



Original Article

The complexity of male reproductive success: effects of nutrition, morphology, and experience

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Many studies have attempted to account for variation in male reproductive success by quantifying a single trait such as an ornament or a behavior, but male reproductive performance may be determined by a number of interacting traits. Although developmental nutrition is often a major determinant of adult body size and secondary sexual trait expression, other factors—such as residual shape variation and prior experience—may also exert independent effects on male reproductive success. Here, we studied how male sexual-trait expression, as manipulated by larval diet quality, and experience in direct male–male competition, affected male reproductive success in the sexually dimorphic neriid fly *Telostylinus angusticollis*. Among competing males matched by body size, individuals with relatively longer antennae (used as weapons) were more likely to win and also achieved matings faster. Unexpectedly, males reared on a poor larval diet and those that had previously lost in male–male combat appeared to invest more in some aspects of reproduction as indicated by a longer mating duration and a higher subsequent egg-hatching rate. Our findings demonstrate the complexity of male reproductive success, indicating that male developmental nutrition as well as morphological variation, and prior adult competitive experience interact in a complex manner to influence overall reproductive performance.

Key words: condition dependence, male–male competition, phenotypic plasticity, reproductive investment, sexual selection.

INTRODUCTION

Males often possess multiple secondary sexual traits, which can serve as signals to females as well as rival males, and several hypotheses have been proposed to explain the evolution and maintenance of these multiple signals (reviewed in Candolin 2003). Multiple signals could evolve due to individual females exhibiting multiple, independent preferences (Brooks and Coullidge 1999) and/or because spatially or temporally heterogeneous environments favor different cues (Bro-Jørgensen 2002), potentially reinforcing an overall signal of quality. Signals themselves, as well as the information they convey, are likely to change with age, reproductive experience, and environmental context (Candolin 2000; Freeman-Gallant et al. 2010). Furthermore, different cues could also be aimed at different recipients as they can function both agonistically, to convey a male's

prowess to rivals, and sexually, to convey his phenotypic or genetic quality to potential mates (Berglund et al. 1996). It is this complexity we aim to address in this study.

Male sexual traits can be reliable indicators of quality, or “honest signals” (Zahavi 1975), if their expression is condition-dependent. Condition has been defined in terms of the quantity of nutritional resources available for investment in vital functions (Andersson 1982; Nur and Hasson 1984), but this definition has been criticized as being too narrow (Hill 2011). Thus, Hill (2011) proposed a more general definition in which condition is the “relative capacity to maintain optimal functionality of vital systems.” According to this definition, many components such as somatic state, environment, genotype, and epigenome interact to contribute to condition, thus extending beyond a simple allocation of stored developmental resources. Hill's view of condition is adopted in this study, in which we examined the contributions of larval diet, morphology, and experience to male reproductive success. In previous experiments, male condition has frequently been manipulated as the quality of developmental nutrition, which significantly alters sexual trait expression in a range of taxa (Cotton et al. 2004). Sexual traits often show stronger condition dependence than nonsexual traits or similar traits in females (David et al. 1998), and the response

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to nutrient availability has also been shown to interact with genotype (David et al. 2000). Larval diet quality should therefore be 1 component reflected in the entire set of signals that influence an individual's potential to succeed in reproductive competition, and females may gain both genetic and nongenetic benefits by using a condition-dependent secondary sexual trait as a mate-choice criterion.

In insects, effects of condition on performance in sexual competition are often mediated by developmental resources (Scheuber et al. 2003; Cotton et al. 2004; Amitin and Pitnick 2007), which can strongly influence adult body size and shape (e.g., Amitin and Pitnick 2007; Bonduriansky 2007) and may thus be an important determinant of male mating success (Otronen 1984; Bonduriansky 2007; Pettinger et al. 2011). Intense intrasexual competition often leads to formation of hierarchies, and male size often determines dominance status in these hierarchies (Otronen 1990; Bonduriansky and Head 2007; Sakaluk and Müller 2008). However, exceptions exist in which the smaller male can gain dominance (Pettinger et al. 2011), and other traits apart from body size might become important in determining the outcome of male–male battles particularly if contestants are equally matched in terms of body size. Exposure to rivals can affect a range of male plastic behaviors before, during, and after mating (Bretman et al. 2011), with significant effects on male fitness (Bretman et al. 2009). Thus, male behavior might depend on a male's past performance, as seen in winner/loser effect experiments (Otronen 1990; Hsu and Wolf 1999). Male behavioral traits might also allow more flexibility in signaling current condition (Kotiaho 2000; Scheuber et al. 2003) as static traits that form during development might misrepresent a male's current condition. Taken together, this suggests that male reproductive performance can be an extraordinarily complex phenotypic trait, affected by genes, epigenetic factors, past and present access to resources, and experience. It remains unclear how these factors interact to influence male performance and whether some factors can be regarded as primary determinants.

