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ORIGINAL PAPER

Frequent mating reduces male mating rate but not offspring quality or quantity in a neriid fly

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Abstract

Frequent mating can deplete sperm and seminal fluid, limiting male ability to sire offspring. Frequent mating could also deplete non-genetic ejaculate components that affect offspring quality. These effects of frequent mating on male reproductive success may be mediated by male condition, or by modification of subsequent male mating behaviour. Using the neriid fly *Telostylinus angusticollis*, we conducted two experiments to examine whether a history of frequent mating affects males' subsequent mating rate and offspring traits. The first experiment tested whether male condition (manipulated by varying larval diet quality) and mating history affects male performance in a subsequent mating with a single novel female whereby we predicted effects of previous mating may be more prevalent in low-condition males. Prior mating resulted in a reduction in mating rate with the novel female, but we did not detect an effect of mating history or male condition on offspring quality or quantity. The second experiment tested whether costs of mating become more evident when males encounter multiple novel females. Surprisingly, while prior mating once again resulted in a reduction in mating rate with the novel females, we still did not detect an effect of condition or mating history on any offspring traits. Therefore, male neriid flies appear to be able to mate many times without suffering a reduction in offspring quality or quantity. The apparent lack of an effect of frequent mating on such traits could be mediated by a reduction in mating rate, reflecting male prudence with ejaculate expenditure.

Keywords Sperm · Seminal fluid · Ejaculate depletion · Ejaculate allocation · Non-genetic · Paternal effects

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Introduction

Frequent mating can deplete the male ejaculate and reduce the ability to sire offspring (e.g., Dewsbury 1982; Preston et al. 2001; Torres-Vila and Jennions 2005; Marcotte et al. 2007; Wigby et al. 2009; Reinhardt et al. 2011; Muller et al. 2016; Hopkins et al. 2019). For example, male Soay rams (*Ovis aries*) become sperm depleted over the course of the breeding season leading to a reduction in the number of lambs sired (Preston et al. 2001). This reduction in offspring quantity with mating can occur rapidly in some species. For example, three to five matings is enough to deplete the ejaculate and reduce offspring number in *Drosophila melanogaster* (Hihara 1981; Linklater et al. 2007; Hopkins et al. 2019). However, male fitness is not only determined by offspring quantity, but can also be affected by offspring quality (Maynard Smith 1977; H Clutton-Brock 1991; Sheldon 2002; Crean et al. 2016).

Offspring quality can vary depending on non-genetic factors in the ejaculate such as proteins, peptides, and epigenetic factors such as non-coding RNAs, DNA methylation and chromatin structure. If frequent mating results in a depletion of these factors—either through a reduction in dose within the ejaculate or through a depletion of sperm and thus a corresponding decrease in the epigenetic factors associated with sperm—males may experience a corresponding decrease in offspring quality. Males of some species transfer large and costly ejaculates such as spermatophores and nuptial gifts that are rich in nutrients (reviewed in Vahed 1998), and frequent mating can reduce the size of spermatophores which corresponds to reductions in female fecundity (Torres-Vila and Jennions 2005) and aspects of offspring quality (e.g., Michaud et al. 2013). Yet, many species do not transfer such large, nutrient rich ejaculates but can still influence the quality of their offspring via non-genetic factors (e.g., Bonduriansky and Head 2007; Delcurto et al. 2013; Mirhosseini et al. 2014; Crean et al. 2016; Evans et al. 2017). However, the potential for frequent mating to reduce offspring quality has yet to be explored in species that do not transfer large and costly ejaculates (see Macartney et al. 2018a).

If the ejaculate components that enhance offspring quality and quantity are depleted with mating, we may expect effects of mating to be more pronounced in males that are less able to invest in ejaculate synthesis. For example, many ejaculate components have been shown to be condition-dependent whereby males in low-condition (such as males from a nutrient poor larval diet) are less able to invest in ejaculate production (reviewed in Macartney et al. 2019). Male investment in offspring quality can also depend on male condition (Delcurto et al. 2013; Bonduriansky and Crean 2017; Evans et al. 2017; Macartney et al. 2018a). Therefore, male condition could interact with male mating history to influence offspring quality and quantity whereby the costs of frequent mating may be exacerbated in low-condition males. For example, a recent study by Polak et al. (2017) found that calorie-restricted *D. melanogaster* that had mated once previously sired offspring with greater embryo mortality, suggesting that even one mating is enough to reduce offspring quality in low-condition *D. melanogaster.*

