

# Sexual Selection on Accessory Glands, Genitalia and Protarsal Pads in the Whirligig Beetle *Dineutus nigrior* Roberts (Coleoptera: Gyrinidae)

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## Abstract

Sexual selection is a potent force in the evolution of morphology in sexually reproducing species. When large size in a trait is favored by sexual selection the trait often exhibits positive allometry. Mating behavior in whirligig beetles consists of males attempting to grasp reluctant females using enlarged protarsi (protarsal pads). Here we use allometry and a mating experiment to investigate sexual selection pressures on accessory glands, intromittant genitalia (aedeagus), and protarsal pads in males of the whirligig beetle *Dineutus nigrior* Roberts. Accessory gland size exhibited positive allometry and males with larger accessory glands were more likely to copulate suggesting that larger size in this trait is favored by sexual selection. Males with larger accessory glands attempted to copulate more often but did not exhibit fewer failed mating attempts before copulating. This suggests that the increased probability of mating in males with large accessory glands is due to higher mating attempt frequency and not to increased ability to overcome female resistance. The length of the aedeagus exhibited negative allometry and males with a longer aedeagus did not have increased mating success. This is consistent with stabilizing selection favoring an intermediate size in this trait. The allometric slope of the protarsal pad did not differ from isometry and males with larger protarsal pads did not have increased mating success. This suggests that larger protarsal pads are not favored by sexual selection.

## Introduction

Sexual selection arises from variance in reproductive success and is a potent force in the evolution of morphology in sexually reproducing species (Andersson 1994). Sexual selection can act on traits related to obtaining mates (pre-copulatory sexual selection), and on traits related to obtaining fertilizations after copulation is achieved (post-copulatory sexual selection) (Simmons 2001). Sexual selection often results in directional selection toward larger trait size. When this is the case, individuals that achieve large overall body size often have disproportionately large traits

resulting in positive allometry (allometric slope  $> 1$ ) (Green 1992, 2000; Kodric-Brown et al. 2006). For example, positive allometry is often present in traits that are associated with attractiveness to females (e.g. Alatalo et al. 1988; Burkhardt et al. 1994) and in traits used in male–male competition (e.g. Simmons & Tomkins 1996; Bean & Cook 2001; Kelly 2005; Kodric-Brown et al. 2006). The evolution of positive allometry in these traits can be explained based on optimal resource allocation during development (Kodric-Brown et al. 2006). Bonduriansky and Day (2003) suggested that sexually selected traits under directional selection could exhibit positive allometry

but other allometric relationships were also possible. In their model, positive allometry was predicted only when fitness is related to body size by an increasing function with diminishing returns, and to trait size by an increasing function without diminishing returns.

As sexually selected traits often exhibit positive allometry, and non-sexual traits generally exhibit isometry or negative allometry, the presence of positive allometry in a trait suggests that the trait may be under the influence of sexual selection (Green 2000; Venzl 2004).

Male genitalia in insects exhibit a high level of morphological diversity and display rapid evolution (Eberhard 1985). Three main hypotheses have been proposed for the evolution of genital morphology: (1) the pleiotropy hypothesis (Mayr 1963), which suggests that genitalia evolve indirectly through selection on non-sexual traits which are genetically correlated with genitalia; (2) the lock-and-key hypothesis, which states that selection for sexual isolation drives genital evolution (reviewed in Shapiro & Porter 1989); and (3) the sexual selection hypothesis. Sexual selection can influence genital morphology through several mechanisms including sperm competition (Parker 1970), cryptic female choice (Eberhard 1985, 1996; Eberhard et al. 1998) and sexual conflict (Lloyd 1979; Alexander et al. 1997; Arnqvist & Rowe 2002).

Although support for the pleiotropy and lock-and-key hypotheses is not particularly compelling (Eberhard 1985; Hosken & Stockley 2004), evidence for the influence of sexual selection on male genital evolution is strong and male genital morphology is generally assumed to be under the influence of sexual selection (Arnqvist 1998; House & Simmons 2003; Hosken & Stockley 2004). However, in contrast to the general pattern in sexually selected traits, male genital traits in invertebrates often exhibit negative allometry (allometric slope  $< 1$ ) (e.g. Eberhard et al. 1998; Uhl & Vollrath 2000; Bernstein & Bernstein 2002; Ohno et al. 2003). Eberhard et al. (1998) suggested that this is due to sexual selection favoring an intermediate male genitalia size that is capable of stimulating most females in the reproductive population, resulting in stabilizing selection on genitalia size ('one-size fits all' hypothesis). However, negative allometry in male genitalia does not seem to be a universal pattern as positive allometry is present in several vertebrate species suggesting directional selection favoring larger genitalia size (Kelly et al. 2000; Miller & Burton 2001; Lupold et al. 2004). Larger male genitalia may be favored by sexual selection if the genitalia is used as a weapon

