ORIGINAL ARTICLE

OPERATIONAL SEX RATIO AND DENSITY DO NOT AFFECT DIRECTIONAL SELECTION ON MALE SEXUAL ORNAMENTS AND BEHAVIOR

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Demographic parameters including operational sex ratio (OSR) and population density may influence the opportunity for, and strength of sexual selection. Traditionally, male-biased OSRs and high population densities have been thought to increase the opportunity for sexual selection on male sexual traits due to increased male competition for mates. Recent experimental evidence, however, suggests that male-biased OSRs might reduce the opportunity for sexual selection due to increased sexual coercion experienced by females. How OSR, density, and any resultant changes in the opportunity for sexual selection actually affect selection on male sexual traits is unclear. In this study, we independently manipulated OSR and density in the guppy (*Poecilia reticulata*) without altering the number of males present. We recorded male and female behavior and used DNA microsatellite data to assign paternity to offspring and estimate male reproductive success. We then used linear selection analyses to examine the effects of OSR and density on directional sexual selection on male behavioral and morphological traits. We found that females were pursued more by males in male-biased treatments, despite no change in individual male behavior. There were no differences in sexual behavior experienced by females or performed by males in relation to density. Neither OSR nor density significantly altered the opportunity for sexual selection. Also, Although there was significant multivariate linear selection operating on males, neither OSR nor density altered the pattern of sexual selection on male traits. Our results suggest that differences in either OSR or density (independent of the number of males present) are unlikely to alter directional evolutionary change in male sexual traits.

KEY WORDS: Density, guppies, microsatellite paternity analysis, operational sex ratio, opportunity for sexual selection, selection analysis, sexual coercion.

Operational sex ratio (OSR: the ratio of sexually receptive males to sexually receptive females, Kvarnemo and Ahnesjö 1996) and population density have often been proposed as important factors in determining the opportunity for, and strength of, sexual selec-

tion (OSR, Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996: density, Eshel 1979; Hubbell and Johnson 1987; Crowley et al. 1991). However, the effects that OSR and density have on the opportunity for, and strength of, sexual selection are varied and are likely to depend on aspects of the mating system in question (Shuster and Wade 2003, Kokko and Rankin 2006). Also, the effects of OSR and density are likely to be closely intertwined, because differences in OSR by their very nature depend on differences in the relative densities of the sexes. In this study we independently

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manipulate OSR and density in an attempt to resolve the effects of these parameters on sexual selection.

The effects of OSR and density on competition for mates appear to be fairly straightforward; most studies have shown that mate competition increases with a bias in OSR toward the competing sex (e.g., pipefish, *Sygnathus typhle*, Vincent et al. 1994; Japanese medaka, *Oryzias latipes*, Grant et al. 1995; Kvarnemo et al. 1995; lobsters *Homerus gammarus*, Debuse et al. 1999) and density (e.g., soldier beetles *Chauliognathus pensylvanicus*, McLain 1982; crickets *Gryllus integer*, Cade and Cade 1992; guppies, *Poecilia reticulata*, Jirotkul 1999a). The effect of these demographic parameters on mate choice, however, is less clear.

Both OSR and density may influence the degree of choosiness expressed by individuals. For mating systems in which females tend to be choosy and males compete for mating opportunities, male-biased OSR and high density may increase choosiness of females (OSR, Lawrence 1986; Gwynne and Simmons 1990; Berglund 1994; Souroukis and Murray 1995; Jirotkul 1999b; density, Gwynne 1984; Palokangas et al. 1992; Shelly and Bailey 1992). This is because under male-biased OSR and high-density conditions the variance in male quality may be greater, offering females increased benefits of choice (Owens and Thompson 1994). Also, increased intersexual encounter rates mean that females may be better able to assess male quality and decrease the costs of mate searching (Crowley et al. 1991). On the other hand, some studies have shown that females become less choosy with increased OSR bias and density (OSR, Lauer et al. 1996; density, Arnqvist 1992; Rowe 1992). This scenario seems likely when females suffer from increased harassment and thus increased costs of being choosy under these conditions (Thornhill and Alcock 1983). Also, males may alter their mating tactics in ways that undermine female mate choice (Crespi 1988; Shine et al. 2003; Byrne and Roberts 2004), perhaps counteracting sexual selection that occurs as a result of conventional mating tactics. For a more in-depth discussion of the effects of population density on mate choice see Kokko and Rankin (2006).

