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Variation in adult sex ratio alters the association between courtship, mating frequency and paternity in the lek-forming fruitfly *Ceratitis capitata*

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Abstract

The intensity with which males deliver courtship and the frequency with which they mate are key components of male reproductive success. However, we expect the strength of the relationship between these traits and a male's overall paternity to be strongly context dependent, for example to be altered significantly by the extent of post-mating competition. We tested this prediction in a lekking insect, *Ceratitis capitata* (medfly). We examined the effect of manipulating the sex ratio from male- to female-biased (high and low male competition, respectively) on courtship behaviour, mating frequency and paternity of focal males. Under high male competition, focal males delivered significantly more courtship but gained lower paternity than under lower competition. Paternity was positively associated with mating frequency and small residual testes size. However, the association between mating frequency and paternity was significantly stronger under low competition. We conclude that manipulation of sex ratio significantly altered the predictors of mating success and paternity. The relationship between pre- and post-mating success is therefore plastic and alters according to the prevailing level of competition. The results highlight the importance of post-copulatory processes in lekking species and illuminate selection pressures placed on insects such as medflies that are mass reared for pest control.

Introduction

Fundamental differences between males and females, rooted in anisogamy, lead to the evolution of contrasting reproductive strategies among males and females (Darwin, 1871; Parker *et al.*, 1972). This is evident in marked sexual dimorphism in traits such as size, plumage colouration, displays, courtship song and pheromone production (Andersson, 1994). Divergence in selection pressures for reproductive traits in males vs. females is thought to arise because of differences in reproductive roles, for example stronger competition between males than between females for access to matings and fertilization opportunities (Johnstone *et al.*, 1996). The basis for differences in reproductive roles has traditionally been thought to be divergence in the relative level of reproductive investment (often assumed higher in females) and in potential reproductive rate (PRR, assumed to be higher in males; Bateman, 1948; Parker, 1970; Parker & Partridge, 1998; Chapman, 2006). However, it is now increasingly realized that reproductive roles and strategies are far more flexible than originally perceived, and that PRR is often a poor indicator of the relative level of reproductive investment (Snyder & Gowaty, 2007; Taylor *et al.*, 2008).

Either sex can express mate choice or be subject to intra sexual competition for mates (Edward & Chapman, 2011). The reproductive strategy adopted by each sex is determined by key factors such as relative reproductive

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investment patterns, the availability of mates, an individual's capacity to mate with all of the available mates and variation in mate quality. Such factors are important as they alter the benefits and costs of each mating. Developing an understanding of how to predict costs and benefits in both sexes can be very informative. For example, it has long been realized that there are likely to be significant disparities in the costs and benefits of multiple mating in males vs. females, especially in cases where males make little or no investment in offspring care such as in lekking species. Under these conditions, sexual selection for traits in males that increase reproductive success should be very strong (Parker & Simmons, 1996). This is borne out in many lekking species of birds, mammals, amphibians and insects, which often show very marked sexual dimorphism, strong male–male competition and female choice. As a result, variation in male reproductive success is often skewed, as relatively few males monopolize females and father most offspring (Kirkpatrick & Ryan, 1991; Rowe & Houle, 1996; Droney & Hock, 1998).

Males can achieve high reproductive success and father significantly more offspring even under intense competition if they are able to monopolize access to females and their ova. They can do this through increasing their investment in premating traits, such as courtship, which may then lead to higher mating frequency. They can also invest more in post-mating traits such as sperm transfer and mechanisms to promote sperm precedence and prevent female remating (Yuval & Hendrichs, 2000). However, despite the realization that many traits can influence male reproductive success, until recently, few studies have investigated the relative contribution of pre- and post-mating traits to a male's overall paternity (Fricke *et al.*, 2010; Pischedda & Rice, 2012). Hence, the relative importance of, and the precise relationship between, pre- and post-mating sexual selection is often unclear. In some species such as *Tribolium castaneum* flour beetles, there is a positive association between a male's pre- and post-mating reproductive success (Lewis & Austad, 1994). In contrast, in the pond skater *Gerris lacustris*, there appears to be a trade-off between investment in pre- and post-mating traits (Danielsson, 2001). Given these varying patterns, the fitness gains of increased investment in either pre- or post-mating traits may not always be straightforward to predict. In addition, perturbation of factors such as the operational sex ratio (the ratio of sexually available males to females, Emlen & Oring, 1977) could alter the expression, and relationship with fitness, of pre- and post-mating traits (Reuter *et al.*, 2008).

Determining the relative contributions of pre- and post-mating sexual selection and a male's overall reproductive fitness is important for understanding the evolution of mating systems in general, but is also of applied relevance. For example, the identification of traits that are key in promoting success during male–male competition could prove useful in improving husbandry regimes for insects such as the medfly, an agriculturally important pest that is mass reared for use in biological control programmes (Mitchell *et al.*, 1997). This knowledge could improve existing biological control methods such as the sterile insect technique (SIT; Knipling, 1955; Dyck *et al.*, 2005), which relies on matings between released sterile males and wild females, and is thereby dependent on the field mating success of mass-reared insects. Interestingly, by releasing only males, medfly SIT also significantly changes the male : female sex ratio in programme areas.

