Sperm investment in relation to weapon size in a male trimorphic insect?

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In the harem polygynous Wellington tree weta (*Hemideina crassidens*), early maturation at the eighth instar by males is associated with smaller mandibular weaponry. Because these males, compared with larger males (i.e., matured at 10th instar), are less successful at holding harems, they appear to have decreased mating success. Therefore, smaller males may acquire mates using tactics different than those of males with larger weaponry. A source of empirical support for this hypothesis is to show that smaller males offset their mating disadvantage by investing more in spermatogenesis to achieve fertilization success. Contrary to expectation, relative investment in testes (controlling for body size) is similar for smaller and larger males. I suggest that this lack of difference in testes size may be due to both morphs experiencing similar risks of sperm competition on Maud Island. Despite having testes similar in size to larger males, smaller males produce significantly larger ejaculates than males with bigger weaponry. This difference in ejaculate size may stem from larger males investing submaximally in current ejaculates in anticipation of their higher future mating success. *Key words:* accessory gland size, allometry, ejaculate size, sexual selection, sperm competition, sperm number, strategic ejaculation, testis size. *[Behav Ecol 19:1018–1024 (2008)]*

Intense male–male competition for females can drive competitively inferior males to evolve alternative reproductive tactics (Taborsky 1994; Gross 1996; Brockmann 2001; Shuster and Wade 2003; West-Eberhard 2003). This typically results in individual males repeatedly adopting either a favored or a disfavored behavioral role (Parker 1990a, 1990b). Males in the favored role (commonly referred to as a guarder, dominant, or major males) usually monopolize mates by guarding females, whereas males in the disfavored role (i.e., sneaker, satellite, subordinate, or minor male) try to acquire fertilizations at the expense of major males (Taborsky 1994; Brockmann 2001). Discrete behavioral roles often lead to an asymmetry in sperm competition risk (Parker 1990b, 1998). Guarder males suffer a lower sperm competition risk because they tend to prevent rivals from copulating with their partner, whereas sneaker males mate with females that are likely to have already mated or will shortly remate a guarder male (Parker 1990b, 1998).

Parker's (1990b) sneak-guard model of sperm competition predicts that males in the role with greater sperm competition risk (i.e., sneaker males) will increase sperm production relative to males paired with females (reviewed in Parker 1998; Simmons 2001; Wedell et al. 2002). That males in the disfavored role increase their investment in sperm production, as indicated by relative testes size, has been demonstrated in mammals (Stockley and Purvis 1993), insects (Simmons et al. 1999, 2000; Tomkins and Simmons 2002), and fishes (e.g., Gage et al. 1995; Taborsky 1998; Oliveira et al. 2001; Neff et al. 2003). Contrary to these examples, some studies have shown no difference in relative testes size between male morphs/behavioral types even when a sneaker strategy clearly exists (Simmons et al. 1999; Neat 2001; Byrne

2004). These null results might reflect cases where there is increased testes investment by guarder males in response to high sperm competition risk (Parker 1990b; Gage et al. 1995). If sneakers are rare, the sperm competition risk for guarders is low, and differential investment in testes between the 2 morphs should be large (Parker 1990b; Gage et al. 1995). However, as the proportion of sneakers in a population increases so too should the risk of sperm competition for guarders, so expenditure on sperm should become more similar for both types of males (Parker 1990b; Simmons et al. 1999). For example, 80% of spawning events in the quacking frog (*Crinia georgiana*) involve both sneaker and guarder males so both types of male experience similar risks of sperm competition (Byrne 2004). Consequently, guarder males seem to have developed larger testes to better compete with sneakers for fertilizations.

