

RICE UNIVERSITY

**Does more choice equal a better choice? Courtship behavior, mating propensity and female fitness in relation to the number and density of potential partners**

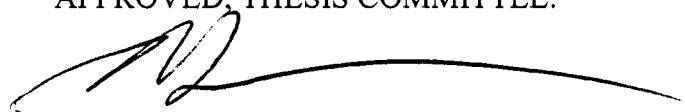
by

**Juli Ann Carrillo**

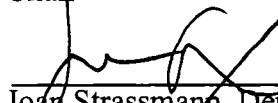
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## ABSTRACT

Does more choice equal a better choice? Courtship behavior, mating propensity and female fitness in relation to the number and density of potential partners

by

Juli Carrillo

The good genes hypothesis predicts that females discriminate among potential mates on the basis of their genetic quality. We measured the indirect benefits received by females with different levels of choice – from no choice to choosing among 5 males – in the housefly, *Musca domestica*, at high and low density. Secondly, we tested how the degree of choice affected the courtship behavior of both sexes and whether this behavior was correlated to female reproductive success. Opportunity for choice did not affect mating propensity or offspring survivorship, but did affect male courtship rate and the number of eggs females laid in their first clutch. Females at low density were more likely to mate, laid more eggs in their first clutches, and had greater egg-to-adult viability than females mated at high density. Overall, the degree of choice affected some aspects of mating behavior and fitness, but the effects were primarily density dependent.

## ACKNOWLEDGEMENTS

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## TABLE OF CONTENTS

I. Introduction.....	1
II. Materials and Methods.....	4
III. Results.....	9
IV. Discussion.....	19
V. List of References.....	23

## INTRODUCTION

Mate choice is the ability of one or both sexes to actively discriminate among potential partners (Andersson 1994, also reviewed in Jennions and Petrie 1997). Mate choice based on a preference for a particular attribute or suite of characteristics can alter the frequency of certain traits in a population through sexual selection. Its study can provide information on the costs, benefits, and mechanisms of partner preferences (Andersson 1994). Understanding the importance of and potential for female mate choice and partner preference to mediate the evolutionary trajectory of a population requires an exploration of female mating behavior and an analysis of the possible benefits obtained through mate choice.

Defined as 'the ratio of sexually receptive males to females,' the operational sex ratio can influence many aspects of mating-systems, such as the direction of sexual selection (e.g. Emlen and Oring 1977, Kokko and Monaghan 2001, Prohl 2002, Head and Brooks 2006) and the intensity of intra- and inter-sexual competition (e.g. Grant and Foam 2002 and Ros *et al.* 2003). Mate choice decisions can shift when males to female ratios change (reviewed in Kvarnemo and Ahnesjö 1996 but see Berglund 1994 & 1995, Souroukis and Kade 1993, Souroukis *et al.* 1995, Jirotkul 1999, Kvarnemo and Simmons 1999 for representative studies), demonstrating potentially strong effects of environment on mate choice. For example, when the operational sex ratio is male biased females have the opportunity to choose among a larger pool of mates that may be more easily or quickly sampled (Kvarnemo and Ahnesjö 1996). Females with the opportunity to choose among more mates should be more likely to choose a better mate than when female mate choice is limited.

Changes in the operational sex ratio may include changes to male and female density that can affect population density overall. That is, increasing the number of one sex in a population to bias the sex ratio will create an equivalent change in population density. Although several studies have examined changes in male courtship and copulatory behavior due to changes in population density and sex ratio (e.g. Warner and Hoffman 1980, Alonso-Pimental and Papaj 1996), few studies have examined the effects that these concurrent changes in sex ratio and density may have on female mating behavior (but see Spence and Smith 2005). No study that we know of has evaluated the separate and interactive effects of density and sex ratio on both mating behavior and the potential benefits obtained through mate choice.

Females can choose mates based on the opportunity for direct and/or indirect benefits. Direct benefits include territory gain, protection, and nuptial gifts while indirect benefits are genetically based, and include increased offspring quality due to choosing males with 'good genes,' that produce 'sexy sons,' that manipulate sensory biases, or that are sexually antagonistic (Andersson 1994, recently reviewed in Kokko *et al.* 2003). To date, most of the work on how sex ratio affects mating behavior and fitness has focused on systems with direct benefits. However, sex ratio may also be important when benefits are less clear and are instead indirect. Unfortunately, despite numerous attempts, few studies have adequately isolated or demonstrated genetic benefits as a consequence of female mate choice (reviewed in Kokko *et al.* 2003, but see Reynolds and Gross 1992 for a representative study). Here, we use the model system of house flies to test how sex ratio affects mate choice when benefits are indirect. In addition, we assess the relative

importance of sex ratio by comparing it to another factor likely to influence mate choice, population density, through simultaneous manipulations of sex ratio and density.

The house fly, *Musca domestica*, is an excellent organism for evaluating the effects that the degree of mate choice may have on a female's receipt of direct and indirect benefits. Female house flies can exhibit mate choice behavior and partner preference (e.g. Meffert and Reagan 2002), and exhibit genetic variation in these traits allowing the opportunity for natural selection to occur. As mate choice behavior is commonly considered costly and non-retainable without compensation in the form of benefits (Andersson 1994), the existence of partner preference in female houseflies suggests they receive some benefit from mate choice. Female houseflies may choose mates on the basis of indirect genetic benefits they expect to receive in the form of increased offspring survivorship. Indirect benefits are likely to be important for two reasons. First, within house fly populations males do not provide the direct benefits of parental care or guard oviposition sites. Second, although recent studies suggest a nutritive effect of accessory seminal substances leading to both increased longevity and fecundity in female house flies (Hicks *et al.* 2004, Arnqvist and Andres 2006), the associated cost of decreased mating frequency due to these seminal products make them an unlikely target of conventional mate choice (Andres and Arnqvist 2001). Therefore, an exploration of possible indirect benefits from female mate choice in house flies may be especially fruitful. Herein, we test a possible genetic benefit of female mate choice in the housefly: increased reproductive output. Specifically, we examined a female's reproductive output (measured by her propensity to mate, latency until copulation, clutch size, and offspring survivorship) under a 1:1, 2:1, and 5:1 male to female sex ratio,



representing three levels of choice. We performed these assays at two densities (high and low) to simulate variation in population density occurring in wild populations, and compare the relative importance (and potential interactions) between sex ratio and density. Secondly, we tested how the degree of choice affected courtship behavior of both males and females and whether this courtship behavior was correlated to female reproductive success. We address the following specific questions. (1) Does sex ratio affect female mating behavior and fitness? (2) Does density affect female mating behavior and fitness? (3) Do interactive effects of sex ratio and density influence female mating behavior and fitness? (4) Do sex ratio and a female's propensity to mate affect the intensity and frequency of male courtship behavior and subsequent female fitness?