Here, we conducted an integrated study of the pre- and post-mating traits that influence a male's overall reproductive success. We focused on medfly as it is both an experimentally tractable model for mating behaviour in lekking species and a key target species for field control by SIT. The natural mating system of this species involves lek formation (Aluja & Norrbom, 2000) where males gather for premating competitions in which pheromone signalling and courtship displays occur and females assess potential mates (Yuval & Hendrichs, 2000). The medfly, like several other lekking species (e.g. Petrie *et al.*, 1992; Kellogg *et al.*, 1995; Lank *et al.*, 2002), exhibits polyandry (Kraaijeveld *et al.*, 2005). In such species, post-copulatory male traits are likely to play a significant role in determining reproductive success, yet this area has received little attention. In this study, we experimentally manipulated the level of male–male competition by varying adult sex ratios to create male- and female-biased treatments (generating high and low male competition, respectively). We did this via proximate manipulations of sex ratio, rather than through experimental evolution as has been employed in studies of nonpest fruitflies (e.g. Snook *et al.*, 2005; Reuter *et al.*, 2008; Crudgington *et al.*, 2009, 2010). Our aim was to test the effect of sex ratio manipulation on courtship, mating frequency and paternity of focal males. This enabled us to identify the traits that most closely predicted a male's pre- and post-mating reproductive success and to discover how the relationships between these traits and a male's overall paternity varied under different competitive conditions. Our prediction was that increasing the level of male–male competition would intensify pre- and post-mating competition, lead to increased sperm competition and potentially uncouple a male's precopulatory reproductive success from the share of paternity gained. A male's precopulatory reproductive success was measured as the frequency of courtship and of mating. Overall reproductive success was measured as paternity gained under competitive conditions over a period of 2 weeks. Body size and testis size were included as covariates.

Materials and methods

Fly stocks and culturing

Wild-type focal males were from a subculture of the 'Cepa petapa' Moscamed mass-reared factory strain from Guatemala, Central America (Rendon, 1996). To assign paternity, we used females and competitor males carrying a recessive *white pupae* (*wp*) pupal colour marker. Hence, pupae fathered by focal wild-type males were brown, and those by *wp* competitor males were white.

Flies were cultured at 25 °C on a 12 : 12 light : dark cycle and 50% relative humidity, in a controlled environment room. Flies were cultured from eggs collected over a 24-h period placed in 500-g standard larval culture medium (400 mL distilled water, 26 g brewer's yeast, 24 mL 1 M HCl, 3 mL formaldehyde (4%), 8 mL methyl paraben (Nipagin, 10% w/v solution in ethanol) and 71 g ground carrot flakes) and placed within 17 cm × 13 cm × 5 cm plastic containers lined with sand to a depth of 1 cm with a mesh lid. At the third instar larval stage, the larvae 'jump' exit the larval culture medium and pupate in the sand. Development time from egg to adult under these conditions is between 16 and 18 days.

Virgin flies were obtained by sorting males and females apart within 24 h of eclosion using light CO_2 anaesthesia and then stored before use in adult holding cages, with plentiful access to sugar–yeast food (3 : 1 w/w brewer's yeast: sugar paste) and water. Focal males were marked with a spot of red paint on the dorsal side of the thorax while anesthetized. Pilot experiments had demonstrated no discernible impact of the paint mark on mating frequency ($\chi^2_1 = 0.322$, $P = 0.57$, $n = 200$). Flies entered the experiments when they were fully sexually mature at 5–7 days old. Males were transferred into the mating arenas 1 day prior to the start of the experiments.

Effect of sex ratio on courtship, mating frequency and paternity

Virgin focal wild-type males were randomly assigned to one of the two groups and placed in 250-mL transparent plastic mating arenas as follows. The 'high male competition' group consisted of 50 replicate pots each of one virgin wild-type male and two virgin *wp* females with five virgin *wp* competitor males. The 'low male competition' group consisted of 50 replicate pots each of one virgin wild-type male and six virgin *wp* females with one virgin *wp* competitor male. Hence, the overall density of flies within each of the pots was the same. Each pot received water through a filter paper wick and sugar–yeast food supplied via the lid of a microfuge tube.

The behaviour of each focal male (courtship or mating) was recorded at 30-min intervals starting at 9 am until 12.30 pm on alternate days. Courtship was scored if a male was seen to be engaged in emission of pheromone by eversion of the rectal ampulla and/or engaged in wing 'buzzing' (Yuval & Hendrichs, 2000). Mating duration is 1.5–3 h in this species, and therefore, sampling at 30-min intervals ensured that all matings were recorded. A mating was scored if individuals in copula were observed on at least two consecutive observation periods. To ensure equal density and equal exposure to females and competitor males throughout the experiment, the sex ratio conditions were maintained by replacing any dead *wp* males or females with spare virgin individuals of the same age. In total, the number of replacements due to deaths were seven females and one male added to six cages under low competition; five males and two females added to seven cages under high competition. The number of such replacements was therefore low and was not biased towards either treatment. After 7 days, all *wp* males and females were replaced by 5–7-day-old virgins, and the mating assay was continued for another 7 days. This was performed to maintain high levels of competition and fertility, so that the reproductive success of the focal males would not be confounded by decreasing fertility of females or competitor males during the experiment.