Using the neriid fly *Telostylinus angusticollis*, we investigated whether frequent mating reduces male ability to sire offspring and/or reduces offspring quality. We also asked whether male condition (manipulated by varying nutrient availability in the larval diet) interacts with mating history to affect offspring quality and quantity. *T. angusticollis* females generally aggregate around food sources (rotting tree bark) and males compete for access to mates through combat. While we do not have precise estimates of mating rates in the wild, both females and males can mate multiply within a short period of time in

captivity (pilot observations have shown that males can mate up to 10 times in a three hour period) and mating duration is very rapid (~60 s) (Bath et al. 2012; Fricke et al. 2015). While male *T. angusticollis* do not transfer large, nutrient-rich ejaculates, previous studies on this species have shown that the seminal fluid can contain non-genetic factors that influence egg-to-adult viability and offspring body size (Bonduriansky and Head 2007; Adler and Bonduriansky 2013; Crean et al. 2014; Macartney et al. 2017). These aspects of offspring quality are likely to be important for male fitness in the wild. Other studies on this species have demonstrated that many sexual traits are condition-dependent, including the secondary sexual traits used in combat (Bonduriansky 2007) and ejaculate traits important for siring offspring (Macartney et al. 2018b). Male larval diet also influences offspring body size whereby high-condition males from a nutrient rich larval diet produce larger offspring (Bonduriansky and Head 2007). Such condition-dependence suggests that these traits are costly, as low-condition males are less able to invest in such traits. Therefore, if the ejaculate components that influence male ability to sire offspring and affect offspring quality are costly and condition-dependent, we may expect these factors to be depleted with mating, and expect depletion to occur more rapidly in males reared on a nutrient-poor larval diet.

We carried out two experiments to address these questions. The first experiment determined whether male condition (larval diet quality) interacts with male mating history to influence male ability to induce female oviposition and fertilise eggs (offspring quantity), and affects offspring viability and body size (offspring quality) when males encounter a single novel female. We predicted that frequent mating would have stronger effects in low-condition males. This experiment showed that a history of frequent mating results in an increased latency to mate with the novel female but did not reveal any effects of frequent mating on offspring quality and quantity. We therefore carried out a second experiment to determine the effects of frequent mating on subsequent mating behaviour when males are presented with multiple novel females. Multiple novel females could motivate males to engage in a greater number of matings (i.e., via a 'coolidge effect'; Dewsbury 1981; Pizzari et al. 2003), and this increased mating effort could reveal effects of ejaculate depletion that were not observed in the single mating assay of Experiment 1. The combination of these two experiments enabled us to test for costs of frequent mating for males of a species that lacks large and nutrient rich ejaculates but exhibits condition-dependent ejaculate investment and non-genetic effects on offspring.

Materials and methods

Experiment 1: frequent mating and male condition

Rearing of experimental flies

Wild flies were collected from Fred Hollows Reserve, Coogee, Sydney (33.91° S, 151.25° E) in March 2016. After two generations of rearing in large stock containers, 40 pairs of virgin males and females were used to collect eggs for a split-brood experimental design.

From each pair, 20 randomly selected eggs were transferred to 100 g of 'nutrient rich' larval diet to create 'high-condition' focal males, and 20 eggs were transferred to 100 g of 'nutrient poor' larval diet to create 'low-condition' focal males. The rich larval diet consisted of 32 g soy protein powder (Nature's Way brand, Pharm-a-care Pty. Ltd.,

Warriewood, NSW, Australia) and 89 g brown sugar (brown sugar; Coles brand, Bundaberg, Australia) per 1 L of cocopeat, and was hydrated with 600 ml of water. The poor larval diet was comprised of 5.5 g protein and 14.8 g brown sugar per litre of cocopeat and hydrated with 600 ml of water. These diets were chosen to create flies of significantly different condition (see Sentinella et al. 2013). We obtained 21 full-sib families with individuals from the rich and the poor larval diets. A further 2000 eggs were transferred from the stock population to the rich larval diet (150 g/50 eggs) to create females for use in the mating history manipulations and reproductive performance assays (described below).

Immediately after adult eclosion of the focal flies, two randomly selected males from the same larval diet and family were paired together in a 250 ml container until the mating history manipulation. This was done because isolated males fail to undergo normal reproductive development (ELM and AJC, unpublished data). Adult males were provided with sugar but not protein to reduce the opportunity for replenishment of depleted ejaculate reserves as reduced adult dietary protein can reduce male ability to synthesise ejaculate components in other Dipteran species (e.g., Droney 1998). Virgin females were housed in groups of 10 in 1 L containers, and fed sugar and yeast. All larval and adult flies were watered periodically and were kept at a constant temperature of 25 °C with a 12 h light/dark cycle.

Male mating history manipulation

Focal males were between 15 and 20 days old (18.49 ± 1.16 SE) at the time of the mating history manipulation. Males from each pair were randomly allocated to either a 'mated' treatment group or a 'control' group ($n = 42$ per mated and control group). Males in the mated treatment were housed with five virgin females in a 1 L container for 3 h where they were free to mate throughout the treatment period. We used five females as other Dipteran studies have found significant costs after mating 3–5 times (e.g., Hihara 1981; Linklater et al. 2007; also see Hopkins et al. 2019). The number of matings per male in the mated treatment group were recorded in the first hour and then every following half hour with half hour breaks between observations (females were not individually identifiable so the number of matings was the total number of matings across all five females). Two males from the mated treatment were not observed mating during the observation period and so these males and their control group siblings were excluded from the reproductive performance assay (resulting in $n = 40$ per mated and control groups). Control males were transferred individually to scintillation vials with a mesh lid and placed in the centre of a 1 L container housing five virgin females for 3 h. This allowed the males to receive visual and pheromonal cues through the mesh but prevented mating.