to force copulation ('weapons' hypothesis), or as an indicator of male quality ('good viability genes' hypothesis) (Eberhard et al. 1998). The positive allometry for male genitalia in three vertebrate species is counter to the general trend in invertebrates. This could suggest that there is a fundamental difference between vertebrates and invertebrates in the evolution of male genital morphology. However, it could also reflect a different use of the genitalia in those particular vertebrate species. For example, females may have very limited ability to assess mate quality other than through male genital morphology (Miller & Burton 2001; Lupold et al. 2004), or larger genitalia may help obtain forced copulations (Kelly et al. 2000). These situations could potentially result in positive allometry in invertebrates in some cases as well.

In insects, male accessory gland proteins (AGP) are transferred to the female with the sperm during copulation and can be important to male reproductive success through several mechanisms including facilitation of sperm transfer, induction of ovulation and oviposition, removal or killing of rival sperm, increasing chance of sperm storage and induction of a refractory period in the female (reviewed in Gillott 2003). The effects of these proteins may be dose dependent (Gillott 2003). Therefore, if males with larger accessory glands are able to produce and store more AGP and deliver more AGP with each ejaculation, then larger accessory glands may be favored by post-copulatory sexual selection. Large accessory glands may also be favored by pre-copulatory sexual selection as the availability of AGP may be a strong constraint on mating frequency as males may become depleted of these materials (Rogers et al. 2005a). A positive correlation between accessory gland size and mating frequency has been shown in *Drosophila melanogaster* (Bangham et al. 2002) and the stalk-eyed fly *Cyrtodiopsis dalmanni* (Baker et al. 2003; Rogers et al. 2005a). This may be due to males with larger accessory glands being able to produce and store greater quantities of AGP and therefore being able to mate more frequently without becoming AGP depleted (Bangham et al. 2002). Males with smaller accessory glands may become AGP depleted during frequent mating. Therefore, subsequent copulations would have low or no reproductive benefit due to inability to transfer sperm or inability to reduce sperm competition or compete effectively in sperm competition. The AGP-depleted individual would incur the costs of courtship without gaining an increase in reproductive success (Bangham et al. 2002). Therefore, lower mating effort or 'sex drive'

may be selected for in males with smaller accessory glands to minimize time and energy wasted on copulations where reproductive benefit is low. Higher 'sex drive' may be selected for in individuals with large accessory glands as they may be able to mate more frequently without depleting AGP reserves. Mating frequency is often correlated with reproductive success (Arnold 1994) and therefore larger accessory glands may be favored by sexual selection.

Females actively resist male mating attempts in some insects (e.g. Allen & Simmons 1996; Lauer et al. 1996; Crean et al. 2000). Active female resistance to male mating attempts may have evolved to avoid the costs of superfluous copulations (Chapman et al. 2003), or to 'screen' potential mates ensuring that only high-quality males are able to mate (Eberhard 2002; Cordero & Eberhard 2003). Regardless of the function of female resistance, male traits that enhance the ability to overcome the resistance would result in more copulations and should be favored by sexual selection. In several groups of insects, males possess structures used to grasp the female during copulation and pre-copulatory resistance (e.g. Stork 1980; Thornhill & Sauer 1991; Arnqvist 1992; Allen & Simmons 1996). Female-grasping traits have been shown to confer an advantage in forcing copulations and in overcoming female resistance to mating (e.g. Arnqvist 1989; Thornhill & Sauer 1991; Sakaluk et al. 1995; Weigensberg & Fairbairn 1996). Males in several groups of beetles have enlarged tarsomeres covered in modified adhesive setae forming a protarsal pad which is used to grasp females during copulation and pre-copulatory resistance (Stork 1980). Protarsal pads are likely important in overcoming pre-copulatory resistance by the female (Aiken & Khan 1992; Bergsten et al. 2001) and there is evidence that larger protarsal pads are favored by sexual selection in a dytiscid beetle (Schulte-Hostedde & Alarie 2006). However, empirical evidence showing increased mating success in males with large protarsal pads is scarce.

