

# Male-biased sex ratio increases female egg laying and fitness in the housefly, *Musca domestica*

Juli Carrillo · Anne Danielson-François ·  
Evan Siemann · Lisa Meffert

Received: 30 June 2011 / Accepted: 16 November 2011  
© Japan Ethological Society and Springer 2011

**Abstract** A biased operational sex ratio (OSR) can have multiple, confounding effects on reproductive fitness. A biased OSR can increase harassment and mating activity directed towards potential mates but may also increase the ability of potential mates to choose a good partner if lower quality mates are screened out through competitive interactions. Additionally, a biased OSR may affect reproductive fitness through changes in male ejaculate content or in female reproductive response. We quantified how a male-biased OSR (1:1, 2:1, or 5:1 male to female) affected the size of a female's first egg clutch and her offspring's survivorship in the housefly, *Musca domestica*. A male-biased OSR increased female fitness: females laid more eggs in their first clutch, had increased offspring survivorship at a 2:1 versus 1:1 OSR, and had equivalent fitness with a 5:1 male to female OSR. Courtship activity increased when the OSR was male-biased but was not a significant predictor of female fitness. Trials where females chose their mates versus trials where a random male was chosen for them had equivalent first clutch sizes and offspring survivorship. These results suggest that there are cryptic effects from a male-biased OSR on female fitness that are most likely driven by pre-copulatory social environment.

**Keywords** Competition · Courtship · Sex ratio · OSR · Sexual conflict · Indirect effects · Clutch size

## Introduction

The operational sex ratio (OSR), the ratio of sexually receptive males to sexually receptive females (Emlen and Oring 1977), can have profound effects on mating behavior and female and male fitness by influencing the intensity of sexual selection (e.g., Prohl 2002; Head and Brooks 2006) and intra- and inter-sexual competition (Berglund 1994; Kvarnemo et al. 1995; Grant and Foam 2002; Ros et al. 2003). When the OSR is biased towards the sex with fewer direct reproductive costs or higher potential reproductive rate, sexual selection theory predicts that the more numerous sex will face increased competition for mates while the limiting sex with higher reproductive costs will face increased intersexual activity and increased selection for mating resistance or mate choice (e.g., Weir et al. 2011). With conventional sex roles, i.e. males competing for choosy females, mating costs of females have been modeled as increasing linearly or even exponentially, while benefits to females saturate due to diminishing marginal returns, leading to an intermediate optimal mating rate (Gavrilets et al. 2001). In contrast, males usually benefit from a higher mating rate, despite costs of ejaculate production, leading to divergence in optimal mating rates between males and females, especially in insects (e.g., Arnqvist and Nilsson 2000; Gavrilets et al. 2001). This is hypothesized to manifest in increased courtship activity and ejaculatory investment of competing males and resistance behavior of choosy females avoiding costly matings.

A male-biased OSR, by increasing male competition, can therefore affect female fitness in at least three ways.

---

L. Meffert: Deceased.

---

J. Carrillo (✉) · A. Danielson-François · E. Siemann ·  
L. Meffert  
Department of Ecology and Evolutionary Biology,  
Rice University, Houston, TX 77005, USA  
e-mail: juli@rice.edu

### Present Address:

A. Danielson-François  
Division of Biology, Department of Natural Sciences,  
University of Michigan-Dearborn, Dearborn, MI 48128, USA

First, increased harassment of females by males. Conflict due to indiscriminate mating activity of males and resisting females is expected to be more pronounced at a biased OSR, and increases in courtship activity at a male-biased OSR have been shown to reduce female longevity in houseflies (Ragland and Sohal 1973) and reproductive fitness in *Drosophila melanogaster* (Holland and Rice 1999; Friberg and Arnqvist 2003). Further, male harassment has been shown to decrease offspring survivorship in adzuki bean beetles (Sakurai and Kasuya 2008) and Trinidadian guppies (Ojanguren and Magurran 2007). A biased OSR can also increase the level of harassment if males change their courtship behavior in the presence of competitors, e.g., increasing the rate or intensity of courtship behaviors or by attempting to sneak or force copulations (Jirotkul 1999; Reichard et al. 2004). Second, increased opportunity for mate choice. Females may be able to more accurately assess or choose among males at a male-biased OSR (e.g., Berglund et al. 1994). At a male-biased OSR, not only is there an increased likelihood of a high quality male being present but also the outcome of male–male competitive interactions may be an important criterion of female mate choice (e.g., Bisazza and Marconato 1988). And, third, changes in ejaculatory investment. Sperm competition is a driving force in many male–male and male–female interactions (Parker 1970), and many animals change ejaculatory investment according to male–male interactions, or perceived sperm competition risk (e.g., Stockley 1997). Importantly, perceived sperm competition risk at high male density may influence the transfer or composition of seminal fluids (e.g., Fedorka et al. 2011). Seminal fluid proteins can affect female behavior and fitness in insects both positively and negatively; for example, by stimulating egg production, increasing oviposition rate towards the current male, inhibiting remating, and even decreasing female lifespan (see Avila et al. 2011 for a review). In *Drosophila melanogaster*, the effects of accessory seminal products (ACPs) have been shown to respond to the presence of other males such that females mated with male-exposed males laid more eggs and took longer to remate, showing a plastic response of males to perceived pre-copulatory sperm competition (Bretman et al. 2009). Adaptations to sperm competition, such as ACPs, are a likely target of selection and can cause substantial sexual conflict, as they are costly to produce for males, affect female fitness, and show rapid evolutionary response (e.g., Stockley 1997; Wolfner 2002).