Male reproductive performance

After the mating history manipulation, focal males were paired with a novel virgin female (assay female) in a scintillation vial until mating was achieved. All assay females were 14 days old and reared on the rich larval diet. For logistical reasons, exact time to mating was not recorded, and males were categorised as 'early mating' (< 30 min until mating) or 'delayed mating' (> 30 min until mating) for analysis of 'latency to mate'. Eight out of 80 males took longer than 90 min to initiate mating, and this was recorded and included in the latency to mate analysis as 'delayed mating', but these males were excluded from the

reproductive performance assays because of the potential for replenishment of ejaculate components during the extended delay.

After mating, the pairs were immediately separated, and the assay females were transferred to individual 250 ml containers with oviposition medium (pre-moulded rich larval food).

The latency of female oviposition was measured (in days). This trait was measured as proteins and peptides in the ejaculate can induce female oviposition in insect species such as *D. melanogaster* (e.g., Chapman 2001). On the first day of egg laying, we collected 20 randomly selected eggs from each female and placed them on damp filter paper (to facilitate quantification of egg hatching success) on top of 100 g of poor larval diet as non-genetic effects of paternal condition may be more pronounced when offspring are reared on a nutrient poor larval diet (Bonduriansky and Head 2007). The number of eggs to have hatched was recorded 42 h after egg laying. Hatched eggs were identified as empty eggshells under a Leica M60 stereo-microscope (Leica Microsystems, Heerbrugg, Switzerland). Larvae were then left to develop at a constant temperature of 25 °C with a 12 h light–dark cycle and watered ad libitum. Egg-to-adult viability was determined by the number of flies to eclose as adults from the 20 eggs transferred to measure egg hatching success.

Morphometric data

To quantify body size, thorax lengths of focal males, the assay females, and of five randomly selected offspring of each sex per brood (where possible) were measured using ImageJ (Rasband 2015) from photos taken under 6.3 × magnification. Photos were taken using a Leica DFC420 camera mounted on a Leica MS5 microscope.

Experiment 2: frequent mating and male prudence

Rearing of experimental flies

New fly stocks were collected from Fred Hollows Reserve in February 2018 and maintained for one generation.

To obtain experimental flies, 4300 eggs were collected from the stock population and all larvae were reared on the rich larval diet (see Experiment 1). Larval diet was not manipulated in Experiment 2 because we did not detect effects of larval diet in Experiment 1. Removing this treatment halved the number of replicates, allowing us to make more detailed behavioural observations during both the mating treatment and male reproductive performance assays. We transferred 50 eggs to 150 g of larval food in each of 86 larval containers. All flies were reared as described above in Experiment 1.

Male mating history manipulation

Focal males aged between 15 and 18 days old (16.95 ± 0.124 SE) were randomly allocated to a mated treatment or control group (n = 40 per treatment), as in Experiment 1. The number of matings was recorded for males across all five females (females were not individually identifiable) from the mated treatment during the first 45 min and the last 45 min of the 3 h treatment period in order to get a measure of observed mating number but also to get an estimate of how male mating rate changed with time.

Male reproductive performance

After the treatment period, focal males from the mated and control groups were individually placed in another 1 L container with five novel virgin females (assay females) for 45 min and the total number of matings across all five females was recorded.

After the assay period, the five assay females per male were placed in a 1 L container with a large oviposition dish in order to measure each male's post-copulatory investment across the five females. The number of eggs laid by the females was counted after 72 h (egg output). We then randomly selected 20 eggs per group of five assay females to measure egg hatching success. Unfortunately, we were unable to measure egg-to-adult viability or offspring body size in this experiment due to very low eclosion of offspring into adults across both treatments (~3% of larvae eclosed into adults—potentially due to an unknown fungus growing on the larval food containers).

Morphometric data

Measurements of focal male thorax length were taken as per Experiment 1. Female thorax length was not measured in this experiment as males could mate with any of the five assay females and the individual females that the males chose to mate with were not identifiable.

Statistical analyses

All analyses were completed in R version 3.3.2, using the *lme4* (Bates et al. 2015) and *LmerTest* (Kuznetsova et al. 2017) packages. *LmerTest* calculates *p* values for Gaussian mixed effects models based on the Satterthwaite approximation for denominator degrees of freedom (Schaalje et al. 2002).

For Experiment 1, effects of larval diet on male thorax length were analysed using a linear mixed-effects model with larval diet as the fixed effect and family as the random effect. The number of times mated treatment males were observed mating in the 3 h treatment period was analysed using a generalised linear mixed-effects model (GLMM) with a Poisson distribution, larval diet as a fixed effect, and family and observation (i.e., a unique level for every data point to correct for overdispersion) as random effects. Male mating latency with the assay females (early mating = 1 versus delayed mating = 0), egg hatching success (hatched eggs = 1 versus not-hatched = 0), and egg-to-adult viability (eclosed offspring = 1 versus not-eclosed offspring = 0) were analysed separately using GLMMs with binomial distributions. The latency to oviposition was analysed using a GLMM with a Poisson distribution, and offspring body size was analysed using a linear mixed-effects model with a Gaussian distribution. In the offspring body size analysis, male and female offspring were pooled and offspring body size (thorax length) was standardised within offspring sex because there were no a priori predictions of treatment on male or female offspring body size and there were no significant interactions of treatments and offspring sex on offspring body size. Models of male mating latency, female latency to oviposition, egg hatching success, egg-to-adult viability, and offspring size included main effects and an interaction term of male larval diet and mating history, as well as male thorax length (standardised within larval diets to avoid co-linearity between the categorical predictor "larval diet" and the continuous predictor "body size") and assay female thorax length as co-variates. Family

was included as a random effect for all analyses and an observation level random effect to correct for overdispersion was included in the male mating latency, egg hatching and egg-to-adult viability analyses.