Of course, investment in sperm production is constrained by trade-offs with other life-history and sexually selected traits (Parker 1998; Simmons and Emlen 2006). Males might face a trade-off between gaining matings versus fertilization success (Preston et al. 2003; Simmons and Emlen 2006). For example, experiments on *Onthophagus* horned beetles show that investment in a trait promoting mating success (horns used as weaponry in combat for females) is traded off against testes size, a trait that elevates fertilization success (Simmons and Emlen 2006). If such trade-offs eventually constrain increased sperm production, guarder males might maximize their fitness by greater investment into other traits or behaviors such as more intense mate guarding that reduce the risk of sperm competition (Alonzo and Warner 2000). This might explain situations in which sneakers comprise a majority of the population but guarders still have relatively smaller testes (e.g., Simmons et al. 2000; Neat 2001).

Despite disfavored males increasing their relative investment in testes, they often have absolutely smaller gonads than favored males and yet favored males will often ejaculate smaller numbers of sperm at a given mating (e.g., Simmons et al. 1999). Because sperm production costs are nontrivial and frequent ejaculation can lead to sperm depletion (Preston

et al. 2001), males with higher future mating opportunities should prudently allocate their sperm reserves across successive matings while males with diminished mating prospects should allocate more sperm to current matings (Parker 1990a, 1998; Ball and Parker 2000; see also Wedell et al. 2002). In other words, to maximize fitness, sneaker or subordinate males are predicted to ejaculate maximally at each mating while guarder or dominant males should ejaculate submaximally (Warner et al. 1995; Simmons et al. 1999; Cornwallis and Birkhead 2006; Rudolfsen et al. 2006).

The Wellington tree weta, *Hemideina crassiden*s (Orthoptera: Tettigonioidea: Anostostomatidae), is a large (ca., 50–70 mm body length), flightless, and nocturnal insect endemic to New Zealand (Gibbs 2001). It is sexually dimorphic with males possessing exaggerated mandibular weaponry, which are used during fights for access to one or more females occupying tree cavities (hereafter termed galleries) (Kelly 2006a). Both sexes seek refuge from diurnal predators in galleries, which are not used for oviposition or feeding (Field and Sandlant 2001). *Hemideina crassidens* appears to exhibit a harem-defence polygynous mating system in which males defend females rather than galleries. Males do not guard females after mating (Kelly 2008b), and male residency time in a gallery is positively correlated with harem size (Kelly 2006c). This suggests that males mate all the females in a gallery and then decamp to search for another gallery (Kelly 2006b).

Sexual selection favors larger weaponry as males with bigger jaws defend significantly larger harems (Kelly 2005, 2008a). There is tremendous variation in mandible size in the wild (Spencer 1995; Field and Deans 2001; Kelly 2005). In fact, adult males exhibit a bimodal (and possibly trimodal) distribution in weapon size (Kelly 2005) apparently because males, but not females, mature at the 8th, 9th, or the 10th instar (Barrett 1991; Spencer 1995; Stringer 2001). Mandible size is positively correlated with ultimate instar number (Spencer 1995). Both field and laboratory evidences suggest that males with smaller weaponry can acquire sneak matings by infiltrating large harems defended by males with bigger weapons. Males with smaller weaponry can also reside with single females in galleries too small to accommodate larger males (Field and Jarman 2001; Kelly 2006d).

As predicted by sperm competition theory, sneaker males usually invest more heavily than guarders into ejaculate production. To investigate whether male tree weta with smaller weaponry represent an alternative mating strategy, I tested the prediction that these males invest relatively more in testes size than males with larger weaponry. This assumes that males with smaller weaponry are in the disfavored role and experience a greater risk of sperm competition. I test this assumption by determining the proportion of males with small weaponry on Maud Island over the course of 6 field seasons; the greater the proportion of males with small weaponry the greater the potential risk of sperm competition faced by males with large weaponry and therefore the smaller the difference in testes investment between the morphs. I also examine whether ejaculate size is positively related to testes size or whether male tree weta prudently allocate sperm in accordance with their potential mating success. Because males with larger weaponry are more likely to have mating access to larger groups of females than males with smaller weaponry, larger males may be required to produce several ejaculates over a short period of time (i.e., several hours). Consequently, larger males may not ejaculate maximally but instead prudently allocate their sperm over several copulations. Therefore, despite having larger testes than smaller males, males with larger weaponry may produce smaller ejaculates.

