitalia attach (D. Gwynne, personal communication). Females of our study species, *H. pallitarsis,* possess the most elaborate accessory organ (Figure 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 personal communication). The female accessory organ has not evolved via sexual conflict to thwart copulation attempts (as in some gerrids: Arnqvist and Rowe 2005) because it is apparently necessary for mating: copulation fails when this device has been experimentally removed (Gwynne 2005).

Extensive interspecific variation in the accessory organ of short-tailed species (Johns 2001; Trewick et al. 2020) suggests they are sexually-selected devices in females, possibly driven by competition for male gifts. This variation is opposite to the usual Darwinian pattern, where male external genitalia vary greatly across closely related species (Gwynne 2005). Variation in female accessory organs is probably not related to species isolation ("lock and key" hypothesis; Mayr 1963; Eberhard 1985) since there is greater support for sexual selection on complex genitalia in males (Eberhard 1985; Gwynne 2005) and, for ground weta, mating

interactions between species are more likely prevented by the species-specific precopulatory drumming behaviour of males and females that precede pairing (Gwynne 2004; Gwynne 2005; Chappell et al. 2012). Furthermore, there is little overlap in ground weta species ranges (Trewick and Bland 2011; Trewick et al. 2020), which makes mis-pairing unlikely.

The accessory organ probably functions to allow sperm and the gift to be transferred, but may also be under sexual selection to secure mates as envisioned by Eberhard (1990). Examples in other animals are the attractive genital structures (e.g., long penes in guppies, *Poecilia reticulata*; Brooks and Caithness 1995, brightly colored penes in some lizards; Bohme 1983) or stimulatory devices (e.g., genital lobes in *Drosophila pachea*; Lefèvre et al. 2021, pneumopophyses in the tsetse fly *Glossina pallidipes;* Briceño et al. 2012, and genital titillators in tettigoniid bushcrickets; Wulff and Lehmann 2016) that increase female receptivity to mating or fertilization. Support for this hypothesis in ground weta is that males make close contact with the accessory organ prior to mating and have been observed to reject females after probing her mid-abdomen (Gwynne 2005). As females with longer accessory organs tend to have a greater number of eggs that develop to the nymphal stage (female body size controlled; Gwynne 2005), this trait may help females obtain matings by signalling quality to males (as in Andersson 1994; Berglund et al. 1997; Barry 2015). Thus, this structure may have evolved via male choice in the context of female mate (and thus food) acquisition (Gwynne 2005),

As the female organ is inserted between two parts of the male genitalia, an alterative hypothesis is that it has evolved to anchor the device between the male genital falci and epiprocts, and thus assure spermatophylax-gift transfer, in a similar manner to the anchoring female penis of *Neotroglea* bark lice that was suggested by Yoshizawa et al. (2014) as a sexually-selected organ to obtain a nutritious sperm capsule. Gwynne's (2005) finding that

accessory organ length correlates with the number of eggs that develop to the nymphal stage is also consistent with this prediction, as it may alternatively suggest that *H. pallitarsis* accessory organs result in longer copulation and more nuptial food. Despite this, there is no direct evidence that any female copulation devices such as accessory organs or female penes are ornaments subject to sexual selection. Here, we test this hypothesis by measuring sexual selection on female ground weta *H. pallitarsis*, and determine whether the accessory organ is a sexual device used to acquire mates. Using estimates of female mating success from microsatellite analysis of stored sperm and measures of lifetime female reproductive success, we determine the degree of sexual selection on females (Bateman gradient) and estimate directional sexual selection on several accessory organ traits. If the accessory organ is sexually-selected in the context of gift acquisition, we predict both directional selection on accessory organ size (length; Gwynne 2005) and that this trait will correlate with female fecundity. Characteristic of sexual selection on a sex (Bateman 1948; Arnold 1994), we also expect there to be a positive relationship between female mating success and reproductive success (Bateman gradient).