For Experiment 2, differences between mated and control males in the number of observed matings across the five assay females after the mating history manipulation were analysed using a generalised linear model (GLM) with a Poisson distribution, mating history manipulation as a fixed effect and male thorax length as a covariate. A decrease in mating rate (i.e. a reduction in mating number over time) was analysed using a GLMM with a Poisson distribution, observation period (i.e., each time point that the males were observed: the two observation periods during the mating history manipulation and the observation period during the reproductive performance assay) as the main effect, male thorax length as a covariate and male identity as a random effect. Effects of the mating history manipulation on male ability to induce female oviposition and fertilise eggs (egg output and egg hatching success) were analysed using GLMMs with treatment as a fixed effect, male thorax length as a covariate and observation (to correct for overdispersion) as a random effect. Egg output was analysed with a Poisson distribution and egg hatching success was analysed with a binomial distribution (hatched eggs = 1 versus not-hatched = 0). In text descriptive statistics are written as means and standard errors. All data and code can be found at https://osf.io/kex4j/.

Results

Experiment 1: frequent mating and male condition

Effects of diet and mating history on mating behaviour

Males reared on the rich larval diet were significantly larger than males reared on the poor larval diet (rich larval diet: 1.706 mm ± 0.065; poor larval diet: 1.263 mm ± 0.063; estimate = 0.418, $t_{48} = 10.56$, $p < 0.001$).

Males reared on the rich larval diet mated significantly more than the males reared on the poor larval diet during the 3 h mating history manipulation (rich larval diet: 7.72 ± 0.794 matings; poor larval diet: 4.48 ± 0.491 matings; estimate = 0.398, $Z_{33} = 2.34$, $p = 0.019$).

During the mating treatment, 16 out of 40 males were not observed mating in the last 30 min of the 3 h treatment period (7/16 of which were from the poor larval diet and 9/16 from the rich larval diet). This suggests that the mating manipulation was successful at depleting male ejaculate reserves in at least some of the males across both diets.

Effects of diet and mating history on subsequent male reproductive performance

Males from the mated treatment took longer to mate with the novel "assay" female ("mating latency") than control males (estimate = 2.113, $Z_{49} = 2.284$, $p = 0.022$). Likewise, males from the poor larval diet had a longer mating latency than did males from the rich larval diet (estimate = 1.878, $Z_{49} = 2.094$ $p = 0.036$). However, there was no significant interaction of mating history and larval diet on mating latency (estimate = − 1.194, $Z_{49} = -1.051$, $p = 0.370$). 8 out of 80 males took > 90 min to initiate mating and, of these, 7/8 were from the mated treatment and 6/8 were from the poor larval diet.

There were no significant main effects or interactions of mating history and larval diet on any of the measures of offspring quality or quantity (latency to lay eggs, egg hatching success, egg-to-adult viability or offspring body size) (Fig. 1, Table S1). Larger males (within diet treatment groups) had increased egg hatching success (estimate = 1.190, $Z_{41} = 2.615$, $p = 0.009$) and larger females produced significantly larger offspring (estimate = 5.030, $t_{119} = 2.038$, $p = 0.044$).

Experiment 2: frequent mating and male prudence

Mated treatment males slowed their mating rate over the course of the initial 3 h exposure to 5 virgin females and when subsequently paired with 5 novel virgin ("assay") females (estimate −0.441, $Z_{113} = -5.149$, $p < 0.001$) (Fig. 2). Male thorax length was not significantly correlated with male mating rate (estimate = −0.237, $Z_{113} = -0.447$, $p = 0.655$).

After the mating history manipulation, control males mated significantly more with the five assay females compared to males from the mated treatment (control: 2.850 ± 0.295 matings; mated treatment: 1.231 ± 0.233 matings; estimate = 0.833, $Z_{78} = 1.137$, $p < 0.001$) (Fig. 2). Male thorax length was not significantly correlated with how many times the males mated with the five assay females (estimate = −0.218, $Z_{78} = -0.495$, $p = 0.620$).

There was no effect of male mating history on egg output or egg hatching success (Fig. 3, Table S2). Male thorax length was positively correlated with egg output and egg hatching success (egg output: estimate = 0.483, $Z_{78} = -1.652$, $p < 0.001$; egg hatching success: estimate = 1.867, $Z_{78} = 0.434$, $p = 0.011$).

