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# The role of functional constraints in non-random mating patterns for a dance fly with female ornaments

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## Abstract

Most hypotheses to explain non-random mating patterns invoke mate choice, particularly in species that display elaborate ornaments. However, conflicting selection pressures on traits can result in functional constraints that can also cause non-random mating patterns. We tested for functional load-lifting constraints during aerial copulation in *Rhamphomyia longicauda*, a species of dance fly that displays multiple extravagant female-specific ornaments that are unusual among sexual traits because they are under stabilizing selection. *R. longicauda* males provide females with a nuptial gift before engaging in aerial mating, and the male bears the entire weight of the female and nuptial gift for the duration of copulation. In theory, a male's ability to carry females and nuptial gifts could constrain pairing opportunities for the heaviest females, as reported for non-ornamented dance flies. In concert with directional preferences for large females with mature eggs, such a load-lifting constraint could produce the stabilizing selection on female size previously observed in this species. We therefore tested whether wild-caught male *R. longicauda* collected during copulation were experiencing load-lift limitations by comparing the mass carried by males during copulation with the male's wing loading traits. We also performed permutation tests to determine whether the loads carried by males during copulation were lighter than expected. We found that heavier males are more often found mating with heavier females suggesting that while *R. longicauda* males do not experience a load-lift constraint, there is a strong relationship of assortative mating by mass. We suggest that active male mate choice for intermediately adorned females is more likely to be causing the non-random mating patterns observed in *R. longicauda*.

Keywords: assortative mating, dance flies, female ornaments, male mate choice, mating constraint, *Rhamphomyia longicauda*, sexual selection

## Introduction

Hypotheses to explain animal mating patterns typically invoke mate choice, but there are other aspects of pair formation that can influence the identity of mating partners. For example, the inability to accurately evaluate a potential mate's quality (Endler & Basolo, 1998, Candolin, 2003, Funk & Tallamy, 2000) can result in maladaptive mate choice (Candolin, 2003, Dubois et al., 2011). Similarly, functional limitations to an individual's mating success (Marden, 1989, Almbro & Kullberg, 2008) can also result in non-random mating patterns, and can prevent pairings regardless of mating preferences (Schluter et al., 1991, Peckarsky et al., 2002).

Functional constraints on pairing may be particularly important for flying animals (Marden, 1989, McLachlan & Allen, 1987, Sawadogo et al., 2013), especially those that engage in aerial copulations (Marden, 1989, Peckarsky et al., 2002). If males carry females during mating, the limits to load-lifting could constrain a male's ability to mate with attractive females because such females are often the heaviest and most fecund (Bonduriansky, 2001). Loading constraints that impact the reproductive biology of a species are likely not only to change observed mating patterns, but could also bring about important ecological and evolutionary consequences such as inbreeding and assortative mating (e.g. see Partridge, 1983, Crespi, 1989).

There are many insect species that mate while flying, and in some of these taxa males provide females with a nutritious nuptial gift (Thornhill & Alcock, 1983, Lewis et al., 2014). Following nuptial gift exchange, many male insects support the female and her meal during an aerial copulation (Kessel, 1955, Marden, 1989, Funk & Tallamy, 2000). In most cases, the larger the nuptial gift, the longer copulation lasts, and long copulations covary with insemination success and an advantage in sperm competition (Thornhill, 1979, Vahed, 1998, Svensson et al., 1990). Loading constraints are potentially intense in nuptial gift giving species because a male must carry the mass of both gift and mating partner. Consequently, load-lift limitations in species with both nuptial gifts and aerial copulation are likely to functionally constrain reproductive success. Sexual selection should favour males that offer large nuptial gifts (to improve the nutrition intake of mates) to the largest females (to improve offspring number, assuming the largest females are also the most fecund). If the size (and importantly, the mass) of the gift and female are under selection to be maximized, males who are at the upper limit of their load-carrying capacity may face a trade-off between the mass of the nuptial gift they offer and the mass of the female with whom they mate.

Dance flies from the subfamily Empidinae (Diptera: Empididae) include species with both nuptial gift giving and aerial copulation; males must carry the combined load of female and nuptial gift for the duration of copulation (Cumming, 1994). Patterns consistent with load-lift constraints have been observed in a previously undescribed species of dance fly belonging to the genus *Hilara* (Marden, 1989). In that study, wild caught mating pairs approached but never surpassed the theoretically predicted load-lifting constraint threshold, suggesting that male *Hilara sp.* were functionally constrained by the mass of female and nuptial gift they could carry (Marden, 1989).

The presence of load-lifting constraints becomes particularly interesting when considering the broad range of mating behaviour among the subfamily to which *Hilara sp.* belongs. In this subfamily, (the Empidinae), not only is mating almost always accompanied by the presentation of nuptial gifts (Cumming, 1994), but adult females are not known to hunt, and must obtain their dietary protein from male nuptial gifts (Hunter & Bussiere, 2018). Presumably as a consequence, females in many species compete intensely for the food gifts provided by males and females have evolved a series of remarkable secondary sex characters that appear to improve their attractiveness to males (Cumming, 1994, Collin, 1961, LeBas et al., 2003, Wheeler et al., 2012, Funk & Tallamy, 2000, Murray et al., 2018). In spite of the strong predicted covariance between sexual trait expression and mating success, studies of sexual selection on female dance flies report remarkable variation in the form and strength of selection (Sadowski et al., 1999, Funk & Tallamy, 2000, LeBas et al., 2003, Bussière et al., 2008, Wheeler et al., 2012). While some studies have shown mating advantages for females with large ornaments (e.g. LeBas et al., 2003, Funk & Tallamy, 2000), Wheeler et al. (2012) showed stabilizing selection on ornaments with intermediately adorned females having the highest mating success. This apparent inconsistency could have several causes, including constraints on the operation of male choice for elaborate (over-) ornamentation by females (Fitzpatrick et al., 1995, Chenoweth et al., 2006, Herridge et al., 2016, Murray et al., 2018). An intriguing alternative is that the lower than expected mating success for the largest females might be produced by load-lifting constraints on males during aerial copulation. To our knowledge, this alternative has never been formally assessed in any ornamented species of dance fly.