The housefly, *Musca domestica*, experiences male-biased sex ratios spatially and temporally. However, we currently have little information on how a male-biased OSR affects male–male competition in the housefly, and whether this influences female fitness. Indeed, although there are striking examples of the effects of sex ratio on male harassment of females, female mate choice, and ejaculatory investment on

female fitness (see preceding sections), we know of no studies that have examined all these together. The courtship behavior of *Musca* is well studied, females choose their mates, and some aspects of male mating activity have been shown to be costly to females. Additionally, male ACPs inhibit females from remating and increase average clutch size and lifetime oviposition rate (Riemann and Thorson 1969; Leopold 1976; Andres and Arnqvist 2001), with potential costs to both current and future female reproduction (Arnqvist and Andres 2006). For these reasons, the housefly is an ideal organism to test the effects of a male-biased OSR on male competition and female fitness.

Male houseflies actively court females through a series of behaviors including buzzing their wings, jumping onto the female's back, lunging over the female's head, and lifting the female's legs (Meffert and Bryant 1991). Females resist courtship by moving their wings perpendicular to their bodies and by kicking at the male with their legs (Meffert et al. 1999; Meffert and Regan 2002). Males also mount other males, who do not perform this resistance behavior, and, at a male-biased sex ratio or at higher male density, males experience higher mortality, wing loss, and flightlessness than at a balanced sex ratio or at lower male density (Ragland and Sohal 1973). Male houseflies do not provide direct benefits of parental care nor do they guard mates post-copulation, though recent work has demonstrated a direct nutritional effect of accessory seminal substances leading to both increased longevity and fecundity in female houseflies, which may reduce costs of mating for females (Hicks et al. 2004; Arnqvist and Andres 2006).

There is evidence of sexual conflict in houseflies. For example, Ragland and Sohal (1973) found that increased activity (presumably mating) resulting from a male-biased sex ratio reduced the longevity of females and increased the rate of female wing loss and flightlessness when males and females were housed together. Indeed, females appear to mitigate some of the damaging effects of mating activity by performing the 'wing-out' behavior that allows them to kick at males, suggesting selection to reduce harm from males. However, we do not yet know whether a male-biased OSR influences the effects of male ACPs, which may be an important source of sexual conflict due to their effects on female reproductive response (e.g., Parker 1979, 2006). Here, we ask if a male-biased OSR increases courtship activity of males and if the increased courtship activity decreases female fitness, i.e. males harass females. We also ask if female mate choice mitigates the effects of male courtship activity by comparing the fitness of females that chose their mate and females who were paired with a random male. Additionally, we ask if there are cryptic effects of a male-biased OSR on female reproductive response after controlling for the effects of courtship

activity and female mate choice that are indicative of changes in ejaculatory investment.

## Materials and methods

### Experimental design

We established a base population of houseflies in the laboratory from approximately 100 presumably mated female individuals collected in a single sampling at a local waste transfer station in Pasadena, TX, in August, 2005. This initial population was flushed to normal laboratory size of approximately 10,000 individuals and maintained as stock (methods from Meffert and Bryant 1991). We collected and cultured eggs from the sixth generation of the stock population and separated pre-reproductive adults by sex 24 h after emergence.

We attempted to counter the effects of body size on female preference or reproductive effort by minimizing such variation by standardizing larval density (larval density is strongly correlated to adult body size; Bryant 1969). We cultured eggs in batches of 80 eggs collected from the stock population that we placed in plastic vials containing 18 g of CSMA (Chemical Specialties Manufacturer's Association) medium and covered with paper towels. We incubated the vials at 25°C with a 12:12 h light:dark schedule. Eclosion began 14 days after incubation, at which point we separated emerging flies by sex every 24 h for 3 days using CO<sub>2</sub> anesthesia. We housed adults of the same sex at equal densities in 1.9-l plastic cages with ventilated screens until mating trials were performed. We fed flies daily with a mixture of evaporated milk and tap water.