In this study, we investigate the separate and interactive effects of larval diet quality, morphology, and success in male–male combat on male reproductive performance. We also ask whether these traits are important in male–female interactions to further assess their link to fitness. We investigated these questions in the neriid fly *Telostylinus angusticollis*, which has become a model for effects of condition on morphology and life-history traits (Bonduriansky 2006, 2007; Bonduriansky and Head 2007; Bath et al. 2012; Adler and Bonduriansky 2013; Adler et al. 2013; Sentinella et al. 2013; Cassidy et al. 2014; Crean et al. 2014). In this species, males fight for control of territories and access to females in aggregations on tree trunks. Fights involve males raising their upper bodies and using their heads, antennae, and forelegs to strike their opponents. The winning males defend the best territories and thus aggregations on tree trunks are spatially structured. Courtship behavior appears to be limited to the male briefly tapping the female abdomen and/or flaring his wings near her. During mating and subsequent mate-guarding, the male stands over the female, usually enclosing her within the span of his legs, and females typically oviposit immediately after each copulation. *Telostylinus angusticollis* shows sexual dimorphism in sexual traits used in male–male competition, particularly in head capsule length and width, antenna length, and foreleg length (Bonduriansky 2006). In these flies, males reared on a nutrient-rich larval diet attain larger adult body size and express enlarged secondary sexual traits (Bonduriansky 2007). Bonduriansky (2007) hypothesized that these sexual traits

are mainly shaped by male–male competition and less so by sexual selection due to female choice. *Telostylinus angusticollis* is thus ideal for this investigation as we can manipulate morphology and condition through larval diet and then test the effects on male–male contests and reproductive success. We expect larger males to be more successful in winning these contests. However, when matched with similarly sized males, we expect body-size-independent variation in morphology (i.e., shape) to determine the outcome of these contests. We further investigate whether females use these same cues that are important in male–male contests as mate-choice criteria and if this translates to higher reproductive success for winning males.

MATERIALS AND METHODS

Culturing methods

Telostylinus angusticollis (Enderlein) flies were collected from a natural population at Fred Hollows Reserve in Sydney, Australia and transferred to the laboratory. Flies were held in cages under controlled temperature and natural lighting in a greenhouse. The bottoms of the cages were lined with moistened shavings from coconut husks (“cocopeat,” Galuku Pty., Sydney, Australia), and adult flies were provided with brown sugar and dried yeast as food sources. First-generation offspring from wild-collected individuals served as the parental generation for our experimental animals.

Larval diet manipulation

Fifty F1 males and females each were combined in a large cage and provided with ad libitum food and water. From this generation, we collected eggs once per week for 4 consecutive weeks, resulting in 4 experimental blocks. F1 individuals aged across this time span, such that F1 age effects may contribute to variation among blocks. As block here can be seen as a temporal factor, we included block as a fixed factor in our models. As block was only significant in the analysis of the egg-hatchability data set, we repeated the model including block as a random factor instead and found qualitatively similar results. We opted to present the model with block as a fixed factor in the results. Each week we collected eggs by placing several 250-mL containers of oviposition material in the cage to encourage egg laying. These containers were then removed and 50 eggs each transferred to 250-mL containers filled with larval medium to establish constant-larval-density cultures. Larvae were allowed to develop in either a nutrient-rich larval environment (“rich” diet), whereby the larval medium was rich in sugar and protein sources, or a nutrient-poor larval environment (“poor” diet), with sugar and protein diluted to one-third of the concentration present in the rich diet (see Bonduriansky 2007 for details). Briefly, the rich diet is a mix of 30-mL molasses, 30-mL barley malt, and 32-g soy protein powder per liter of dry cocopeat. As is typical for these diets (Bonduriansky 2007; Bonduriansky and Head 2007), males reared on a rich larval diet had larger body size and expressed relatively more elongated secondary sexual traits (head capsule, antennae, and legs) than males reared on a poor larval diet. A total of 3 containers (i.e., 150 eggs) were prepared for each larval diet per block, whereby all 3 containers were filled from the same batch of the respective medium. We allowed larvae to develop by placing all 6 containers together on the same rack in a climate chamber at 27 °C and 60% relative humidity to minimize environmental variation apart from diet. At eclosion, virgin males and females were collected twice per day for a week and held in single-sex groups of up to 20 individuals