To measure the paternity of focal males, we collected egg samples from each mating pot on days 1 and 4 of each of the 2 weeks of the experiment. Females laid eggs through the gauze in the lid of each mating pot, and these were brushed lightly, using strips of filter paper, into separate pots containing 150 g larval culture media. The two samples of eggs collected per week were placed in excess food (the maximum larval density was 0.5 g per egg compared with standard mass-rearing densities of 0.04 g per egg, Fay, 1988). This ensured that all larvae were reared under low-density conditions during development. This reduced the potential for differential larval survival given that 2 vs. 6 females were contributing to egg production in the high vs. low competition treatments, respectively. We recorded the number of offspring fathered by the focal males from the four samples taken over the 2 weeks.

Associations of morphological covariates with courtship, mating frequency and paternity

We recorded body and testis size of focal males from the high and low male competition treatments as covariates in our analyses and tested for associations with pre- and post-mating reproductive success. Males were frozen at the end of the observation period for subsequent dissection. The wings were first removed and placed into phosphate-buffered saline (PBS) and sealed under a cover slip. The wingless males were then transferred to a cavity slide containing 15 μL PBS and the abdomen removed to dissect out the testes. These organs were then placed in 5 μL of PBS, and the digital images of the testes and wings recorded using an Olympus BX41 microscope (Olympus Corp., Tokyo, Japan) fitted with a JVC KY-F70B digital camera (JVC Kenwood Group, Yokohama, Japan) (under × 10 magnification for testes × 4 magnification for wings). We recorded landmarks on each wing to calculate wing area, measuring along the radial vein, median flexion-line and the anal fold (Gullan & Cranston, 1994). Testis area was recorded by tracing the perimeter of the recorded images, and areas were then calculated using Image J software (Rasband, 1997).

Data analysis

Courtship, mating frequency and paternity data were non-normally distributed, and Mann–Whitney tests were therefore used in initial tests to determine the effect of high and low male competition on these traits. However, to include analyses of covariates and test for interactions, we then used generalized linear models (GLMs) with quasipoisson error distributions, to identify significant predictors of courtship, mating frequency or paternity. We included in each model, where appropriate, measures of courtship, mating frequency, body size (i.e. wing area) and testis size.

As courtship and mating cannot occur simultaneously, we calculated courtship frequency as the proportion of time spent courting for males that were not mating. For example, if the total number of observations = 60, with 32 of these being mating, then 20 courtship observations would yield a courtship frequency value of 20/(60–32) = 0.71. There was a highly significant correlation between wing and testis area (mm^2) across males ($r^2 = 0.433$, $P < 0.001$). We therefore used relative testis size as the covariate for our analyses, as calculated from the residuals of a linear regression of testis on body size.

The number of females varied between each pot for the high and low competition treatments, respectively. This required a correction factor to be applied to the mating frequency and paternity data to enable direct comparisons between the reproductive performance of high and low competition focal males. The mating frequency and paternity data for each focal male were therefore divided by the number of females present in each cage (i.e. high competition counts divided by two, low competition counts divided by six). This gave mating frequency and paternity data that were standardized per female. Initial maximal models were constructed to include all main effects and interactions. These models were subsequently simplified to attain the minimal adequate model by stepwise removal of the least significant terms. Terms were removed from the model only if removal did not significantly ($P > 0.05$) influence the fit of the model when comparing previous and reduced models in an analysis of deviance. Analyses were performed using R v2.11.1 (R Development Core Team., 2010) and PASW v18.0.0 (PASW Statistics v.18., 2009).

Results

Effect of high vs. low male competition on courtship, mating frequency and paternity

The initial analyses showed that males held under high competition delivered significantly more courtship than did low competition males ($u_{79} = 554$, $P = 0.018$; Fig. 1a). The significant differences in courtship delivery did not translate into differences in mating frequency per female, as there were no significant differences in the number of focal male matings per female ($u_{79} = 697.5$, $P = 0.325$; Fig. 1b). Therefore, the increased courtship activity under elevated male competition did not increase the focal males' matings per female beyond that seen in the low male competition group. In terms of absolute paternity, low competition males (that delivered significantly lower levels of courtship) had significantly higher paternity per female ($u_{79} = 558$, $P = 0.02$; Fig. 1c).