Virgin flies were separated into the following OSR treatments: 'no competition' (1:1 male to female sex ratio), 'intermediate competition' (2:1 male to female sex ratio), and 'high competition' (5:1 male to female sex ratio). These sex ratios are within the normal range of bias experienced by some populations (Tomita and Wada 1989; Avancini and Silveira 2000; Cakir and Kence 2000; Feldmeyer et al. 2008). Trials began when single females were isolated from stock cages with a glass vial and released into a small, 30-ml mating chamber, which is a density within the range experienced by natural populations (J. Carrillo, personal observation). One, two, or five males were then similarly isolated from stock cages and released into the mating chamber simultaneously.

### Courtship behavior

We videotaped and then analyzed the first 30 min of mating trials for pre-copulatory courtship behavior using

The Observer<sup>®</sup> (Noldus Information Technology) event recording software. We recorded the total and per capita number of male–female interactions, male–male interactions, and a particular female behavior thought to be associated with female rejection of courting males, 'wing-out,' for every trial at each level of male–male competition until copulation commenced or until 30 min elapsed. Males interacted with both females and other males by attempting a repertoire of courtship behaviors including buzzing their wings, lifting the female's front legs, and lunging over the female's body (for detailed description, see Meffert and Hagenbuch 2005). Females perform the behavior 'wing-out' almost exclusively when courted by males by placing their wings perpendicular to their body, and then kicking at the male with her legs. This is thought to signal to the male that she is unwilling or unable to mate or to prevent the male from securing copulatory access (Meffert and Regan 2002). Additionally, we recorded the duration and interval between each of these behaviors, as well as the time until the first courtship occurred. Courtship began when a male attempted to mount a female, and ended when the male was dislodged by the female, by another male, or flew away. We calculated the rate of courtship by dividing the number of courtships by the time until copulation commenced or by 30 min, whichever occurred first.

### Female mate choice

If copulation commenced within 30 min (approximately 50% of females did not mate within the 30-min timeframe, irrespective of treatment), we recorded the time and then isolated and relocated the mating pair into cages conducive to egg-laying (males generally do not dislodge other males after copulation begins and pairs are easy to remove at this time). Pairs remained in copula during this process. We housed the isolated mating pair together in 300-ml ventilated plastic cups inverted onto plastic petri plates with egg-laying medium for 24 h. Females houseflies can lay multiple clutches after a single mating, but we were particularly interested in the number of eggs laid in the first clutch as male ACPs (1) inhibit females from remating, but receptivity can sometimes be regained after oviposition of the first clutch (Riemann et al. 1967; Riemann and Thorson 1969), (2) differences in sizes of the first clutch may represent context dependent responses to cryptic male seminal products, and (3) offspring survivorship of the first clutch is positively correlated with lifetime fitness in laboratory-reared houseflies (Reed and Bryant 2004). Subsequently, we censored egg laying by only culturing the first clutch laid or the minimum density of eggs from multiple clutches necessary for successful cultivation (~40 eggs; J. Carrillo, personal observation). The eggs were counted and cultured in the same manner described previously at the ratio of

0.23 g of CMSA medium per egg. Offspring survivorship was calculated as the number of adults emerged from the total number of eggs laid in the first clutch. Females that did not mate within 30 min were noted and all but one male was removed from the chamber (a random male was chosen for 2:1 and 5:1 male to female treatments). We housed these isolated male/female pairs together for 24 h to be given a subsequent chance at mating and eggs were collected as described above. These mating pairs served as a control where there was courtship activity without mate choice (2:1 and 5:1 trials), either by females or through male competitive interactions. We excluded females that laid eggs with no survivorship because we could not be certain that they had actually completed copulation without dissection.

#### Statistical tests

We first examined variation in courtship traits (number, frequency, and duration of male–female interactions, male–male interactions and female ‘wing-out’ behavior) among OSR treatments and between females that chose a mate or had a random male chosen for them using the general linear model procedure (SAS Institute, 2000) to perform a two-way analysis of variance (ANOVA). For female fitness, we conducted a two-way ANOVA in the number of eggs laid in the first clutch and offspring survivorship among OSR treatments and between females that chose a mate or had a random male chosen for them to determine the effects of OSR, mate choice, and the interaction between them. We also included an ANCOVA with courtship rate to determine if reproductive fitness was affected by sex ratio apart from the effects of courtship activity or mate choice, which would indicate cryptic effects of sex ratio on female reproductive responses. Male behaviors were averaged for all males within an individual trial—we did not track individual males. We square- or cube-root-transformed courtship data for both males and females to correct left skew of the data; duration of courtship attempts and the time until mating began (for females mating within 30 min) were log transformed. After transformations, all data fit assumptions of normality and homogeneity of variances.