per cage for 2 weeks prior to use in experiments. Even though egg-to-adult survival is not consistently different on the rich versus the poor diet (Bonduriansky 2007), fewer adults eclosed from the poor larval diet treatment. Sample sizes, therefore, varied from $n = 40$ males reared on a rich diet to $n = 20$ males reared on a poor diet for some treatment combinations.

Male–male contests

After the 2-week maturation period, we allowed pairs of virgin males within each larval diet treatment to establish hierarchies in direct male–male contests. Males were matched by body size (matching was done by eye and confirmed by 2 independent observers, and exact measurements of body size were performed after the experiment ended) in a 1-L cage and allowed to compete over an oviposition source and a random stock female. The mating history of the stock female was not known, but any variation in female mating history should have been more or less equally distributed across male treatment groups as females were allocated at random. Males were left to interact undisturbed and we did not observe interactions. After 6 hours to provide males with ample time to establish a hierarchy and settle in their roles and allow, for example, physiological adjustments (Bretman et al. 2010), we determined male status. “Winners” were identified as those males that had monopolized the oviposition source and/or the female (i.e., located in close proximity to the female, often standing over her or directly next to her). The “loser” male would usually be found at the opposite end of the cage. We observed cages for up to 5 minutes to establish that the male hierarchy was settled and no further battles over ownership of territory or the female occurred. In the few cases (fewer than 5 cases) where we could not without uncertainty determine male status, we allowed males to interact overnight. If the males still failed to establish a stable hierarchy, they were excluded from the analysis. Winners and losers were then separated into individual cages containing a fresh dish of larval medium, supplemented with brown sugar and dried yeast, as food.

No-choice mating trials

The next morning, each male was provided with an experimental virgin female from the rich or poor larval diet treatment in a full-factorial design of male and female larval diet as well as male status (winner/loser). Pairs were placed into 250-mL cages with a dish of fresh oviposition medium. We noted the time of introduction and the start and end of mating (if any). Pairs were observed continuously until mating occurred or for a maximum of 2 hours, but allowed to interact unobserved for an additional 4 hours after the initial observation period. Matings also took place during this period. At the end of the 6-hour interaction time, we transferred each female to a new cage with a fresh oviposition dish for another 2 days. The 2 oviposition dishes per female were collected, the eggs laid in each dish were counted, and dishes were subsequently incubated for 2 days. Larvae were then counted to provide an estimate of egg hatchability.

For subsequent morphological measurements, males were frozen after the 6-hour mating trial and females after the 48-hour oviposition period. We measured thorax length of each individual as a proxy of body size (see Bonduriansky 2007). For males, we also measured head capsule length and width, antenna length, foretibia length, and the length of the R_{4+5} wing-vein from the r-m cross-vein to the wing margin, as described in Bonduriansky (2007). Flies were glued to insect pins and images were taken of all

traits of interest using a Leica DFC digital camera mounted on a Leica MS5 stereomicroscope. Wings and legs were severed at the base and mounted on stickers glued to glass slides before imaging. Some individuals' wings were severely damaged and could not be measured (see Results for actual samples sizes). Measurements were taken from images using image analysis software (ImageJ, National Institute of Health).