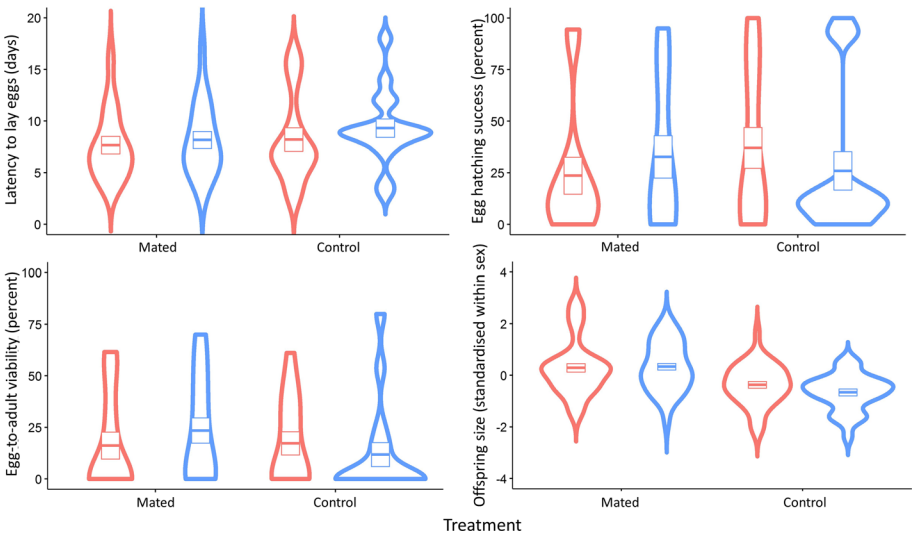

Fig. 1 Violin plots showing the effects of the mated and control treatments, and the poor larval diet (red) and rich larval diet (blue) on male fecundity and offspring traits in Experiment 1. Offspring size is standardised within sex. Bars represent mean ± SE

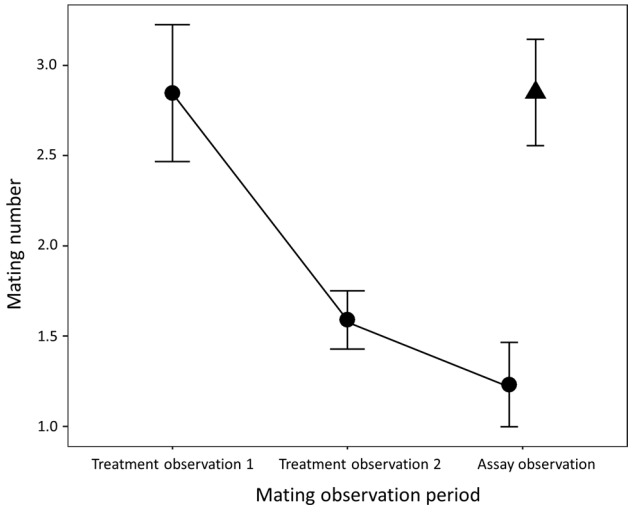

Fig. 2 The observed mean ± SE of the total number of matings across five females in each observation period in Experiment 2. Males in the mated treatment were observed twice while in the treatment, and both control and mated males were observed with five novel females during the assay period. Mated treatment = circles, control = triangle

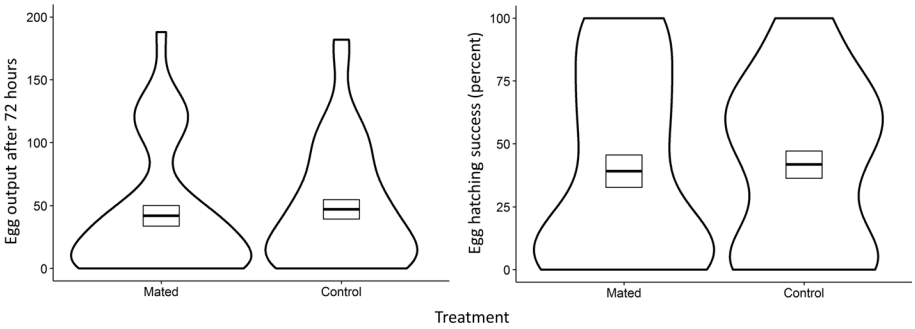

Fig. 3 Violin plots showing the effects of the mated and control treatments on the number of eggs laid by the assay females and egg hatching success in Experiment 2. Bars represent mean ± SE

Discussion

Using the neriid fly *T. angusticollis*, we investigated if males suffered a reduction in offspring quality and quantity after a bout of frequent mating. We also examined whether males reared on a nutrient poor larval diet would suffer a greater reduction in offspring quality and quantity after a bout of frequent mating due to a greater relative cost of ejaculate synthesis in low-condition males compared to high-condition males. In Experiment 1, we did not detect an effect of male mating history or larval nutrient availability on male ability to induce female oviposition, fertilise eggs, or produce viable and high-condition (i.e., large) offspring with a single novel female, even though males reared on the poor larval diet were significantly smaller than those reared on the rich larval diet. However, previously mated males had a longer latency to mate with the novel female compared to control (virgin) males. In Experiment 2, we presented mated and control males with five

novel females to determine if costs of prior mating would become more apparent when previously mated males encountered greater mating opportunities. We found that males in the mated treatment group reduced their mating rate over time, including when given the opportunity to mate with five novel, virgin females (i.e., they did not show renewed interest in novel females via a 'coolidge effect; Dewsbury 1981; Pizzari et al. 2003). Yet, we still found no effect of prior mating on male ability to induce oviposition or fertilise eggs when encountering five novel females. Thus, *T. angusticollis* males appear to adjust mating behaviour rather than investment in each mating event in response to frequent mating.

