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The effects of social experience with varying male availability on female mate preferences in a wolf spider

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Abstract While female preferences may vary depending on population-level differences in density or sex ratio, factors affecting mate choice may act at the individual level, i.e., females may encounter males with varying frequency or encounter multiple males simultaneously. The "socially cued anticipatory plasticity" hypothesis suggests that females may bias mate preferences based on prior experience. In the wolf spider, *Schizocosa ocreata*, males typically mature before females, allowing females to experience male courtship before maturation. Using video playback, we simulated differences in the encounter rate and the number of males simultaneously encountered to examine effects on female preference for a secondary sexual character (foreleg tufts). Penultimate females were exposed to video playback of zero, one, or three courting males either once every 2 days (low encounter rate) or twice per day (high encounter rate). At adulthood (week 2 post-maturity), females were presented video playback of courting males with small or large tufts to test for preferences in no-choice and two-choice designs. In two-choice (but not no-choice) presentations, female receptivity varied significantly with treatment. Females exposed to three males simultaneously at a higher encounter rate during their penultimate stage exhibited greater receptivity to large-tufted than small-tufted males as adults. Subsequent analyses revealed that females were more selective as adults if they encountered cumulatively more males during their penultimate stage, which was a repeatable trend when re-testing some individuals

3 weeks later. This study adds to the growing literature that demonstrates that invertebrates exhibit plasticity in mating preferences depending on social experience.

Introduction

Across the animal kingdom, females often prefer males with brighter or larger secondary sexual characteristics (Andersson 1986; Kuijper et al. 2012). This selectivity is often argued to result in either indirect (Andersson 1994; Jennions and Petrie 2000) or direct (Kirkpatrick 1996; Iwasa and Pomiankowski 1999) fitness benefits for females choosing males that invested resources into increased trait expression. Female mating preferences may vary, however, based on intrinsic factors such as nutritional stress and condition (Hunt et al. 2005; Burley and Foster 2006; Byers et al. 2006; Hebets et al. 2008; Holveck and Riebel 2010), reproductive status (Lynch et al. 2005; Uetz and Norton 2007; Judge et al. 2010), and age (Gray 1999; Kodric-Brown and Nicoletto 2001; Coleman et al. 2004). Female mating preferences may also vary depending on extrinsic factors such as predation risks (Forsgren 1992; Hedrick and Dill 1993; Johnson and Basolo 2003), the presence of competitors (Fawcett and Johnstone 2003), social experience (Hebets 2003; Tudor and Morris 2009; Rutledge et al. 2010; Bailey 2011), and the physical environment (Gordon and Uetz 2011). Specifically, the effects of previous social experience on subsequent mate preferences, an example of "socially cued anticipatory plasticity" or SCAP (Kasumovic and Brooks 2011), have been increasingly studied over the last decade due to the impact of female preferences on the evolution

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of mating signals (Jennions and Petrie 1997; Kasumovic and Brooks 2011; Verzijden et al. 2012).

The density of potential mates and the operational sex ratio (OSR) are two demographic factors in an individual's social environment that influence the overall availability of potential mates. Results from studies across a variety of taxa demonstrate variation in mating preferences based on the availability of potential mates. Females have been shown to be more selective (i.e., choose high-quality males vs low-quality mates) when male densities are high (e.g., Berglund 1995; Bondurianksy 2001; Kokko and Rankin 2006) or when OSRs are male-biased (e.g., Berglund 1994; Souroukis and Murray 1995; Baslhine-Earn 1996; Jirotkul 1999; Tinghitella et al. 2013). When male densities are low or when OSRs are female-biased, females are predicted to be less selective in order to avoid missing opportunities to mate. However, while OSR and male density are often quantified at the population level (e.g., Gwynne 1984; Palokangas et al. 1992), individual females presumably do not assess them at that level. Instead, individuals may have past experience with differences in the encounter rate (how frequently a female may encounter a male) and/or the number of males encountered simultaneously (e.g., encountering one or more males at a time), which is affected by the population density and OSR. SCAP in mating preferences based on the availability of potential mates would presumably be selected for over time, as long as social conditions of the past (e.g., high encounter rates or encounters with multiple males) accurately convey what the future social environment will be like. Consequently, SCAP would lead to behaviors (e.g., maintaining strict preferences for males with particular phenotypes or relaxing preferences) that are advantageous during mating decisions (Kasumovic and Brooks 2011). This study aims to determine whether SCAP in female selectivity is best predicted by previous experience with varying encounter rates and/or simultaneous encounters with multiple males.