In the current study, we investigate the role of functional constraints in the evolution of non-random mating patterns in *Rhamphomyia longicauda,* the long-tailed dance fly. *R. longicauda* display several elaborate female-specific ornaments, which remain perplexing both in terms of their evolutionary origin (e.g. see Cumming, 1994, Funk & Tallamy, 2000) and for their relationship with mating success (Wheeler et al., 2012). *R. longicauda* forms mating swarms at dusk and dawn in which females display two sex-specific ornaments: pinnate leg scales and inflatable abdominal sacs (Newkirk, 1970, Funk & Tallamy, 2000). Males enter the mating swarm carrying a prey item as a nuptial gift, and approach females from below. When mated pairs first form, they engage in the apparently cumbersome task of transferring the nuptial gift from the male to the female as they exit the mating swarm. This transfer typically involves a loss of altitude for the pair, and some initial attempts at gift transfers are unsuccessful, with the male and female separating before pair formation is complete, and each returning to the mating swarm (R. Murray personal observation). For pairs that successfully transfer the nuptial gift, copulation occurs on the wing, with the male carrying the female in a stereotypically circular or figure-8 pattern while she feeds on the nuptial gift he has provided (Funk & Tallamy, 2000).

Previous studies have shown that *R. longicauda* females with the largest ornaments are more likely to attract a male (Funk & Tallamy, 2000, Wheeler, 2008, Murray et al., 2018) suggesting positive directional sexual selection on ornamentation. However, a cross-sectional comparison of the phenotypic traits of successful (paired) and unsuccessful (unpaired) females found no evidence for directional selection on either of two ornamental traits (Wheeler et al., 2012). Instead, Wheeler et al. (2012) found support for the hypothesis that ornaments were under stabilizing selection, consistent with the supposition that over-ornamented females were being rejected because ornament cost reduces fecundity (Chenoweth et al., 2006). Wheeler et al. (2012) acknowledged that the heightened success of females displaying intermediate-sized ornaments could also reflect differences in selection acting on two separate episodes in the *R. longicauda* mate pairing process. While large ornaments could be important for initially attracting males to potential mates within the *R. longicauda* mating swarm (Murray et al., 2018), the actual mating success of a female might be mediated by behavioural interactions that follow the initial attractiveness stage.

Males might prefer to avoid overly ornate females at early stages of ovarian maturity, knowing that such females are likely to mate repeatedly both because they are attractive and to obtain further nuptial meals. These subsequent matings by females could displace a male's ejaculate long before fertilization and oviposition potentially causing a male to lose out on a large fraction of the paternity share (Parker, 1970).

An additional alternative hypothesis for the depressed mating success of over-ornamented females (Wheeler et al., 2012), is that these females are often heavier individuals, and despite being more attractive (Murray et al., 2018), their mass constrains the range of possible partners to a subset of males (Bussière et al., 2008, Wheeler et al., 2012). If females displaying the largest ornaments are also the heaviest (because large ornaments tend to covary, albeit imperfectly, with increased mass; Wheeler, 2008), attractive, high-mass females may be more difficult to carry during prey transfer (when we often observe a loss of altitude during pair formation) and aerial copulation.

In the current study, we performed three analyses to test for load-lift functional constraints *Rhamphomyia longicauda*. First, we tested for a relationship between female mass and nuptial gift mass and hypothesized that a load-lift constraint would result in a negative correlation. Second, we tested for a relationship between the total mass carried during aerial copulation and a male's own wing traits. Here, we hypothesized that males with larger wings would be able to pair with more massive females, and that males with a large wing load (males who are heavy relative to their wing size) would pair with lighter females. Third, we performed a permutation test to create a null distribution of mean load-lift ratios (total mass carried/male wing area). If males in the wild experience a load-lift constraint that contributes to non-random mating patterns, we hypothesized that we would observe a lower load-lift ratio from our data compared to the simulated null distribution that assumes random mating.

## Methods

### *Sample Collection*

We collected *R. longicauda* samples on the banks of the Credit River in June 2012 near Glen Williams (Halton Co., Ontario, Canada: 43°41’117”N, 79°55’34”W). This site has been used for several previous studies on *R. longicauda* (Bussière et al., 2008, Gwynne et al., 2007, Gwynne & Bussière, 2002, Wheeler et al., 2012, Murray et al., 2017, Murray et al., 2018). At each swarming event, we collected mated pairs from nuptial flight using an entomological sweep net. We placed pairs and their nuptial gifts individually in collection tubes. We were unable to retrieve all nuptial gifts from each mated pair because the female sometimes dropped the gift upon capture. All samples were frozen at -20C in a laboratory freezer within two hours following collection.