MATERIALS AND METHODS

This study was conducted during April–May 2004 and 2006 on Te Hoiere/Maud Island (41°02′S, 173°54′E), a 309-Ha scientific reserve in the Pelorus Sound, New Zealand.

I collected male tree weta (2004: $n = 64$; 2006: $n = 75$) by scanning vegetation at night. For each test male, I measured the left and right hind femur lengths and total head length to the nearest 0.05 mm with digital callipers (Mitutoyo Digimatic). All weta were uniquely marked with bee tags (H. Thorne Ltd, Market Rason, UK) to facilitate identification during the experiment. I assigned males to either of 2 categories according to their weapon size. Based on field surveys from 2001 to 2003 (Kelly 2005), 2004, 2006, and 2007, I considered head lengths of <19 mm and >23 mm to define males with smaller and larger weaponry, respectively (Figure 1). Adult male *H. crassidens* cannot be unambiguously categorized with regard to final instar number; however, it is likely safe to assume that the smallest (<19 mm) and largest (>23 mm) individuals represent 8th and 10th instar males, respectively (Kelly 2005). On the other hand, not only are intermediate-sized males more rare on Maud Island than either smaller or larger individuals but also because 9th instar males overlap considerably in weapon size with the other 2 morphs (Kelly 2005), it is more difficult to assign them to a putative instar number. Consequently, intermediate-sized males were excluded from some analyses. Head length was measured from the top of the head capsule to the tip of the left mandible. A significantly greater proportion of males on Maud Island have smaller (putative eighth instar: mean ± standard error [SE] proportion of males, 0.44 ± 0.017 males, $n = 6$ years) rather than larger (putative 10th instar: 0.34 ± 0.017 males, $n = 6$ years) weaponry in each year the population was surveyed (paired t-test, $t = 3.74$, degrees of freedom [df] = 5, $P = 0.013$) (see also Figure 4 in Kelly 2005).

This study is focused on the effects of "mean" sperm competition risk on investment in sperm production, so I controlled for a male's perceived "immediate" risk (see Engqvist and Reinhold 2005) by isolating $n = 41$ test males individually in plastic containers (12 cm diameter × 10 cm height) for 2 days prior to each trial. Sets of 7 or 8 stimulus females were housed together for several days in a large plastic cage
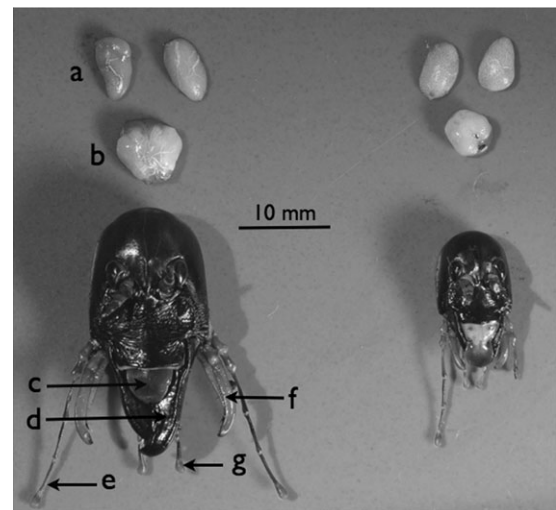

Figure 1
Photo of testes (a), accessory glands (b), and head (c–g) of males representing the larger (left) and smaller (right) weapon size categories in *Hemideina crassidens*. The labels on the head denote the labrum (c), mandibles (d), maxillary palp (e), galea (f), and labial palp (g).