Most studies that have examined the effects of OSR and density on sexual selection have measured the opportunity for sexual selection (e.g., Jones et al. 2004). The opportunity for sexual selection is estimated as:

$$I = \frac{V_W}{W^2}$$

where V_W is the variance in relative fitness of males and W is the mean fitness of males (Wade 1979; Wade and Arnold 1980).

Although variation in fitness is essential for selection to occur, it does not provide evidence that selection is occurring. This is because a large proportion of fitness variation may be random with respect to phenotype (Sutherland 1985). For selection to occur, mating or reproductive success must covary with phenotypic differences among individuals (Fincke 1986).Therefore, it is necessary to consider the strength of selection acting on the traits of interest when examining differences in selection.

In this study we conduct a manipulative experiment to disentangle the effects of OSR and density on sexual selection in guppies. We use microsatellite data to assign paternity to offspring, and then quantify the opportunity for sexual selection within each of our experimental treatments. Last, we employ linear selection analyses (Lande and Arnold 1983) and new approaches for statistically comparing fitness surfaces (Bowerman and O'Connell 1990, Chenoweth and Blows 2005) to test whether OSR or density alter the strength or direction of directional selection operating on sexual traits in male guppies.

Guppies offer an ideal species for experimentally testing the effects of OSR and density on mating behavior and sexual selection. Male guppies may employ one of two tactics to obtain matings (Houde 1997). First, a male may perform a sigmoid courtship display in which he shows off his colored flanks to a female, and she exercises choice by either mating with him or not. Alternatively, a male may "sneak" copulate, by approaching a female from behind and inserting his gonopodium into her gonopore. Females often flee from sneak-mating attempts suggesting that this mating tactic may be costly to females. Sneak copulation in guppies is facultative and the rate at which sneak copulations are performed varies depending on ecological and demographic parameters (e.g., predation risk, Godin 1995; OSR, Jirotkul 1999b). The selection that sneak copulations impose on male phenotype has been suggested to be different from that imposed by female choice (Becher and Magurran 2004). Hence, both solicited and sneak copulations are likely to contribute to male reproductive success and their relative contributions may differ depending on OSR and male density leading to differing selection on male sexual traits associated with these demographic parameters.

Methods
STUDY SYSTEM

The guppies used in this experiment were collected from second-generation laboratory stock, which descended from 500 wild-caught feral guppies from Alligator creek, near Townsville, Australia.

To ensure that females for this experiment were virgins they were collected from tanks containing only virgin females. Females in these tanks had been sexed under a microscope between 30 and 40 days of age, based on the presence of female egg spots (Houde 1997). The use of virgins in our experiment ensured that offspring were sired by males within our treatments, as female guppies are able to store sperm over several reproductive cycles (Houde 1997). Theory predicts that virgins should seldom be choosy (Kokko and Mappes 2005), and observations on virgin guppies in the first

hours after the introduction to males confirm this (Houde 1997). However, this initial lack of choosiness is unlikely to have affected either our behavioral data or our genetic data because females were allowed time to mate and overcome the short time of virginal nonchoosiness prior to behavioral data being collected (see below), and females mate multiply, exerting postcopulatory choice for preferred males within a reproductive cycle (Pilastro et al. 2004), so any indiscriminate virgin mating is likely to be negated.

Males were collected from mixed sex stock tanks with approximately equal sex ratios (1:1) and were distributed randomly with respect to treatment. This controlled for any possible differences in male-mating behavior that were due to the social environment in which males were reared (Evans and Magurran 1999).