### *Morphological Measurements*

Because male mate choice for larger females is typically linked to those females also having more, or higher quality, eggs (e.g. Bonduriansky, 2001), we dissected each female’s abdomen to measure egg length (as a proxy for egg quality) and count the number of mature eggs. We measured ornamentation (pinnate leg scales) for each female so that we could relate measures of fecundity (egg number) and mass to female ornamentation measures. We also recorded mating status for each female as the presence or absence of sperm in the spermatheca; we wanted to account for any potential effect of females having not mated (or consumed a nuptial gift) prior to capture because it could change a female’s mass and/or egg number/size.

We photographed morphological traits using an AmScope MA500-CK digital microscope camera attached to a dissecting microscope and measured the digital images using ImageJ software (Abràmoff et al., 2004). For morphological measurements, we dissected each frozen specimen and photographed only the body part of interest. We took the following morphological measurements: leg length (femora and tibia length summed together), wing length (the longest point from the axillary area to the apex), thorax length (centre of the neck to the centre of the abdominal-thorax connection), leg area (including pinnate leg scales in the female, see below for details), wing area, number of eggs and the egg length of five eggs per individual (see Figure S1). Wing measurements were collected to assess male fly traits associated with lift and flight endurance (see below), while leg and thorax measurements were collected as proxies for body size and/or ornamentation. To obtain a count of the number of eggs within each female (as a proxy for fecundity) we dissected the abdomen. The eggs are immediately obvious in the dissected abdomens of frozen, adult females. We measured female ornamentation (pinnate leg scales) as the summed leg area of the fore, mid and hind legs of each individual. For paired characters we measured both right and left sides and took the mean. When this was not possible because of damage to one side, we measured only the undamaged side.

### *Female ornamentation and mating status*

In order for a loading constraint to contribute to the observed stabilizing selection pattern on *R. longicauda* ornaments (Wheeler et al., 2012), female mass must positively covary with female ornaments. We performed a linear regression of female leg ornamentation on female mass; because our specimens were frozen, the inflatable abdominal ornament was deflated and impossible to measure. To assess whether female mass was an accurate measure of fecundity in *R. longicauda*, we also performed a linear regression of oocyte count and size against female mass.

To assess a female's previous mating behaviour (and nuptial gift consumption), we dissected the spermatheca (sperm storage organ) from females and recorded the presence (pale opaque substance) or absence (empty) of sperm to determine whether or not the female had mated (and consumed a nuptial gift) previously. Because individuals can change their behaviour when they are hungry (e.g. Sandhu et al., 2018), we wanted to account for this potential source of variation. We note that a female may have received sperm from the male she is paired with during collection, and consequently we may be underestimating the number of virgin females. We recorded male mass, female mass and, when possible, nuptial gift mass from frozen specimens within 24 hours of collection.

### *Male loading measures*

We computed two indices of the load on an insect's wings arising from its own body mass: wing load (WL), the ratio of male mass to wing area, and aspect ratio (AR), the ratio of the squared wingspan to wing area (e.g. see Berwaerts et al., 2002, Gyulavári et al., 2014). Males displaying a high WL (relatively large mass to wing size) will have less lift compared to males with a low WL (reviewed in Dudley, 2002) and therefore are more likely to be constrained by the mass of females they can carry and copulate with. Males displaying a high AR (relatively long, narrow wings) will have better flight endurance but lower maneuverability relative to males with a low aspect ratio (reviewed in Dudley, 2002). We might expect maneuverability to be more important during initial nuptial gift transfer (e.g., to improve pairing success), but flight endurance might be important for sustained aerial copulation (to improve sperm transfer success).

Below we use the term ‘total mass carried’ (TMC) to refer to the mass lifted (i.e. the mass of the female and, where possible, the nuptial gift) by a male during aerial copulation. Within our permutation tests (see below), we use the term ‘total aerial mass’ to refer to the combined mass of the male, female and nuptial gift. Finally, the term ‘load-lift ratio’ refers to the total aerial mass (g) per unit ($mm^2$) of wing area during our permutation tests.

### *Statistical Analyses*

Area measurements (legs and wings) were square root transformed and mass measurements were cube root transformed prior to inclusion in any statistical models. All independent variables were also z-transformed before analyses to improve the interpretability of coefficients (Schielzeth, 2010). Because *R. longicauda* swarm composition can vary temporally (Wheeler, 2008), and trying to measure composition as a covariate requires disrupting the swarm, for each set of statistical models we tested for an effect of “date” that each sample was collected as a random effect in a mixed model, but found the results from mixed and fixed models were qualitatively the same; date tended to account for little variation among observations (<10%). For simplicity, the results we report here all come from fixed models.

High positive correlations between phenotypic traits are expected whenever groups of traits are functionally related, and such correlations can pose problems for the estimation of model coefficients. The extent to which variance inflation is problematic is controversial (Morrissey & Ruxton, 2018), but we favoured model simplicity and confidence in parameter estimates over strict estimates of partial effects, especially since some of our traits represent one of several alternative indices of underlying phenomena. To test for collinearity in our models we examined variance inflation factors (VIF) using the ‘vif’ function in the car package (Fox, 2011). If any VIF in our linear model exceeded three, we removed the variable with the highest VIF value and reran the model until all VIF values were below three. We note that this is a conservative VIF cut-off (Zuur et al., 2010), but given the potential for collinearity between the wing traits used as predictor variables, we wanted to limit correlations between phenotypic traits as much as possible.