Recent research across a variety of arthropods has demonstrated that invertebrates are not rigid in their mating preferences, making them useful study organisms to investigate SCAP (fruit flies: Dukas 2005; katydids: Fowler-Finn and Rodriguez 2012; crickets: Bailey and Zuk 2008; Bailey 2011; Kasumovic et al. 2012; Bailey and Macleod 2014; wolf spiders: Hebets 2003; Hebets and Vink 2007; Hebets 2007; Rutledge et al. 2010). In particular, *Schizocosa ocreata* is a useful organism to study the plasticity of female mate preferences for several reasons. Female *S. ocreata* have been shown to prefer males with large tufts (conspicuous dark tibial bristles on the forelegs) (McClintock and Uetz 1996; Scheffer et al. 1996) and can use this trait to choose high-quality males, because tuft size is a condition-dependent trait (Uetz et al. 2002). These previous studies, though, have been conducted on females without any prior experience with courting males, despite evidence from other studies in *Schizocosa* spp.

demonstrating that previous experience affects mate preferences in some contexts (Hebets 2003; Hebets and Vink 2007; Rutledge et al. 2010). Studies have yet to investigate how variation in the availability of potential mates impact female mate preferences for tuft size. This is an interesting question to ask in *S. ocreata*, as variation in male densities across microhabitats exists and OSRs change as the mating season progresses (Roberts and Uetz, unpublished data).

During a period of asynchrony of maturation (males mature on average 2 weeks before females: Roberts and Uetz, unpublished data), female *S. ocreata* would be expected to encounter male courtship during their penultimate stage (i.e., the instar or growth stage before molting to maturity) for several reasons. Because males may court in response to chemical cues from penultimate females (though at a significantly lower level in comparison to male courtship levels in response to mature female silk), female *S. ocreata* may encounter male courtship prior to maturity (Roberts and Uetz 2005). There is also an overlap of juvenile and mature females, so males may court in response to silk from mature females while within the visual field of neighboring subadults. Additionally, males may court, though at a significantly reduced level, in response to no chemical cues at all (Roberts and Uetz 2005), providing additional opportunities for females to be exposed to male courtship. The ability to estimate the availability of potential mates during their penultimate stage (i.e., when they may be exposed to male courtship) would be particularly advantageous for species such as *S. ocreata*, in which females are monogamous and would not have the opportunity to re-mate (Norton and Uetz 2005).

In this study, we use video playback to give penultimate females experience with courting males at an individual level to examine the impact on female preferences of a wolf spider that exhibits asynchrony of maturation. We hypothesize that wolf spiders exhibit SCAP in mating preferences (Kasumovic and Brooks 2011) as a consequence of exposure to male courtship and that encounter frequency and/or simultaneous encounters with males experienced by penultimate females should influence female mate preferences as adults. Based on this hypothesis, we predict that when female *S. ocreata* either encounter males more frequently (twice per day) and/or encounter males simultaneously (three males per exposure) during their penultimate stage, they will increase their receptivity to large-tufted males relative to receptivity to small-tufted males. On the other hand, females that encounter males less frequently (every other day) or do not encounter males simultaneously (one male per exposure) will demonstrate relaxed preferences (i.e., equal receptivity to small-tufted and large-tufted males).

Using a subset of individuals, we also tested whether female preferences arising from social experience would be maintained at a later age (fifth week post-maturity). Previous studies have shown that in individuals without any previous social experience, age affects mate preference; i.e., at an age of 5 weeks post-maturity, female *S. ocreata* no longer maintain

their earlier preferences for males with large tufts (Uetz and Norton 2007). However, if prior experience affects adult mate preferences as hypothesized above, we predict that female preferences will be repeatable and that relationships between experience during their penultimate stage and adult mate preferences will be maintained across multiple trials over time.