EXPERIMENTAL DESIGN

We independently manipulated OSR and density as follows. All of our treatments had the same number of males, but differed in the number of females and size of the tank. By keeping the number of males the same in each treatment we avoided confounding effects of male number on our estimates of the opportunity for sexual selection, as well as avoiding confounding effects of male pattern rarity (e.g., Hughes et al. 1999) and familiarity (e.g., Zajitschek et al. 2006). Our low-density treatment contained six males and four females in a 135-L tank (75 (length) × 45 (width) × 40 (height) cm), giving a density of 0.07 fish/L, whereas our female-biased treatment contained six males and eight females in a 135-L tank, giving a density of 0.11 fish/L. To test the effects of OSR and density each of these were compared to our control treatment that was male-biased and high density (0.11 fish/L), that is, six males and four females in a 94-L tank (52.5 (length) × 45 (width) × 45 (height) cm). These differences in OSR and density are similar to those that are experienced by wild guppies from this population (Head 2005) and Trinidadian populations (Reznick and Endler 1982; Rodd and Reznick 1997; Reznick et al. 2001; Pettersson et al. 2004). We used six males in each treatment because this number provided females with enough males to choose between, while still allowing us to economically assign paternity. The tanks were lined with a gravel substrate but otherwise contained no furnishings. This habitat is similar to the habitat in Alligator creek.

The number of replicates differs between our behavioral data and genetic data, as not all males survived until the end of the experiment and thus paternity analyses could not be performed for these replicates. For our selection analyses only males with measures for all traits were included. For behavioral analyses we had 11 replicates of the low-density treatment, 11 replicates of the female-biased treatment, and 14 replicates of the control. Although for our selection analyses we had nine replicates of the low-density treatment, 10 replicates of the female-biased treatment, and eight replicates of the control treatment.

Nineteen days prior to treatment, females were marked using fluorescent red elastomer (Northwest Marine Technology Inc., Olympia, WA), so that we could identify individual females during behavioral observations. The elastomer was injected into the tail muscle using a syringe. We injected the elastomer into one or two different positions (out of six possible positions), so that each female in a tank had a unique marking. Pilot studies in our laboratory have shown that females recover full swimming ability within a week of being injected with elastomer and that elastomer does not affect female mate choice decisions (M. L. Head, unpubl. data).

The day prior to being placed in treatment, males and females were photographed so that we could identify individuals during behavioral trials and later record male color patterns. Each fish was anaesthetized by being dipped in an ice slurry for a few seconds, and then laid flat on white waterproof paper on its right side. We then photographed its left side, and used a ruler for calibration, using a digital camera. We used Measuremaster 3.44 (Leading Edge Pty Ltd, Adelaide, Australia) to trace and calculate male body area and each color spot area from the photograph. For males we then calculated the proportion of body area covered by black, orange, and iridescent spots.

BEHAVIOR

Fish were placed in their respective treatment tank the day before behavioral observations began, to allow them to acclimate. Behavioral observations were conducted on the two mornings after being placed in the tank, because this is when females are fertile and responsive to males (Houde 1997; Head and Brooks 2006). On each day focal observations involved watching each male and female (in random order) for 5 min. After all fish had been observed this was repeated in a different random order, so that after two-day observations we had observed each individual fish for 20 min. During behavioral trials light was provided using three reading lamps, one placed 1.5 m above each of the tanks (60W daylight incandescent bulbs). The mean ± SD total irradiance (400–700nm, Skye PAR meter) over the three tanks was 1.93 ± 0.15 μmol m^{-2} s^{-1}. Fish were kept in treatment for 14 days.

During focal observations we recorded both male and female sexual behaviors. These behaviors included the number of male harassment behaviors such as following (i.e., when a male orients toward and remains close to a female without disrupting the females current activity), chasing (i.e., when a male pursues a female and the female tries to move away from the male), jockeying (i.e., when two or more males pursue a female each trying to be closer to the female than the other males, while the female tries to get away), (Magurran and Seghers 1994; Houde 1997), as well as mating behaviors such as sneak copulations (i.e., when the male approaches the female from behind and attempts to insert his gonopodium in her gonopore with no prior courtship) and

sigmoid displays (i.e., when the male performs an "S" shaped display in front of the female, showing off his brightly colored flanks). We also recorded whether a female showed a positive response to a display (i.e., whether the female ceased her current activity and glided toward the male or not), or whether the display was interrupted by another male (Houde 1987, 1997).