We performed a series of analyses to test for evidence of loading constraints in *R. longicauda* mated pairs. First, we tested for a relationship between female mass and nuptial gift mass. Second, we investigated the relationship between the load a male carries during aerial copulation and male wing traits. We performed linear models on both datasets (for the subset of observations including a nuptial gift as well as the larger dataset with only female measures), fitting predictor variables that previously have been shown to influence loading constraints and insect flight performance (Berwaerts et al., 2002, Peckarsky et al., 2002, Gyulavári et al., 2014). We fit the total mass carried during aerial copulation as the response, and morphological measures for male thorax length, male WL (male mass/wing area), AR (wingspan$^2$/wing area) and wing length as predictor variables. Because female dance flies feed on the nuptial gift during copulation, the mass of the gift will decrease in proportion to how much the female has fed. Similarly, the female's mass will increase as she feeds. Therefore, the combined mass of both the female and the nuptial gift is a superior measure of the estimated total mass carried by the male for testing the constraint hypothesis. Third, we tested whether the observed mass that a male carries during aerial copulation was less than expected by chance (i.e. if mating was random). We used resampling methods to generate the null expectations under the assumption of random pairing for the total mass carried (TMC) per mm$^2$ of male wing area. For every iteration of our resampling procedure, we randomly paired each male wing area measure in our dataset with a female mass and nuptial gift mass, and then recomputed the mean ratio of total mass carried to male wing area. This exercise produced 9999 means from the null distribution, where female mass, gift mass, and male wing area were randomly assigned and therefore had no bearing on pair formation. We then added our observed mean to these 9999 trials, and assessed its rank among the total distribution of 10000 as the probability that our collected samples could have arisen through random pairing in nature. We repeated this procedure but omitted nuptial gift mass to take advantage of the more numerous samples collected without nuptial gifts. If load-lifting constraints are important for the non-random mating patterns observed in *R. longicauda*, we might expect that the load-lift ratios from our actual dataset would fall within the lower 2.5% (for a significance cut off of $p<0.05$) of the random distribution of loading ratios. A lower than expected ratio would indicate that the actual TMC by wild *R. longicauda* males during aerial copulation is less than would be expected under random pairing.

## Results

We collected 112 *R. longicauda* mated pairs from the wild in June 2012. For 57 of those pairs we also collected their nuptial gifts. The remaining 55 pairs dropped the nuptial gift during collection. Summaries of our morphological measurements for female and male traits can be found in Table 1. Only 5% of the females collected had no sperm in their spermatheca, and while we recognize that this might be an underestimate, we can only be sure that four females were collected during their first mating event. Because of the small fraction of our samples that had no sperm in the spermatheca, we did not include this proxy of previous mating activity in any further analyses.

### *Female mass correlates with fecundity and ornamentation*

2We tested whether or not female mass covaried with female fecundity, oocyte size, or ornamentation. We found that female mass positively predicts leg ornamentation (Figure 1A; estimate=0.12±0.016 $F_{(1, 105)}$=58.35, $P<0.001$). We also found that female mass positively covaries with female fecundity (Figure 1B; estimate= 6.029±1.579, $F_{(1, 105)}$=14.33, $P<0.001$) but not oocyte size (estimate= 8.45±8.31, $F_{(1, 105)}$=1.035, $P=0.31$).

### *Testing for load-lift constraints*

First, we tested for a relationship between the female mass and the nuptial gift. We found that the nuptial gift accounts for 4-40% of the total mass carried by a male (mean: 14.26 ± 0.97%; Table 1). We found that the nuptial gift mass positively covaried with female mass in mated pairs (estimate=0.31±0.12, $F_{(1, 56)}$=6.85, $P=0.01$).

Second, we tested for a relationship between TMC and male wing traits. We found that the only morphological trait that predicted the TMC during aerial copulation was a male's WL (male mass/wing area) for both datasets (with and without nuptial gift; Tables 2 and S1). We also found that males that had a high WL paired with heavier females both in the absence of nuptial gift measure (Figure 2; estimate=0.62±0.12, $F_{(1, 105)}$=26.95, $P<0.001$) and for the subset of data where nuptial gift masses were available (Figure S2; estimate=0.86±0.19, $F_{(1,56)}$=19.6, $P<0.001$).

Third, we performed a permutation test to determine the null distribution of mean loads a male might carry if foraging and mating were completely random. We performed 9999 iterations without resampling and found a null distribution of load-lift ratios that had a mean of 0.607 ± 0.016 mg/mm$^2$ (Figure 3A). The mean load-lift ratio we observed in nature is 0.606 ± 0.022 mg/mm$^2$, which was not significantly smaller than expected by our null distribution (P = 0.24). We performed a similar permutation test without nuptial gifts included in the load measurement (so that the potential sample to draw from was larger) and retrieved the same result: the null distribution of wing loads had a mean of 0.538 ± 0.014 mg/mm$^2$ (Figure 3B) while our data had a mean of 0.535± 0.013 mg/mm$^2$ (P=0.13).

## Discussion

Previously observed patterns of sexual selection in *Rhamphomyia longicauda* suggested that females were more likely to mate if they displayed intermediate-sized ornaments (Wheeler et al., 2012). One hypothesis for why females displaying the largest class of ornaments are mated less frequently is that these females are too heavy or cumbersome for males to carry during aerial copulation. We tested wild-caught *R. longicauda* mated pairs for a load-lifting functional constraint that might be contributing to non-random mating patterns. We found that male wing load, but no other male wing traits, covaried positively with the mass of female and nuptial gift carried by a male during aerial copulation. We also performed permutation tests to assess the null distribution of load-lift ratios when random foraging and mating are assumed. We found that our observed load-lift ratio did not differ from the null distribution (Figure 3) providing no evidence that load-lifting functional constraints are contributing to the non-random mating patterns observed in *R. longicauda*.