## MATERIALS AND METHODS

### *Variation in Female Mate Choice*

Previous house fly studies have highlighted the genetic variation in female preference for male courtship displays (see Meffert and Hagenbuch 2005 for a detailed description of courtship behavior). In controlled pairings of males and females, this variation manifests as time differences in copulation initiation (Meffert and Bryant 1991). Females requiring less elaborate courtship displays mate significantly faster than those females that are more 'choosy' about their mates and require more complex courtships (Meffert and Bryant 1991), with most controlled pairings occurring within 30 minutes and lasting for approximately 60 minutes (Meffert, pers. comm.).

### *Experimental Design*

We established a base population of house flies in the laboratory from

approximately 100 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 derived two experimental groups from this population, CR-A (high-density matings) and CR-B (low-density matings). Eggs from the sixth generation of the stock population were collected, cultured and separated by sex 24 hours after emergence (see below for greater detail) to form the first experimental line (CR-A), which consisted of approximately 1,241 individuals. Likewise, eggs from the seventh generation of the stock population were collected, cultured and 24 hours after emergence were separated by sex to form the second experimental line (CR-B), which consisted of approximately 2,412 individuals. The experimental populations were maintained only for the duration of the experiment.

To counter any possible effects of size on female preference or reproductive effort, we controlled adult body size by standardizing egg collection and larvae rearing across experimental populations. Approximately 80 eggs collected from the stock population were placed in plastic vials containing 18g of CMSA medium and covered with paper towels. The tubes were incubated at 25° C with a 12:12 light:dark schedule. Eclosion began after 14 days of incubation, and we separated emerging flies by sex every 24 hours using light CO<sub>2</sub> anesthesia over a 1-3 day period. Adult flies were housed in same-sex 1.9 liter plastic cages with ventilated screens until mate tests were performed. All flies were fed daily with a mixture of evaporated milk and water.

### *Density*

Staged matings occurred in mating chambers of two sizes: high and low density.

The CR-A population line mated in small, 30 ml mating chambers, leading to higher mating density as compared to the CR-B population line, which was mated in larger, 950 ml, mating chambers (low mating density). Two lines were used because all individuals of the CR-A line (sixth generation of CR stock population) were used for mate-choice trials, leaving none to create subsequent generations. Therefore, a second line was formed from the CR stock population in the seventh generation (CR-B) to continue mate-choice and density trials. Pre- and post-mating housing was the same for both populations.

#### *Sex-ratio*

Virgin flies were mated in three ‘choice’ treatments at each density (Fig. 1). ‘No-choice’ was defined by a 1:1 male to female sex ratio and ‘limited-choice’ by a 2:1 or 5:1 male to female sex ratio. Single females were isolated from stock cages with a glass vial and released into the mating chamber. One, two, or five males were then similarly isolated from stock cages and released into the mating chamber simultaneously. Equal mating trials were staged for each ratio, but approximately twice as many trials were staged at low-density due to the relative ease of releasing flies into a large mating chamber as compared to a small mating chamber. Latency until copulation was recorded for each trial.

Once copulation commenced, the mating pair was isolated and removed from the mating chamber so that eggs could be collected, counted, and cultured. The isolated mating pair was housed together in 300ml ventilated plastic cups inverted onto plastic petri plates until at least 40 eggs were collected from the female or until the

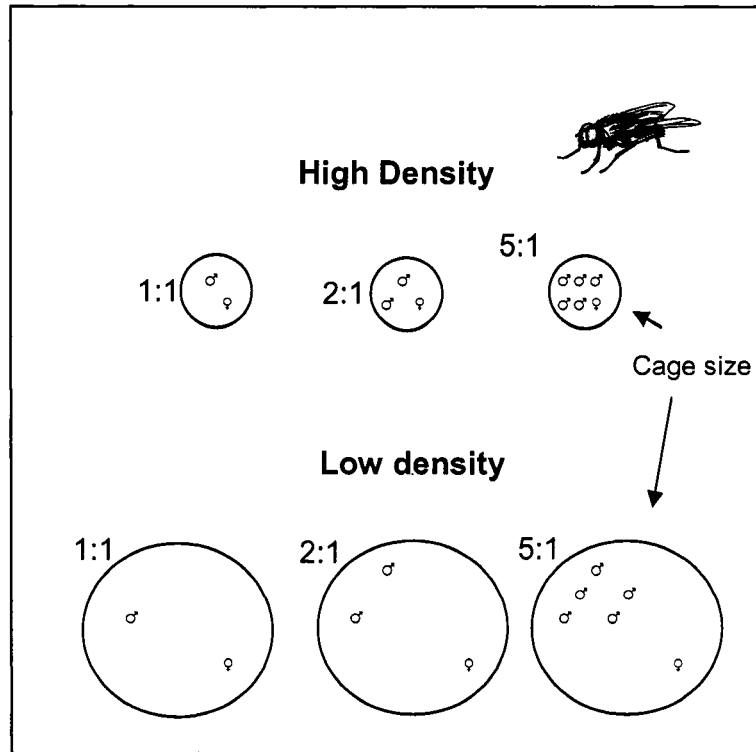


FIGURE 1. Experiment schematic: CR-A mated at high density, CR-B at low density, each under three sex ratios, 1:1, 2:1, and 5:1. Not to scale.

female's death. The eggs were cultured in the same manner described previously at the ratio of 0.23 g of medium/egg. Pairs that took longer than thirty minutes to mate were considered were isolated and removed from the mating chamber along with the male from the staged encounter (a random male was chosen for 2:1 and 5:1 treatments). These male/female pairs were housed together to be given a subsequent chance at mating until at least 40 eggs were collected from the female or until the female's death (approximately 10% of all females mated died before egg collection). Additionally, we recorded the overall number of females taking longer than thirty minutes to mate for each ratio and either density treatment to ascertain a female's propensity to mate under each particular condition. Overall mating propensity was measured by calculating the percentage of

females that mated within thirty minutes for each sex ratio and between density treatments.

### *Fitness tests*

To measure offspring survivorship, we calculated egg to adult viability for each female. To do this, we counted the total number of emerged adults produced by each female and divided this number by the total number of eggs cultured for that female. This gave the percentage of eggs that hatched and survived to adulthood, or the overall survivorship of offspring.