In comparison to other insect species, including other Diptera, male neriid flies achieved a high number of matings without any detectable costs to offspring quality or quantity (see Hihara 1981; Linklater et al. 2007; Reinhardt et al. 2011; Michaud et al. 2013; Perry and Tse 2013; Hopkins et al. 2019). Furthermore, it is interesting that we did not detect any condition-dependent differences in male ability to fertilise eggs or produce large and viable offspring given that many ejaculate traits are expected to be condition-dependent (reviewed in Macartney et al. 2019), and a nutrient poor larval diet has been shown to reduce several ejaculate traits in *T. angusticollis* (Macartney et al. 2018b), as well as offspring body size (Bonduriansky and Head 2007). This may be because males reared on the poor larval diet were observed mating fewer times compared to males from the rich larval diet while in the mated treatment. This difference in mating number may mean that males from the poor larval diet conserved more of their smaller ejaculate stores, preventing us from detecting differences in diet on offspring quality and quantity. Additionally, male neriid flies transfer a very small ejaculate, estimated at ~ 1/10,000 of male body volume (Bonduriansky and Head 2007), and this may enable males of both high- and low-condition to maintain sperm and seminal fluid stores. Furthermore, we did not detect any effect of larval diet on offspring body size, as shown previously in this species (Bonduriansky and Head 2007; Crean et al. 2014). The absence of such an effect may be due to differences in the age of females used in the experiments. Previous studies paired males with immature females whose developing (i.e., uncorionated) eggs may be more permeable to non-genetic factors in the seminal fluid (e.g., Crean et al. 2014), whereas the current experiments used mature females that carried mature (i.e., corionated) eggs. Alternatively, it could be due to differences in mating opportunities between studies as previous studies housed pairs together for 24 h (e.g., Crean et al. 2014). Further research is needed to determine whether female age and/or number of matings with the same female can modulate non-genetic effects of paternal environment. Such studies would provide more information on how non-genetic effects of paternal environment are conferred in this species and how important such effects would be for fitness in wild populations of *T. angusticollis.*

The lack of an effect of previous mating on offspring quality and quantity but a clear reduction in mating rate suggests a trade-off between mating rate and ejaculate transfer in *T. angusticollis.* Such trade-offs between mating frequency and ejaculate expenditure have been suggested in the theoretical and empirical literature (Pitnick and Markow 1994; Parker and Ball 2005; Parker and Pizzari 2010; Reinhardt et al. 2011), and this trade-off can depend on the level of sperm competition (Parker and Pizzari 2010). Females of *T. angusticollis* are highly promiscuous and can store sperm from multiple males (Wylde et al. 2020). Therefore, high sperm competition in this species may have selected for an ability to transfer a full ejaculate at each mating. If reducing ejaculate transfer per mating would result in a very low fertilisation probability, the best strategy could be to mate fewer times while investing fully in each mating. However, our male reproductive performance assays were conducted under non-competitive environments and it is possible that the presence of competitor males would cause males to transfer more sperm and seminal

fluid at each mating—potentially increasing the rate of ejaculate depletion (see Linklater et al. 2007; Douglas et al. 2020 for effects of mating and male perception of competitors on offspring quantity).

Furthermore, reproductive investment can also incur latent costs. A number of studies have shown that frequent mating reduces longevity (Cordts and Partridge 1996; Kotiaho and Simmons 2003; McNamara et al. 2008; Perry and Tse 2013), and may also affect reproductive aging (Koppik et al. 2018). While we did not measure such latent costs in this study, it is possible that frequent mating reduces lifespan. However, early life reproductive investment and short-term costs are likely to be more relevant in this species as life expectancy is short in natural populations of *T. angusticollis* (Kawasaki et al. 2008).

In summary, in *T. angusticollis* males, frequent mating does not appear to reduce subsequent ability to induce female oviposition, fertilise eggs or produce viable, high-condition offspring, and costs of frequent mating on offspring quality and quantity do not become more evident in low-condition males. Our results suggest that males might avoid costs of ejaculate depletion by changing their mating behaviour. Previously mated males increased mating latency and reduced their mating rate, suggesting that males may be selected to prioritise full ejaculate transfer per mating, even if this necessitates a reduction in the number of matings achieved.