Methods

Specimen Collection: Bateman Gradient

To determine Bateman gradients for female ground weta *(H. pallitarsis*), females were collected in 2002 (n=10) and 2017 (n=17) from two main sites on the North Island of New Zealand: Kiriwhakapapa trail near Masterton (-40.807627, 175.546532) and private gardens in Palmerston North (-40.413909, 175.662814). Collections took place from mid-February to early March, nearing the end of the ground weta mating season (Gwynne 2004). Live specimens were transported back to our lab in Toronto, Canada to oviposit eggs to obtain measures of lifetime

reproductive success. This was a lengthy process that involved placing females in artificial brood chambers formed from potter's clay (2002) or soil (2017) and allowing 2-3 months for females to lay eggs, as well as an additional 5-6 months for eggs to mature. Once eggs began hatching, offspring were counted (including the total number of eggs laid and the number of developed eggs: hatched nymphs or eggs with visible eye spots), and mothers were preserved with their broods at -20°C. We then dissected females to remove the spermatheca, containing the sperm stored after each mating. We isolated the sperm contents from the spermatheca using >70% ethanol, which causes sperm to harden into a pellet (Tripet et al. 2001) and stored them at -20°C. We then imaged females under a dissecting scope fitted with a digital camera and measured pronotum length as an estimate of body size (as in Taylor Smith 2015). Unfortunately, four broods were excluded from the study because females died and rapidly decayed, preventing any further analysis.

Specimen Collection: Sexual Selection on Accessory organs

To measure sexual selection on accessory organs, an additional *H. pallitarsis* females (n=58) were in the years 2012, 2016, 2017, and 2019 from the same two sites: Kiriwhakapapa and Palmerston North. Following capture, specimens were killed via freezing and placed in strong ethanol (70-95%) to be returned to Toronto, Canada. We then imaged all females under a dissecting scope and measured them using ImageJ software. We measured pronotum length (body size; Taylor Smith 2015) as well as accessory organ length and width (Figure 4.1). Prior to measurement, all accessory organs were removed and soaked in a 5% Decon 90 solution for 1 hour at 40ºC. This was done in order to soften and restore the structures (Banks and Williams 1972), as prolonged preservation caused many to become bent and brittle. We then dissected the

spermathecae from all females, isolated the sperm contents using strong ethanol (Tripet et al. 2001), and stored them at -20°C. During the dissection, we also counted the number of developing ova in the female's abdomen.

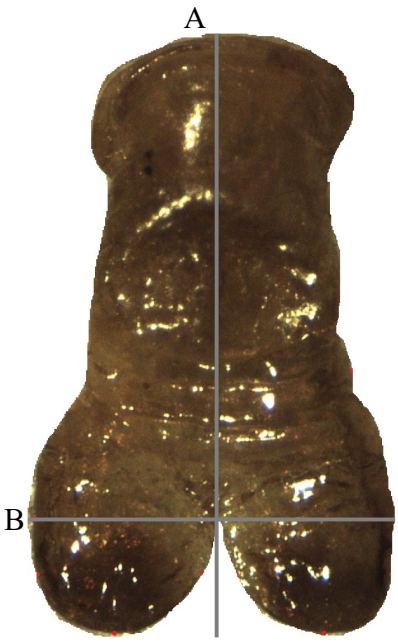

Figure 4.1 Modified 6th sternite, the accessory organ, on the abdomen of female *H. pallitarsis* ground weta. Image shows the structure removed from the body and the two measurements taken: A) accessory organ length, from the point where the organ attaches to the body to the furthest point on the distal end and B) accessory organ width, left to right on the widest point of the distal end.

Microsatellite analysis

We extracted DNA from stored sperm using the Qiagen DNeasy Blood and Tissue Extraction kit. We followed a modified version of the recommended protocol, which involved adding 12µL DTT to each sample to improve lysis of sperm cells and dissolving DNA in a reduced volume (50µL) of elution buffer. We also extracted DNA from offspring tissue (nymph

or egg) using a Proteinase K based extraction method with multiple ethanol washes. The resulting concentration of DNA was much higher for offspring relative to sperm and so we diluted offspring samples to roughly 50ng/μL.

We then conducted polymerase chain reaction (PCR) on each sample using a set of three fluorescence-labelled microsatellite markers (wet80, wet81, and wet89; See Chapter 1 for primer details and characterization of loci) developed by Genetic Marker Services in Brighton, UK. Primers were combined into two separate PCR reactions, each containing 5.75μL sterile filtered water, 1μL 10X PCR reaction buffer, 0.2μL 10mM dNTP mixture, 0.5μL of forward primer (each with fluorescent labels), 0.5μL reverse primer, and 1μL of template DNA. Thermocycling was done using an Eppendorf Mastercycler with the following temperature regime: 2 minutes at 94°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 15 seconds at 72°C, then an additional 3 minutes at 72°C. PCR products were sent to The Centre for Applied Genomics in Sick Kids Hospital in Toronto, ON for fragment analysis on an Applied Biosystems 3730xl capillary sequencer. The resulting electropherograms were examined using GeneMarker V1.97, which allowed us to identify fluorescence peaks, representing alleles.