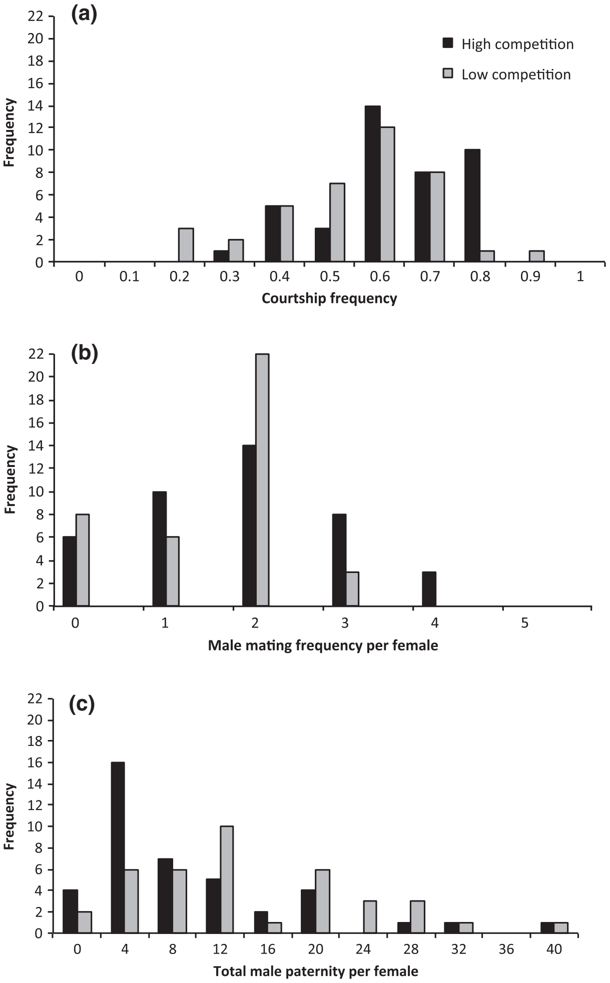

Fig. 1 Frequency distributions of (a) the proportion of time spent courting by focal males under high and low levels of male competition (high competition median = 0.66, SD = 0.15, $N = 41$; low competition median = 0.63, SD = 0.16, $N = 39$), (b) the total number of mating observations per female for focal males (high competition median = 1.41, SD = 1.03, $N = 41$; low competition median = 1.35, SD = 0.70, $N = 39$), (c) the total focal male paternity per female (high competition median = 1.76, SD = 0.82, $N = 41$; low competition median = 2.21, SD = 0.82, $N = 39$).

Effect of high vs. low male competition on interactions between premating success and paternity

Consistent with the above, the GLM analyses suggested that there was no significant effect of high or low male competition on mating frequency per female (Table 1). There was a marginally significant interaction of body size and courtship frequency ($P = 0.048$) and a marginally nonsignificant effect of courtship delivery ($P = 0.066$; Table 1). Body size was significantly negatively correlated with mating frequency per female (Table 1; $r^2 = 0.101$, $P = 0.013$).

We then examined whether precopulatory success (mating frequency) explained a significant proportion of the variance in post-copulatory success (i.e. paternity gained per female), and whether this differed across high and low male competition treatments. We indeed found a significant interaction between mating frequency per female and high or low competition ($P = 0.030$, Table 2) in addition to a marginally significant main effect of mating frequency itself ($P = 0.050$, Table 2). The positive relationship between mating frequency per female and paternity per female was therefore significantly stronger under low in comparison with high competition. This is consistent with the idea that increased levels of competition between males led to increased post-mating competition and hence a breakdown in the association between premating success and paternity (Fig. 2).

We then analysed which of the different covariates (courtship, body size, mating frequency and relative testes size) best explained the variation in paternity per female across high and low competition treatments (Table 3). Consistent with the analysis above, the most significant predictor of paternity was the interaction between high and low male competition and mating frequency ($P < 0.001$). A significant interaction was also found between mating frequency and testis size ($P = 0.038$; Table 3), independent of male competition. Residual testes size also emerged as a significant predictor of paternity ($P = 0.026$), though interestingly, this was a negative correlation, with smaller residual testes size associated with increased paternity. There was no significant interaction of testis size with the level of male competition ($P = 0.309$).

Table 1 Analysis of the variation in mating frequency per female for focal males held under high or low levels of male competition. Competition level was a fixed effect, and courtship and body size were covariates in the analysis. The data were analysed using generalized linear models (GLM) with a quasi-poisson error distribution. Removal of the nonsignificant interactions had no effect on the overall results of the model.

Factor	Deviance	*F*	d.f.	*P*
Competition (high or low)	1.685	0.651	1,75	0.517
Courtship	4.640	1.866	1,77	0.066
Body size	1.090	2.578	1,76	0.013
Competition × Courtship	1.080	0.261	1,72	0.795
Competition × Body size	0.535	0.767	1,73	0.445
Courtship × Body size	1.636	2.010	1,74	0.048

Table 2 Analysis of the proportion of variance in post-mating reproductive success (paternity per female achieved by focal males) explained by premating reproductive success (mating frequency per female), for males held under high and low level of competition. Competition level was a fixed effect, and mating frequency was a covariate in the analysis. Data were analysed using a generalized linear model (GLM) with a quasi-poisson error distribution.

Factor	Deviance	*F*	d.f.	*P*
Competition (high or low)	1.040	0.299	1,77	0.765
Mating frequency	2.737	1.952	1,77	0.050
Competition × mating frequency	5.133	2.205	1,76	0.030

Discussion

The main findings were that manipulation of sex ratio from female to male biased (low to high male competition, respectively) had a significant effect on courtship delivery and offspring production. The strength of the association between mating frequency and the number of offspring fathered was also stronger under low in comparison with high levels of competition.