*Testing for load-lift constraints*

*R. longicauda* mated pairs copulate in-flight with the male carrying the female while she feeds on the nuptial gift he provides. It is unlikely that the female is able to contribute to flight during copulation, leaving the male to bear the weight of the entire load (Marden, 1989). We performed three analyses to test for load-lift functional constraints and found no evidence that a load-lifting constraint during aerial copulation exists in *R. longicauda*. First, we tested for a relationship between the female mass and nuptial gift mass carried by a male during aerial copulation. We hypothesized that a male experiencing a loading constraint might have to trade-off female mass with nuptial gift mass and expected a negative relationship. Second, we tested for a relationship between the total mass carried by a male during copulation and a male's wing traits. We hypothesized that male's experiencing a load-lift constraint might have smaller wings, and would carry smaller females. We found that female mass did not covary with male wing length and male wing load did not trade-off with the mass of the female he paired with, but was instead positively correlated with female mass. Third, we performed a permutation test to create a null distribution of mean load-lift ratios (total mass carried/male wing area) assuming random mating and foraging. If males experience a load-lift functional constraint during aerial copulation, we expected our observed mean load-lift ratio to be less than the null distribution of mean load-lift ratios. Once again, we found no evidence for a load-lifting constraint in *R. longicauda*; the observed load-lift ratios did not differ from null distributions (Figure 3).

Previous work on load-lifting constraints in a different dance fly species showed that males do experience a load-lift constraint during in-flight copulation. Marden (1989) found a negative relationship between female mass and nuptial gift mass, and a significant difference in the observed load-lift ratio compared to a simulation of random pairings. Marden (1989) concluded that a similar functional constraint might be an important influence of mating patterns in other taxa where the male carried the female during copulation. Our data provide no support for this hypothesis, but we did find evidence for assortative mating by mass in *R. longicauda*; males with a high wing load (heavy for their wing area) paired with heavier females (Table 2 Figure 2). Both *R. longicauda* and *Hilara sp.* (Marden, 1989) display elaborate mating behaviour that is common in dance fly species, including lek-like mating swarms, nuptial gift-giving, and in-flight copulation (Collin, 1961, Cumming, 1994, Marden, 1989, Funk & Tallamy, 2000). However, mating in *R. longicauda* and *Hilara sp.* (where load-lifting functional constraints were observed) differ in two ways: First, in contrast to *R. longicauda* (Funk & Tallamy, 2000), *Hilara sp*. does not have female-specific ornaments. Second, swarms of *Hilara sp.* are male-biased (Marden, 1989) while those of *R. longicauda* are consistently female-biased (Funk & Tallamy, 2000, Gwynne, 1991, Bussière et al., 2008, Murray et al., 2017). Given the variation in ornamentation and contrasting operational sex ratios, sexual selection and mate choice in the two species are expected to be quite different (Emlen & Oring, 1977, Clutton-Brock, 2009, Hare & Simmons, 2018, Kokko et al., 2012, Herridge et al., 2016). In *R. longicauda* there is male mate choice and female ornamentation (Funk & Tallamy, 2000, Murray et al., 2018) whereas the majority-male swarms of the non-ornamented *Hilara sp*. that experiences load-lifting constraints (Marden, 1989) suggests that males compete strongly for access to mates.

### *Direction of sexual competition and load-lift constraints*

We found contrasting patterns of load-lift constraints between *R. longicauda* (female ornaments, female-biased OSR measures) and *Hilara sp.* (no ornaments, male-biased OSR measures; Marden, 1989). One hypothetical explanation is that the effort to carry a load in flight limits in-flight copulation duration, thus number of sperm transferred (Thornhill, 1979, Simmons & Siva-Jothy, 1998, Simmons, 2001, Schluter et al., 1991) and the number of offspring a male sires (Parker & Pizzari, 2010). Longer in-flight copulation duration will cause higher energy expenditure from the male because he has to carry the mass of the female and the nuptial gift for the duration (Marden, 1989). Therefore, in species with female-biased mating swarms, males might choose to reject females at the upper limits of their load-lifting abilities if it means they are unable to transfer as much sperm as necessary to ensure paternity. However, in species where females are the choosier sex and mating opportunities are limited, such as in *Hilara sp*., mating with a heavy female for a shorter copulation duration (and potentially transferring a small number of sperm) is undoubtedly better for a male than not mating at all.

*Assortative mating by mass*

While a previous study found no size-assortative mating in *R. longicauda* (Bussière et al., 2008), our results suggest assortative-mating by mass across mated pairs (Figures 2 and S2). However, the two findings need not be contradictory because paired females can be heavier but not larger in linear dimensions than unpaired females (Woodhead, 1981). Assortative-mating by body mass has been observed in diverse taxa (Alcock & Gwynne, 1987, Ridley & Thompson, 1979, Speakman et al., 2007) and loading constraints have been proposed as a possible mechanism to explain this pattern in species where males carry females. However, following a comparative study across diverse arthropod taxa, Crespi (1989) concluded that functional constraints were far less likely to explain observed assortative-mating patterns than mate choice. Our data align well with this conclusion and suggest mate choice is responsible for observed patterns of mating in *R. longicauda*. We show that mass covaries positively with ornamentation (Figure 1A) and fecundity (Figure 1B) in *R. longicauda.* Therefore, high-condition males could be choosing more fecund mates (Bonduriansky, 2001). While there is variation around the linear estimates for both ornamentation and fecundity regressed on female mass (arguably two measures of female mate quality), on average choosing an ornamented female will also result in a heavier, more fecund female.