### *Courtship Behavior*

Mating trials at high density were videotaped and analyzed using *The Observer* event recording software for pre-copulatory courtship behavior. 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' were recorded for every trial at each sex ratio. Additionally, the intervals between and duration of each of these behaviors were recorded as well as the time until the first courtship occurred. Rate of courtship was calculated by dividing the number of courtships by the time until copulation commenced. These behaviors were then compared across sex ratios and between trials where females either did or did not mate within thirty minutes to ascertain if propensity to mate and/or female reproductive fitness are linked with the number, rate, or duration of courtship behavior.

### *Statistical tests*

Variation in four response variables – mating propensity, latency until copulation, number of eggs laid in the first clutch, and egg to adult viability – were examined among sex ratio treatments and between densities using the general linear model procedure (SAS Institute 2000) to perform an analysis of variance (ANOVA) and to examine interactive effects. Likewise, analysis of variance was conducted among sex ratios and between propensities for the number, frequency and duration of male-female interactions, female-female interactions, female ‘wing-out’ behavior and time until first courtship. These data were rank transformed (see Conover and Iman 1981). After transformations, all data fit assumptions of normality and homogeneity of variances. Spearman rank correlation analyses were conducted on latency until copulation data and two variables: first clutch size and egg-to-adult viability for and across both densities. Spearman rank correlation analyses were also conducted on the number, rate, and duration of courtship behaviors and female fitness for mating trials conducted at high density. Variance in mating propensity among ratios and between densities was calculated using heterogenic chi-square tests.

## RESULTS

We determined a female’s reproductive fitness using four measures: Propensity to mate within thirty minutes, latency until copulation, the number of eggs laid in a female’s first clutch, and egg-to-adult viability. However, creating a composite measure of fitness incorporating these variables proved difficult, as the relative importance of each measure in determining a female’s overall reproductive fitness is unknown. For this reason, we analyzed the effects that sex ratio, density, and the interactive effects of sex ratio and density, had on each fitness measure separately. Likewise, we examined changes in the

number, frequency and duration of courtship behaviors by looking at the effects of sex ratio, a female's propensity to mate, and the combined effects of sex ratio and female propensity (see Tables 1,2).

*Does sex ratio affect courtship behavior and female reproductive fitness?*

The number and rate of male courtship attempts towards females varied among sex ratios, with significantly fewer courtships occurring less often at the 'no-choice' 1:1 sex ratio (Figs. 2a,b, ANOVA  $F_{2, 151} = 8.61$ ;  $P = 0.0003$ ,  $F_{2, 151} = 3.94$ ,  $P = 0.022$ ). The per capita number of courtship attempts was highest at the 'limited-choice' 2:1 sex ratio (Fig. 3a, ANOVA  $F_{2, 151} = 13.06$ ,  $P < 0.0001$ ), while the per capita number of male-male interactions and the rate of male-male interactions was highest at the 5:1 sex ratio (Fig. 3b, ANOVA  $F_{1, 100} = 4.85$ ,  $P = 0.029$ ;  $F_{1, 100} = 4.11$ ,  $P = 0.045$ ). There were no significant differences among sex ratios in the total number of male-male interactions (ANOVA  $F_{1, 100} = 0.32$ ,  $P = 0.573$ ), the duration of courtship attempts and male-male interactions (ANOVA  $F_{2, 151} = 0.79$ ,  $P = 0.045$ ;  $F_{1, 100} = 0.22$ ,  $P = 0.64$ ), or the time until the first courtship occurred (ANOVA  $F_{2, 151} = 0.79$ ,  $P = 0.276$ ).

The female courtship behavior 'wing-out,' a potential indicator to males of a female's willingness or unwillingness to mate, varied among sex ratios as well. Wing-out occurred fewer times and less often at the unbiased 'no-choice' sex ratio of 1:1 (ANOVA  $F_{2, 151} = 8.75$ ,  $P = 0.0003$ ;  $F_{2, 151} = 3.72$ ,  $P = 0.027$ ), varying almost in lock-step with the total number and rate of male courtship attempts towards females (Spearman rank correlation:  $r_s = 0.997$ ,  $P < 0.0001$ ,  $n = 152$ ;  $r = 0.996$ ,  $P < 0.0001$ ,  $n = 152$ ).

**Table 1.** Analysis of variance of the effect of operational sex ratio and density on female fitness

	Among-ratio contrasts			Between-density contrasts			Interactions (OSR*density)		
	df	F	P	df	F	P	df	F	P
Latency	2, 221	1.24	0.291	1, 221	0.30	0.582	2, 221	2.80	0.063
Egg number in first clutch	2, 352	1.28	0.279	1, 352	2.67	0.103	2, 352	3.14	<b>0.045</b>
Egg-to-adult viability	2, 352	1.13	0.326	1, 352	28.89	<b>&lt;.0001</b>	2, 352	0.06	0.941

**Table 2.** Analysis of variance of the effect of operational sex ratio and female mating propensity on courtship (CS) behavior

	Among-ratio contrasts			Between-propensity contrasts			Interactions (OSR*propensity)		
	df	F	P	df	F	P	df	F	P
Time until 1 <sup>st</sup> CS*	2, 151	1.30	0.276	1, 151	1.51	0.221	2, 151	1.11	0.332
♂/♀ CS (total #)*	2, 151	8.61	<b>0.0003</b>	1, 151	52.56	<b>&lt;.0001</b>	2, 151	2.71	<b>0.070</b>
♂/♀ CS/# ♂s*	2, 151	13.06	<b>&lt;.0001</b>	1, 151	4.94	<b>0.027</b>	2, 151	1.39	0.260
Rate of ♂/♀ CS	2, 151	3.94	<b>0.022</b>	1, 151	0.3	0.587	2, 151	0.68	0.506
Avg. duration of ♂/♀ CS*	2, 151	0.79	0.455	1, 151	2.67	0.105	2, 151	0.65	0.522
♂/♂ CS (total #)*	1, 100	0.32	0.573	1, 100	34.1	<b>0.0001</b>	1, 100	0.13	0.722
♂/♂ CS/# ♂s*	1, 100	4.85	<b>0.029</b>	1, 100	0.04	0.834	1, 100	6.66	<b>0.011</b>
Rate of ♂/♂ CS	1, 100	4.11	<b>0.045</b>	1, 100	1.07	0.303	1, 100	0.00	0.975
Avg. duration of ♂/♂ CS*	1, 100	0.22	0.64	1, 100	0.87	0.35	1, 100	0.79	0.37
Wingout*	2, 151	8.75	<b>0.0003</b>	1, 151	51.1	<b>&lt;.0001</b>	2, 151	2.64	<b>0.075</b>
Rate of wingout	2, 151	3.72	<b>0.027</b>	1, 151	0.30	0.585	2, 151	2.12	0.124
Avg. duration of wingout*	2, 151	0.51	0.603	1, 151	0.10	0.757	2, 151	0.53	0.590