After treatment we stripped males of sperm, following the methods of Matthews et al. (1997), 24 h after being removed from treatment. Male guppy bodies were then kept in ethanol for later genetic analysis. Females were kept in individual 5-L tanks until they gave birth or for 90 days if they did not give birth. After giving birth both females and their offspring were euthanized in an ice slurry and their bodies were kept in ethanol for genetic analysis.

BEHAVIORAL DATA ANALYSIS

Male behaviors directed toward females, including pursuit of females (following, chasing, and jockeying), sneak copulations, sigmoid displays as well as the proportion of these displays that were successful or interrupted were analyzed. These behaviors were analyzed separately for males and females. This is because, with varying sex ratio and density, the number of behaviors a female experiences will not necessarily equal the number of behaviors a male performs. These two aspects of behavior vary in their importance for males and females. To avoid pseudoreplication of measures within tanks we pooled data for individual males and females to create tank means and analyzed these using multivariate analysis of variance (MANOVA) (SPSS ver. 12.0). Because we were interested only in differences depending on density or OSR we conducted separate analyses testing for these differences (i.e., low density versus control and female-biased versus control), rather than conduct post hoc analyses to see which treatments differed.

DNA MICROSATELLITE PATERNITY ANALYSIS

DNA was isolated from the tail muscle for adults and the whole body for offspring, by salt precipitation, using Puregene tissue kit (Gentra, Minneapolis, MN), according to the manufacturer's instructions. Four mothers from each replicate, all possible fathers, and three offspring (unless a female had fewer than three offspring, then all offspring were genotyped) from each brood were genotyped using seven polymorphic microsatellite loci. Microsatellite loci analyzed, the number of alleles, and their observed and expected heterozygosities are given in Table 1. Primers were labeled with fluorescent markers and PCR products were run on an ABI 3730 DNA Analyzer. Fragment lengths were determined using Genemapper software. There was a mean of 5.9 alleles per loci. For the D21 loci there was one null allele, so all homozygotes were treated as if they possessed the null allele. This is a conservative approach to dealing with null alleles. Expected heterozygosity for all loci combined was 0.542 and the exclusionary power was 0.922.

Table 1. The seven DNA microsatellite loci used and their properties including observed (H_o) and expected (H_e) heterozygosities.

Locus	H_o	H_e	Alleles	n	Reference
D15	0.490	0.609	5	157	Seckinger et al. 2002
D21	0.204	0.621	4	255	Seckinger et al. 2002
D6	0.264	0.259	2	235	Seckinger et al. 2002
Pr39	0.743	0.601	3	171	Becher et al. 2002
Pr67	0.634	0.575	7	235	Becher and Magurran 2004
Pr80	0.345	0.411	3	252	Becher et al. 2002
TTA	0.721	0.716	14	258	Taylor 1999

Paternity was assigned using CERVUS software (Marshall et al. 1998). Paternity was assigned with 95% confidence in 205 of 295 genotyped offspring (69%) and with a relaxed 80% confidence in an additional 28 cases (10%). LOD scores (log-likelihood that putative parent is a true parent relative to other candidates) of the second most probable father was negative in 10 of these cases, discounting their assignment as sires (Marshall et al. 1998). For all other offspring paternity was not assigned (62 cases or 21%).

ESTIMATING THE OPPORTUNITY FOR SEXUAL SELECTION (I)

We used analysis of variance (ANOVA) (SPSS ver. 12.0) to compare the opportunity for sexual selection between treatments. The opportunity for sexual selection was estimated as the variance in male paternity for each tank over the mean paternity squared for each tank. Paternity analysis for each treatment was conducted on the offspring from four broods in each replicate to avoid our estimates of the opportunity for sexual selection being biased by the number of females sampled. This allows us to estimate differences in *I* that are due to variation in mating behavior and not simply the number of females present.