Statistical analysis

Statistical analyses were performed using R v2.15 (Ihaka and Gentleman 1996). We fit mixed effects models when appropriate with male and female larval diet, male status as winner or loser, and block as fixed factors and including male pair ID (each male pair was given a unique identifier) as a random factor using the lme command from the nlme package. For the egg count and egg-hatchability data sets, we used generalized linear mixed effects models with suitable error structure, as appropriate, to our data. Here, we used the glmer function and the lme4 package. As data were overdispersed, we gave each replicate a unique number and included this observation level random effect in our model (Harrison 2014). Unless otherwise stated, we first started with the full model and tested for the significance of parameters by excluding them in turn, and compared models in an analysis of deviance. For mixed effects models, we used maximum likelihood to compare models with this approach (see Crawley 2005, p. 636). Because larval diet has a strong effect on adult body size, we standardized our morphological measurements by replacing raw trait size with standardized size (z -score) computed within larval diet treatment in order to eliminate collinearity between the categorical larval diet effect and the continuous effect of morphology. We used these standardized values in multivariate selection analysis (e.g., Blows et al. 2003) to test for linear and nonlinear selection on each trait by performing multiple regression analysis to estimate selection differentials (Phillips and Arnold 1989; Stinchcombe et al. 2008). First, we constructed a minimal model containing only male larval diet as a fixed factor and then compared this to a model including all standardized morphological traits. This was followed by excluding each trait individually to determine which traits significantly determine male dominance status. We performed multivariate selection analysis only for male–male contest data (see male–male contests) as we did not have enough power to also meaningfully extend this type of analysis to data on male–female interactions (see Results for details).

To test whether male morphological traits identified to be under selection in male–male contests are also important for male reproductive fitness, we included these in subsequent models of male–female interaction data (mating latency and duration in the no-choice mating trials) and female reproductive output. We included the z -score standardized morphological values to eliminate collinearity as outlined previously. Female and male larval diets were included as fixed factors as was male status (winner/loser) in male–male contests and the interactions between these factors plus block. We report mean \pm standard error values throughout.

RESULTS

Male–male contests

To better understand which morphological traits might be beneficial in male–male contests, we employed multivariate selection analysis to test for linear and nonlinear selection. We used male status (winner/loser, coded as a binary response variable and

standardized) as the measure of fitness. A linear mixed effects model containing all 6 z-score standardized morphological traits significantly improved the fit of our model compared with our minimal model including only male larval diet as a fixed factor, indicating linear selection is acting on 1 or more of these traits in addition to overall condition ($LogRatio = 13.16$, degrees of freedom [df] = 6, $P = 0.041$). After model simplification, we retained only 2 significant traits: standardized antenna length ($LogRatio = 6.10$, df = 1, $P = 0.014$) and standardized wing length ($LogRatio = 6.44$, df = 1, $P = 0.011$). We also included male thorax length to control for body size, despite male thorax length not significantly improving the fit of the model by itself. Inclusion of these 3 traits significantly improved the fit of the model compared with the minimal one that included only larval diet ($LogRatio = 10.84$, df = 3, $P = 0.013$). We next employed multiple regression analysis separately for males reared on poor and rich larval diets. We found significant linear selection acting on antenna length and wing length in males reared on a rich diet, and whereas results are not significant for poor-diet males, the direction of the relationship is the same as for rich-diet males (Table 1). Males with increased antenna length and reduced wing length had an advantage in winning male–male battles at least when reared on a rich diet. There was no evidence of selection acting on male thorax length, which indicates that we matched males well for body size (as confirmed by a significant correlation of winner against loser thorax sizes: $r = 0.706$, $N = 65$, $P < 0.0001$; mean relative size: rich winners = 1039.60 ± 5.98 ; rich losers = 1035.60 ± 5.41 and poor winners = 690.89 ± 11.22 ; poor losers = 690.21 ± 9.72) and thus strengthened our power to detect selection on body shape. We did not find evidence for nonlinear selection acting on these traits ($LogRatio = 5.11$, df = 6, $P = 0.53$) and thus did not further extend our analysis beyond this point.

Male–female interactions

A linear mixed effects model showed that males reared on a rich diet were overall faster in gaining a mating than poor-diet males (mean time to mating: rich-diet males = 2529 ± 353 seconds, $N = 71$; poor-diet males = 3683 ± 585 seconds, $N = 30$; $LogRatio = 4.96$, df = 1, $P = 0.026$). Male status as winner or loser in the prior male–male contest marginally significantly affected time to mating ($LogRatio = 3.98$, df = 1, $P = 0.046$), with losers taking longer. This was mostly due to poor-diet males who were also losers taking longer to gain a mating than other combinations of larval diet and status (see Figure 1). This pattern was independent of female larval diet ($LogRatio = 0.09$, df = 1, $P = 0.759$; all interactions nonsignificant; thus, we here present the results from the reduced model).