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References

Adler MI, Bonduriansky R (2013) Paternal effects on offspring fitness reflect father's social environment. Evol Biol 40:288–292. https://doi.org/10.1007/s11692-012-9211-6

Bates D, Maechler M, Bolker B, Walker S (2015) lme4: linear mixed-effects models using Eigen and S4. J Stat Softw 67:1–48

Bath E, Tatarnic N, Bonduriansky R (2012) Asymmetric reproductive isolation and interference in neriid flies: the roles of genital morphology and behaviour. Anim Behav 84:1331–1339

Bonduriansky R (2007) The evolution of condition-dependent sexual dimorphism. Am Nat 169:9–19. https://doi.org/10.1086/510214

Bonduriansky R, Crean AJ (2017) What are parental condition-transfer effects and how can they be detected? Methods Ecol Evol. https://doi.org/10.1111/2041-210X.12848

Bonduriansky R, Head M (2007) Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). J Evol Biol 20:2379–2388. https://doi.org/10.1111/j.1420-9101.2007.01409.x

Chapman T (2001) Seminal fluid-mediated fitness traits in Drosophila. Heredity (Edinb) 87:511–521. https://doi.org/10.1046/j.1365-2540.2001.00961.x

Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton

Cordts R, Partridge L (1996) Courtship reduces longevity of male *Drosophila melanogaster*. Anim Behav 52:269–278

Crean AJ, Adler MI, Bonduriansky R (2016) Seminal fluid and mate choice: new predictions. Trends Ecol Evol 31:253–255. https://doi.org/10.1016/j.tree.2016.02.004

Crean AJ, Kopps AM, Bonduriansky R (2014) Revisiting telegony: offspring inherit an acquired characteristic of their mother's previous mate. Ecol Lett 17:1545–1552. https://doi.org/10.1111/ele.12373

Delcurto H, Wu G, Satterfield MC (2013) Nutrition and reproduction: links to epigenetics and metabolic syndrome in offspring. Curr Opin Clin Nutr Metab Care 16:385–391. https://doi.org/10.1097/MCO.0b013e328361f96d

Dewsbury DA (1982) Ejaculate cost and male choice. Am Nat 119:601–610. https://doi.org/10.1086/283938

Dewsbury DAA (1981) Effects of novelty on copulatory behavior: the Coolidge effect and related phenomena. Psychol Bull 89:464–482. https://doi.org/10.1037/0033-2909.89.3.464

Douglas T, Anderson R, Saltz JB (2020) Limits to male reproductive potential across mating bouts in *Drosophila melanogaster*. Anim Behav 160:25–33. https://doi.org/10.1016/j.anbehav.2019.11.009
Droney DC (1998) The influence of the nutritional content of the adult male diet on testis mass, body condition and courtship vigour in a Hawaiian Drosophila. Funct Ecol 12:920–928. https://doi.org/10.1046/j.1365-2435.1998.00266.x
Evans JP, Lymbery RA, Wiid KS et al (2017) Sperm as moderators of environmentally induced paternal effects in a livebearing fish. Biol Lett 13:10–13. https://doi.org/10.1098/rsbl.2017.0087
Fricke C, Adler MI, Brooks RC, Bonduriansky R (2015) The complexity of male reproductive success: effects of nutrition, morphology, and experience. Behav Ecol 26:617–624. https://doi.org/10.1093/beheco/aru240
Hihara F (1981) Effects of the male accessory gland secretion on oviposition and remating in females of *Drosophila melanogaster*. Zool Mag 90:307–316
Hopkins BR, Sepil I, Thézénas M-L et al (2019) Divergent allocation of sperm and the seminal proteome along a competition gradient in *Drosophila melanogaster*. Proc Natl Acad Sci 116:17925–17933. https://doi.org/10.1073/pnas.1906149116
Kawasaki N, Brassil CE, Brooks RC, Bonduriansky R (2008) Environmental effects on the expression of life span and aging: an extreme contrast between wild and captive cohorts of *Telostylinus angusticollis* (Diptera: Neriidae). Am Nat 172:346–357. https://doi.org/10.1086/589519
Koppik M, Ruhmann H, Fricke C (2018) The effect of mating history on male reproductive ageing in *Drosophila melanogaster*. J Insect Physiol 111:16–24. https://doi.org/10.1016/j.jinsphys.2018.10.003
Kotiaho JS, Simmons LW (2003) Longevity cost of reproduction for males but no longevity cost of mating or courtship for females in the male-dimorphic dung beetle *Onthophagus binodis*. J Insect Physiol 49:817–822. https://doi.org/10.1016/S0022-1910(03)00117-3
Kuznetsova A, Brockhoff P, Rune H (2017) lmerTest package: tests in linear mixed effects models. J Stat Softw 82:1–26
Linklater JR, Wertheim B, Wigby S, Chapman T (2007) Ejaculate depletion patterns evolve in response to experimental manipulation of sex ratio in *Drosophila melanogaster*. Evolution (N Y) 61:2027–2034. https://doi.org/10.1111/j.1558-5646.2007.00157.x
Macartney EL, Crean AJ, Bonduriansky R (2018a) Epigenetic paternal effects as costly, condition-dependent traits. Heredity (Edinb) 121:248–256. https://doi.org/10.1038/s41437-018-0096-8
Macartney EL, Crean AJ, Bonduriansky R (2017) Adult dietary protein has age- and context-dependent effects on male post-copulatory performance. J Evol Biol 38:42–49. https://doi.org/10.1111/jeb.13087
Macartney EL, Crean AJ, Nakagawa S, Bonduriansky R (2019) Effects of nutrient limitation on sperm and seminal fluid: a systematic review and meta-analysis. Biol Rev 94:1722–1739. https://doi.org/10.1111/brv.12524
Macartney EL, Nicovich PR, Bonduriansky R, Crean AJ (2018b) Developmental diet irreversibly shapes male post-copulatory traits in the neriid fly *Telostylinus angusticollis*. J Evol Biol 31:1894–1902. https://doi.org/10.1111/jeb.13384
Marcotte M, Delisle J, McNeil JN (2007) Effects of different male remating intervals on the reproductive success of *Choristoneura rosaceana* males and females. J Insect Physiol 53:139–145. https://doi.org/10.1016/j.jinsphys.2006.11.005
Maynard Smith J (1977) Parental investment: a prospective analysis. Anim Behav 25:1–9. https://doi.org/10.1016/0003-3472(77)90062-8
McNamara KB, Elgar MA, Jones TM (2008) A longevity cost of re-mating but no benefits of polyandry in the almond moth, Cadra cautella. Behav Ecol Sociobiol 62:1433–1440. https://doi.org/10.1007/s00265-008-0573-9
Michaud JP, Bista M, Mishra G, Singh O (2013) Sexual activity diminishes male virility in two Coccinella species: consequences for female fertility and progeny development. Bull Entomol Res 103:570–577. https://doi.org/10.1017/s0007485313000199
Mirhosseini MA, Michaud JP, Jalali MA, Ziaaddini M (2014) Paternal effects correlate with female reproductive stimulation in the polyandrous ladybird Cheilomenes sexmaculata. Bull Entomol Res 104:480–485. https://doi.org/10.1017/s0007485314000194
Muller K, Arenas L, Thiéry D, Moreau J (2016) Direct benefits from choosing a virgin male in the European grapevine moth, *Lobesia botrana*. Anim Behav 114:165–172. https://doi.org/10.1016/j.anbehav.2016.02.005
Parker GA, Ball MA (2005) Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. Biol Lett 1:235–238. https://doi.org/10.1098/rsbl.2004.0273
Parker GA, Pizzari T (2010) Sperm competition and ejaculate economics. Biol Rev 85:897–934. https://doi.org/10.1111/j.1469-185X.2010.00140.x