\*data were rank transformed



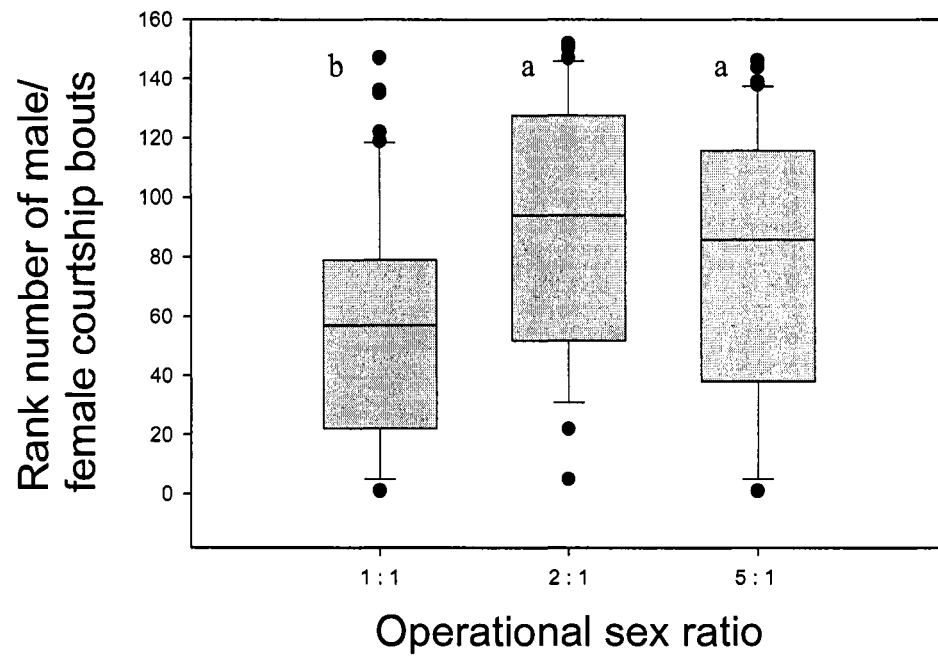


FIGURE 2.a. Rank average of male/female courtship attempts at high density. Among sex ratios, the number of courtships is significantly low at the 'no-choice' 1:1 sex ratio (ANOVA  $F_{2, 151} = 8.61$ ,  $P = 0.0003$ ). Box plot with quartiles shown. Means with different letters are significantly different.

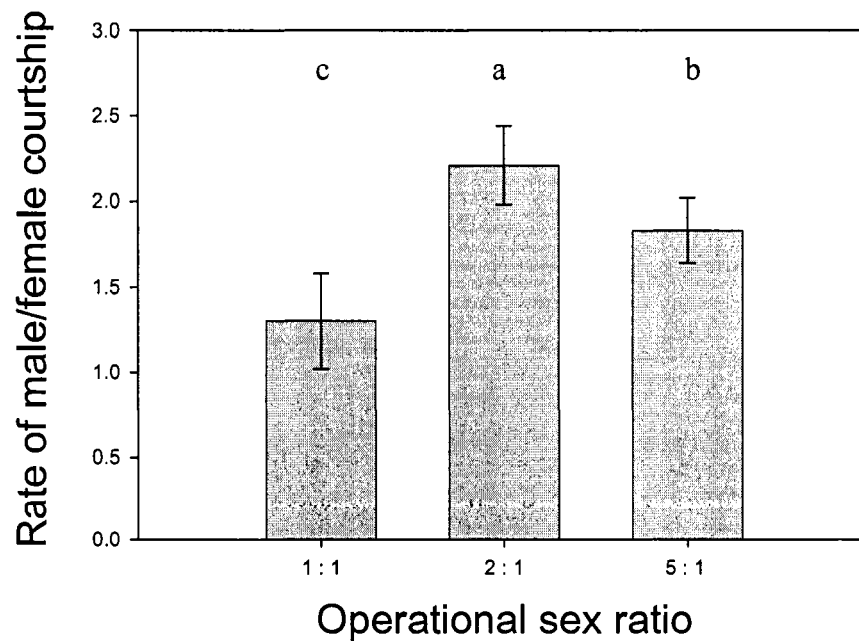


FIGURE 2.b. Rate of male/female courtship attempts (number of courtships/minute) at high density. Among sex ratios, the rate of male female courtships is significantly low at

the 'no-choice' 1:1 sex ratio (ANOVA  $F_{2, 151} = 3.94$ ,  $P = 0.022$ ). Means with different letters are significantly different. Standard error bars shown.

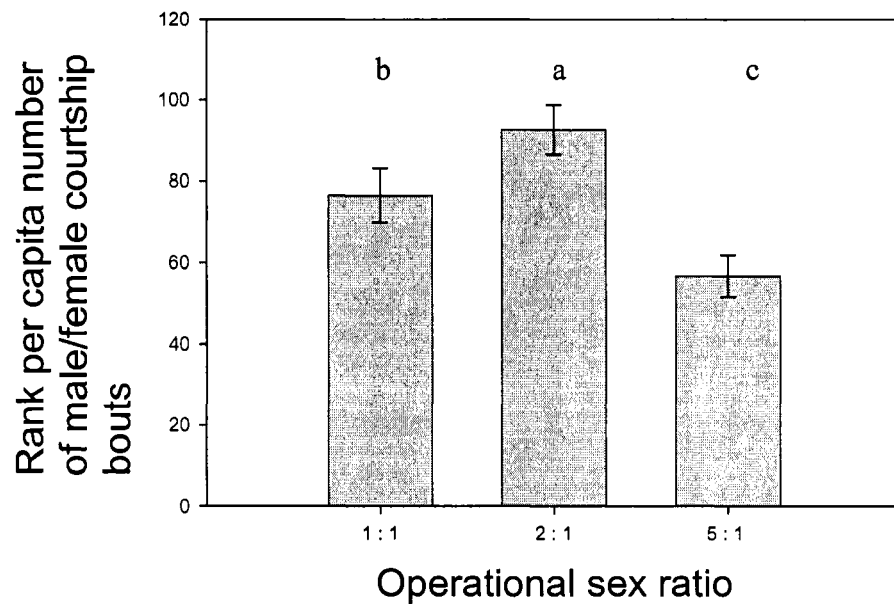


FIGURE 3a. Rank per capita number of male courtship attempts towards females at high density. The per capita number of courtship attempts was highest at a 'limited-choice' 2:1 sex ratio (ANOVA  $F_{2, 151} = 13.06$ ,  $P < 0.0001$ ). Means with different letters are significantly different. Standard error bars shown.