Whirligig beetles (Coleoptera: Gyrinidae) are gregarious, surface-inhabiting, water beetles. The mating behavior in this group consists of males attempting to grasp the dorsal surface of the female with protarsal pads equipped with modified adhesive setae. There is no obvious courting behavior. Females often resist male mating attempts by quickly swimming away. This resistance appears to be successful in preventing copulation in some cases as many mating attempts fail to result in copulation (Y. Alarie, pers. obs.). Here we investigate aspects of

sexual selection on male accessory glands, protarsal pads, and external genitalia in the whirligig beetle *Dineutus nigrior* Roberts. While the total sexual selection pressure on these traits is the sum of pre-copulatory + post-copulatory selection, we focus largely on pre-copulatory selection in this study. We perform a mating experiment to investigate whether these traits are related to mating success. We also investigate patterns of allometry to provide further support for the results of the mating experiment as well as to provide some insight into potential post-copulatory sexual selection pressures.

First, we investigate whether sexual selection favors large accessory glands. We predict that accessory gland size will exhibit positive allometry and that males with larger accessory glands will have higher 'sex drive'. Second, we test the prediction from the 'one-size fits all' hypothesis that male external genitalia size exhibits negative allometry and has an allometric slope less than other traits. We also test the 'weapons' and 'good viability genes' hypotheses for male external genital evolution by determining if males with larger genitalia are more likely to copulate and less likely to have mating attempts end unsuccessfully.

Finally, we test the hypothesis that larger protarsal pads allow for a greater ability to grasp and hold the female resulting in increased mating success. We predict that males with larger protarsal pads will be more likely to copulate and will have fewer mating attempts end unsuccessfully. We also predict that this trait will exhibit positive allometry.

## Methods

### Collection

Sexually mature *D. nigrior* were collected from Swan Lake, a small (ca 6 ha surface area, 8.5 m maximum depth) lake on the Precambrian shield near Sudbury (Ontario, Canada). The lake was historically acidified by local sulfur deposition and is presently fishless. All beetles were collected between June 21 and June 24 of 2005 which is during the reproductive season (Y. Alarie, pers. obs.).

Nets were used to opportunistically sample beetles that were observed swimming on the water surface. The specimens were sexed in the field and males and females were isolated from each other during transport to the laboratory. To prevent injuries we did not overcrowd the collection containers. Water from Swan Lake was also transported to the laboratory.

### Mating experiment

In the laboratory, lake water was transferred to a plastic storage box (62 cm × 41.3 cm × 16 cm). Fifty females were randomly selected from the specimens collected earlier the same day and placed in the storage box containing the lake water. Mating history (i.e. virgin or non-virgin) was not known for the individuals used in the experiments. However, specimens were collected in the middle of the breeding season and all adult individuals in the population were sexually mature, as adults emerge in late summer (Y. Alarie, pers. obs.). Due to the high mating rate in this species, we believe that most individuals would likely be experienced sexually.

Ten males were randomly selected and were weighed to the nearest 0.001 g on an OHAUS balance (model TS400D) (OHAUS, Pine Brook, NJ, USA) after being blotted with paper towel to remove water. All males were marked on either left or right elytron with a unique combination of colored nail enamel (formaldehyde and toluene free) so that individual males could be readily identified. Ten males were then introduced into the container with the 50 females and a 2-h observation period was immediately commenced. We included 50 females and 10 males in each trial as this reflected the highly female-biased sex ratio present in this population during the breeding season (Y. Alarie, pers. obs.). The number of attempted copulations and successful copulations were recorded for each male. After the observation period the male beetles were killed and preserved in vials of 70% ethanol. Female beetles were returned alive to the collection site. This procedure was repeated five times between June 21 and June 24. All trials were performed between 11:00 and 16:00 hours. The same researcher recorded the behavior for all trials.

### Measurements

Linear morphological measurements were obtained from all 50 males used in the mating experiment using a micrometer-equipped Olympus SZH10 research stereo microscope (Olympus, Tokyo, Japan). Before each trait was measured, the specimen was oriented so that the trait of interest was as close to parallel to the plane of the objective lens as possible. The morphological characters used in this study were as follows.

*Pronotum length*: measured dorsally along the midline.

*Elytra length*: measured dorsally along the midline.