Female mating success

For measures of the Bateman gradient, we used a combination of paternity data and analysis of stored sperm to estimate female mating frequency. Based on offspring genotypes, we first determined the minimum number of sires in each brood using the parentage program GERUD 2.0 (Jones 2005). If any alleles were discovered in a female's sperm stores that were not present in the offspring genotypes, we assumed that they belonged to additional mates that inseminated the female but failed to sire any of the offspring analyzed. we used a method

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

We used this same method (probable mate number; Bretman and Tregenza 2005) of estimating female mating frequency to measure sexual selection on accessory organs. In this case we determined the most probable mate number using all the male alleles detected in stored sperm. In cases where an allele was found in sperm stores that was not previously detected in the population, it was assumed to be rare and assigned a frequency equal to the least common allele in the population. Further, we were unable to estimate the mate number for four females due to poor amplification at all three loci.

Statistical Analysis: Bateman Gradients

We measured the Bateman gradient for female *H. pallitarsis* using our estimates of mating frequency and the number of offspring in single broods each female cares for before dying (lifetime reproductive success). We calculated standardized Bateman gradients (β'_{ss};

Bateman 1948; Arnold 1994) using separate ordinary least squares (OLS) regressions of the relative number of mates and relative number of offspring- both the number of eggs laid, and the number of eggs that developed to the nymphal stage (determined by hatching or visible eye spots). Decomposition of several females prevented me from controlling for body size in our regression model, however, we used the remaining females to test whether the inclusion of pronotum length (an indicator of body size e.g., Taylor Smith 2015) altered our results and whether body size alone explained significant variation in reproductive success. Significance of β'_{ss} was determined using a generalized linear model (GLM) with Poisson error distribution of the unstandardized traits (similar to Gerlach et al. 2012; Martin et al. 2015). We tested whether measures of sexual selection varied by collection year or population by including interaction terms in our regression model.

Statistical Analysis: Sexual selection on female accessory organs

We assessed the relationship between phenotype (body size, accessory organ length, accessory organ width) and number of mates in female ground weta using multiple least squares regression. We first z-score standardized all trait values and converted the fitness response variable to relative mating success by dividing by the mean. Low sample size limited the complexity of our regression model; thus, we was unable to include quadratic and correlational terms (Lande and Arnold 1983) in our analysis. Our estimates of sexual selection only included linear selection gradients, indicative of directional selection. Apparent differences in the strength of sexual selection between the two collection sites led us to examine sexual selection in each population separately. We re-standardized the data within each population and again regressed the trait values against female mating success. Additionally, we tested if the accessory organ has

the potential to signal female fecundity by measuring the relationship between female phenotype and the number of developing eggs in their abdomen.

Results

Bateman Gradients

Consistent with our prediction that female ground weta are subject to sexual selection, the standardized Bateman gradient (table 1; Appendix) was positive for both measures of female reproductive success, but only reached significance when using the number of developed eggs. Estimates of β'_{ss} were similar (0.16 for eggs laid; 0.43 for eggs laid and developed) when we controlled for body size using the available pronotum data. Consistent with Gwynne (2005), female body size alone had no significant effect on either measure of reproductive success ($R^2 = 0.136$, $p = 0.120$ for eggs laid; $R^2 = 0.09$, $p = 0.563$ for eggs developed). When using number of eggs to estimate reproductive success, Bateman gradients varied significantly between populations (interaction term from GLM; $p<0.0001$). While mating success apparently had no effect on the number of eggs laid had in the Palmerston North population, this relationship was much stronger in Kiriwhakapa. Notably, estimates of β'_{ss} were significant in Kiriwhakapa regardless of the measure of reproductive success used (eggs laid or eggs developed; table 1; Appendix).