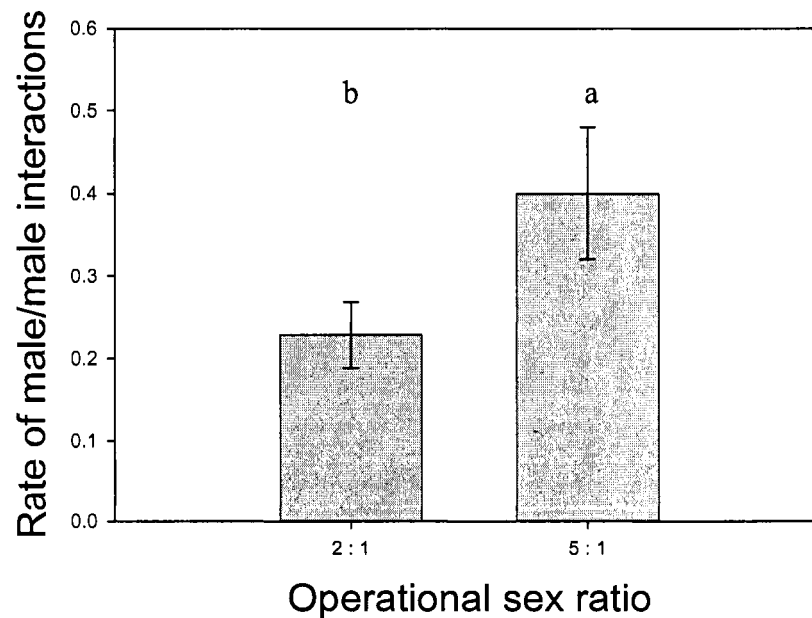


FIGURE 3b. Rate of male/male interactions at high density. The rate of interactions was highest at a 5:1 sex ratio ( $F_{1, 100} = 4.11$ ,  $P = 0.045$ ). Means with different letters are significantly different. Standard error bars shown.

Sex ratio did not affect a female's propensity to mate within thirty minutes (Table 3,  $\chi^2_{2,353} = 0.05$ ,  $P = 0.973$ ) or, for females mating within thirty minutes, latency until copulation (ANOVA  $F_{2,221} = 1.24$ ,  $P = 0.291$ ). Similarly, sex ratio alone did not affect the number of eggs a female laid in her first clutch (ANOVA  $F_{2,352} = 1.13$ ,  $P = 0.326$ ), or the percentage of eggs laid surviving to adulthood for each female (Fig. 4, ANOVA  $F_{2,352} = 1.28$ ,  $P = 0.279$ ).

TABLE 3. Comparison of mating propensity among sex ratios, across densities for individual ratios, and between densities for all ratios. No difference in mating density was found among sex ratios. Across densities, the 5:1 treatment showed significant variance in mating propensity. Between densities (H=high, L=low), females had a higher propensity to mate at low mating density. OSR = Operational sex ratio, df = degrees of freedom, N = total number of mating pairs, Fo = number of matings taking longer than thirty minutes. 1 = from first ratio reported, 2 = from second ratio reported. Bolded entries indicate  $p < 0.05$ .

Density	OSR	df	N <sub>1</sub>	N <sub>2</sub>	Fo <sub>1</sub>	Fo <sub>2</sub>	$\chi^2$	P
High	1:1/2:1	2	37	44	14	21	0.24	0.621
High	2:1/5:1	2	44	42	21	22	1.32	0.250
High	1:1/5:1	2	37	42	14	22	0.45	0.503
Low	1:1/2:1	2	125	116	44	39	0.17	0.683
Low	2:1/5:1	2	116	122	39	31	2.70	0.101
Low	1:1/5:1	2	125	122	44	31	1.47	0.225
H vs L	1:1/1:1	1	37	125	14	44	0.03	0.861
H vs L	2:1/2:1	1	44	116	21	39	1.17	0.279
H vs L	5:1/5:1	1	42	122	22	31	8.10	<b>0.004</b>
H vs L	ALL/ALL	1	123	363	57	114	5.62	<b>0.018</b>

*Is a female's propensity to mate related to courtship activity?*

Courtship activity of both males and females was similar between trials where females did and did not mate within thirty minutes. Although the total number of male courtship attempts towards females, female wing-out, and male-male interactions were