Perry JC, Tse CT (2013) Extreme costs of mating for male two-spot ladybird beetles. PLoS ONE 8:1–5. https://doi.org/10.1371/journal.pone.0081934
Pitnick S, Markow TA (1994) Male gametic strategies - sperm size, testis size, and the allocation of ejaculate among successive mates by the sperm-limited fly Drosophila pachea and its relatives. Am Nat 143:785–819. https://doi.org/10.1086/285633
Pizzari T, Cornwallis CK, Hanne L et al (2003) Sophisticated sperm allocation in male fowl. Nature 426:70–74. https://doi.org/10.1038/nature02025.1
Polak M, Simmons LW, Benoit JB et al (2017) Nutritional geometry of paternal effects on embryo mortality. Proc R Soc B Biol Sci 284:1–9. https://doi.org/10.1098/rspb.2017.1492
Preston BT, Stevenson IR, Pemberton JM, Wilson K (2001) Dominant rams lose out by sperm depletion. Nature 409:681–682. https://doi.org/10.1038/35055617
Rasband WS (2015) ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. http://imagej.nih.gov/ij/
Reinhardt K, Naylor R, Siva-Jothy MT (2011) Male mating rate is constrained by seminal fluid availability in bedbugs, Cimex lectularius. PLoS ONE 6:1–8. https://doi.org/10.1371/journal.pone.0022082
Schaalje GB, McBride JB, Fellingham GW (2002) Adequacy of approximations to distributions of test statistics in complex mixed linear models. J Agric Biol Environ Stat 7:512–524. https://doi.org/10.1198/108571102726
Sentinella A, Crean A, Bonduriansky R (2013) Dietary protein mediates a trade-off between larval survival and the development of male secondary sexual traits. Funct Ecol 27:1134–1144. https://doi.org/10.1111/1365-2435.12104
Sheldon BC (2002) Relating paternity to paternal care. Philos Trans R Soc B Biol Sci 357:341–350. https://doi.org/10.1098/rstb.2001.0931
Torres-Vila LM, Jennions MD (2005) Male mating history and female fecundity in the Lepidoptera: do male virgins make better partners? Behav Ecol Sociobiol 57:318–326. https://doi.org/10.1007/s00265-004-0857-7
Vahed K (1998) The function of nuptial feeding in insects: review of empirical studies. Biol Rev Camb Philos Soc 73:43–78. https://doi.org/10.1017/s0006323197005112
Wigby S, Sirot LK, Linklater JR et al (2009) Seminal fluid protein allocation and male reproductive success. Curr Biol 19:751–757. https://doi.org/10.1016/j.cub.2009.03.036
Wylde Z, Crean A, Bonduriansky R (2020) Effects of condition and sperm competition risk on sperm allocation and storage in neriid flies. Behav Ecol 31:202–212. https://doi.org/10.1093/beheco/arz178