(ca., $100 \times 40 \times 50$ cm) prior to being used in a trial. Each stimulus female was used once. All animals were fed dry cat food, freshly chopped carrot, and apple ad libitum. For each mating trial ($n = 41$), I placed a male and female into the test arena (a $19 \times 11 \times 12$ cm plastic container) approximately 1 h after sunset and observed the pair until they successfully mated or 1 h had elapsed. In addition, I videotaped all matings in order to accurately determine copulation duration. *Hemideina* tree weta lack elaborate courtship (Field and Jarman 2001); however, the mechanics of copulation are well studied making it easy to determine copulation duration (Field and Jarman 2001; Kelly 2008b). Briefly, males engage in palpation, antennation, and genital probing of females (males curl the abdomen ventrally into a C-shape and apply their genitalia to the female's body in an apparent attempt to find the female's genital opening) prior to copulation (Field and Jarman 2001). Males open the female's subgenital plate by using a gin trap—a grasping device comprised of small hooks on the anterior margin of the epiproct (dorsal lobe of the 10th abdominal tergite) and a lip of thickened cuticle on the posterior margin of the 9th tergite (Field and Jarman 2001). After lifting the female's subgenital plate, the male inserts his phallus and transfers a spermatophore. At this time, the male's antennae and palps become motionless and the female becomes quiescent, and the only movements performed by the male at this time are rhythmic contractions of his abdomen (Kelly CD, personal observation).

Procedure for estimating ejaculate size

In tree weta, as in other orthopteran species, sperm are packaged in bundles called spermatodesms and transferred to the female in a spermatophore (see also Boldyrev 1915; Viscuso et al. 1998; Gwynne 2001). In many orthopterans, males transfer a nuptial meal (spermatophylax) for the female, but in tree weta, the spermatophylax is greatly reduced and apparently does not provision the female (Gwynne 1997; Field and Jarman 2001; Stringer 2001) and is basically just a sperm-filled ampulla. The spermatophore sits in the female's genital opening under the subgenital plate for several hours after mating. During this time, the spermatophore contents are squeezed into the female and individual spermatozoa are released from the spermatodesms.

Within 1 min after copulation, I removed the spermatophore from the female using fine forceps. The spermatophore was immediately placed into an Eppendorf tube with 1.5 ml of water and crushed with forceps to break up the spermatodesms. I thoroughly mixed the sample by manually vortexing and repeatedly uptaking/expelling 1-µL samples with a Gilson pipettor. I then smeared a 1-µL sample of the sperm solution over an entire microscope slide using the side of the pipette tip. Slides were then allowed to air-dry. I then took 10 photographs at random locations within the sample using a digital camera-mounted compound microscope (area: 1.0915 mm^2). Blind counts of sperm heads were then made either by me or a technician. We were blind to the identity of test males.

Testes and accessory gland mass

The day after collection from the wild ($n = 98$) or after collection and then mating ($n = 41$), males were euthanized and their testes were dissected and placed in an Eppendorf tube with 70% ethanol (Figure 1). Testes were then dried in an oven at 55 °C for 2 days and weighed to the nearest 0.1 mg on a Mettler AE240 balance.

Ensifera possess 2 types of accessory glands: smooth glands, which produce the sperm ampulla, and coarse glands, which produce the spermatophylax. The accessory glands of *Hemi-deina* spp. are predominantly smooth, and their size provides an indirect measure of ejaculate (ampulla) size. In 2006 only, I removed the accessory glands from the males dissected for their testes ($n = 75$) and then stored and weighed them in the same fashion as testes.

Estimating sperm competition risk

In 2006, I estimated the (past) risk of sperm competition in the Maud Island population of *H. crassidens* by assessing the mating status of wild-caught females (captured in similar fashion as males, see above). I dissected 54 wild-caught adult females 24 h after capture and removed their spermathecae. I pinched the base of the spermatheca with fine forceps to stop the contents leaking and then cut through its base with microscissors. I estimated the number of sperm stored in the spermatheca following the protocol used for spermatophores (see above).