Methods

Animal care

Female *S. ocreata* were collected from the Cincinnati Nature Center, Clermont County, Ohio, USA, as virgin juveniles prior to the researchers encountering any mature males in the field. Each female was isolated in a plastic container with available water in a room with controlled humidity, temperature, and light cycle (L/D 11:13 h). All females were fed two to three crickets (10-day old) twice per week.

Overall design

In brief, we gave penultimate females repeated encounters (every other day or twice per day) with (zero, one, or three) visually courting males of average tuft size and tested female preferences for small-tufted and large-tufted males as adults during week 2 (see Fig. 1 for timeline). Week 2 trials were conducted in no-choice (days 1 and 2) and two-choice (day 3) experimental designs, and a subset of females was re-tested in two-choice trials during week 5 (days 4–7).

Video playback

Digital playback of courting male *S. ocreata* on LCD screens has been shown to elicit female receptivity behaviors (see review in Uetz and Roberts 2002). The use of video playback

techniques allows precise control over interactions that an individual has with potential mates, while simultaneously providing a standardized stimulus that controls for individual variation in male phenotypes (in this study: body size, tuft size, and courtship vigor). These controlled variables are additionally important when measuring the repeatability of female preferences. Thus, we used video playback to vary the experience of penultimate females with males under lab-simulated conditions to determine whether adult females demonstrate plasticity in their receptivity according to male traits (foreleg tuft size). The courting male stimuli have been previously used in other studies (McClintock and Uetz 1996; Roberts et al. 2007; Moskalik and Uetz 2011). All courting male stimuli have a mean body size based on the population average from Roberts et al. (2007) and had identical courtship vigor (number of taps per minute). The courting male stimulus during the experience of penultimate females was a male with average tuft size (also based on population averages from Roberts et al. 2007). Digital stimuli of small-tufted and large-tufted males used to measure female selectivity during adulthood were previously manipulated to represent the lower and upper 95 % confidence intervals (see Roberts et al. 2007).

Manipulation of social experience

Exposure to male courtship began the day after females molted to their penultimate stage and ended as females matured. Each exposure lasted 5 min and took place in arenas with three iPod Classics placed equilaterally behind clear acetate sheets, facing the center of the arena. All iPods displayed identical leaf litter backgrounds during the exposures. However, we varied the number of iPods that displayed a video of a courting male with an average tuft size in the foreground (zero, one, or three) and the frequency of exposures (once every 2 days or twice per day). This resulted in six treatment groups: females ($n=12$) exposed to zero males every other day, females
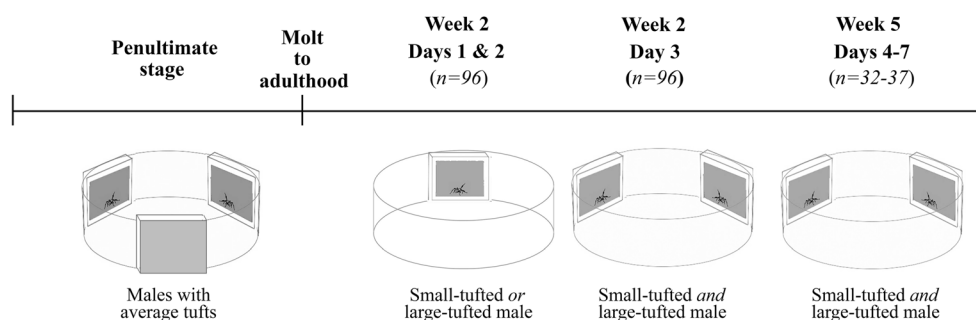
Fig. 1 The experimental design, arenas, and sample sizes used in this study. The *top row* indicates the stage or age in which trials were conducted, while the *bottom row* depicts the arrangement of the iPod units in the clear plastic arenas (diameter 15.5 cm, height 6.5 cm) that were used in these trials. The first arena had three iPod units placed equilaterally around the edge and was used to provide females with experience during their penultimate stage. Once mature, females were

presented a small-tufted or large-tufted male in separate no-choice trials on days 1–2 (one trial per day). On day 3 of week 2, females were simultaneously presented a small-tufted and large-tufted male in an arena with two iPods. The same two-choice arena was used again for a subset of individuals week 5 for three to four consecutive days to test the repeatability of female preferences