In contrast, copulation duration was significantly affected not only by male larval diet but also by male winner/loser status and

female diet (see Table 2, Figure 2). Although loser males, independent of their larval diet, tended to mate for longer, loser males reared on a poor larval diet mated for significantly longer with females reared on a rich diet compared with all other combinations (see Table 2, Figure 2). However, the length of copulation was also influenced by male morphology as indicated by a significant effect of male antenna length when controlling for male and female body size. There is a potentially interesting diet-dependent relationship between male antenna length and winner/loser status as rich winner males copulated for longer if they had elongated antennae ($r = 0.359$, $n = 36$, $P = 0.032$, nonsignificant for all other groups).

Female reproductive output

Female reproductive output was significantly dependent on an interaction between her own larval diet and the larval diet of her mate (Table 3). Females mated to rich-diet males produced more eggs than females mated to poor-diet males (mean number of eggs: rich-diet mate = 73.50 ± 6.42 , $N = 88$; poor-diet mate = 58.02 ± 7.48 , $N = 43$), whereas rich-diet females produced more eggs than poor-diet females (mean number of eggs: rich diet = 73.72 ± 6.05 , $N = 100$; poor diet = 49.91 ± 7.25 , $N = 32$). Also, within larval diet treatments, female size significantly influenced female reproductive output (Table 3). Across both larval diet treatments, larger females laid more eggs ($r = 0.230$, $P = 0.008$). However, this effect of thorax length within larval diet treatments is driven entirely by females reared on a poor diet (poor diet: $r = 0.432$, $N = 31$, $P = 0.015$; rich diet: $r = 0.113$, $N = 100$, $P = 0.263$).

Interestingly, egg-hatching success depended on male larval diet (Table 4). Hatching success was higher in poor-diet females (poor diet: $70.6\% \pm 8.1\%$, $N = 32$; rich diet: $48.9\% \pm 5.0\%$, $N = 100$) and was also higher in females mated to poor-diet males (poor diet: $68.4\% \pm 7.0\%$, $N = 44$; rich diet: $46.6\% \pm 5.3\%$, $N = 88$; Figure 3). This difference in hatchability compensates for the higher egg output females show after mating with a rich-diet male (mean number of larvae: rich-diet mate = 34.25 ± 3.96 , $N = 88$; poor-diet mate = 39.58 ± 5.86 , $N = 43$). Hatching success is the only trait that showed significant variation across the 4 blocks (Table 4). This could be due to variation in the quality of food provided or random variation in light and temperature regime. However, a tendency for poor-diet males and females to achieve higher hatching success was observed in all blocks.

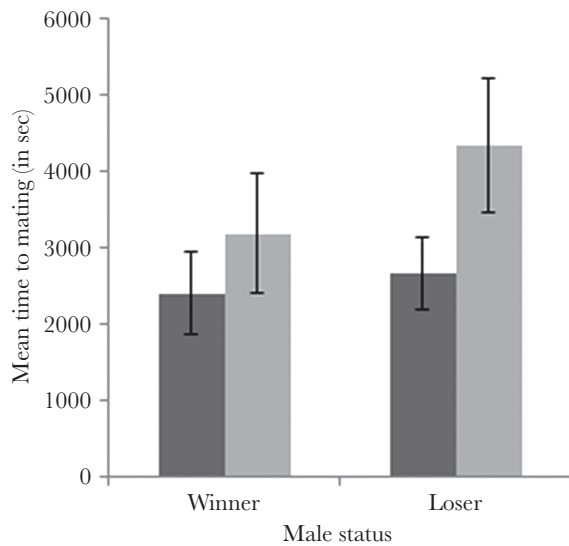
DISCUSSION

Our results demonstrate that, in *T. angusticollis*, male performance in male–male and male–female interactions is affected by complex interactions of male larval diet, morphology, and experience. Male larval diet, which affects body size and secondary sexual trait expression in this species, exerted strong effects on mating behavior

Table 1

Multiple regression analysis testing for the direction of selection acting on male morphological traits (standardized by the mean and standard deviation for each larval diet) that determine a male's status in direct male–male contests, whereby β represents the selection gradient for these traits

Source	Poor larval diet			Rich larval diet		
	β	t	P	β	t	P
Thorax length	−0.005	−0.58	0.568	0.004	0.95	0.346
Antennae length	0.017	1.73	0.093	0.008	2.03	0.047
Wing length	−0.009	−0.52	0.604	−0.019	−2.53	0.014