We found a significant difference in the amount of courtship delivered, with focal males from the high competition treatment exhibiting significantly more courtship behaviour in comparison with low competition males. Courtship delivery therefore exhibited plasticity, with males adjusting their courtship delivery according to the level of competition with males and likelihood of matings with females. This finding complements previous work from experimental evolution in *Drosophila* (Crudgington *et al.*, 2010). It also adds to a growing body of evidence documenting behavioural plasticity in responses to rivals across a wide range of taxa, although the ultimate significance of such plasticity is not yet fully understood (Bretman *et al.*, 2011). Courtship frequency was a marginally nonsignificant predictor of mating frequency across high or low male competition. This could indicate that although investment in courtship may be necessary in order for females to assess male quality (for instance using morphological measures such as body size or fluctuating asymmetry), it is not itself a reliable predictor of female choice. The idea that female medflies may use courtship to assess the attractiveness of morphological traits has been considered, but results are so far conflicting (Mendez *et al.*, 1999; Hunt *et al.*, 2002). Alternatively, quality of the courtship song itself or the threshold level of courtship needed for males to be competitive may increase as more males are present (Snook *et al.*, 2005). Although the number of matings

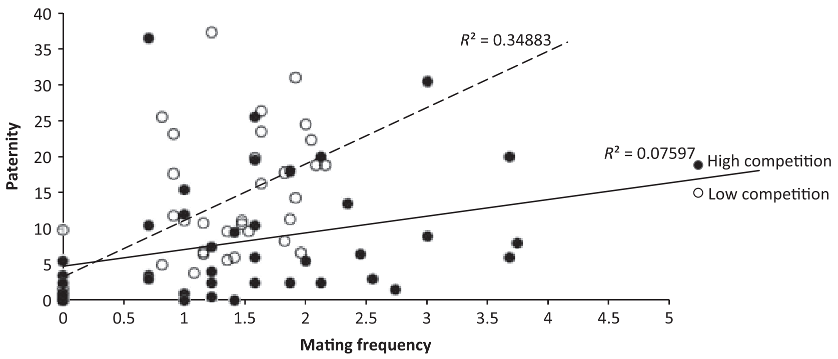

Fig. 2 Correlations between the number of matings and total paternity per female for focal males held under high (filled circles, solid line, $N = 41$) and low (open circles, dashed line, $N = 39$) levels of male competition.

Table 3 Analysis of the proportion of variation in paternity per female achieved by focal males under high or low levels of male competition due to competition, mating frequency and residual testis size. Competition level was a fixed effect, and courtship, mating frequency and residual testis size were covariates in the analysis. Analysis of the variation in mating frequency and residual testis size on paternity achieved by focal males under high and low levels of male competition. Data were analysed using generalized linear models (GLM) with a quasi-poisson error distribution.

Factor	Deviance	*F*	d.f.	*P*
Competition (high or low)	0.364	1.327	1.75	0.189
Residual testis size	11.065	2.274	1.75	0.026
Mating frequency	0.236	2.342	1.75	0.022
Mating frequency × residual testis size	6.789	2.116	1.73	0.038
Competition × residual testis size	5.373	1.024	1.73	0.309
Competition × mating frequency	0.661	3.666	1.73	< 0.001

per female was not significantly different across treatments, males produced more offspring per female under low in comparison with high competition. Under relaxed levels of male–male competition, therefore, focal males gained higher reproductive success per female. The stronger correlation between the number of matings and number of offspring fathered per female under low but not high competition is consistent with the idea that strong post-copulatory effects under high competition result from increased sperm competition or cryptic female choice (Zeh & Zeh, 1994).

In terms of the morphological correlates, we found a negative effect of male body size on mating frequency per female. This is in contrast to some previous reports where larger males were reported to mate more frequently (Rodriguero *et al.*, 2002; Anjos-Duarte *et al.*, 2011); however, in such studies, which were comparisons between wild and laboratory flies, any effect of body size may often be an artefact of female preference for wild (and typically larger) males (Rodriguero *et al.*, 2002). Body size may be important in the establishment of territories at lekking sites (Niyazi *et al.*, 2008). The precise mechanism of competition between males for preferred lek positions is not known, although disparities in body size between two competing males may play a role (Kaspi & Yuval, 1999). Under laboratory conditions, medflies may not form a natural lek. This could remove the presence of 'hotspots' of female or male activity that may be present under more natural conditions and hence reduce or possibly even reverse the potential role of body size on precopulatory success, as observed in this study.

Our results showed a paternity advantage to small residual testis size. It is possible that this reflects greater sperm depletion rates in successful males, or that testis size or sperm production costs (Sella & Lorenzi, 2003) trade off against other life history traits such as growth, lifespan and manoeuvrability. In addition, absolute testis size could be constrained by the size of other reproductive tissues such as the accessory glands (Nijhout & Emlen, 1998), which could themselves be stronger targets of sexual selection (Crudgington *et al.*, 2009). It would therefore be useful to develop an accurate method for measuring short and long male accessory gland size or to quantify seminal fluid production and transfer (Sirot *et al.*, 2009). With such techniques, it would then be possible to test for associations of small residual testis size with other reproductive traits to understand why small testes did not apparently limit male reproductive success. Evidence from comparative studies shows that testis size is a reliable indicator of the level of promiscuity; the interpretation is that males with larger testes can produce more sperm and can achieve reproductive success even in the face of strong sperm competition (Møller, 1989; Simmons *et al.*, 1993; Møller & Briskie, 1995). However, the medfly typically exhibits low to moderate rates of remating in the wild (e.g. 7.1%; Bonizzoni *et al.*, 2002), which predicts that wild males should have a small residual testes size. Under selection for increased post-mating competition in the laboratory, males with relatively larger testes, capable of producing large numbers of sperm, should therefore be favoured. However, this was the opposite of what was observed, suggesting that even in the laboratory, factors other than sperm numbers (e.g. seminal fluid proteins, sperm morphology or possibly cryptic female choice) are important in securing paternity.