We conducted 137 observations total, with the following breakdown for treatments: 45 (1:1 ♂:♀ OSR), 47 (2:1 ♂:♀ OSR), 45 (5:1 ♂:♀ OSR). Thirty-five females died (irrespective of treatment) or escaped before all fitness data were collected. We utilized all available data for each step of the mating process. For this reason, different statistical analyses contain different sample sizes (e.g.,  $n = 137$  for pre-copulatory male/female courtship behavior,  $n = 112$  for fitness variables).

## Results

### OSR and courtship behavior

The OSR affected some aspects of male courtship behavior. At a 2:1 and 5:1 male-biased OSR, males courted females more and at a greater rate than in unbiased OSR trials (Fig. 1a, b). The average duration of male–female courtships was not significantly different among OSR treatments (Fig. 1c). When there was more than one male, males did not interact with other males significantly more at more biased OSRs (Fig. 1d) nor did they interact with other males at a significantly greater rate (mean  $\pm$  SE bouts/min: 2:1 ♂:♀  $0.22 \pm 0.06$ , 5:1 ♂:♀  $0.40 \pm 0.06$ ). When a male courted other males, the duration of male–male interactions was similar between intermediate and high competition OSR trials [mean  $\pm$  SE duration (s): 2:1 ♂:♀  $0.28 \pm 0.06$ , 5:1 ♂:♀  $0.36 \pm 0.06$ ].

Females responded to increases in male courtship from a 1:1 OSR to 2:1 and 5:1 OSR by performing more ‘wing-out’ behavior (number of ‘wing-out’: ANOVA  $F_{2,131} = 6.59$ ,  $P = 0.0019$ , mean  $\pm$  SE: 1:1 ♂:♀  $24.48 \pm 5.20$ , 2:1 ♂:♀  $51.36 \pm 4.99$ , 5:1 ♂:♀  $38.18 \pm 5.10$ ; ‘wing-out’ rate: ANOVA  $F_{2,131} = 4.48$ ,  $P = 0.01$ , mean  $\pm$  SE: 1:1 ♂:♀  $1.36 \pm 0.26$ , 2:1 ♂:♀  $2.23 \pm 0.25$ , 5:1 ♂:♀  $1.85 \pm 0.26$ ). A female’s propensity to mate in 30 min did not depend on the OSR [ $n = 137$ :  $\chi^2$  (2 df) = 1.57,  $P = 0.45$ ], and courtship behaviors did not depend on whether a female mated in 30 min or not (results not presented) so these data were pooled in Table 1.