**Figure 1**

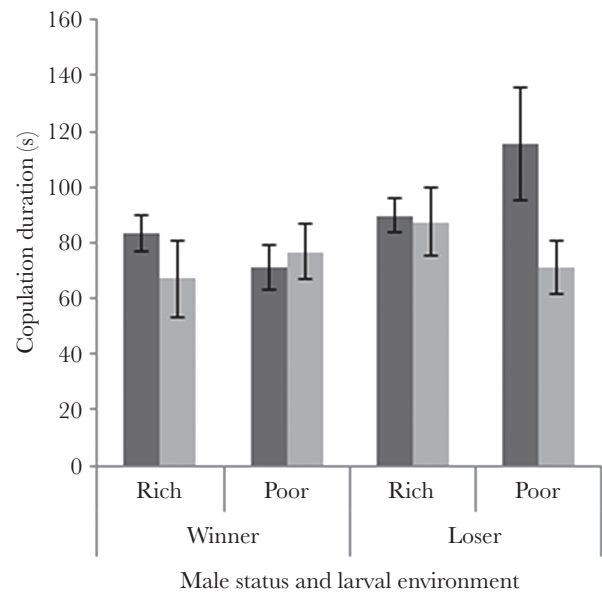
Mean (\pm standard error) time to mating depending on male status as a winner or loser as determined in male–male contests and larval diet. Dark gray bars represent males raised on a rich larval diet and light gray bars represent males raised on a poor larval diet.

Table 2

A mixed effect model of copulation duration, with male and female larval diet and male status included as fixed factors in the model. A unique ID for each loser and winner male pair and a unique ID for each female were included as random factors in the model. Male and female body size as well as male antenna length were included as z-score standardized covariates computed within larval diet treatments

Source	df	LogR	P
Male food	1	0.04	0.845
Male status	1	6.25	0.012
Female food	1	1.33	0.249
Female body size	1	0.99	0.319
Male body size	1	2.75	0.097
Antenna length	1	3.96	0.047
Male food \times female food	1	0.22	0.643
Male food \times status	1	2.76	0.097
Female food \times status	1	0.03	0.863
Male food \times status \times female food	1	8.24	0.004

as well as reproductive output. Males reared on a rich diet were faster in gaining a mating, but conferred lower egg-hatching success to females. Likewise, copulation duration was affected by male larval diet, but there was also an effect of prior experience during male–male contests. Although male body size is known to be important for the establishment of dominance hierarchies (Bonduriansky and Head 2007), we found that body shape (i.e., relative antenna and wing length) also contributed to variation in performance, independently of effects of body size and larval diet. Although shorter wings were favored only in rich-diet males, longer antennae (relative to thorax length) were selected for in both rich-diet and poor-diet males during direct male–male contests. In addition, male experience (winner/loser status in male–male contests) affected male mating performance in subsequent interactions with females, that is, loser males took longer to gain a mating and stayed longer in copula. Our results suggest that male reproductive performance is a highly complex and multifaceted trait, and that small and subordinate males may be able to compensate to some extent

**Figure 2**

Mean (\pm standard error) duration of copulation depending on male and female larval diet as well as male status as determined in male–male contests. Dark gray bars represent females reared on a rich larval diet and light gray bars represent females reared on a poor larval diet.

Table 3

Female egg output was analyzed using a generalized linear mixed effects model with Poisson errors. Male and female larval diet and male status were included as fixed factors. Male morphological traits and female body size were included as z-score standardized covariates calculated within larval diet treatments. Male–male pair identity and female identity were included as random factors in the model

Source	df	χ^2	P
Male food	1	2.074	0.150
Male status	1	1.115	0.291
Female food	1	0.312	0.577
Female body size	1	4.342	0.037
Male body size	1	1.760	0.185
Antenna length	1	0.610	0.435
Wing length	1	0.110	0.740
Male food \times female food	1	5.291	0.021
Male food \times status	1	0.214	0.643
Female food \times status	1	0.006	0.937
Male food \times status \times female food	1	0.596	0.440

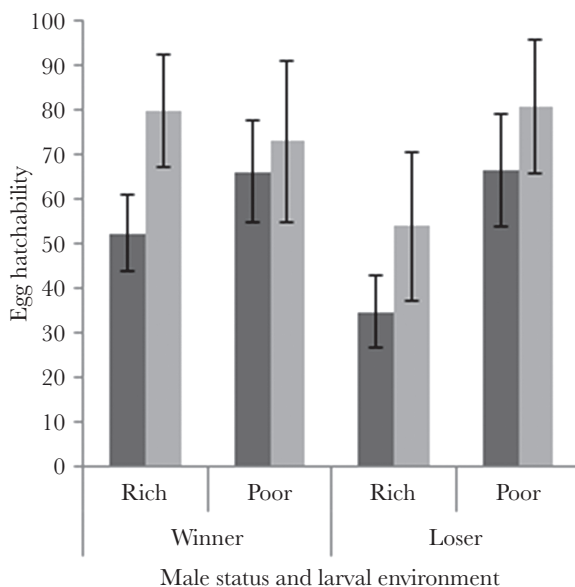
for their poor precopulatory performance through increased investment in mating and offspring viability.