LINEAR SELECTION ANALYSIS

To determine directional selection acting on male sexual traits we estimated linear selection gradients using the multiple regression approach proposed by Lande and Arnold (1983). We used the proportion of all offspring sampled from a tank, sired by a given male as our measure of male fitness. Our fitness measure was standardized within treatments so that differences in paternity between treatments would not interfere with our estimates of selection on traits. Whether fitness was standardized within or across treatments did not affect the outcome of either our estimates of selection gradients or our comparison of selection between the treatments.

In total we had seven predictor variables consisting of male sexual traits including, color traits, sperm number, body size, as well as male sexual behaviors. We standardized all predictor variables to units of standard deviation from the mean phenotype

within treatments, because the means of three male traits (sperm number, proportion of black coloration, and display rate) significantly differed between the treatments. We used SPSS 14.0 (SPSS Inc., Chicago, IL) to fit multiple regression models to estimate linear selection gradients (β) of the seven predictor variables (Lande and Arnold 1983).

COMPARING SELECTION BETWEEN TREATMENTS

To test for significant overall patterns of selection and to compare the strength of selection acting on male sexual traits between the three treatments we used partial F-tests (Bowerman and O'Connell 1990; Chenoweth and Blows 2005). First we fit a model containing only treatment as a fixed effect (model 1), we then added all linear terms as covariates (model 2) and then included all linear covariate by treatment interactions (model 3). To determine whether the addition of the linear covariates significantly improved the fit of our model we estimated partial F for the comparison of model 2 against model 1. To test for overall treatment by linear interactions we estimated partial F for model 3 versus model 2.

Although the number of males in each replicate is small (six), the number of replicates of each treatment (low density = 9, female biased = 10, control = 8) provides increased power to detect treatment differences. The overall number of individuals on which our selection analysis is based (152) is comparable to several published studies of linear multivariate selection (Janzen 1993; Dudley 1996; LeBas et al. 2004; Calsbeek and Sinervo 2007).

Results
BEHAVIOR

The frequency with which males followed, chased, and jockeyed females were all highly correlated and are likely to be tightly linked both in male motivation to perform these behaviors and in their affects on females experiencing these behaviors. For these reasons we combined these behaviors into a single measure, which we call "pursuit," using separate principal components analyses for the data as experienced by females and as performed by males. All measured components of pursuit experienced by females loaded strongly and positively on the first principal component (follow = 0.875, chase = 0.906, jockey = 0.832), which accounted for 75.9% of the variation. All components of pursuit performed by males loaded strongly and positively on PC1 (follow = 0.745, chase = 0.836, jockey = 0.697), accounting for 57.9% of the variation.

Multivariate analyses of variance showed that there was a significant difference in the behaviors experienced by females with respect to OSR, but not density (Table 2). The frequency of male pursuit experienced by females was less in the female-biased treatment than in the control. There was also a lower proportion of displays interrupted in the female-biased treatment (Table 2). There was no multivariate difference in male behavior with respect to OSR or density (Table 3).

THE OPPORTUNITY FOR SEXUAL SELECTION (I)

There was no difference in the opportunity for sexual selection, I, with regard to OSR or density (mean $\pm$ SE: low density = 1.800 $\pm$ 0.575, female-biased = 1.368 $\pm$ 0.530, control = 2.074 $\pm$ 0.826: $F_{2,22} = 0.838$, $P = 0.446$). Treatment explains less than 3% of the variation in opportunity for sexual selection, $\hat{\eta}^2 = 0.028$).

SELECTION ANALYSIS

There was significant linear selection on male traits ($F_{7,142} = 2.48$, $P = 0.019$). Standardized linear selection gradients and the associated univariate significance tests are presented in Table 4. Significant directional selection for decreased sperm number was detected with marginal evidence that selection favored males performing fewer sneak copulations and larger areas of iridescent coloration. There were no significant differences in multivariate linear selection on male sexual traits with regard to OSR or male density ($F_{14,128} = 1.472$, $P = 0.131$).