Our findings are broadly relevant to the control of insect pests using methods such as SIT (Knipling, 1955;

Dyck *et al.*, 2005) and the Release of Insects with a Dominant Lethal (RIDL) (Thomas *et al.*, 2000; Gong *et al.*, 2005; Fu *et al.*, 2007; Morrison *et al.*, 2011). In these techniques, the insects are mass reared, sterilized (under SIT) and released into the wild to mate with wild females. These matings are either sterile (SIT) or pass a dominant lethal gene to offspring (RIDL). Both outcomes lead to a subsequent reduction in the pest population size (Knipling, 1955; Thomas *et al.*, 2000; Gong *et al.*, 2005; Fu *et al.*, 2007; Morrison *et al.*, 2011). Insights into methods for improving SIT-based programmes can be gleaned by understanding that the traits that increase male reproductive success are dependent on the relative abundance of potential mates and rivals, and how those traits are affected by selection for mass rearing (Cayol, 2000). Our data suggest that there could be relaxation in sexual selection for large body size under mass rearing. Selection under mass rearing may instead focus on rapid development time or early sexual maturation, which could be accompanied by reduced body size. In this case, the screening of lines for body size could indicate the intensity of artificial selection and help to determine the likely efficacy of a line in SIT or RIDL programmes. Changes in selection pressure on body size could also partly contribute to the finding that mass-reared males consistently under-perform in the field against wild-type males (Cayol & Zarai, 1999; Lux *et al.*, 2002; Shelly *et al.*, 2007) together with degraded male courtship behaviour under mass-rearing conditions (Briceño *et al.*, 1996; Briceño & Eberhard, 1998, 2002).

Our findings are also useful in contributing to a better understanding of how selection operates under mass-rearing regimes used for SIT (Cayol, 2000). For example, under natural conditions, females visit leks for mating, but then leave to oviposit into ripe fruit (Yuval & Hendrichs, 2000). Under high density confined conditions escape is restricted, and this, together with the presence of many males, may result in decreased courtship, more frequent mating attempts by males and a higher probability of mating disruption. Successive generations of exposure to these conditions are predicted to focus sexual selection on post-copulatory processes rather than on precopulatory morphology and behaviour (Reuter *et al.*, 2008). This may have consequences for the efficient implementation of pest control mechanisms such as SIT and RIDL, which rely on maintaining the natural reproductive repertoire of mass-reared males.

Our findings are also relevant to the evolution of mating systems in lekking insects. We show that male reproductive success is context dependent and can be significantly affected by post-copulatory traits even in lekking species, where the emphasis has traditionally been on precopulatory traits. We found that associations between pre- and post-mating success became uncoupled when male competition increased and the potential for sperm competition and/or female cryptic choice was intensified. This will now allow better predictions for how selection will act on male reproductive traits as the operational sex ratio changes.

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References

Aluja, M. & Norrbom, A.L. 2000. *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior*. CRC Press, Boca Raton, USA.

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.

Anjos-Duarte, C.S., Costa, A.M. & Joachim-Bravo, I.S. 2011. Sexual behaviour of the Mediterranean fruit fly (Diptera: Tephritidae): the influence of female size on mate choice. *J. Appl. Entomol.* **135**: 367–373.

Bateman, A.J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* **2**: 349–368.

Bonizzoni, M., Katsoyannos, B.I., Marguerie, R., Guglielmino, C.R., Gasperi, G., Malacrida, A. *et al.* 2002. Microsatellite analysis reveals remating by wild Mediterranean fruit fly females, *Ceratitis capitata*. *Mol. Ecol.* **11**: 1915–1921.

Bretman, A., Gage, M.J.G. & Chapman, T. 2011. Quick-change artists: male plastic behavioural responses to rivals. *Trends Ecol. Evol.* **26**: 467–473.

Briceño, R.D. & Eberhard, W.G. 1998. Medfly courtship duration: a sexually selected reaction norm changed by crowding. *Ethol. Ecol. Evol.* **10**: 369–382.

Briceño, R.D. & Eberhard, W.G. 2002. Decisions during courtship by male and female medflies (Diptera: Tephritidae): Correlated changes in male behavior and female acceptance criteria in mass-reared flies. *Fla. Entomol.* **85**: 14–31.

Briceño, R.D., Ramos, D. & Eberhard, W.G. 1996. Courtship behavior of male *Ceratitis capitata* (Diptera: Tephritidae) in captivity. *Fla. Entomol.* **79**: 130–143.

Cayol, J.P. 2000. Changes in sexual behavior and life history traits of tephritid species caused by mass-rearing processes. In: *Fruit Flies (Tephritidae): Phylogeny, Evolutionof.Behavior* (M. Aluja & A.L. Norrbom, eds), pp. 846–860. CRC Press, Boca Raton, USA.

Cayol, J.P. & Zarai, M. 1999. Field releases of two genetic sexing strains of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) in two isolated eases of Tozeur governorate, Tunisia. *J. Appl. Entomol.* **123**: 613–619.

Chapman, T. 2006. Evolutionary conflicts of interest between males and females. *Curr. Biol.* **16**: 744–754.