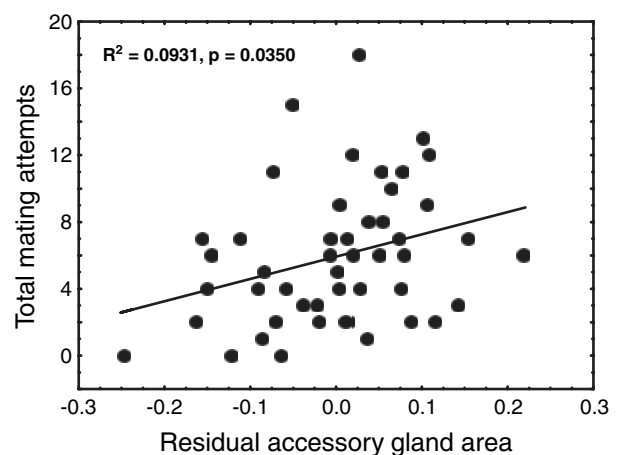
*Length, width, and area of the protarsal pad*: the protarsal pad is comprised of five articles or tarsomeres. Length (maximum linear distance along midline) and width (maximum transverse distance measured transversally) of each article was measured. Protarsal pad length was determined by obtaining the sum of the lengths of the five tarsomeres. Protarsal pad width was represented by the width of the widest tarsomere. The area of the protarsal pad was calculated by finding the sum of the area of each of the five tarsomeres. As the tarsomeres were approximately rectangular the area of each tarsomere was found by multiplying the length by the width. Measurements for the right pad were used in this study.

*Length of aedeagus*: the male genitalia is comprised of two lateral parameres and one median aedeagus. The length of the aedeagus was measured at the maximum linear distance between the two extreme ends.

*Length, width, and area of accessory gland*: male *D. nigrior* possess two large accessory glands. Accessory gland length was measured at the maximum linear distance along midline. Accessory gland width was measured at the maximum transverse distance measured transversally. The area of the accessory gland was determined by multiplying the length by the width and dividing by two as the gland is approximately diamond shaped (see Fig. 1 in Breland & Simmons 1970). Measurements of the right accessory gland were used in this study.

### Statistical analysis

Sexually selected traits are often thought to have high phenotypic variation and therefore high



**Fig. 1:** Relationship between accessory gland area and total mating attempts when controlling for condition, elytra length, protarsal pad area, aedeagus length, and trial number;  $n = 48$  male *Dineutus nigrior*

coefficients of variation (CV), while traits under stabilizing selection typically have low phenotypic variation and low CV (Pomiankowski & Moller 1995). We determined the CV by dividing the standard deviation by the mean and multiplying by 100. The CV was determined for untransformed measurements. All subsequent analyses were performed on  $\log_{10}$  transformed data to improve normality.

Allometric relationships were found by determining the slope of  $\log_{10}$  transformed measures of the traits of interest plotted against a  $\log_{10}$  transformed measure of body size (elytra length). Elytra length is a large, relatively flat trait that can be measured accurately and precisely and therefore is an appropriate choice as a measure of overall body size (Bernstein & Bernstein 2002). Univariate outliers ( $>3$  SD) were removed from all traits before determining allometric relationships. We used the square root of area measurements to equalize biological scales. There is debate about which regression method is most appropriate for determining allometric slopes. Ordinary least squares regression is used in several studies (e.g. Eberhard et al. 1998; Schmitz et al. 2000), but this method has been criticized because the assumption of no measurement error in the independent variable (X) is likely violated in morphological data (Sokal & Rohlf 1995; Green 1999). Model II regression methods are often advocated because variation in the independent variable is incorporated into the sum of squares (Sokal & Rohlf 1995; Green 1999). We used reduced major axis regression (RMA), a model II method, to determine allometric slopes. We chose pronotom length as a control trait not involved in reproduction to compare with the slopes of the traits of interest. We determined if RMA slopes differed significantly from isometry (allometric slope = 1) by testing if the residual and axis scores are uncorrelated, when they are calculated using 1 as the slope (Warton et al. 2006).

Mating experiment data was analyzed using general linear models (GLM) and generalized linear models (GLZ). We included accessory gland area, aedeagus length, and protarsal pad area as independent variables in all models. To control for potential effects of body size and body condition we also included elytra length and body condition in all models. We used residuals from a regression of mass on body size (elytra length) as a measure of body condition (Schulte-Hostedde et al. 2005). We assumed that size-corrected mass is indicative of the amount of energy reserves present and is therefore an indicator of condition. For example, residual mass

is positively correlated with fat reserves in the carabid beetle *Pterostichus melanarius* (Oestman et al. 2001). As males in the different trials experienced different environments (e.g. different females and different male competitors), trial number was added as a categorical predictor in all models.