Table 2. Multivariate analyses of variance on behaviors experienced by females.

	Control (mean±SE)	Female biased (mean±SE)	OSR effects				Low density (mean±SE)	Density effects			
			Wilks' λ	df	F	P		Wilks' λ	df	F	P
Multivariate			0.494	5,18		0.018		0.947	5,20		0.948
Pursuit	0.297±0.271	−0.664±0.198		1,24	7.711	0.011	0.264±0.293		1,24	0.007	0.935
Sneak	0.721±0.179	0.335±0.066		1,24	3.560	0.072	0.582±0.121		1,24	0.416	0.525
Display	2.317±0.474	1.696±0.226		1,24	1.243	0.277	1.865±0.248		1,24	0.713	0.407
Proportion of interrupted displays	0.198±0.031	0.071±0.020		1,24	11.215	0.003	0.206±0.062		1,24	0.015	0.904
Proportion of successful displays	0.322±0.073	0.389±0.053		1,24	0.504	0.485	0.317±0.049		1,24	0.003	0.959

Table 3. Multivariate analyses of variance on behaviors performed by males.

	Control (mean±SE)	Female biased (mean±SE)	OSR effects Wilks' λ	df	F	P	Low density (mean±SE)	Density effects Wilks' λ	df	F	P
Multivariate			0.702	5,18		0.231		0.912	5,20		0.852
Pursuit	0.064±0.196	0.306±0.195		1,24	0.014	0.907	−0.089±0.137		1,22	0.354	0.557
Sneak	0.625±0.079	0.587±0.101		1,24	0.089	0.768	0.519±0.067		1,22	1.041	0.318
Display	1.651±0.342	2.405±0.408		1,24	2.041	0.167	1.500±0.271		1,22	0.119	0.733
Proportion of interrupted displays	0.264±0.070	0.084±0.024		1,24	5.126	0.034	0.188±0.041		1,22	0.870	0.360
Proportion of successful displays	0.321±0.056	0.414±0.049		1,24	1.521	0.231	0.304±0.044		1,22	0.056	0.814

Discussion

OSR and density have often been shown to influence male and female-mating behavior. For this reason it is commonly predicted that one or both of these demographic parameters will influence the opportunity for and strength of selection on male traits (Emlen and Oring 1977; Eshel 1979; Hubbell and Johnson 1987; Crowley et al. 1991; Kvarnemo and Ahnesjö 1996). Our results show that although females in male-biased OSRs are pursued more by males and male displays are interrupted more often, these changes in the behaviors experienced by females do not alter directional selection on male sexual traits. We also found no behavioral or selection differences with respect to density.

THE EFFECTS OF OSR AND DENSITY ON MATING BEHAVIOR

Increased population density and male-biased OSRs are expected to increase encounter rates with competitors (Kokko and Rankin 2006). Accordingly the degree to which males employ different mating strategies, such as "sneak" mating, forced copulations, and mate guarding can differ in relation to these demographic parameters. This has been found in previous studies investigating the effects of population density and OSR on male-mating behavior (e.g., Jirotkul 1999a; Mills and Reynolds 2003). Here we show, however, that individual male-mating behavior is not affected by

Table 4. Overall linear selection gradients (β) for male sexual traits based on 152 individuals.

	β (SE)	P
Sperm	−0.194 (0.082)	0.019
Body area (mm²)	0.005 (0.082)	0.949
Black (proportion of body area)	0.001 (0.082)	0.988
Orange (proportion of body area	0.047 (0.084)	0.576
Iridescent (proportion of body area)	0.150 (0.084)	0.077
Sneak copulations (#/5min)	−0.162 (0.083)	0.052
Courtship displays (#/5min)	0.042 (0.084)	0.618

either OSR or density. One reason for the difference between our study and previous studies of this kind, could be due to the range of densities and OSRs used. We were careful to keep these demographic parameters within the range that guppies experience in the wild, whereas other studies have often used much stronger contrasts. Although these studies provide valuable information on whether such relationships are possible, they provide little information on how important these relationships are in shaping sexual traits in natural populations.