($n=8$) exposed to zero males twice per day, females ($n=18$) exposed to one male every other day, females ($n=18$) exposed to one male twice per day, females ($n=22$) exposed to three males every other day, and females ($n=18$) exposed to three males twice per day. Originally, 130 penultimate females were randomly assigned to these six treatment groups. During the exposures, females escaped or developed parasites, resulting in reduced sample sizes. While there was natural variation in the length of a female's penultimate instar (growth stage prior to maturity) during experience trials (i.e., females varied in how many days it took females to molt from their penultimate stage to their adult stage), there was not a significant difference in the penultimate instar length based on whether females viewed a digitally courting male or not ($t_{96}=1.0853$; $p=0.2805$) or across the four treatment groups that viewed digitally courting males ($F_{3,73}=1.2613$, $p=0.2940$). Additionally, there were no significant correlations between the length of a female's penultimate instar stage and female selectivity in the no-choice or two-choice trials, and there were no significant correlations between the number of times a female was placed into the arena during the penultimate stage and female selectivity in no-choice or two-choice trials ($p>0.05$ for all analyses). Therefore, any observed differences in female selectivity should be due to variation in whether or not female encountered males simultaneously (hereafter referred to as "simultaneous encounters") and/or the encounter rate.

Measuring adult mate preferences

All females were fed two to three crickets the day before trials. A series of three 5-min trials initially took place when females were 10–15 days post-maturity (week 2, days 1–3). On days 1 and 2, females were placed in an arena facing a 5 min video of a courting male with small or large tufts in a no-choice trial (Fig. 1). The order of the males (i.e., small-tufted or large-tufted) was determined using systematic randomization to allow an equal number of females within each treatment group to view each male first. On day 3, females were placed in an arena and simultaneously presented two 5 min videos of courting males (Fig. 1), identical to those seen in days 1 and 2. Systematic randomization was used to allow an equal likelihood that the large-tufted male and the small-tufted male were randomly assigned to the iPod on the left side of the arena or the iPod on the right side. The center of the iPods was 12 cm from each other, and iPods were angled at 90° from each other, facing the center of the arena. Some females ($n=37$) were tested again across a 4-day period (days 4–7) during their fifth week post-maturity to test for repeatability of mate preferences in two-choice trials using the same setup (Fig. 1). All trials were video recorded with a digital video camcorder (Canon ZR960). The observer was blind to which treatment group each female belonged to during video analysis. The number of female receptivity behaviors commonly observed

in *S. ocreata* (tandem leg extensions, slow pivots, and settles) was recorded as a behavior directed to a particular male video only if the female was directly in front of the video. These receptivity behaviors have previously been shown to lead to copulation in *S. ocreata* (Uetz and Denterlein 1979; Scheffer et al. 1996; Montgomery 1903; Norton and Uetz 2005). Female receptivity displays are important for successful mating to take place for *S. ocreata* as they may signal to males that they will not be cannibalized. Additionally, mating does not typically proceed until a female signals receptivity.

Data analysis

All statistical analyses were conducted using the R statistical package (R Core Team 2013, version 2.15.3). Female "selectivity" refers to the cumulative number of receptivity displays to the courting male with large tufts relative to the cumulative number of receptivity displays to the courting male with small tufts. Statistically speaking, in week 2 trials, the number of receptivity displays to the small-tufted male and those to the large-tufted male were bound together using the "c-bind" function in R to define an individual's selectivity in no-choice and (separately) two-choice trials. Consequently, when a generalized linear model (family=binomial) was conducted, the response variable included a matrix with two columns: the number of receptivity displays toward large-tufted males (i.e., successes) and the number of receptivity displays toward small-tufted males (i.e., failures). Initially, generalized linear models (family= binomial) were constructed using combinations of encounter rate, simultaneous encounters, and their interaction (encounter rate×simultaneous encounters). Each model was compared to an intercept-only model and compared to other models using likelihood ratio tests. This allowed us to test the initial hypothesis, for example, of whether the encounter rate (every other day or twice per day) or simultaneous encounters with males (zero, one, or three males per encounter) best predicted female selectivity. However, given the nature of the study (e.g., the instar length for penultimate females may vary among individuals), females were exposed to a wide range of males. Consequently, additional generalized linear models (family=binomial) were constructed using encounter rate, simultaneous encounters, encounter rate×simultaneous encounters, and total males (the cumulative number of courting males that a female saw during her entire penultimate stage) in all appropriate combinations for both no-choice and two-choice analyses. Each model was compared to an intercept-only model and compared to other models using likelihood ratio tests. Akaike information criterion (AIC) values were then compared to determine the combination of independent variables