Data analysis

A total of 64 and 75 males spanning the entire weapon size range were measured and dissected in 2004 and 2006, respectively. Of these, 41 males were categorized as possessing either small (2004: $n = 5$; 2006: $n = 16$) or large (2004: $n = 7$; 2006: $n = 13$) weaponry. The relationships between body size (log femur length) and testes mass or accessory gland mass were examined by using major axis regression. Prior to regression analysis, weight measures were cube-root transformed to convert them to the same scale as the length measure. I compared testes and ejaculate size (log$_{10}$ sperm count) between male morphs using analysis of covariance (ANCOVA) with male morph (smaller vs. larger weaponry) as the factor and log body size or testes mass as covariates. ANCOVA is the most effective method to control for allometric relationships when testing for differences between size classes (Tomkins and Simmons 2002), but it is only a reliable way to compare relative investment of males adopting different tactics when the relationship between the covariate (body size or testes mass) and the response variable (testes mass or sperm number) is the same for both size classes (weapon size). I therefore tested whether the slopes differed. If interaction terms were not statistically significant (i.e., slopes are homogeneous), they were omitted from the analysis and an ANCOVA was performed. Body size (1-way analysis of variance [ANOVA]: $F_{1,108} = 0.16$, $P = 0.69$), testes weight ($F_{1,107} = 2.00$, $P = 0.16$), or ejaculate size ($F_{1,39} = 0.17$, $P = 0.69$) did not differ between 2004 and 2006. Means are presented $\pm$ 1 SE.

RESULTS

Allometry of testis size

Testes size showed positive allometry with body size (femur length) (MA regression slope = 2.39, 95% confidence interval [CI]: 1.49–4.80; $n = 139$ males spanning the continuum of weapon sizes on Maud Island; Figure 2). By using a subset of males (i.e., excluding $n = 30$ intermediate-sized males), I analyzed differences in testes investment in relation to weapon size category. In terms of absolute size, males with smaller weaponry had significantly smaller testes ($1.53 \times 10^{-2} \pm 0.001$ g, $n = 55$) than those with larger weaponry ($1.85 \times 10^{-2} \pm 0.001$ g, $n = 54$) (general linear model [GLM] ANOVA using log cube-root–transformed testes mass, $F_{1,106} = 4.32$, $P = 0.04$). Contrary to prediction, males with smaller weaponry did not have relatively larger testes (GLM ANCOVA with log femur length as covariate, weapon size category: $F_{1,106} = 1.02$, $P = 0.32$; femur length: $F_{1,106} = 5.97$, $P = 0.016$; interaction from heterogeneity of slopes test:
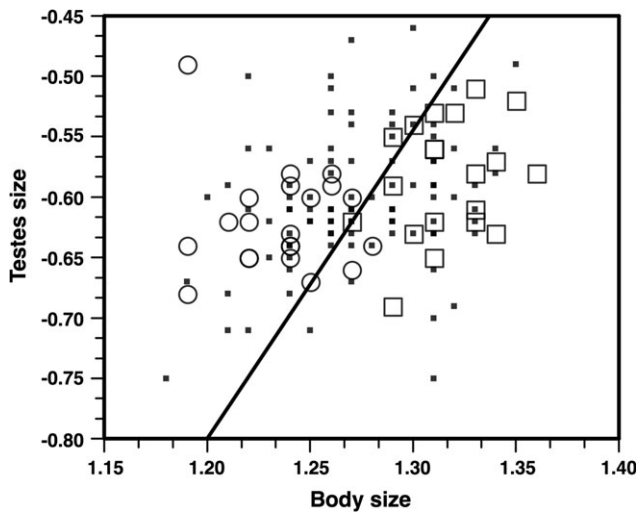
Figure 2
The allometric relationship between testes size ($\log_{10}$ cube-root weight, milligrams) and body size ($\log_{10}$ femur length, millimeters) for $n = 139$ male *Hemideina crassidens* (MA regression slope, $b = 2.39$, 95% CI: 1.49–4.80, $P < 0.05$). Males used in the mating experiments that examined investment in testes and ejaculates are shown as either open circles ($n = 21$ males categorized as having smaller weaponry, <19 mm head length) or open squares ($n = 20$ males categorized as having larger weaponry, >23 mm head length). Filled squares represent males that were euthanized and measured without having their ejaculate size estimated.