Despite a lack of difference in individual male-mating behavior with respect to OSR and density, we did see an increase in the number of pursuit behaviors experienced by females in the male-biased OSR. Increased harassment of female poeciliids in male-biased environments has often been proposed to be costly to females (e.g., Magurran and Seghers 1994; Brewster and Houde 2003; but see Head and Brooks 2006; Smith and Sargent 2006). Increased sexual harassment in the form of pursuit and sneak matings has been shown to decrease female foraging rates and has been suggested to circumvent female mate choice (Magurran and Seghers 1994). Whether increased harassment actually poses fitness costs for females, however, has not been demonstrated. In a previous study on this Alligator creek population, we found no evidence of any direct fitness costs due to increased harassment (Head and Brooks 2006). This raised the question of whether female guppies sacrifice mate choice to avoid the direct costs associated with increased harassment. When the costs of rejecting males outweigh the benefits gained by being choosy, females may mate indiscriminately to avoid harassment (Thornhill and Alcock 1983; Lee and Hayes 2004). If this were the case we would predict an increase in female mating rate and a decrease in the opportunity for sexual selection and strength of sexual selection. A lack of difference in these parameters between our treatments indicates that female guppies do not mate indiscriminately to avoid harassment, suggesting that the costs of harassment for females may be negligible compared with the benefits of being choosy and that increased harassment may not pose fitness costs for females.

A number of theoretical studies suggest that if mate choice is to evolve via indirect benefits then the direct costs that females suffer as a consequence of being choosy must be negligible (e.g., Kirkpatrick and Barton 1997). In addition, Kokko et al. (2002) model sexual selection via indirect benefits and show that the costs of mate choice play a primary role in determining the relationship between male quality, attractiveness and other fitness components. At one end of their "sexual selection continuum" cheap choice favors the evolution of strong mate choice such that high-quality males advertise so strongly that they experience higher mortality than low-quality males. The principal benefit of mate choice under this scenario is the elevated attractiveness of sons alone. Kokko et al. (2002) suggest the reason that few empirical studies have presented results consistent with this end of the continuum is that choice may rarely be cheap. One of the few studies to have shown a negative relationship between attractiveness, and longevity was conducted on this population of guppies (Brooks 2000). If harassment costs associated with mate sampling are small, as suggested by the current study and Head and Brooks (2006), it would provide circumstantial evidence in favor of the predictions made by Kokko et al. (2002).

THE EFFECTS OF OSR AND DENSITY ON SEXUAL SELECTION

We found no effect of either OSR or density on the opportunity for sexual selection or on the strength or direction of linear selection on male morphological or behavioral traits. The scale of our study did not permit a large number of replicate tanks per treatment, and future studies with more replication at this level may find significant treatment effects where ours did not. Nonetheless, our results suggest that if OSR and density affect the strength and direction of sexual selection, then this affect is small.

Previous studies looking at the effects of OSR on the opportunity for sexual selection, have had varied results. Studies using behavioral observations of mating success to estimate the opportunity of sexual selection have found that the opportunity for sexual selection may increase (e.g., guppies, *P. reticulata*, Jirotkul 2000; frogs, *Dendrobates pumilio*, Prohl 2002) or decrease (e.g., bitterlings, *Rhodeus sericeus*, Mills and Reynolds 2003) under more male-biased OSRs. Relatively few studies have used genetic estimates of the opportunity of sexual selection, even though these are likely to give more accurate estimates because postcopulatory processes which may be influenced by OSR also contribute to reproductive success. One such study conducted on newts (Jones et al. 2004) found that in male-biased OSRs the opportunity for sexual selection increased. One difference between our study and that of Jones et al. (2004) is how females were sampled for paternity analysis. Although we conducted paternity analysis on the same number of females from the male-biased and female-biased treatments to investigate how differences in mating behavior affect sexual selection, Jones et. al. (2004) sampled all females from both treatments, so that their estimate of the opportunity for sexual selection may be due either to differences in mating behavior or the number of females in the population. For this reason, our experiment does not imply that OSR does not affect sexual selection in this species, but that it does not do so through differences in individual female-mating behavior.