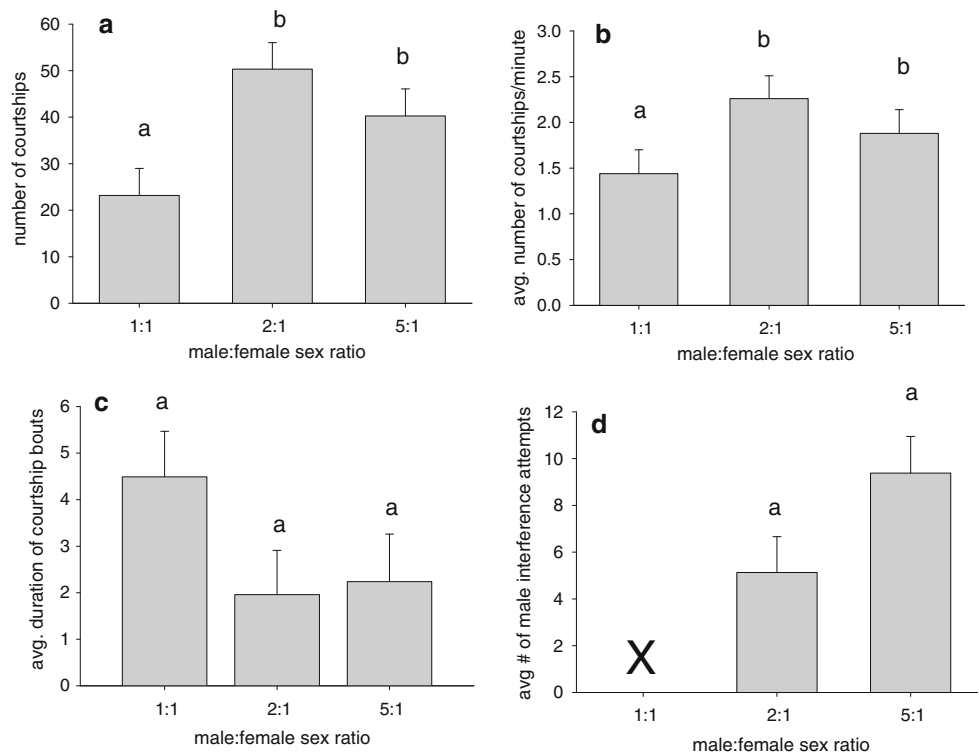
### Sex ratio and female fitness

Females laid fewer eggs in their first clutch after mating in trials with an unbiased OSR trials compared to trials with a 2:1 and 5:1 male to female OSR (Fig. 2a). However, offspring survivorship was greater when females had two versus one potential mate (Fig. 2b). These patterns remained after controlling for the effects of courtship activity and mating choice, i.e. females laid similar numbers of eggs in their first clutches and had similar offspring survivorship whether or not they chose a mate in the 30-min timeframe (Table 2).

## Discussion

The OSR is hypothesized to affect many aspects of mating behavior and subsequent female fitness. As predicted, we found an increase in overall courtship activity with an increasingly male-biased OSR. Despite this increase in activity, we found little evidence that males overtly compete, or that, with greater male–male competition, females

**Fig. 1** Mean  $\pm$  SE shown.  $n = 137$ . Bars with different letters are significantly different (Fishers PLSD). **a** Number of courtships (all males included), **b** courtship rate (all males included), **c** average duration of a single courtship bout in seconds (average of individual males within a trial), **d** number of male/male interference attempts (all males included)



**Table 1** ANOVA of pre-copulatory behavior

Source	df	Number <sup>a</sup>		Rate <sup>b</sup>		Duration <sup>c</sup>	
		F	P	F	P	F	P
<b>♂♀ Courtship</b>							
Sex ratio	2	5.68	<b>0.004</b>	4.24	<b>0.02</b>	0.82	0.44
Error	134						
<b>♂♂ Interaction</b>							
Sex ratio	1	1.58	0.21	3.21	0.08	0.07	0.79
Error	90						

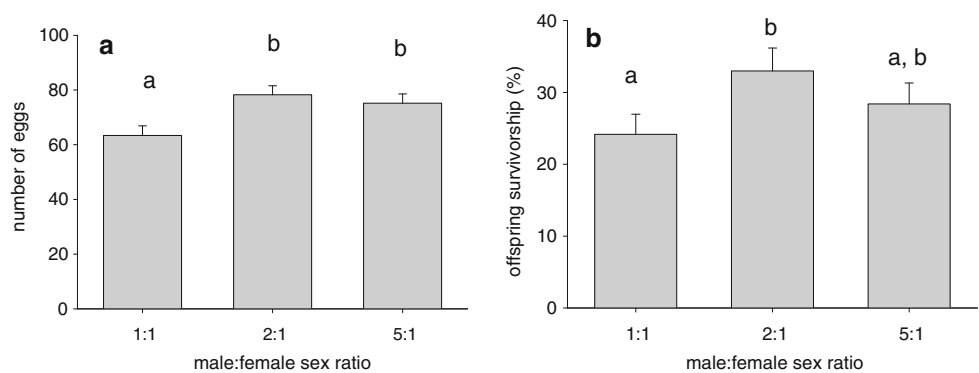
Significant results in bold

<sup>a</sup>  $X = X^{(1/3)}$

<sup>b</sup>  $X = X^{(1/2)}$

<sup>c</sup>  $X = \log(X)$

**Fig. 2** Mean  $\pm$  SE shown.  $n = 112$ . Bars with different letters are significantly different (Fishers PLSD). **a** Number of eggs in the first clutch, **b** offspring survivorship of the first clutch



suffer fitness consequences from male harassment or change their pre-copulatory mate choice behavior. Surprisingly, females did not benefit from choosing their mate, as females in trials where a random male was chosen for them had similar numbers of eggs allocated to the first

clutch and offspring survivorship of the first clutch compared to females that chose their mates. However, we found that females benefited from an increase in male-male competition through male OSR bias in terms of fitness of the first clutch, and that females laid significantly

**Table 2** Female fitness for first clutch

	Source	df	Number of eggs		Offspring survivorship	
			F	P	F	P
ANOVA	Sex ratio	2	5.25	<b>0.01</b>	3.40	<b>0.04</b>
	Mate choice <sup>a</sup>	1	0.14	0.71	0.00	0.95
	Sex ratio × mate choice <sup>a</sup>	2	0.84	0.43	2.05	0.13
	Error	106				
ANCOVA	Sex ratio	2	6.25	<b>0.003</b>	5.11	<b>0.02</b>
	Mate choice <sup>a</sup>	1	0.31	0.58	0.05	0.34
	Sex ratio × mate choice <sup>a</sup>	2	0.58	0.45	0.71	0.66
	Courtship rate <sup>b</sup>	1	1.73	0.18	1.85	0.16
	Error	93				

Significant results in bold

<sup>a</sup> Mated in 30 min or not<sup>b</sup>  $X = X^{(1/2)}$ 

fewer eggs in their first clutch when females mated in unbiased versus at a 2:1 and 5:1 male to female OSR.