$F_{1,105} = 0.23$, $P = 0.88$) (Figure 3). Males categorized as having smaller weaponry had significantly smaller body sizes (femur length) than males with larger weaponry (17.28 ± 0.13 mm vs. 20.54 ± 0.13 mm; $F_{1,106} = 307.89$, $P < 0.0001$).

Relative weapon size and copulation duration

Males could spend considerable time attempting to copulate, with some males chasing and applying genitalia to females for more than an hour. Once the male lifted the female's subgenital plate he required 32.25 ± 2.60 s ($n = 24$) to begin transferring the ejaculate. Copulation duration did not differ significantly between male types (small weapons: 69.30 ± 4.02 s, $n = 20$; large weapons: 74.37 ± 3.92 s, $n = 19$; $t = 0.90$, df $= 37$, $P = 0.37$). Sample sizes are smaller for this analysis because data were not available for a small and a large male.

Relative weapon size and ejaculate expenditure

I examined ejaculate expenditure in relation to weapon size category by using a subset of males that had successfully copulated in the laboratory mating trials. Males with smaller weapons produced absolutely larger ejaculates (15.28 ± 1.61 sperms per mm^2, $n = 21$) than those with larger weapons (10.96 ± 1.36 sperms per mm^2, $n = 20$; $F_{1,39} = 5.27$, $P = 0.027$). Similarly, males with smaller weapons produced relatively larger ejaculates than males with larger weapons after controlling for testes size (ANCOVA, weapon size category: $F_{1,38} = 4.18$, $P = 0.048$; testes size: $F_{1,38} = 0.03$, $P = 0.87$; interaction from heterogeneity of slopes test: $F_{1,37} = 1.34$, $P = 0.26$; Figure 4).

Accessory gland mass was not significantly related to body size (femur length) (MA regression slope $= 0.32$, 95% CI: -0.23 to 1.13; $n = 75$). The accessory glands of males with smaller weapons (16.39 ± 0.83 mg, $n = 16$) were of similar size to those of males with larger weapons (18.32 ± 0.94 mg, $n = 13$; $F_{1,27} = 1.86$, $P = 0.18$).

Estimation of sperm competition risk in the wild

Eighty percent of the sampled female tree weta were storing sperm, and the number of sperm stored per female ranged from 0 to 81 sperms per mm^2.

DISCUSSION

Weapon size and investment in testes

Male tree weta with larger weaponry have absolutely larger testes than smaller males, but relative testes size did not differ.
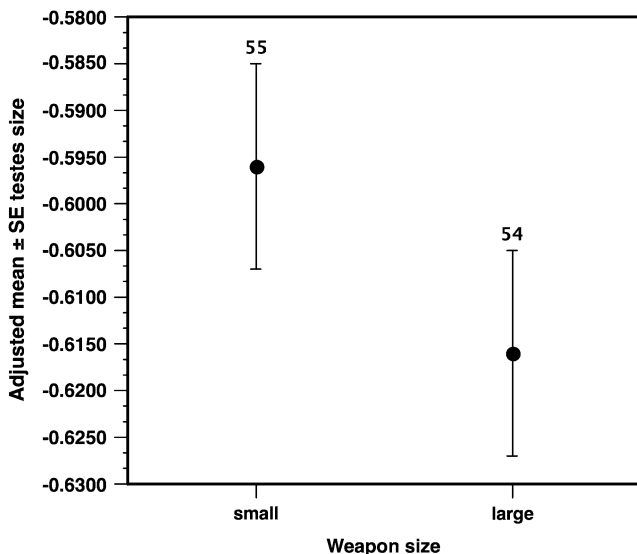