### *Male mate choice for female-specific ornaments*

We set out to test the hypothesis raised by two studies measuring sexual selection in *R. longicauda* (Bussière et al., 2008, Wheeler et al., 2012) that functional loading constraints were contributing to non-random mating patterns. We found no evidence that *R. longicauda* males are limited by the mass of the load they can carry during aerial copulation, and therefore conclude that functional constraints do not explain the reduced mating success of highly-ornamented female *R. longicauda* (Wheeler et al., 2012). What does explain this observation remains unclear, but one possibility is active mate choice by males for females with moderate ornaments, which is predicted if males avoid females that overinvest in ornaments at the expense of fecundity (Fitzpatrick et al., 1995, Chenoweth et al., 2006). Another potential explanation is that males avoid particularly popular females (who pose high risks of sperm competition). Such preferences would more sensibly be based on cues of actual mating success rather than ornaments *per se*, lest they undermine the ornamental signal value. However, ornament expression might easily be correlated with previous mating success in observational studies.

An alternative explanation for why we observe selection for intermediately ornamented *R. longicauda* females is antagonistic coevolution (Arnqvist & Rowe, 2005). Females may be deceiving males about their potential fecundity by displaying larger ornaments, resulting in male resistance to antagonistically seductive female characters (Arnqvist & Rowe, 2005), and preferences for more modestly adorned females. It is important to note, however, that it is currently unclear whether there is a fecundity cost associated with ornamentation in *R. longicauda* females. Here, and in previous studies we consistently see a positive relationship between ornamentation and fecundity (Figure 1B; Wheeler, 2008, Funk & Tallamy, 2000). However, correlations among phenotypic traits do not always reveal underlying tradeoffs because animals vary in both resource acquisition and allocation (Van Noordwijk & de Jong, 1986), and we are unable to measure resource acquisition and allocation directly (Houslay & Bussière, 2012). We note, though, that in a comparison between *R. longicauda* and *Rhamphomyia sociabilis*, a dance fly with no inflatable abdomen or other female ornament, the abdomen size is a much better predictor of fecundity in unornamented *R. sociabilis* (Figure 9 in Funk & Tallamy, 2000) suggesting that there could be a fecundity cost that ornamented females pay relative to non-ornamented species.

At present we have not been successful in rearing or maintaining any dance fly species in captivity. However, if successful in the future, laboratory-controlled mating trials would allow us to directly assess male mate choice for female ornaments in *R. longicauda*. Through male mate choice trials we could also tease apart the timing of when during the mating process males are choosing intermediately-ornamented females (Wheeler et al., 2012) over the large-ornamented females they are initially most attracted to (Funk & Tallamy, 2000, Murray et al., 2018).

## Conclusions

We tested for a load-lift constraint in shaping non-random mating patterns in the dance fly *Rhamphomyia longicauda.* We used morphological measures from wild-caught mating pairs and permutation tests and found no evidence for a load-lift constraint. Instead, we saw the opposite pattern: positive assortative mating for mass. We conclude that our findings suggest male mate choice for ornamented females as a probable explanation for the previously observed non-random mating patterns in *R. longicauda*.

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## Tables

Table 1. Means and standard errors for morphological traits of paired *R. longicauda* females and males (N=112).

| **Trait** | **Mean** | **Standard Error** |
|---|---:|---:|
| ***females*** | | |
| thorax length (mm) | 1.60 | 0.012 |
| squareroot wing area (mm) | 98 | 0.021 |
| wing length (mm) | 6.74 | 0.040 |
| mass (mg) | 4.63 | 0.13 |
| hind leg length (femora and tibia: mm) | 6.46 | 0.36 |
| squareroot hind leg ornament area (mm) | 2.23 | 0.014 |
| fecundity (oocyte count) | 66.3 | 1.67 |
| oocyte size (mm) | 0.31 | 0.01 |
| ***males*** | | |
| thorax length (mm) | 1.57 | 0.012 |
| squareroot wing area (mm) | 71 | 0.023 |
| wing length (mm) | 6.31 | 0.039 |
| mass (mg) | 2.68 | 0.085 |
| hind leg length (femora and tibia: mm) | 5.77 | 0.033 |
| squareroot hind leg area (mm) | 1.29 | 0.008 |
| nuptial gift mass (mg)* | 0.80 | 0.068 |

*N=57

Table 2. Male wing traits that predict the mass carried by a male during copulation in *R. longicauda*. Estimates are standardised coefficients of a multiple regression. Mass carried is female mass (mg).

| **Trait** | **Estimate** | **Standard Error** | **z** | **p** |
|---|---:|---:|---:|---:|
| intercept | 5.6 | 0.19 | 28.82 | <0.001 |
| male wing length | -0.056 | 0.13 | 4.76 | 0.68 |
| male wing load<br>(mass/wing area) | 0.62 | 0.13 | 2.39 | <0.001 |
| aspect ratio<br>(wingspan$^2$/wing area) | 0.18 | 0.12 | 1.15 | 0.14 |