Our data most closely fit an environmentally dependent response of oviposition in the first clutch, although the mechanism is unclear. A likely explanation is that male-biased sex ratios trigger an increase in ACP production. Ejaculatory investment has been shown to be dependent on male–male interactions and sperm competition risk across diverse taxa (e.g., yellow dung fly, Simmons 2001; Hosken and Ward 2001; Norway rat, Pound and Gage 2004; cricket, Simmons et al. 2007). A growing body of evidence suggests that accessory proteins in particular are dependent on male–male interactions. For example, males raised at high male density or male exposure during sexual development can determine accessory reproductive gland size (Lemaître et al. 2010) and seminal fluid protein production (Fedorka et al. 2011). In *Drosophila*, exposure to rival males pre-mating increased the transfer of the seminal fluid proteins ovulin and sex peptide to females (Wigby et al. 2009). Our work demonstrates that pre-copulatory social environment at the time immediately preceding mating, independent of the rate of courtship and female mate choice, is an important determinant of a female's first clutch size and survivorship which are likely affected by similar ACPs.

Male-biased sex ratios have been shown to increase copulation duration in the walnut fly (Alonso-Pimentel and Papaj 1996) and male insistence in water striders (Lauer et al. 1996). If similar processes occur in the housefly, an increase in the transfer of semen and ACPs due to such behavioral changes may explain the variation in oviposition rates seen among our OSR treatments. One other explanation is that females that mated after experiencing greater male–male competition allocate more to early egg production than females mated at no competition as an evolutionary response to high levels of male harassment. As male harassment has previously been shown to decrease longevity in houseflies and other insects (e.g., Ragland and

Sohal 1973), allocating more to the first clutch of eggs after experiencing high levels of harassment may be optimal given the likely reduction in female longevity. Heubel et al. (2008) found support for OSR-dependent reproductive decisions in common gobies (*Pomatoschistus microps*) where females increased the size of their first clutch when the sex ratio was female-biased, presumably in response to uncertainty over future matings. Although our result of females reducing the size of their first clutch in response to fewer males appears opposite to these findings, females are known to allocate more to early reproduction when early mortality is likely (see Heubel et al. 2008 for a brief summary, and references therein) or at high harassment levels (Cordoba-Aguilar 2009), which may be likely at male-biased OSRs in systems dominated by sexual conflict.

There are several reasons why the fecundity of the first clutch may be an important reproductive response in houseflies. First, as remating in female house flies before oviposition is infrequent due to the effects of ACPs (2–14%; Leopold 1976), a small first clutch may represent a female strategy for gaining higher reproductive fitness by delaying oviposition of a large clutch until after mating with a more preferred male. This may explain why we observed small first clutches in trials where females never had a choice among mates (1:1 ♂:♀). Secondly, male ACPs have also been shown to increase oviposition rate (Leopold 1976; Riemann and Thorson 1969). We observed that, in mating trials with only one male, females oviposited small first clutches. This indicates that the effects of ACPs are environmentally dependent and that the effects of male-biased OSR on ejaculate content or cryptic female mate choice influence the number of eggs laid in a female's first clutch. Andres and Arnqvist (2001) demonstrated divergence of the seminal signal receptor system in genetically differentiated populations of houseflies, such that females exhibited the weakest response in terms of oviposition rate and refractory period to males from their source population. Our results suggest that either there is variation in the

production of these accessory products or that females can alter their response to these products depending on pre-copulatory competitive environment. However, further research is needed to detect if these effects go beyond fitness of the first egg clutch, and data on how OSR affects subsequent clutches would improve our understanding of female fitness.

In an analysis of fitness traits in singly mated isolated pairs of the housefly, Reed and Bryant (2004) found that offspring survivorship of the first clutch was positively correlated to lifetime offspring survivorship in the housefly and that there was no tradeoff among first and subsequent clutches, although they argue that true fitness can only be measured over the lifespan of the female. When courtship rate was included as a covariate in our analysis, we still observed positive effects of a male-biased OSR on female fitness; however, analysis of lifetime fitness could show differences in reproductive fitness related to the decrease in female lifespan due to mating activity. Female lifespan is reduced at high mating density (Ragland and Sohal 1973), and lifetime fitness should better reflect the effects of high mating density and increased competition on reproductive fitness. However, lifetime fitness in a laboratory setting will undoubtedly be an overestimate of lifetime fitness in a natural setting. As little is known about the ecology of the housefly, such as remating rate, egg production, or mortality between mating/oviposition events, an incorporation of ecologically important parameters with experimental treatments will be especially informative. Although the relative importance of positive and negative direct and indirect effects on fitness and trait selection is still poorly understood (but see Cameron et al. 2003), understanding how environmental parameters alter lifetime fitness functions is necessary to understand the biological relevance of sexual conflict and mate choice. With fluctuations in both density and sex ratio during and across reproductive periods, a context-dependent response to mating conditions could increase lifetime reproductive success. In general, female houseflies were not negatively affected by increased mating activity and appear to benefit (greater fecundity and offspring survivorship) with some male–male competition, though our findings point to complex interactions between environment (competition) and reproductive response.