To determine if traits were correlated with the probability of copulating we used a GLZ with binomial distribution and logit-link function. Mating success (0, no copulation; 1, at least one copulation) was the dependent variable.

We used a GLM with the number of failed mating attempts before the first copulation as the dependent variable to determine if traits were associated with increased ability to overcome female resistance. To investigate the effect of the traits on mating effort or 'sex drive' we used the total number of mating attempts (failed copulation attempts + successful copulations) as the dependent variable in a GLM. Measurements were not available for the area of the protarsal pad from two individuals and therefore  $n = 48$  males for the probability of copulating GLZ model and the total mating attempts GLM. For the mating attempts before copulation model,  $n = 35$  as only individuals that achieved a copulation were included.

We used a Microsoft Excel spreadsheet written by Dr David Warton (<http://web.maths.unsw.edu.au/~dwardon/programs.html>) to perform tests for the difference of RMA slopes from isometry. All other statistical analyses were performed using STATISTICA 6.1 (StatSoft, Inc., Tulsa, OK, USA).

## Results

### Coefficient of variation

The measures of accessory gland size were the traits with the highest CV consistent with sexually selected traits. The CV for aedeagus length was the lowest of any trait consistent with stabilizing selection. Elytra length, pronotom length, and measures of the size of the protarsal pads had intermediate CV values (Table 1).

### Allometry

There was a significant positive correlation between all traits and body size (elytra length) ( $F > 4.53$ ,  $p < 0.05$ ). The length, width, and area of the accessory gland all exhibited significant positive allometry, indicative of a sexually selected trait. Aedeagus length exhibited negative allometry and the

**Table 1:** Descriptive statistics of traits used in this study based on untransformed measurements. All measurements in mm or mm<sup>2</sup>

| Trait                  | n  | $\bar{x}$ | Range         | SD    | CV (%) |
|------------------------|----|-----------|---------------|-------|--------|
| Elytra length          | 50 | 8.06      | 7.12 – 8.73   | 0.421 | 5.22   |
| Pronotom length        | 50 | 1.94      | 1.68 – 2.38   | 0.119 | 6.12   |
| Protarsal pad length   | 48 | 1.43      | 1.30 – 1.55   | 0.063 | 4.41   |
| Protarsal pad width    | 48 | 0.374     | 0.298 – 0.408 | 0.022 | 5.84   |
| Protarsal pad area     | 48 | 0.494     | 0.362 – 0.564 | 0.043 | 8.66   |
| Aedeagus length        | 50 | 2.30      | 2.20 – 2.45   | 0.063 | 2.74   |
| Accessory gland length | 50 | 4.03      | 2.86 – 5.33   | 0.691 | 17.15  |
| Accessory gland width  | 50 | 1.09      | 0.751 – 1.32  | 0.121 | 11.16  |
| Accessory gland area   | 50 | 2.21      | 1.07 – 3.29   | 0.518 | 23.46  |

**Table 2:** Reduced major axis regressions of traits on body size (elytra length). 95% confidence limits calculated according to McArdle (1988). F and P values from test of the difference of the slope from isometry. N = 47–50

| Morphological trait    | R <sup>2</sup> | Slope | LCL   | UCL   | F      | p-value |
|------------------------|----------------|-------|-------|-------|--------|---------|
| Pronotom length        | 0.213          | 0.987 | 0.763 | 1.28  | 0.01   | 0.923   |
| Protarsal pad width    | 0.257          | 0.966 | 0.748 | 1.25  | 0.07   | 0.791   |
| Protarsal pad length   | 0.235          | 0.828 | 0.641 | 1.07  | 2.16   | 0.148   |
| Protarsal pad area     | 0.349          | 0.841 | 0.663 | 1.07  | 2.15   | 0.149   |
| Aedeagus length        | 0.163          | 0.510 | 0.392 | 0.633 | 30.24  | <0.001  |
| Accessory gland length | 0.103          | 3.28  | 2.50  | 4.31  | 118.85 | <0.001  |
| Accessory gland width  | 0.088          | 1.93  | 1.46  | 2.55  | 38.02  | <0.001  |
| Accessory gland area   | 0.132          | 2.30  | 1.76  | 3.00  | 48.21  | <0.001  |

allometric slope was lower than all other traits consistent with predictions from the 'one-size fits all' hypothesis (Eberhard et al. 1998). The length, width, and area of protarsal pads were not significantly different from isometry and therefore provide no evidence that the size of the protarsal pads is sexually selected (Table 2).