(encounter rate, simultaneous encounters, encounter rate × simultaneous encounters, and total males) that best fit variation in female selectivity in no-choice trials and two-choice trials during week 2.

Given the importance of total males (see "Results") in two-choice trials from week 2, we statistically analyzed female receptivity in response to an increasing number of total males on a categorical level (receptive or not receptive) using logistic regression analyses. This allowed us to examine the effects of exposure to an increasing number of males (total males) on the probability of a female demonstrating receptivity to a male with small tufts or a male with large tufts.

We used the lme4 package (Bates et al. 2012) in R to build separate-slope, separate-intercept, and mixed (i.e., separate slope and intercept) models to examine aspects of the repeatability of female preferences. In these analyses that include week 5 results, female selectivity (response variable) was statistically defined as the difference in receptivity displays (i.e., the number of displays toward large-tufted males minus those toward small-tufted males). First, we determined if female selectivity (response variable) changed over time (fixed effect) in two-choice trials (days 3–7) after accounting for differences in individual variation (female ID: random effect). Additionally, a linear mixed-effects analysis was used to determine the relationship between the cumulative number of males that females encountered during their penultimate stage (fixed effect) and adult female selectivity (response variable), after accounting for differences in individual variation (female ID: random effect) and time (days 3–7: random effect). In all cases, a visual inspection of residual plots did not demonstrate any notable deviations from homoscedasticity or normality. Finally, a generalized linear model was run to examine the relationship between the cumulative number of males encountered during their penultimate stage and the differences in female receptivity scores averaged across all two-choice trials during week 5 (days 4–7).

We calculated the repeatability of female preferences to compare: (1) female selectivity from week 2 to week 5 and (2) female selectivity across all 4 days of week 5. In both cases, we used the "rptR" package (Nakagawa and Schielzeth 2010; Schielzeth and Nakagawa 2011) within the R statistics program. To calculate the repeatability of week 2 compared to week 5, we ran a correlation between the differences in female receptivity displays (those to large-tufted males minus those to small-tufted males) in week 2 and the mean differences in female receptivity displays of the four trials in week 5. This allowed us to calculate the correlation-based repeatability (R_C; see Nakagawa and Schielzeth 2010 for further discussion). To compare female selectivity across multiple days within week 5, we calculated the ANOVA-based repeatability (R_A; see Nakagawa and Schielzeth 2010 for further discussion).

Results

No-choice trials, week 2, days 1 and 2

Overall, there were no significant effects of any aspect of male availability on female selectivity in no-choice trials (Fig. 2). Specifically, likelihood ratio tests showed no significant effect of any combination of encounter rate, simultaneous encounters, and encounter rate × simultaneous encounters (Table 1). Including total males to any model still resulted in a non-significant improvement upon the intercept-only model (Table 1). Because female experience did not significantly explain variation in female selectivity during these no-choice trials, no further statistical analyses were conducted.