Table 1 Standardized Bateman Gradients (β'_{ss}) for female ground weta, *H. pallitarsis*. Estimates 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*

β'_{ss} = Standardized Bateman gradient

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

Sexual selection on accessory organs

Female mating success ranged from 0-6 mates (mean: 2.3 +/- 1.4 SD) and increased significantly with accessory organ length (Table 2). Larger body size and narrower accessory organs were also associated with increased mating success; however, these trends were non-significant. Although there was no significant interaction between phenotype and population (OLS; body size p=0.112; accessory organ length p=0.749; accessory organ width p=0.129), we found that selection appeared to be stronger on all traits in Palmerston North when the populations were analyzed separately (Table 2). Although the magnitude of selection was higher in Palmerston North, the best predictor of female mating success was accessory organ length in both populations, followed by body size and accessory organ width (Table 2; Appendix). Interestingly, females with wider accessory organs had lower mating success in the Palmerston North population, but marginally higher mating success in the Kiriwhakapapa population.

Table 2 Relationship between female phenotype (traits z-score standardized) and mating success (relative number of mates) in ground weta (*H. pallitarsis*). Shown overall and by 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

β= standardized directional selection gradients

* Denotes significant p-value determined by OLS regression

Accessory organs as a signal of fecundity

None of the measured traits significantly predicted the number of eggs in a female's abdomen although those with wider accessory organs tended to be more fecund (Table 3). Again, trends differed between collection sites. In Palmerston North, larger females tended to have somewhat greater fecundity and in Kiriwhakapapa, wider accessory organs were associated with higher fecundity (Table 3).

Table 3 Relationship between female phenotype (traits z-score standardized) and relative 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

β_R = standardized regression coefficients

* Denotes significant p-value determined by OLS regression

Discussion

In *H. pallitarsis*, sexual competition for male-provided nutrition (spermatophylaxes) prior to an extended maternal care period without food probably creates an opportunity for sexual selection on females. Morphological evidence that females are subject to sexual selection comes from the existence of an apparent female ornament, a secondary-genitalic device known as an accessory organ (Gwynne 2002; Gwynne 2005). Here, we provide empirical evidence that *H. pallitarsis* females are subject to sexual selection, by showing a significant positive relationship between female mating success and lifetime reproductive success (Standardized Bateman gradient; β'_{ss}). Although estimates of the Bateman gradient were not as high as some species where sexually selected females compete for mates, such as bean beetles or pipefish (reviewed in Hare and Simmons 2018; Fromonteil et al. unpublished), they were higher than the non-significant relationship between number of sires and absolute hatching success found for females of another anostostomatid weta, *Hemideina crassidens,* where males are sexually competitive (males fight using very large mandibular weaponry) (β= 0.04; Nason and Kelly 2020). Measures of β'_{ss} for female *H. pallitarsis* ground weta tended to be stronger when using the number of developed eggs to estimate reproductive success rather than just the number of eggs laid. Because these females do not eat for several months while providing care, we suggest that the nutritional benefits from each mating may have greater effects on female fitness via effects on her survival and quality of care rather than on fecundity (total eggs laid). A comparable example is found in birds, where mate-feeding increases hatching success by reducing a mother's energetic constraints and thus improving her nest attentiveness throughout the incubation period (Galván and Sanz 2011).

Further evidence of sexual selection on female ground weta comes from our finding that accessory organs are subject to directional selection. There was a significant positive relationship between the length of the accessory organ and female mating success, supporting the hypothesis that this this structure functions as an ornament that ensures successful mating (there was also a non-significant trend for larger females to mate more frequently). Sexual selection on accessory organs appeared to be greater at the Palmerston North site, as all female traits had a stronger effect on mating success than in Kiriwhakapapa. Despite this, none of these estimates were significant, likely due to the small sample size in the Palmerston North population. Notably, body size appeared to have very little effect on mating success in Kiriwhakapapa and although non-significant, accessory organ width had an opposite (positive) effect on female mating success relative to Palmerston North. It is interesting that the strength of sexual selection, and potentially even the traits subject to sexual selection, appear to differ between the two study populations. This may be influenced by variation in the environment or population density between the two sites (Mobley and Jones 2007; Siepielski et al. 2009). However, morphological differences between populations of *H. pallitarsis* are not uncommon (Chappell et al. 2012). Chappell et al. (2012) found that some populations of *H. pallitarsis* differed in size as well as the drumming pattern performed by males prior to mating. While this variation was not congruent with genetic differences among populations (suggesting that widely separated *H. pallitarsis* populations comprise one interbreeding species; Chappell et al. 2012), it is possible that patterns of selection and mate attraction differ slightly between populations of ground weta in Palmerston North compared to Kiriwhakapapa. Regardless, the accessory organ appears to be involved in acquiring mates and in particular, the length of the device appears to play a role in mating success in both populations.