### Mating experiment

All males obtained either zero, one, or two copulations during the 2-h observation period. Thirty-five of the 50 males (70%) were able to obtain at least one copulation in the 2-h observation period and nine individuals (18%) mated twice. In total, there were 299 copulation attempts, of which 44 resulted in copulation (14.72%). The mean number of copulations per male was 0.88, the mean number of failed mating attempts per male was 5.1, and the mean number of total mating attempts per male was 6.0. Trial number was not significant in any model ( $p > 0.068$ ). Accessory gland area was positively correlated with the probability of obtaining at least

**Table 3:** Generalized linear model with binomial distribution and logit-link function. Dependent variable is mating success

| Trait                | d.f. | Estimate | SE    | Wald   | p-value |
|----------------------|------|----------|-------|--------|---------|
| Condition            | 1    | -3.92    | 26.35 | 0.022  | 0.882   |
| Elytra length        | 1    | -25.12   | 35.33 | 0.505  | 0.477   |
| Protarsal pad area   | 1    | 0.467    | 15.66 | <0.001 | 0.976   |
| Aedeagus length      | 1    | 74.69    | 61.70 | 1.47   | 0.226   |
| Accessory gland area | 1    | 16.13    | 7.33  | 4.84   | 0.028   |
| Trial                | 4    | -        | -     | 6.89   | 0.142   |

one copulation after controlling for other traits (Table 3). No other traits were correlated with probability of obtaining a copulation (Table 3). Accessory gland area was also positively correlated with total mating attempts after controlling for other traits ( $\beta = 0.338$ ,  $F = 5.56$ ,  $p = 0.024$ ) (Fig. 1). No other traits were correlated with the number of mating attempts ( $F < 1.18$ ,  $p > 0.284$ ). None of the traits were significantly correlated with the number of failed mating attempts before first copulation ( $F < 2.19$ ,  $p > 0.152$ ).

## Discussion

### Accessory glands

Our results support the hypothesis that larger accessory glands are favored by sexual selection. Sexually selected traits often exhibit positive allometry (e.g. Alatalo et al. 1988; Burkhardt et al. 1994; Simmons & Tomkins 1996; Bean & Cook 2001; Kelly 2005) and have higher CV than other body traits (Pomiankowski & Moller 1995). Both of these characteristics were present in the size of the accessory gland in *D. nigrior* lending support to the hypothesis that larger size in this trait is favored by sexual selection.

Our results also support the hypothesis that one advantage of large accessory glands is the ability to mate more frequently. When mating opportunities are frequent, the availability of AGP is likely to constrain mating frequency (Rogers et al. 2005a,b). Mating opportunities are probably numerous in *D. nigrior*. Male *D. nigrior* have access to females in mixed sex aggregations throughout the breeding season and our study population has a female-biased sex ratio, suggesting that males are probably able to find potential mates. Also, mating attempts and successful copulations were observed throughout the day (11:00–16:00 hours) and the mating season lasts for several weeks (Y. Alarie, pers. obs.). Moreover, in this laboratory study, we found that most males (70%) were able to obtain at least one copulation in a 2-h period,

and 18% were able to mate twice in that period. This suggests that overcoming female resistance may not be a strong constraint on mating frequency in this species. Therefore, the availability of AGP is a potential constraint on mating frequency in this species. If males with larger accessory glands are able to produce and store greater quantities of AGP they may be less susceptible to having mating frequency constrained by the availability of AGP and larger accessory glands would be favored by sexual selection.

Males with larger accessory glands in our study had a higher probability of obtaining a copulation in the 2-h observation period. We interpret this as reflecting the fact that those individuals have a higher probability of obtaining a copulation in any given time period, and therefore mate more frequently. This is consistent with the positive correlation between accessory gland size and mating frequency in *D. melanogaster* (Bangham et al. 2002) and the stalk-eyed fly *C. dalmanni* (Baker et al. 2003; Rogers et al. 2005a). Moreover, a line of *C. dalmanni* artificially selected for high mating frequency had larger accessory glands than a line artificially selected for low mating frequencies (Rogers et al. 2005b), further supporting the idea that accessory gland size is important in regards to mating frequency.