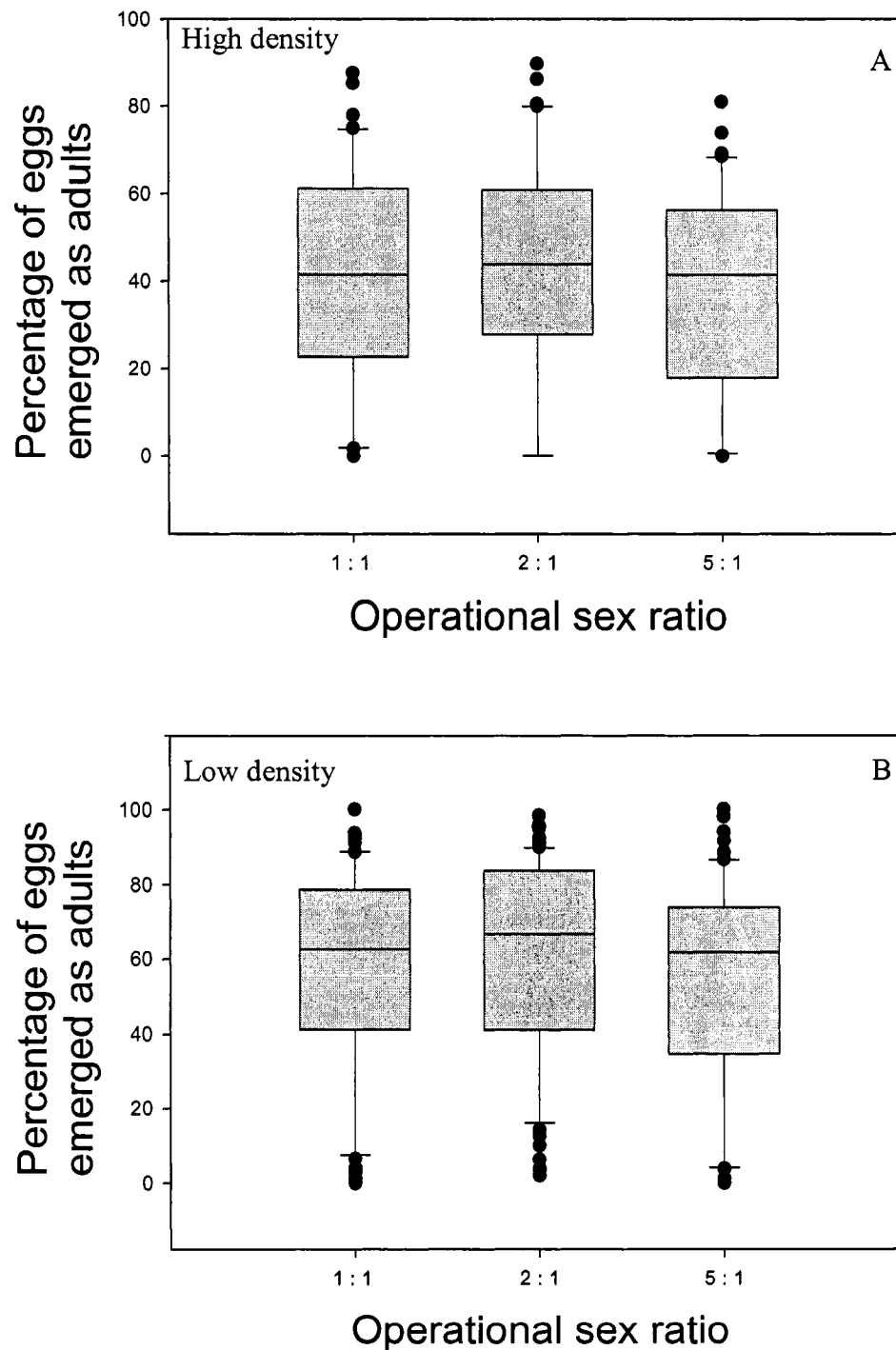


FIGURE 4. Egg-to-adult viability among sex ratios at high density (A) and low density (B). The percentage of eggs emerged as adults did not differ significantly among sex ratios (ANOVA  $F_{2,352} = 1.28$ ,  $P = 0.279$ ). Box plot with quartiles shown.

significantly higher in trials where females did not mate in thirty minutes (ANOVA  $F_{1,151} = 52.56$ ,  $P < 0.0001$ ;  $F_{1,151} = 51.11$ ,  $P < 0.0001$ ;  $F_{1,100} = 34.06$ ,  $P = 0.0001$ ), the rate and duration of these three activities were similar between these trials (ANOVA  $F_{1,151} = 0.30$ ,  $P = 0.587$ ;  $F_{1,151} = 0.10$ ,  $P = 0.757$ ;  $F_{1,100} = 1.07$ ,  $P = 0.303$ ). Likewise, the time until the first courtship occurred did not significantly differ between trials where the females mated within thirty minutes and those that took longer to mate (ANOVA  $F_{1,151} = 1.51$ ,  $P = 0.221$ ).

*Does density affect female mating behavior and reproductive fitness?*

Density had strong effects on some aspects of female mating behavior and fitness. Females were much more likely to mate within thirty minutes at low density (Table 3,  $\chi^2_{1,353} = 5.67$ ,  $P = 0.017$ ). Egg-to-adult viability was also higher for females that mated at lower density, at a 16% increase in the number of eggs emerging as adults (Fig 5, ANOVA  $F_{1,352} = 28.89$ ,  $P < 0.0001$ ). The number of eggs a female laid in her first clutch was larger from females mated at lower density, although the magnitude of difference was not large (ANOVA  $F_{1,352} = 3.13$ ,  $P = 0.078$ ). Latency until copulation did not vary significantly between densities (ANOVA  $F_{1,221} = 0.30$ ,  $P = 0.582$ ).

*Do interactive effects of sex ratio and density influence female mating behavior and fitness?*

Some aspects of female mating behavior and reproductive fitness were affected by the combined effects of sex ratio and density. Females were most likely to mate at a 5:1

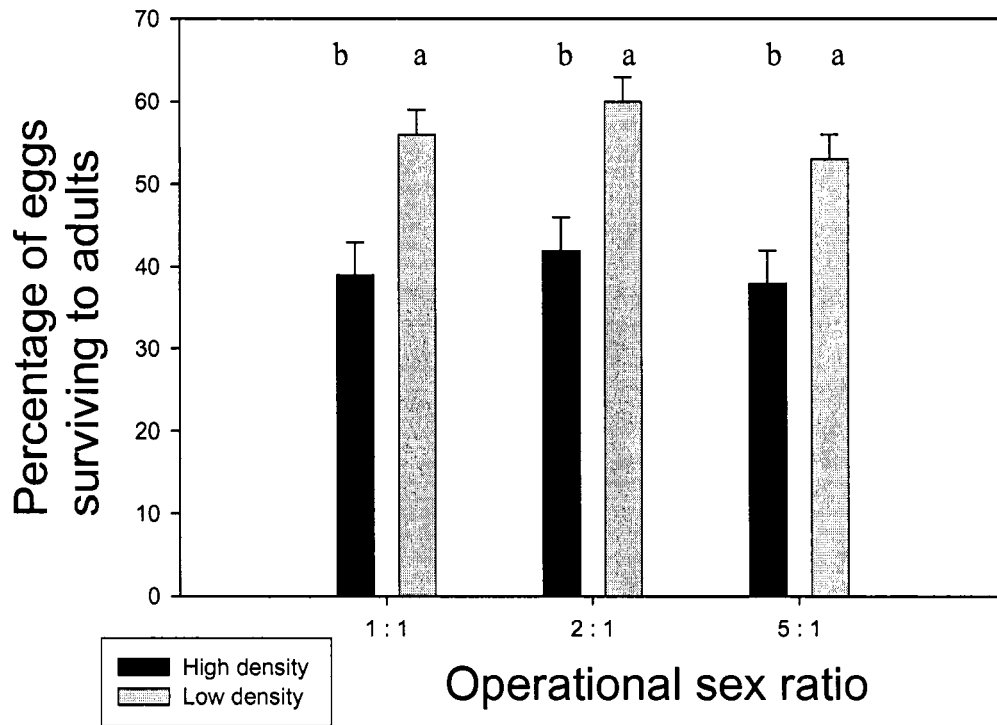


FIGURE 5. Egg-to-adult viability is higher for females mated at lower density (ANOVA  $F_{1, 352} = 28.89$ ,  $P < 0.0001$ ). Means with different letters are significantly different. Standard error bars shown.