Figure 3
Mean ($\pm$SE) testes size ($\log_{10}$ cube-root–transformed mass, grams) of males with smaller and larger mandibular weaponry adjusted for body size using ANCOVA.
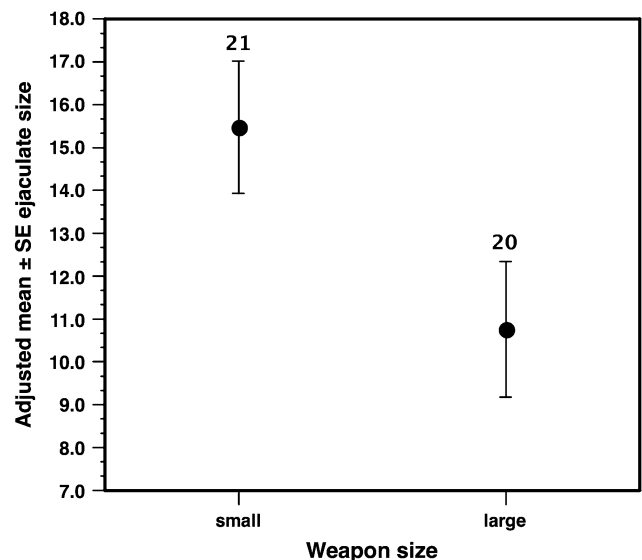

Figure 4
Adjusted mean ($\pm$SE) ejaculate size (number of sperms per mm^2) of males with smaller and larger mandibular weaponry (ejaculate size was adjusted for testis size using ANCOVA).

Contrary to expectation, these data suggest that smaller males do not offset their mating disadvantage by investing more into sperm production to increase their fertilization success. Although this counters my original prediction, it does not refute the hypothesis that males adopt alternative reproductive strategies based on weapon size. This is because Parker (1990b) also predicted that as the frequency of sneaks in a population increases, the sperm competition risk becomes more similar for sneaks and guards so that any disparity in expenditure between them should decrease. Indeed, sperm investment in *H. crassidens* resembles that in the dung beetle *Onthophagus taurus*, a species with distinct alternative mating tactics. In *O. taurus*, minor males are hornless and adopt a mating strategy, whereby they sneak into breeding tunnels and copulate with guarded females (Emlen 1997; Hunt and Simmons 1997; Moczek and Emlen 2000). Both major and minor morphs show equal investment in testes size and sneakers account for approximately 60% of the population suggests that guarders have responded to an increased risk of sperm competition by increasing relative testes size (Simmons et al. 1999).

Two lines of evidence support the hypothesis that similar investment in sperm production by smaller (eighth instar) and larger (10th instar) tree weta males on Maud Island is due to both morphs experiencing similar sperm competition. First, approximately 44% of the males in this population possess smaller weaponry (<19 mm head length), whereas only 34% of males have larger weapons (>23 mm head length) (see also Kelly 2005). Second, a significant proportion of adult female tree weta (ca., 80%) on Maud Island have sperm stored in their spermatheca. Moreover, females in the wild, as well as those housed in field cages, will mate with different males over consecutive days, and several days to weeks can pass between bouts of oviposition (Kelly CD, personal observation). This means that females tend to store sperm from several males (Ordish 1992), and so there is a high likelihood that any given male will be faced with competing against the sperm of a rival conspecific in his next copulation (assuming virgin and mated females exhibit similar mating activities). Taken together, these points suggest that males in the favored role (i.e., 10th instar males) likely experience high levels of sperm competition and should increase their investment in sperm production accordingly. One way to test this hypothesis would be to compare relative testes size among populations of *H. crassidens* that differ in the proportion of males with small weaponry.

Consistent with studies across many taxa (Gage et al. 1995; Pitnick 1996; Tomkins and Simmons 2002; Neff et al. 2003; Byrne 2004; Hosken et al. 2005; Stoltz et al. 2005), I found that, like weaponry (Kelly 2005), testes size shows positive allometry in *H. crassidens*. This positive allometry suggests that larger males invest proportionately more into both competition in fertilization (testes size) as well as access to mates (weapon size) compared with smaller males. Selection may favor greater investment in both pre- and postmating traits (i.e., weaponry and testes, respectively) if, for example, males with larger weaponry require absolutely larger testes because they have more mating opportunities (e.g., Gage et al. 1995; Preston et al. 2003) and thus require greater spermatogenesis (e.g., Schärer and Vizoso 2007).