Males with larger accessory glands were not better able to manipulate the female into copulating (i.e. males with larger accessory gland size did not require fewer failed mating attempts before the first successful copulation). This suggests that the association between larger accessory gland and probability of mating was not due to increased ability to overcome female resistance or to increased attractiveness to females. However, there was a positive correlation between accessory gland size and total number of mating attempts when other traits were controlled for, suggesting that males with larger accessory glands are more likely to copulate simply because they attempt to mate more frequently. The positive correlation between accessory gland size and total mating attempts also supports the hypothesis that males with larger accessory glands have higher mating effort or 'sex drive'.

It is also possible that larger accessory glands are favored by post-copulatory sexual selection. If the AGP in this species function to increase sperm competition success or decrease the risk of engaging in sperm competition, and if the effects are dose-dependent, then males with larger accessory glands may gain an advantage in post-copulatory success. Therefore, it is possible that both pre-copulatory and post-copulatory sexual selection are contributing to

the pattern of positive allometry and high CV in this trait.

### Aedeagus length

Male genitalia often exhibit negative allometry (e.g. Eberhard et al. 1998; Uhl & Vollrath 2000; Bernstein & Bernstein 2002; Ohno et al. 2003) and in some cases the size of male genitalia is not correlated with body size at all (e.g. Schulte-Hostedde & Alarie 2006). The 'one-size fits all' hypothesis suggests that this pattern is the result of stabilizing sexual selection via cryptic female choice. An intermediate genitalia size is favored because it is capable of stimulating most females in the reproductive population (Eberhard et al. 1998). Consistent with the prediction of this hypothesis the aedeagus length in *D. nigrior* exhibited negative allometry and had an allometric slope less than other traits. Furthermore, males with a larger aedeagus were not more likely to be successful in obtaining a copulation and did not experience more mating failures before achieving a copulation which suggests that males do not use the aedeagus to force the female into copulating (counter to 'weapons' hypothesis) and that females do not prefer to mate with males with a longer aedeagus (counter to 'good viability genes' hypothesis) (Eberhard et al. 1998). This suggests that longer genitalia are not favored by pre-copulatory sexual selection.

Although the negative allometry and low CV exhibited by aedeagus length is not consistent with the general pattern seen in traits where sexual selection favors larger size, we cannot rule out the possibility that longer genitalia are favored by post-copulatory sexual selection. For example, male genitalia exhibited negative allometry in a tortoise beetle but males with longer genitalia had increased reproductive success (Rodriguez et al. 2004).

We focus on the length of genitalia in this study. However, other aspects of genital morphology may be influenced by sexual selection as well. For example, the shape of the male intromittant genitalia is correlated with fertilization success in two water striders species (Arnqvist & Danielsson 1999; Danielsson & Askenmo 1999).

### Protarsal pad

Despite evidence that female-grasping traits can confer a mating advantage (e.g. Arnqvist 1989; Thornhill & Sauer 1991; Sakaluk et al. 1995) that larger protarsal pad size is sexually selected in water beetles (Schulte-Hostedde & Alarie 2006), and that

male-biased traits result from sexual selection (e.g. Tseng & Rowe 1999; Kelly 2005; Teder 2005), we found no evidence that larger protarsal pad size is sexually selected in *D. nigrior*. The length, width, and area of the protarsal pads exhibited allometric slopes slightly less than, and not significantly different from, isometry. Furthermore, males with larger pads did not have a higher probability of copulating and did not require fewer failed attempts before successful copulation. There are several reasons why larger protarsal pads may not be selected in this species. One possibility is that the protarsal pad is not related to overcoming female resistance. Another possibility is that this trait is involved in overcoming female resistance but the advantage is conferred by aspects other than larger size. For example, a particular size protarsal pad that fits the morphology of the average female elytra may provide greater adhesion than larger pads that do not fit as well. It is also possible that the protarsal pad is involved in tactile stimulation of the female and a precise fit to the female anatomy offers better stimulation. The antennal clamp structures in male *Cerotoma salvini* (Chrysomelidae: Galerucinae) serve non-intromittant, close range, tactile functions during courtship and exhibit isometry. These hypotheses do not necessarily contradict the observed sexual size dimorphism in this trait as the optimal size for grasping or stimulating the female may be larger than the optimal tarsal size for females.

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