male-biased sex ratio at low mating density and least likely to mate at a 5:1 male-biased sex ratio at high mating density (Table 3,  $\chi^2_{1, 353} = 8.10$ ,  $P = 0.0004$ ). Females at high mating density laid fewer eggs in their first clutch at the ‘no-choice’ 1:1 sex ratio compared to females with more choice at high density, while females at low mating density laid fewer eggs in the somewhat ‘limited-choice’ 2:1 sex ratio than other females mated at low density (Fig. 6, ANOVA  $F_{2, 53} = 3.83$ ,  $P = 0.028$ ;  $F_{2, 76} = 5.62$ ,  $P = 0.005$ ). Latency until copulation and egg to adult viability were not significantly affected (ANOVA  $F_{2, 221} = 2.80$ ,  $P < 0.0001$ ;  $F_{2, 352} = 0.06$ ,  $P = 0.941$ ), although for females mating at low density at a 1:1 sex ratio, latency and the number of eggs laid in the first

clutch were negatively correlated (Fig. 7, Spearman rank correlation:  $r_s = -0.416$ ,  $P = 0.0024$ ,  $n = 51$ ).

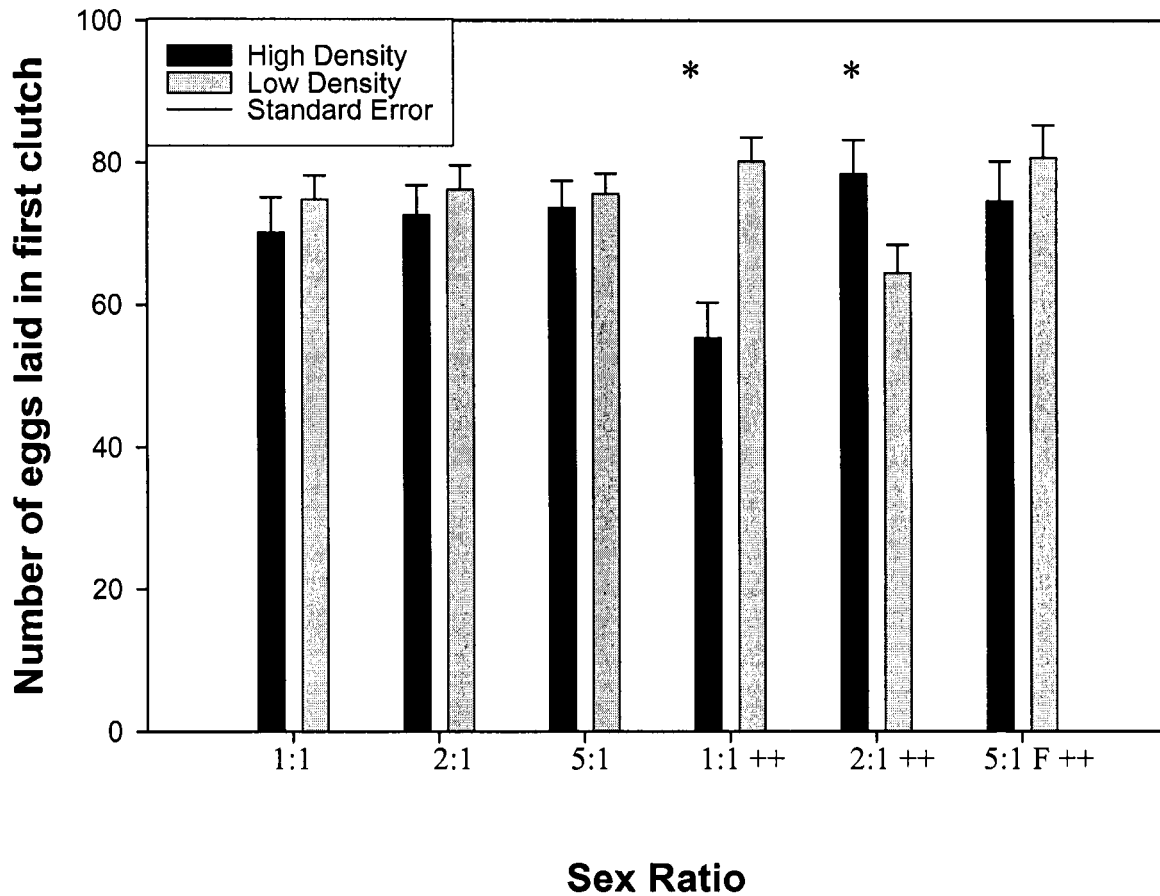


FIGURE 6. Variation among sex ratios across densities. First clutch size was significantly lower for females with no choice taking longer than thirty minutes to mate (1:1 ++) than for other ratios mated at high density and those females taking less than thirty minutes to mate, while first clutch size was significantly lower for females with limited choice taking longer than thirty minutes to mate (2:1 ++) than for other ratios at low density and those females taking less than thirty minutes to mate (ANOVA  $F_{2,53} = 3.83$ ,  $P = 0.028$ ;  $F_{2,76} = 5.62$ ,  $P = 0.005$ ). Standard error bars shown. \* indicates significant difference between densities.