Two-choice trials, week 2, day 3

In contrast to the no-choice trials, when adult females were offered a choice between two males courting simultaneously, significant differences in female selectivity were seen across treatments (Fig. 3). A model containing encounter rate, simultaneous encounters, and their interaction was significantly better than an intercept-only model (likelihood ratio test $\chi^2_5 = 16.825$, $P = 0.0048$; Table 2). Subsequent paired t tests within each treatment group revealed that females that saw three males twice per day (male-biased ratio, high frequency) had significantly more receptivity displays to males with large tufts than males with small tufts (paired t test $t_{17} = 4.1231$, $P = 0.0007$). All other paired t tests within each treatment group were not significantly different ($P > 0.05$ in all cases). Further, the model including only simultaneous encounters was significantly better than the model including only encounter rate (likelihood ratio test: $\chi^2_1 = 6.061$, $P = 0.0138$). The model with only simultaneous encounters was also significantly better than the intercept-only model (likelihood ratio test: $\chi^2_2 = 8.947$, $P = 0.0114$), while the model with only encounter rate was not (likelihood ratio test: $\chi^2_1 = 2.886$, $P = 0.0894$).

While a comparison of the effects of encounter rate and simultaneous encounters tests the original hypothesis,
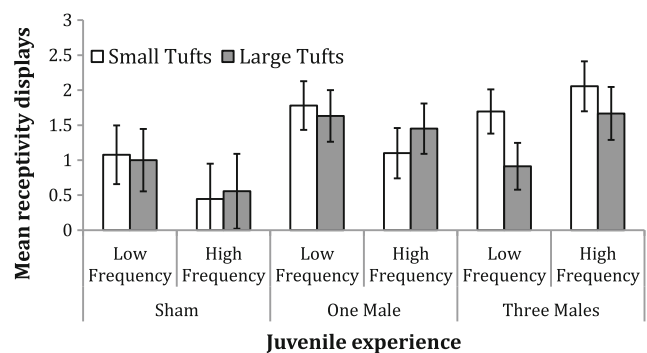
Fig. 2 The mean (±SE) number of female receptivity displays to males with small tufts and males with large tufts across different treatment groups in no-choice trials

Table 1 A comparison of models including different independent variables and the degree to which they account for variation in female selectivity in no-choice trials

Included variables	Deviance[a]	P value (>chi)[b]	AIC	ΔAIC[c]
Encounter rate+total males	5.4715	0.0649	210.53	0
Simultaneous encounters+encounter rate	5.8666	0.1183	212.14	1.61
Encounter rate	1.6212	0.2029	212.38	1.85
Simultaneous encounters	3.5094	0.1730	212.49	1.96
Total males	0.3637	0.5464	213.64	3.11
Simultaneous encounters+total males	4.2085	0.2398	213.80	3.27
Simultaneous encounters+encounter rate+total males	6.2042	0.1844	213.80	3.27
Simultaneous encounters×encounter rate[d]	5.8853	0.3175	216.12	5.59
Simultaneous encounters×encounter rate[d]+total males	6.6085	0.3586	217.40	6.87

[a] Deviance refers to amount of variation explained by the variables included in the model, calculated as the difference between the null deviance and the residual deviance values provided by the generalized linear models (binomial family) in R

[b] P values were calculated by comparing each generalized linear model (binomial family) to an intercept-only model

[c] Relative to the model with the lowest AIC value

[d] Model includes each individual variable ("simultaneous encounters" and "encounter rate") as well

additional tests with inclusion of the cumulative number of males that females saw during their penultimate stage (total males) as a covariate significantly improved all statistical models (Table 1; all $P<0.05$). Consequently, AIC values were then compared for each model (Table 2) to determine which factor of experience best predicts female receptivity. The best model has the lowest AIC value, which in this case includes simultaneous encounters and total males as factors predicting female selectivity. However, traditionally, any other model that is within two AIC units of the lowest AIC value should not be dismissed. More recently though, it has been suggested that models within 2–7 AIC units should have some support as well and analyses should often look at the AIC values for all statistical models to make a strong inference as to the importance of the factors in each model (Burnham et al. 2011). The five models with the lowest AIC values all include total males as a factor and removing this factor from any model increases the AIC value by >7 AIC units. Taken together, the

cumulative number of males that penultimate females encountered best predicts female selectivity as adults in two-choice trials.