We did not explicitly set out to test different hypotheses of multiple ornament evolution here, but we found evidence that some male shape components are under selection via both male–male combat and potentially also male–female interactions in *T. angusticollis* flies. As we size-matched males to minimize body-size differences between contestants, we were able to identify shape components that are also under selection. A previous study in this species showed that male body size conferred an advantage in male–male contests when the size difference between rivals exceeded a lower limit (Bonduriansky and Head 2007). Below this minimum body-size difference, body shape (i.e., relative antenna and wing length) appears to influence combat success. Male antennae are employed as weapons in male–male combat (Bonduriansky

Table 4

Egg-hatchability analysis, with male and female larval diet and male status included as fixed factors in a linear mixed effects model with binomial errors. Male morphological traits and female body size were included as z-score standardized covariates calculated within larval diet treatments. Male–male pair identity and female identity were included as random factors in the model

Source	df	χ^2	<i>P</i>
Male food	1	5.97	0.015
Male status	1	1.02	0.313
Female food	1	0.60	0.440
Block	3	16.77	0.001
Female body size	1	1.45	0.228
Male body size	1	0.49	0.485
Antenna length	1	1.20	0.274
Wing length	1	1.37	0.241
Male food × female food	1	0.07	0.789
Male food × status	1	0.11	0.743
Female food × status	1	2.84	0.092
Male food × status × female food	1	0.33	0.565

**Figure 3**

Mean (\pm standard error) hatchability of eggs depending on male and female larval diet and male status as determined in male–male contests. Dark gray bars represent females reared on a rich larval diet and light gray bars represent females reared on a poor larval diet.

2006), and antenna length is also strongly sexually dimorphic and condition-dependent in males in this species (Bonduriansky 2007), fulfilling the prerequisite for an honest sexual ornament. Because *T. angusticollis* males tend to engage in combat with similar-sized rivals (Bonduriansky R, unpublished observations), variation in body shape may have considerable consequences for male fitness.

It was striking that low-condition males outperformed high-condition males in some ways. Loser males reared on a poor diet mated for longer, and egg hatchability was higher for poor-diet males than for rich-diet males. This pattern tended to be pronounced when the female was also reared on a rich larval diet and was therefore more fecund. However, we found no clear link between copulation duration and egg hatchability. Previous work in the guppy *Poecilia reticulata* showed a positive correlation between ornament expression and

production of healthier and more fertile sperm (Locatello et al. 2006; Pitcher et al. 2007) as males in good condition might be better able to protect their sperm from oxidative stress as seen in the great tit *Parus major* (Helfenstein et al. 2010). In our study, male *T. angusticollis* reared on a poor larval diet showed higher fertility. A possibility is that stronger survival selection on larvae in the poor-diet treatment (which yielded fewer adult flies per egg transferred) might have favored certain genotypes with more fertile sperm. However, previous work showed that nutrient-poor larval diet is in general a benign larval environment for this species (Sentinella et al. 2013), thus not necessarily exerting stronger selection pressures. Instead, the observed pattern could occur if extended engagement in male–male contests of rich-diet males leads to a trade-off with production or protection of sperm, or production of accessory gland products, resulting in lower quality ejaculates. Similarly, rich-diet males could trade-off investment in secondary sexual trait expression with investment in testis or accessory gland growth, as found in horned beetles *Onthophagus* spp. (Simmons and Emlen 2006).