Figures

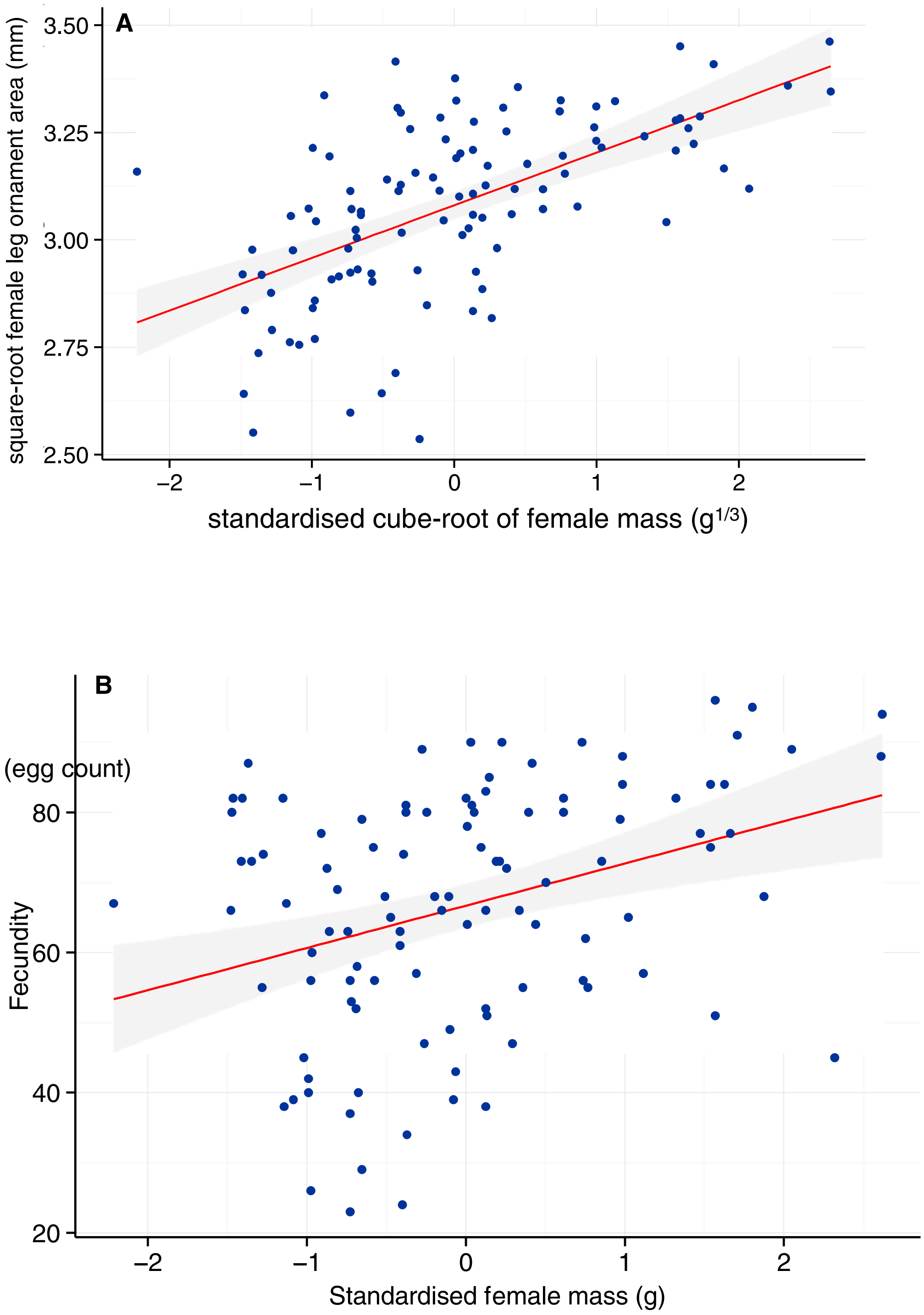

Figure 1. Female body mass correlates positively with and ornamentation and fecundity in *Rhamphomyia longicauda*. A) Linear regression of square-rooted female leg ornament area

(mm) on standardised cube-rooted female mass ($g^{1/3}$; estimate=0.12±0.016, $F_{(1, 105)}$=58.35, P<0.001). B) Linear regression of female fecundity (measured as oocyte count) against standardised female body mass (estimate= 6.029±1.579, $F_{(1, 105)}$=14.33, P<0.001). The shaded area represents the standard error measure around the linear estimate (shown in red).