There was a significantly positive relationship between the total number of males that penultimate females saw and how selective (i.e., receptivity displays to large-tufted vs small-tufted males) they were as adults (Fig. 4; Pearson correlation: $R^2=0.0976$, $N=96$, $P=0.0019$). At a more categorical level (receptive or not receptive), there was a significantly increased likelihood for females to be receptive to males with large tufts if they saw more cumulative males during their penultimate stage (Fig. 5; logistic regression: $Z=2.126$, $P=0.0335$). While the relationship between total males and the likelihood for females to be receptive to males with small tufts is negative, it is not significant (Fig. 5; logistic regression: $Z=1.586$, $P=0.1130$).

Repeatability of female preferences in two-choice trials

In analyses of female selectivity across all 5 days of two-choice trials (days 3–7), there was no effect of time on female selectivity (likelihood ratio test: $X_{1,141}=1.0544$, $P=0.3045$), as shown when comparing a model with a separate intercept for each individual female with and without "day" as a factor. The model accounting for a separate intercept was used due to its lower AIC value (626.65) in comparison to the separate-slope (AIC 628.79) and mixed (AIC 632.79) models.

When analyzing female selectivity as a function of the cumulative number of males, there was a significant effect of total males seen as a penultimate on adult female selectivity when accounting for multiple measures on individuals over time and accounting for difference in slopes due to the test day (likelihood ratio test: $X_{1,136}=11.571$, $P=0.0007$). The model accounting for a separate slope only was chosen due to its lower AIC value (613.04) in comparison to the
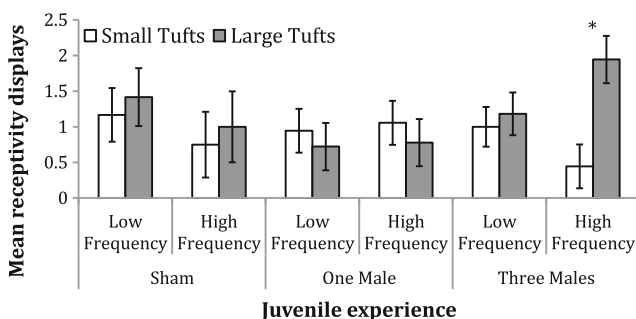
Fig. 3 The mean (±SE) number of female receptivity displays to males with small tufts and males with large tufts across different treatment groups in two-choice trials. An *asterisk* indicates a significant difference in the number of receptivity displays to males with small tufts and males with large tufts within a particular treatment group

Table 2 A comparison of models including different independent variables and the degree to which they account for variation in female selectivity in two-choice trials

Included variables	Deviance[a]	P value (>chi)[b]	ΔAIC[c]	AIC	Z value for total males[d]	P value for total males[d]
Simultaneous encounters+total males	23.44	0.00003	0	190.83	3.554	0.0001
Simultaneous encounters+encounter rate+total males	25.369	0.00004	0.07	190.90	3.394	0.0007
Simultaneous encounters×encounter rate[e]+total males	27.951	0.00009	1.49	192.32	3.030	0.0024
Total males	17.111	0.00004	2.33	193.16	3.822	0.0001
Encounter rate+total males	18.023	0.00012	3.41	194.24	3.649	0.0003
Simultaneous encounters×encounter rate[e]	16.825	0.00484	10.61	201.44	–	–
Simultaneous encounters+encounter rate	12.455	0.00597	10.98	201.81	–	–
Simultaneous encounters	8.947	0.01141	12.49	203.32	–	–
Encounter rate	2.886	0.08935	16.55	207.38	–	–

[a] The variation explained by variables included in the model, calculated as the difference between the null and residual deviance values from generalized linear models (binomial family) in R

[b] Calculated by comparing each model to an intercept-only model

[c] Relative to the model with the lowest AIC value

[d] Z and P values are included for "total males" only due to its demonstrated importance