Although determining the opportunity for sexual selection provides us with estimates of the maximum amount of possible sexual selection, it does not tell us which traits are being selected or the mode of selection on these traits. We found no differences in linear selection on male morphological, or behavioral traits, with respect to OSR or density. These results suggest that there would be no directional change in male sexual traits in populations that differ in OSR or density. Similarly, LeBas et al. (2004) found no differences in multivariate selection when they compared their data on male dancefly mating success collected over the whole breeding season to that collected only during times when the population was male biased. Although their analysis did not formally test for differences due to OSR their results imply that OSR did not affect sexual selection on male traits.

Due to the diverse and often inconsistent effects of OSR and density on sexual selection, alternative population measures that are better at predicting sexual selection have been sought. It has been suggested that there are a number of parameters (mate encounter rates, costs of breeding, costs of searching, and variability in mate quality) that are important in determining choosiness (Kokko and Monaghan 2001; Kokko and Johnstone 2002). Although all of these parameters are influenced by OSR and density, Kokko and Monaghan (2001) note that these parameters may affect sexual selection in a way that is not reducible to either of these demographic parameters. This may provide clues as to why neither OSR nor density affected sexual selection in our experiment. Although our treatments are likely to have altered mate encounter rates and costs of searching, they are unlikely to have influenced the costs of breeding, which have been suggested to be particularly important in determining choosiness (Kokko and Monaghan 2001; Kokko and Johnstone 2002; but see, Simmons and Kvarnemo 2006). Also, because we altered the number of females to create different OSRs, and the tank size to create different densities, while keeping the number of males same in each treatment we did not alter variability in mate quality. This factor may be important in producing differences in behavior and selection in differing social environments (Owens and Thompson 1994).

SEXUAL SELECTION ON MALE SEXUAL TRAITS

Our overall selection analysis showed negative selection for sperm number. Although negative selection on sperm number may at first seem paradoxical, because sperm number may be expected to be positively related to fertilization success of a male, this result

could arise in a number of ways. First, male sperm number is not necessarily a good predictor of fertilization success (Evans and Magurran 2001), because males may deliver varying amounts of their available sperm to females when mating (Pilastro and Bisazza 1999). Second, sperm number may trade-off with sperm quality. Multiple regression techniques of selection analysis are useful because they control for correlations between traits that are included in the model (Lande and Arnold 1983), however, it is possible that traits implicated in selection are correlated with traits not included in the analysis and are only indirectly selected and may not be important in determining fitness. For instance, it is possible that negative selection on sperm number is actually caused by positive selection on sperm quality, a trait not included in this analysis that may have also influenced reproductive success. Third, in many studies negative selection on sperm number may be the result of a trade-off between gamete production and mating rate. For example in bluehead wrasse, *Thalassoma bifasciatum*, Warner et al. (1995) found that males with the highest daily mating success produce the fewest sperm.

We also found marginally significant effects of the frequency of sneak copulation attempts and the area of iridescent coloration on paternity. The negative association ($P = 0.052$) between sneak frequency and paternity may indicate that sneaking is a strategy employed by unattractive or otherwise unsuccessful males. Whether this is due to an underlying issue of phenotypic or genetic quality or a facultative response by males to a short-term lack of success in obtaining matings, we cannot tell from our data. The marginally greater paternity ($P = 0.077$) of males with larger areas of iridescence is consistent with previous findings that iridescence is among the most important determinants of attractiveness and mating success in Alligator creek guppies (Brooks and Endler 2001; Blows et al. 2003).

Conclusions

In this study we corroborate previous findings that females receive more male attention at male-biased sex ratios. A lack of similar effects due to density suggests that this OSR effect is due to the relative number of males and females and not mate encounter rate. Despite differences in the mating behaviors experienced by females we found no differences in the opportunity for sexual selection or the strength or sign of directional selection acting on male sexual traits.

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