**Acknowledgments** J.A.C. received support from an Alliance for Graduate Education and the Professoriate Research Fellowship (National Science Foundation Cooperative Agreement HRD-0450363), the Ford Foundation, and a National Science Foundation pre-doctoral fellowship. This work was supported by National Science Foundation DEB-0128855 to L.M. We would like to thank four anonymous reviewers for their insightful comments. All experiments complied with the current laws of the country in which they were performed.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Alonso-Pimentel H, Papaj DR (1996) Operational sex ratio versus gender density as determinants of copulation duration in the walnut fly, *Rhagoletis juglandis* (Diptera: Tephritidae). *Behav Ecol Sociobiol* 39:171–180
- Andres JA, Arnqvist G (2001) Genetic divergence of the seminal signal-receptor system in houseflies: the footprints of sexually antagonistic coevolution? *Proc R Soc Lond B* 268:399–405
- Arnqvist G, Andres JA (2006) The effects of experimentally induced polyandry on female reproduction in a monandrous mating system. *Ethology* 112:748–756
- Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. *Anim Behav* 60:145–164
- Avancini RMP, Silveira GAR (2000) Age structure and abundance in populations of muscoid flies from a poultry facility in Southeast Brazil. *Mem Inst Oswaldo Cruz* 95:259–264
- Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF (2011) Insect seminal fluid proteins: identification and function. *Annu Rev Entomol* 56:21–40
- Berglund A (1994) The operational sex-ratio influences choosiness in a pipefish. *Behav Ecol* 5:254–258
- Bisazza A, Marconato A (1988) Female mate choice, male-male competition and parental care in the river bullhead, *Cottus gobio* L. (Pisces, Cottidae). *Anim Behav* 36:1352–1360
- Bryant EH (1969) Fates of immatures in mixtures of 2 housefly strains. *Ecology* 50:1049–1069
- Bretman A, Fricke C, Chapman T (2009) Plastic responses of male *Drosophila melanogaster* to the level of sperm competition increase male reproductive fitness. *Proc R Soc Lond B* 276:1705–1711
- Cakir S, Kence A (2000) Polymorphism of M factors in populations of the housefly, *Musca domestica* L., in Turkey. *Genet Res* 76:19–25
- Cameron E, Day T, Rowe L (2003) Sexual conflict and indirect benefits. *J Evol Biol* 16:1055–1060
- Cordoba-Aguilar A (2009) A female evolutionary response when survival is at risk: male harassment mediates early reallocation of resources to increase egg number and size. *Behav Ecol Sociobiol* 63:751–763
- Emlen ST, Oring LW (1977) Ecology, sexual selection, and evolution of mating systems. *Science* 197:215–223
- Fedorka KM, Winterhalter WE, Ware B (2011) Perceived sperm competition intensity influences seminal fluid protein production prior to courtship and mating. *Evolution* 65:584–590
- Feldmeyer B, Kozielska M, Kuijper B, Weissing FJ, Beukeboom LW, Pen I (2008) Climatic variation and the geographical distribution of sex-determining mechanisms in the housefly. *Evol Ecol Res* 10:797–809
- Friberg U, Arnqvist G (2003) Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. *J Evol Biol* 16:797–811
- Gavrillets S, Arnqvist G, Friberg U (2001) The evolution of female mate choice by sexual conflict. *Proc R Soc Lond B* 268:531–539
- Grant JWA, Foam PE (2002) Effect of operational sex ratio on female–female versus male–male competitive aggression. *Can J Zool* 80:2242–2246
- Head ML, Brooks R (2006) Sexual coercion and the opportunity for sexual selection in guppies. *Anim Behav* 71:515–522
- Heubel KU, Lindstrom K, Kokko H (2008) Females increase current reproductive effort when future access to males is uncertain. *Biol Lett* 4:224–227
- Hicks SK, Hagenbuch KL, Meffert LM (2004) Variable costs of mating, longevity, and starvation resistance in *Musca domestica* (Diptera: Muscidae). *Environ Entomol* 33:779–786