Crudgington, H.S., Fellows, S., Badcock, N.S. & Snook, R.R. 2009. Experimental manipulation of sexual selection promotes greater male mating capacity but does not alter sperm investment. *Evolution* **63**: 926–938.

Crudgington, H.S., Fellows, S. & Snook, R.R. 2010. Increased opportunity for sexual conflict promotes harmful males with elevated courtship frequencies. *J. Evol. Biol.* **23**: 440–446.

Danielsson, I. 2001. Antagonistic pre- and post-copulatory sexual selection on male body size in a water strider (*Gerris lacustris*). *Proc. Roy. Soc. B Biol. Sci.* **268**: 77–81.

Darwin, C. 1871. *The Descent of Man and Selection in Relation to Sex*. John Murray, London.

Droney, D.C. & Hock, M.B. 1998. Male sexual signals and female choice in *Drosophila grimshawi* (Diptera: Drosophilidae). *J. Ins. Behav.* **11**: 59–71.

Dyck, V., Hendrichs, J. & Robinson, A.S. 2005. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*. Springer, The Netherlands.

Edward, D.A. & Chapman, T. 2011. The evolution and significance of male mate choice. *Trends Ecol. Evol.* **26**: 647–654.

Emlen, S.T. & Oring, L.W. 1977. Ecology, sexual selection, and evolution of mating systems. *Science* **197**: 215–223.

Fay, H.A.C. 1988. A starter diet for mass-rearing larvae of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). *J. Appl. Entomol.* **105**: 496–501.

Fricke, C., Martin, O.Y., Bretman, A., Bussière, L.F. & Chapman, T. 2010. Sperm competitive ability and indices of lifetime reproductive success. *Evolution* **64**: 2746–2757.

Fu, G., Condon, K.C., Epton, M.J., Gong, P., Jin, L., Condon, G.C. *et al.* 2007. Female-specific insect lethality engineered using alternative splicing. *Nat. Biotech.* **25**: 353–357.

Gong, P., Epton, M.J., Fu, G., Scaife, S., Hiscox, A., Condon, K.C. *et al.* 2005. A dominant lethal genetic system for autocidal control of the Mediterranean fruitfly. *Nat. Biotech.* **23**: 453–456.

Gullan, P.J. & Cranston, P.S. 1994. *The Insects: an Outline of Entomology*, 3rd edn. Blackwell, Oxford, UK.

Hunt, M.K., Roux, E.A., Wood, R.J. & Gilburn, A.S. 2002. The effect of supra-fronto-orbital (SFO) bristle removal on male mating success in the Mediterranean fruit fly (Diptera: Tephritidae). *Fla. Entomol.* **85**: 83–88.

Johnstone, R.A., Reynolds, J.D. & Deutsch, J.C. 1996. Mutual mate choice and sex differences in choosiness. *Evolution* **50**: 1382–1391.

Kaspi, R. & Yuval, B. 1999. Mediterranean Fruit Fly leks: factors affecting male location. *Funct. Ecol.* **13**: 539–545.

Kellogg, K.A., Markert, J.A., Stauffer, J.R. & Kocher, T.D. 1995. Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proc. Roy. Soc. B Biol. Sci.* **260**: 79–84.

Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* **350**: 33–38.

Knipling, E.F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol.* **48**: 459–462.

Kraaijeveld, K., Katsoyannos, B.I., Stavrinides, M., Kouloussis, N.A. & Chapman, T. 2005. Remating in wild females of the Mediterranean fruit fly, *Ceratitis capitata*. *Anim. Behav.* **69**: 771–776.

Lank, D.B., Smith, C.M., Hanotte, O., Ohtonen, A., Bailey, S. & Burke, T. 2002. High frequency of polyandry in a lek mating system. *Behav. Ecol.* **13**: 209–215.

Lewis, S.M. & Austad, S.N. 1994. Sexual selection in flour beetles – the relationship between sperm precedence and male olfactory attractiveness. *Behav. Ecol.* **5**: 219–224.

Lux, S.A., Munyiri, F.N., Vilardi, J.C., Liedo, P., Economopoulos, A., Hasson, O. *et al.* 2002. Consistency in courtship pattern among populations of medfly (Diptera : Tephritidae): comparisons among wild strains and strains mass reared for SIT operations. *Fla. Entomol.* **85**: 113–125.

Mendez, V., Briceño, R.D. & Eberhard, W.G. 1999. Functional significance of the capitate supra-fronto-orbital bristles of male medflies (*Ceratitis capitata*). *J. Kans. Entomol. Soc.* **71**: 164–174.

Mitchell, W.C., Andrew, C.O., Hagen, K.S., Hamilton, R.A., Harris, E.J., Maehler, K.L. *et al.* (1997) The Mediterranean fruit fly and its economic impact on Central American countries and Panama. In: *UC/AID Pest Management and Related Environmental Protection Project at the University of California*, pp. 189. University of California, Berkeley.

Møller, A.P. 1989. Ejaculate quality, testes size and sperm production in mammals. *Funct. Ecol.* **3**: 91–96.

Møller, A.P. & Briskie, J.V. 1995. Extra-pair paternity, sperm competition and the evolution of testis size in birds. *Behav. Ecol. Sociobiol.* **36**: 357–365.