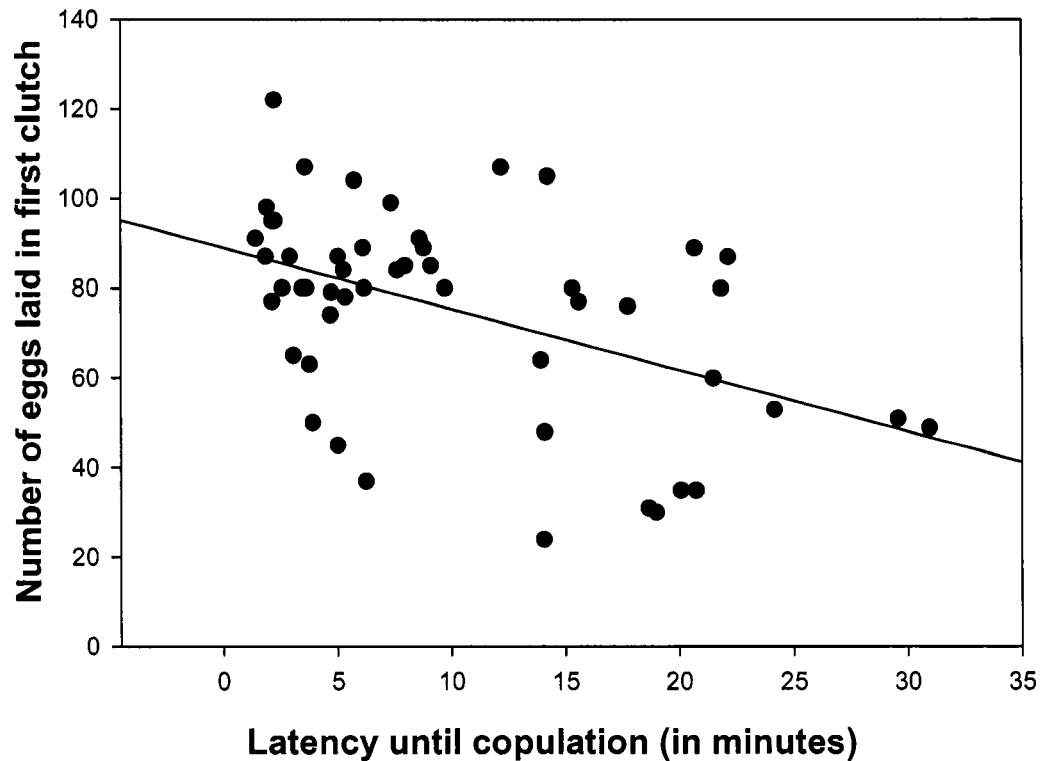


FIGURE 7. Negative correlation between latency until copulation and number of eggs laid in the female's first clutch under no choice treatment (1:1) at low mating density (Spearman correlation coefficient:  $r_s = -0.4159$ ,  $P = .0024$ ).

## DISCUSSION

Sex ratio affects some aspects of female mating behavior and fitness, but these effects were density dependent. In the high density population, females that mated at 1:1 sex ratios (no choice) and that took longer than thirty minutes to mate laid significantly fewer eggs in their first clutch than other females mating at high density. At low density, females facing 2:1 sex ratios (limited choice) and that did not mate within thirty minutes laid fewer eggs in their first clutches than females mating at 1:1 and 5:1 sex ratios. This



trend for 'choosier' females (those that take longer to mate or with a low mating propensity) to lay fewer eggs in their first clutch suggests a possible strategy for a female to gain higher reproductive fitness. That is, female house flies may regulate the number of eggs they oviposit in their first clutch, and try to remate with a different, better quality, male. As remating in female house flies before oviposition is infrequent (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.

Density affected female mating propensity and several fitness measures, no matter the sex ratio. Females were much more likely to mate within thirty minutes when courted at low mating density. This was possibly reflected in two fitness measures: egg-to-adult viability and the number of eggs laid in the first clutch, which were both significantly higher for females mating at low density. Other studies of mating density indicate that high mating density has the potential to drastically reduce female lifespan and offspring survivorship (e.g. Ragland and Sohal 1973, White and Bell 1993, Sehgal and Toor 1995, Friberg and Arnqvist 2003), usually due to decreased female fitness when exposed to the greater, and potentially more stressful, mating activity. At higher mating densities males have a great opportunity to harass and may injure females to try to attain matings, and females can exert considerable time and energy resisting or coping with male mating efforts (recently reviewed by Arnqvist and Rowe 2005).

Among sex ratios, no differences in mating propensity were found, suggesting that females are equally apt to mate under a 1:1, 2:1, or 5:1 sex ratio. On the other hand, a female's mating propensity was influenced by the interactive effects of the density and

sex ratio at which she was courted. Comparing individual sex ratios across densities revealed that females were most likely to mate when presented with many partners (five males) at low density, and least likely to mate when presented with many partners (five males) at high density. One explanation for the decrease in a female's probability of mating observed at higher mating densities may be that flies experience greater stress when population density is high, and may be unwilling or unlikely to mate. Video analysis of male harassment and courtship behavior at *both* high and low mating densities should illuminate whether males, females, or both reduce courtship activity at higher densities and aid in determining density effects on a female's propensity to mate.

Surprisingly, egg-to-adult-viability was not significantly different for females among sex ratios; consequently, we found no support for the idea that a greater degree of mate-choice leads to increased offspring survivorship or that females are choosing among mates based on egg-to-adult-viability. However, females may benefit indirectly through mate-choice with an increase in reproductive fitness aside from egg-to-adult-viability. For example, the offspring of females with a greater degree of mate-choice may have greater longevity, or be more attractive to females and thus mate more frequently (Fisher 1930). Or, if costs of mate choice increase with increasing population density, benefits of mate choice at greater male densities may be matched with concomitant costs, masking or blurring the line between mate choice and sexual conflict.

It is important to note that the rate and number of courtships experienced by females was lowest within the 1:1 sex ratio at high density, the same ratio at which first clutch size was significantly small. So, even though females may encounter significantly more harassment at higher mating densities it may be necessary for females to participate

in a certain level of courtship behavior before a mate choice decision can be made. That is, mate choice behavior and subsequent fitness may be a balance between having enough courtship activity and potential partners to make an accurate assessment of partner quality and the potential fitness costs of harassment at higher partner densities.

This study revealed several interesting areas for future research: To better understand potential benefits of reducing the size of the first clutch, remating, and ovipositing more eggs, fitness should be measured for females with the opportunity to remate. Also, measuring both female longevity and variance in first clutch size in a more natural setting may be useful in determining the appropriateness of this female mating strategy. Specifically, comparing first clutch size in environments with high predation risk, where females may not live long enough to oviposit a second clutch, to environments with less dangerous conditions may be especially fruitful in evaluating the importance of such behavior. Likewise, additional mating behaviors, such as courtship activity and male harassment, should be analyzed at both high and low density to see if there is a reduction in mating behavior at higher densities that would possibly account for the lower propensity to mate observed in this study. Finally, a more thorough analysis of the effects stress and male harassment has on mate choice is needed, especially due to changes in population and mating density. Stressful or suboptimal mating circumstances may significantly affect female mate choice decisions and the overall fitness of the female and her offspring, so simultaneous manipulation experiments of density and sex ratio should be the norm when testing the effects of sex ratio on mating behavior and fitness.

Although this preliminary study did not find evidence of indirect benefits to female mate choice in the housefly, it does suggest that females may act strategically in their post-copulatory egg-laying behavior. By laying small first clutches, females may have the opportunity to have the rest of their eggs fertilized by a more preferred or higher quality male. However, determining quality in the male housefly has proven difficult as, thus far, no benefits (direct or indirect) of mate choice have been demonstrated within this system even though costly mate-choice behavior is retained. This leads us to contend that further analysis of the costs and benefits of female mate choice for the housefly is warranted.

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