- Holland B, Rice WR (1999) Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc Natl Acad Sci USA* 96:5083–5088
- Hosken D, Ward P (2001) Experimental evidence for testis size evolution via sperm competition. *Ecol Lett* 4:10–13
- Jirotkul M (1999) Operational sex ratio influences female preference and male-male competition in guppies. *Anim Behav* 58:287–294
- Kvarnemo C, Forsgren E, Magnhagen C (1995) Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. *Anim Behav* 50:1455–1461
- Lauer MJ, Sih A, Krupa JJ (1996) Male density, female density and inter-sexual conflict in a stream-dwelling insect. *Anim Behav* 52:929–939
- Lemaître J-F, Ramm SA, Hurst JL, Stockley P (2010) Social cues of sperm competition influence accessory reproductive gland size in a promiscuous mammal. *Proc R Soc Lond B* 278:1171–1176
- Leopold RA (1976) The role of male accessory glands in insect reproduction. *Annu Rev Entomol* 21:199–221
- Meffert LM, Bryant EH (1991) Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. *Evolution* 45:293–306
- Meffert LM, Hagenbuch KL (2005) The genetic architecture of house fly mating behavior. In: *Current topics in developmental biology*, vol 66. Elsevier, San Diego, pp 189–213
- Meffert LM, Hicks SK, Regan JL (2002) Nonadditive genetic effects in animal behavior. *Am Nat* 160:S198–S213
- Meffert LM, Regan JL (2002) A test of speciation via sexual selection on female preferences. *Anim Behav* 64:955–965
- Meffert LM, Regan JL, Brown BW (1999) Convergent evolution of the mating behaviour of founder-flush populations of the housefly. *J Evol Biol* 12:859–868
- Ojanguren AF, Magurran AE (2007) Male harassment reduces short-term female fitness in guppies. *Behaviour* 144:503–514
- Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45:525–567
- Parker GA (1979) Sexual selection and sexual conflict. In: Blum MS, Blum NA (eds) *Sexual selection and reproductive competition in insects*. Academic, New York, pp 123–166
- Parker GA (2006) Sexual conflict over mating and fertilization: an overview. *Philos Trans R Soc Lond B* 361:235–259
- Prohl H (2002) Population differences in female resource abundance, adult sex ratio, and male mating success in *Dendrobates pumilio*. *Behav Ecol* 13:175–181
- Pound N, Gage MJG (2004) Prudent sperm allocation in Norway rats, *Rattus norvegicus*: a mammalian model of adaptive ejaculate adjustment. *Anim Behav* 68:819–823
- Ragland SS, Sohal RS (1973) Mating behavior, physical activity and aging in housefly, *Musca-domestica*. *Exp Gerontol* 8:135–145
- Reed DH, Bryant EH (2004) Phenotypic correlations among fitness and its components in a population of the housefly. *J Evol Biol* 17:919–923
- Reichard M, Jurajda P, Smith C (2004) Male-male interference competition decreases spawning rate in the European bitterling (*Rhodeus sericeus*). *Behav Ecol Sociobiol* 56:34–41
- Riemann JG, Thorson BJ (1969) Effect of male accessory material on oviposition and mating by female house flies. *Ann Entomol Soc Am* 62:828–834
- Riemann JG, Moen DJ, Thorson BJ (1967) Female monogamy and its control in houseflies. *Insect Physiol* 13:407–418
- Ros AFH, Zeilstra I, Oliveira RF (2003) Mate choice in the Galilee St. Peter's fish, *Sarotherodon galilaeus*. *Behaviour* 140:1173–1188
- Sakurai G, Kasuya E (2008) The costs of harassment in the adzuki bean beetle. *Anim Behav* 75:1367–1373
- Simmons LW (2001) *Sperm competition and its evolutionary consequences in the insects*. Princeton University Press, Princeton
- Simmons LW, Denholm A, Jackson C, Levy E, Madon E (2007) Male crickets adjust ejaculate quality with both risk and intensity of sperm competition. *Biol Lett* 3:520–522
- Stockley P (1997) Sexual conflict resulting from adaptations to sperm competition. *Trends Ecol Evol* 12:154–159
- Tomita T, Wada Y (1989) Multifactorial sex determination in natural-populations of the housefly (*Musca-Domestica*) in Japan. *Jpn J Genet* 64:373–382
- Weir LK, Grant JWA, Hutchings JA (2011) The influence of operational sex ratio on the intensity of competition for mates. *Am Nat* 177:167–176
- Wigby S, Sirot LK, Linklater JR, Buehner N, Calboli FCF, Bretman A, Wolfner MF, Chapman T (2009) Seminal fluid protein allocation and male reproductive success. *Curr Biol* 19:751–757
- Wolfner MF (2002) The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* 88:85–93