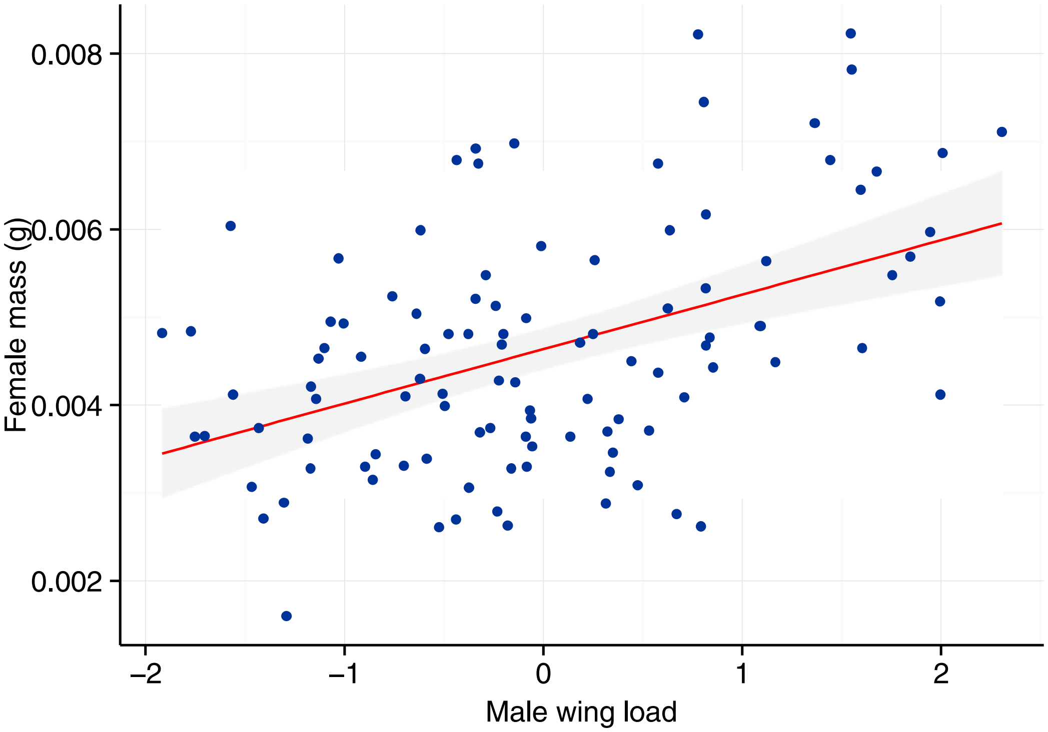

Figure 2. Linear regression of the female mass (g) on standardised male wing load in *R. longicauda* mated pairs. Wing load is calculated as mass/wing area (see text for details). The shaded area represents the standard error measure around the linear estimate (shown in red). (estimate=0.00062± 0.00012, $F_{(1, 105)}$=26.95, P<0.001).

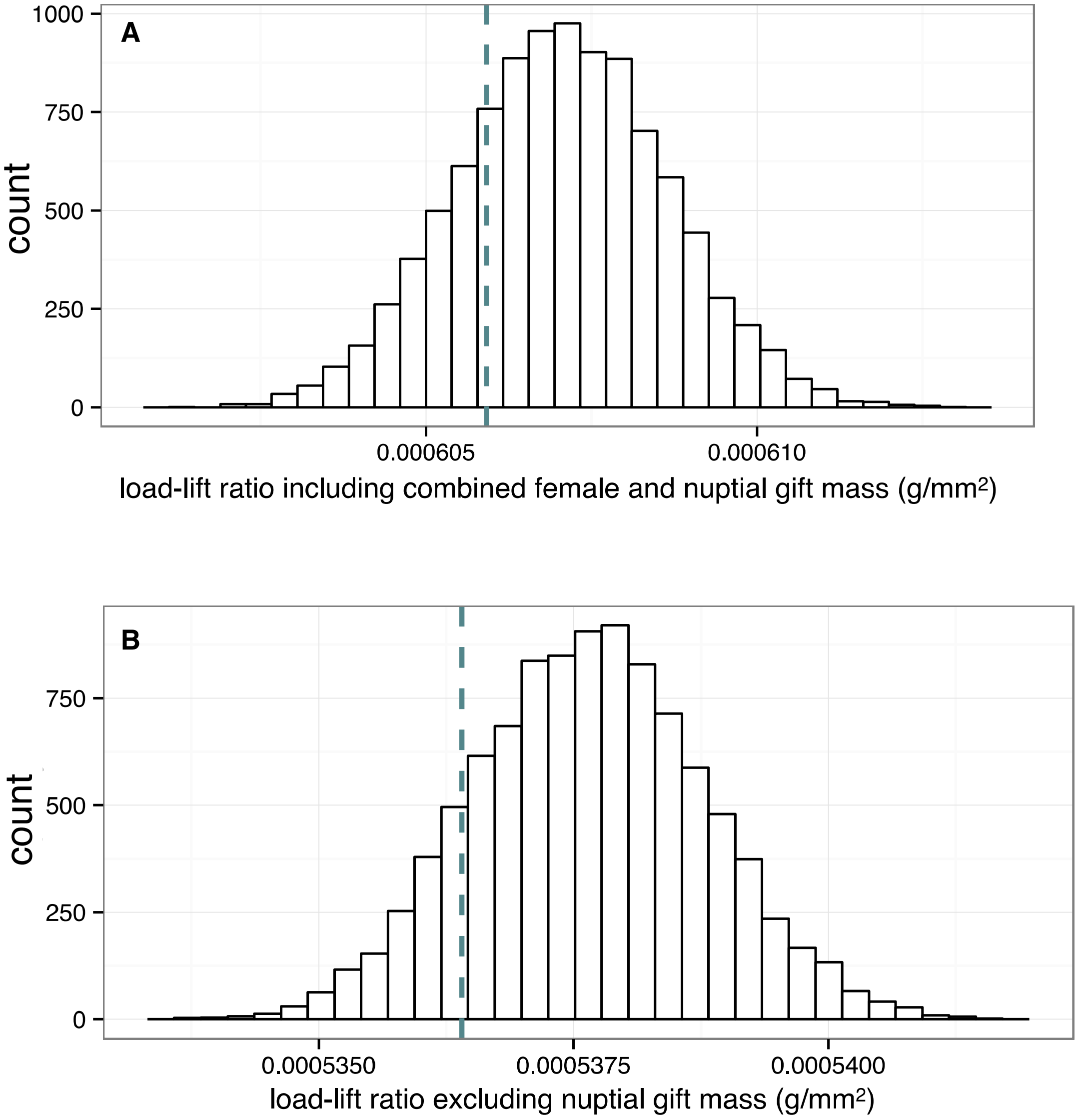

Figure 3. Null distribution of the load-lift ratio from permutation tests with 9999 iterations, assuming random mating and male foraging. Load-lift ratio is the total aerial mass (g) / male wing area (mm$^2$). The total aerial mass carried by the male is made up of non-randomised male mass values and (A) randomised female and nuptial gift mass values or (B) randomised female mass values excluding nuptial gift (see text for details). The observed mean of the load-lift ratio from our data is displayed as dashed lines (A: P=0.24; B: P=0.13).

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