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

As noted previously, the accessory organ's role in competing successfully for copulations does not exclude the possibility that the device has a mechanical function as well (many male genitalia function in sperm transfer as well as stimulating mates; Eberhard 1990). Given the presumed ancestral state where males used genitalic hooks to latch onto a female's unmodified 6th sternite, the accessory organ could have either evolved in the context of mate assessment (in the region that interacts closely with sensitive male genitalia) or as an anchoring device between the male genital falci and epiprocts, that assures full spermatophylax-gift transfer. The association between paired male genitalic structures (falci, paraprocts, and the end of his 8th sternite; D. Gwynne personal communication) almost certainly achieve stability while first the sperm capsule (ampulla) and then the spermatophyax gift is delivered. It is possible, however, that like many male structures (Eberhard 1990), different parts of the accessory organ serve different functions. We note for example that the distal end of the accessory organ has a very

prominent bilobed shape that appears to align with the lobes of the males' paired paraprocts (in several *Hemiandrus* species: Gwynne 2002). Because our measures of the accessory organ were simple linear ones, we may not have captured the full complexity of this trait that may be important to successful mating. Future studies should focus on quantifying the shape of this structure and its role in mating.

Another limitation of the study is that we were only able to measure directional selection on accessory organs. Due to our limited sample size, we did not compute estimates of non-linear or correlational selection, meaning we may have an incomplete understanding of the type of selection acting on these structures (Lande and Arnold 1983). In particular, it would be valuable to test for stabilizing sexual selection on female accessory organs as preference for intermediately ornamented females has been predicted in some species (typically those with more elaborate and costly traits) due to the energetic costs of investing in these traits (Fitzpatrick 1995; Chenoweth et al. 2006; Wheeler et al. 2012). Further, genital structures may be expected to be under stabilizing sexual selection in cases where they have evolved to fit the average male (Eberhard 1998).

In conclusion, we show evidence of sexual selection on female ground weta, *H. pallitarsis* generally, and also on a secondary genitalic structure known as the accessory organ. Directional selection for longer accessory organs suggests this device is a sexual ornament or a coercive device used to increase mating success, however the strength of selection differed between the two collection sites. Although this structure does not correlate with measures of 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 when females engage in long and risky maternal care periods.

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Appendix: Visualization of directional sexual selection on female *H. pallitarsis*

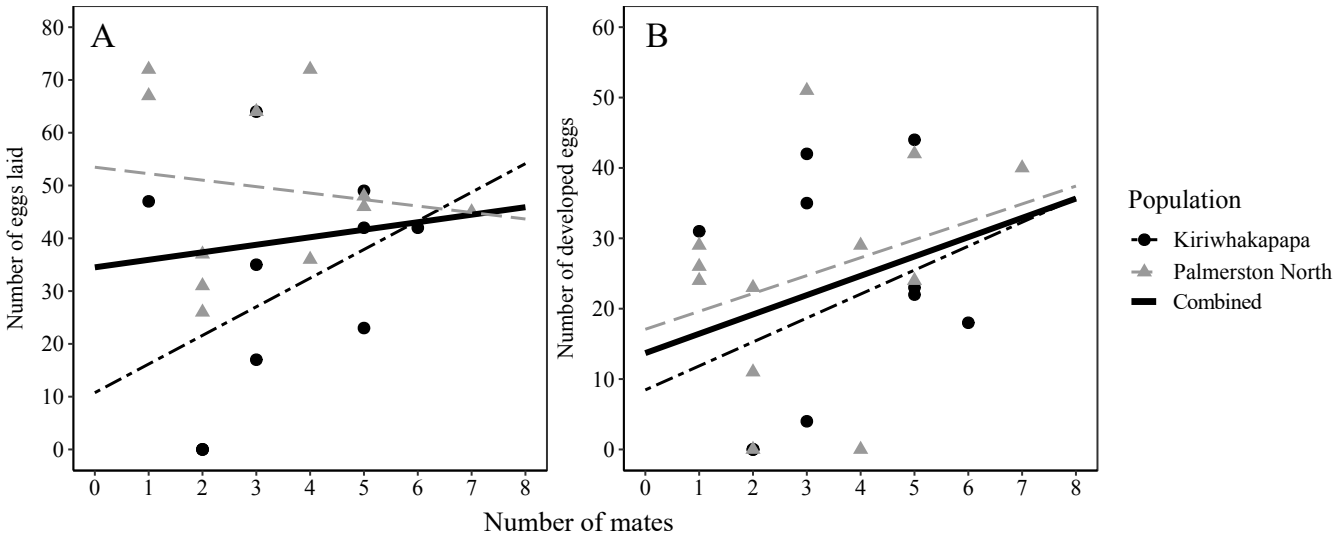

Figure A4.1 Raw Bateman Gradients for female *H. pallitarsis* showing relationship between number of mates and either A) number of eggs laid or B) number of developed eggs across populations