Weapon size and ejaculate expenditure

In addition to predicting how males in the disfavored role should invest in sperm production, Parker and coworkers (Parker 1990a, 1990b, 1998; Gage et al. 1995) predicted how males should invest in the current ejaculate. Sperm competition theory argues that males in the disfavored role should produce relatively more sperms per ejaculate than those in the favored role. In my study, male tree weta hypothesized to occupy the disfavored role (i.e., smaller weaponry) produced more sperms per ejaculate than males putatively in the favored role (i.e., larger weaponry). This supports the hypothesis that male tree weta adopt mating tactics based on morphotype.

Given the difference in absolute testes size, it is surprising that males with smaller weaponry also ejaculated absolutely more sperm than males with larger weaponry. This suggests that larger males ejaculate submaximally (assuming ejaculate size is dependent on testes size; Parker 1998; Schärer and Vizoso 2007). This might occur if males strategically adjust ejaculate size based on their probability of future mating opportunities, which in turn, is dependent on male phenotype. Parker (1982) argued that selection should favor males that ensure an amount of sperm is reserved that is related to the probability that they will subsequently encounter females (see also Fryer et al. 1999; Reinhold et al. 2002; Wedell et al. 2002). Males with smaller weaponry are less likely to hold large harems (Kelly 2005), so they are unlikely to mate with several females in a short period of time. In contrast, males with larger weaponry tend to defend harems. Therefore, smaller males might be able to ejaculate maximally in each mating, whereas larger males have to be more conservative. This might also explain why testes show strong allometric scaling in *H. crassidens*; if larger males mate more often, they should require larger testes to enable frequent production of competitively sized ejaculates (sensu Schärer and Vizoso 2007).

Two alternative hypotheses could also explain the significantly larger ejaculates produced by males with smaller weaponry. First, males in each treatment group may have systematically perceived different levels of immediate sperm competition intensity (Engqvist and Reinhold 2005). Sperm competition theory predicts that when males are certain of immediate competition, they always increase their ejaculate size with maximum-sized ejaculates produced when one rival is present and then decreasing monotonically as the number of rivals increases. Hence, in my experiment, smaller males may have perceived the presence of rivals, whereas larger males did not perceive any competition. This is unlikely as all experimental males were individually isolated for 48 h prior to mating and were never in visual, olfactory, or acoustic contact with other males. Second, optimal sperm allocation might depend on the size of competing ejaculates. A recent model by Engqvist and Reinhold (2007) predicts that males with larger sperm reserves should ejaculate more sperm as sperm competition intensity increases, whereas males with smaller reserves should allocate fewer sperm as intensity rises. This model could explain my results if males with smaller weaponry have larger sperm reserves in addition to perceiving higher intensities of sperm competition. This is a reasonable supposition for situations in the wild because males with smaller weaponry probably mate less frequently and should thus have larger sperm reserves in addition to facing higher intensities of sperm competition because of their subordinate mating role. However, my experimental males should have had their maximum reserves, with smaller males having smaller maximum reserves given their smaller absolute testis size compared with larger males, given that they were isolated for 48 h before mating. Again, this premating isolation should have also reduced each male's perceived intensity of sperm competition. Thus, if sperm reserves alone drives ejaculate expenditure, then larger males should have had bigger ejaculates than smaller males in my experiment.

In conclusion, my study supports 2 main predictions of sperm competition theory. First, the lack of differential investment between male tree weta morphs in testes size matches that predicted by Parker (1990b) for populations in which

males in favored and disfavored roles experience similar risks of sperm competition—a likely scenario given the larger proportion of small versus large males on Maud Island. Second, my study supports Parker's (e.g., 1990a) prediction that sneaker males (i.e., smaller weaponry) should ejaculate relatively more sperms per copulation than guarder males (i.e., larger weaponry).

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