Morrison, N.I., Franz, G., Koukidou, M., Miller, T.A., Saccone, G., Alphey, L.S. *et al.* 2011. Genetic improvements to the Sterile Insect Technique for agricultural pests. *Asian-Pacific J. Mol. Biol. Biotechnol.* **18**: 275–295.

Nijhout, H.F. & Emlen, D.J. 1998. Competition among body parts in the development and evolution of insect morphology. *Proc. Natl Acad. Sci. USA* **95**: 3685–3689.

Niyazi, N., Shuker, D.M. & Wood, R.J. 2008. Male position and calling effort together influence male attractiveness in leks of the medfly, *Ceratitis capitata* (Diptera: Tephritidae). *Biol. J. Linn. Soc.* **95**: 479–487.

Parker, G.A. 1970. Sperm competition and its evolutionary consequences in insects. *Biol. Rev.* **45**: 525–567.

Parker, G.A. & Partridge, L. 1998. Sexual conflict and speciation. *Phil. Trans. R. Soc. B Biol. Sci.* **353**: 261–274.

Parker, G.A. & Simmons, L.W. 1996. Parental investment and the control of sexual selection: predicting the direction of sexual competition. *Proc. Roy. Soc. B Biol. Sci.* **263**: 315–321.

Parker, G.A., Baker, R.R. & Smith, V.G.F. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. *J. Theor. Biol.* **36**: 529–553.

PASW Statistics v.18. 2009. *PASW for Windows*. SPSS Inc., Chicago, IL, USA.

Petrie, M., Hall, M., Halliday, T., Budgey, H. & Pierpoint, C. 1992. Multiple mating in a lekking bird – why do peahens mate with more than one male and with the same male more than once? *Behav. Ecol. Sociobiol.* **31**: 349–358.

Pischedda, A. & Rice, W.R. 2012. Partitioning sexual selection into its mating success and fertilization success components. *Proc. Natl Acad. Sci. USA* **109**: 2049–2053.

R Development Core Team. 2010. *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org.

Rasband, S.N. (1997) Image J. In: *Image J.* Graphic measuring program. U.S. National Institutes of Health, Bethesda, USA, http://rsb.info.nih.gov/ij/

Rendon, P.A. (1996) Development and evaluation of a temperature sensitive lethal (TSL) genetic sexing strain of the

Mediterranean Fruit Fly. *Ceratitis capitata*. PhD. Manchester University, Manchester, UK.

Reuter, M., Linklater, J.R., Lehmann, L., Fowler, K., Chapman, T. & Hurst, G.D.D. 2008. Adaptation to experimental alterations of the operational sex ratio in populations of *Drosophila melanogaster*. *Evolution* **62**: 401–412.

Rodriguero, M.S., Vilvardi, J.C., Vera, M.T., Cayol, J.P. & Rial, E. 2002. Morphometric traits and sexual selection in medfly (Diptera : Tephritidae) under field cage conditions. *Fla. Entomol.* **85**: 143–149.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. B Biol. Sci.* **263**: 1415–1421.

Sella, G. & Lorenzi, M.C. 2003. Increased sperm allocation delays body growth in a protandrous simultaneous hermaphrodite. *Biol. J. Linn. Soc.* **78**: 149–154.

Shelly, T.E., Edu, J. & Pahio, E. 2007. Age-dependent variation in mating success of sterile male Mediterranean fruit flies (Diptera : Tephritidae): implications for sterile insect technique. *J. Econ. Entomol.* **100**: 1180–1187.

Simmons, L.W., Craig, M., Llorens, T., Schinzig, M. & Hosken, D. 1993. Bush-cricket spermatophores vary in accord with sperm competition and parental investment theory. *Proc. R. Soc. B Biol. Sci.* **251**: 183–186.

Sirot, L.K., Buehner, N.A., Fiumera, A.C. & Wolfner, M.F. 2009. Seminal fluid protein depletion and replenishment in the fruit fly, *Drosophila melanogaster*: an ELISA-based method for tracking individual ejaculates. *Behav. Ecol. Sociobiol.* **63**: 1505–1513.

Snook, R.R., Robertson, A., Crudgington, H.S. & Ritchie, M.G. 2005. Experimental manipulation of sexual selection and the evolution of courtship song in *Drosophila pseudoobscura*. *Behav. Genet.* **35**: 245–255.

Snyder, B.F. & Gowaty, P.A. 2007. A reappraisal of Bateman's classic study of intrasexual selection. *Evolution* **61**: 2457–2468.

Taylor, M.L., Wigmore, C., Hodgson, D.J., Wedell, N. & Hosken, D.J. 2008. Multiple mating increases female fitness in *Drosophila simulans*. *Anim. Behav.* **76**: 963–970.

Thomas, D.D., Donnelly, C.A., Wood, R.J. & Alphey, L.S. 2000. Insect population control using a dominant, repressible, lethal genetic system. *Science* **287**: 2474–2476.

Yuval, B. & Hendrichs, J. 2000. Behavior of flies in the genus *Ceratitis* (Dacinae: Ceratitidini). In: *Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior* (M. Aluja & A.L. Norrbom, eds), pp. 429–456. CRC Press, Boca Raton, USA.

Zeh, J.A. & Zeh, D.W. 1994. Last-male sperm precedence breaks down when females mate with 3 males. *Proc. R. Soc. B Biol. Sci.* **257**: 287–292.

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