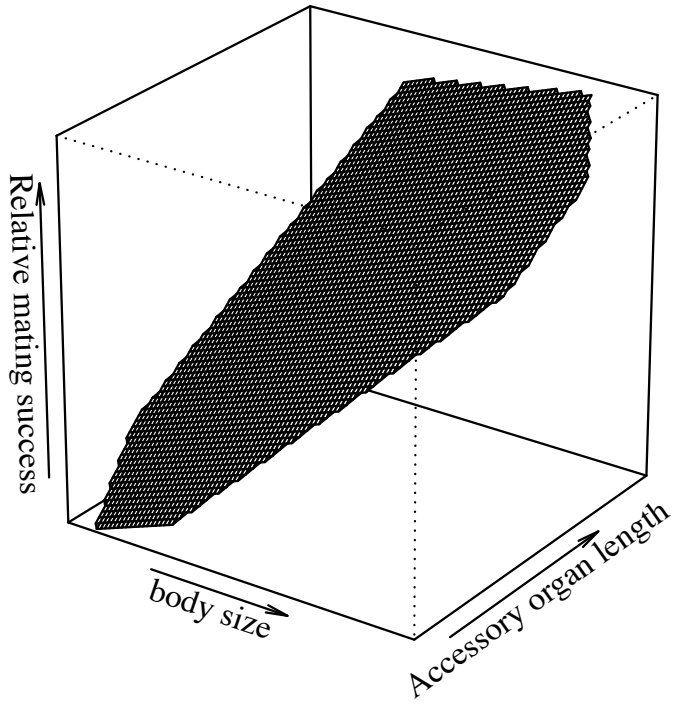

Figure A4.2 Thin-plate spline visualization of female body size and accessory organ length and their effect on relative mating success in female ground weta (*H. pallitarsis*) collected from Kiriwhakapapa. Relative mating success was estimated from the number of alleles found in a female's stored sperm.

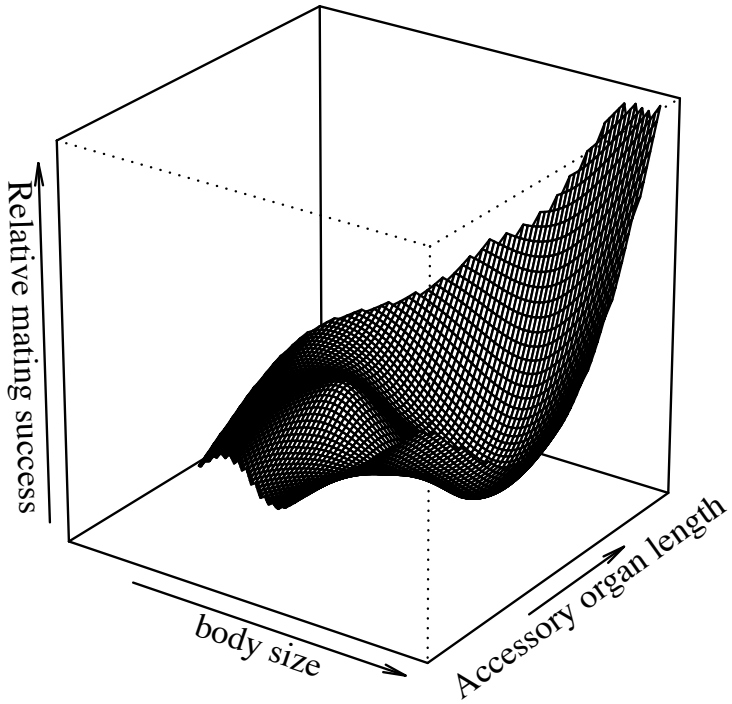

Figure A4.3 Thin-plate spline visualization of female body size and accessory organ length and their effect on relative mating success in female ground weta (*H. pallitarsis*) collected from Palmerston North. Relative mating success was estimated from the number of alleles found in a female's stored sperm.