Alternatively, the observed effect on hatching success could be due to males exhibiting plasticity in sperm allocation. We suggest that males may adjust their mating investment depending on their larval diet as well as social experience (Bretman et al. 2011; Kasumovic and Brooks 2011). Males might change the composition of their ejaculate (Wigby et al. 2009) or transfer more sperm (Bretman et al. 2011) when exposed to rivals, and may allocate resources to mating in expectation of future mating opportunities. This explanation might be particularly likely in this species, in which male fitness in the wild appears to be strongly dependent on relative position in the dominance hierarchy (M.I.A. and R.B., personal observations). Thus, males with a good prospect of mating multiply might prudently allocate their ejaculates across time and females (Wedell et al. 2002). In contrast, males that lack large secondary sexual traits or have previously lost in mating competition may instead invest in maximizing the output from the few mating opportunities they are likely to achieve. Mating in this species appears to require female acquiescence, but is often initiated while females are feeding, suggesting convenience polyandry may occur, as females apparently cannot effectively feed and avoid males at the same time. Thus, dominant males may be more likely to easily achieve mating both because they are preferred by females and because they guard food resources, allowing them easier access to females while feeding. Subordinate males are perhaps more likely to pursue a “sneaker” strategy, but their lower success rate in achieving matings suggests that investing more when an opportunity arises would be a prudent strategy.

Despite the higher egg-hatching success induced by the poor-diet males, females showed no preference for poor-diet males. Although we did not allow females to exert mate choice beyond delaying or refusing mating with the 1 male offered to them, we found that females might still exert mating preferences, as indicated by the faster acceptance of a mating with rich-diet males as well as a tendency to reject loser males. Additionally, rich-diet females might invest more in matings with larger males, as those matings resulted in more eggs being laid. However, our experimental design does not allow us to disentangle male and female effects. Thus, it is possible that rich-diet males achieved matings more quickly simply because they were more vigorous and caused females to lay more eggs by transferring more or better fecundity-stimulating factors in the ejaculate, with no active discrimination on the females’ part. Nonetheless, female preference to mate with rich-diet males in this system could potentially confer indirect benefits, as rich-diet males produce larger offspring, which are likely to attain higher fitness

(Bonduriansky and Head 2007; Adler and Bonduriansky 2013; Crean et al. 2014). Thus, female choice could play a role in this system, and it would be interesting to establish whether females reinforce or oppose intrasexual selection (Candolin 2004).

Even with male larval diet and morphology included in models, we found that male status (winner/loser) in a previous male–male interaction affected subsequent performance in male–female interactions. Males that lost contests against other males took longer to gain a mating and, when successful, mated for longer than males that had previously won. These effects were amplified when loser males developed on a poor larval diet. Variation in male performance in male–male contests that is not accounted for by larval diet and morphology may reflect uncontrolled environmental variation and genotype. Like antenna length, male performance in combat may therefore signal both phenotypic and genetic quality. However, as females did not witness the male–male contests, the effects of male status must have been mediated by variation in the behavior of the males themselves in their interaction with females. In particular, loser males may have perceived their mating prospects as being poor and, thus, each mating as more valuable, and so attempted to mate longer (and perhaps transfer more or higher quality sperm) when paired with females. This finding also suggests that stochastic events (e.g., the order in which a male encounters rivals) could influence his performance in future intersexual and intrasexual interactions via lingering winner/loser effects (Hsu and Wolf 1999). Alternatively, loser males may have mated for longer than winner males because winner males may have mated with the stock female they monopolized during the contest. Thus, our design very likely introduced a difference in male mating history between winner and loser males, which is ecologically realistic in this species. *Telostylinus angusticollis* males compete regularly to establish dominance hierarchies. Males that win these contests guard feeding and oviposition sites, and monopolize access to females at these sites. Thus, dominant males tend to have many mating opportunities, whereas subordinate males likely have few or none. Nonetheless, because our design did not control for differences in male mating history, it might have amplified sequence effects of winner and loser males in the sense that differences in reproductive success might be more pronounced due to the likelihood that winner males had mated prior to the mating with the test females and in nature losers males might never get the opportunity to mate at all.

Our results show that male larval diet and the expression of secondary sexual traits were key in gaining a dominant position during male–male contests. The complex interplay of factors affecting male mating strategies suggests that benefits to a female based on the identity of the male she mates with are not straightforward. Although females exerted a preference for rich-diet males, males reared on a poor larval diet appeared to invest more into each mating and conferred higher egg hatchability. Our findings demonstrate that male reproductive success is not determined solely by the nutritional environment, nor fixed at the point of adult sexual maturity. Rather, male reproductive performance is a dynamic property that integrates the effects of nutrition, additional variation in morphology that is not accounted for by nutrition, and perhaps adult experience in social encounters.

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