[e] Model includes individual variables ("simultaneous encounters" and "encounter rate") as well

intercept-only (AIC 621.20) and mixed (AIC 620.29) models. Despite this evidence that the slope varies depending on which day females were tested, there was, in general, a positive relationship between the cumulative number of males that females saw during their penultimate stage and adult female selectivity across each test day for two-choice trials (Pearson correlations: week 2, day 3 $R^2=0.0976$, $n=96$, $P=0.0019$; week 5, day 4 $R^2=0.0065$, $n=37$, $P=0.6364$; week 5, day 5 $R^2=0.1118$, $n=35$, $P=0.0400$; week 5, day 6 $R^2=0.1067$, $n=37$, $P=0.0484$; week 5, day 7 $R^2=0.0641$, $n=32$, $P=0.1622$). Mean female selectivity across all five two-choice trials (days 3–7) significantly increased as females were exposed to an increasing cumulative number of males during their penultimate stage (Pearson correlation $F_{1,35}=9.5311$, $r^2=0.2140$, $P=0.0039$). This relationship remained significantly positive if only two-choice trials from week 5 (days 4–7) were included (Pearson correlation $F_{1,35}=6.7794$, $r^2=0.1623$, $P=0.0134$).

There was a significantly positive correlation between female selectivity during week 2 and mean female selectivity during week 5 (Pearson correlation $N=36$, $r^2=0.1260$, $P=0.0311$). The repeatability of female selectivity from week 2 to week 5 was 0.2350. The repeatability of female selectivity across week 5 (days 4–7) was 0.1620.

Discussion

Female mate preferences in adult *S. ocreata* were influenced by past experience in their social environment, providing evidence for socially cued anticipatory plasticity (SCAP) in mating preferences. Specifically, females displayed more receptivity displays toward large-tufted males than small-tufted males based on some aspects of past experience during their penultimate stage. Initially, experience with simultaneous encounters with males, but not encounter rate, best predicted adult female selectivity. However, females became increasingly more selective as adults if they were exposed to cumulatively more courting males during their penultimate stage, performing more receptivity displays toward large-tufted males than small-tufted males. These results support other studies in which females are predicted to be less selective when males are rare, but more selective when male abundance is high (see Eshel 1979; Hubbell and Johnson 1987, and Crowley et al. 1991 for further discussion).

As mentioned, the density-dependent effect seen in two-choice trials was due to cumulative exposure, as other variables did not have an effect on female selectivity once the cumulative number of males was incorporated into analyses. While many studies examine the effects of sex ratio and population density separately, other studies demonstrate the importance of examining sex ratio and density simultaneously in
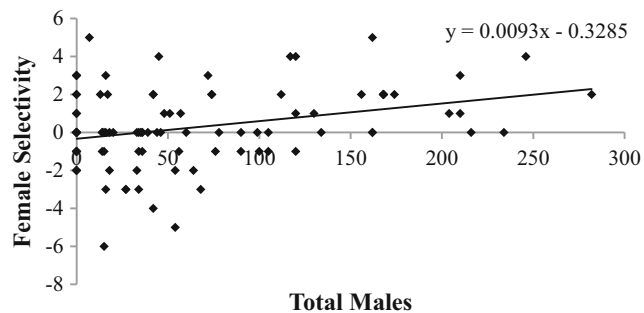
Fig. 4 The relationship between the total number of males that penultimate female *Schizocosa ocreata* saw (total males) and female selectivity (number of receptivity displays to males with large tufts− number of receptivity displays to males with small tufts). There was a significantly positive relationship between the total number of males that females saw during their penultimate stage and how selective they are as adults (Pearson correlation $R^2=0.0976$, $N=96$, $P=0.0019$)

the context of male courtship (Spence and Smith 2005), copulation duration (Alonso-Pimentel and Papaj 1996), and the opportunity for sexual selection (Wacker et al. 2013). Male zebrafish, *Danio rerio*, alter their courtship rates in response to changes in the adult sex ratio, but only when the overall population density is high (Spence and Smith 2005). In the walnut fly, *Rhagoletis juglandis*, male and female density both significantly impacted copulation duration (Alonso-Pimentel and Papaj 1996). However, overall density had no significant effect once OSR was taken into account (Alonso-Pimentel and Papaj 1996). These studies demonstrate the difficulty of teasing apart effects of sex ratio and mate density on mating behaviors and suggest, along with this study, that future studies should focus on investigating the effects of what occurs at the individual level (e.g., the cumulative number of males encountered by females in a high-density or male-biased population).
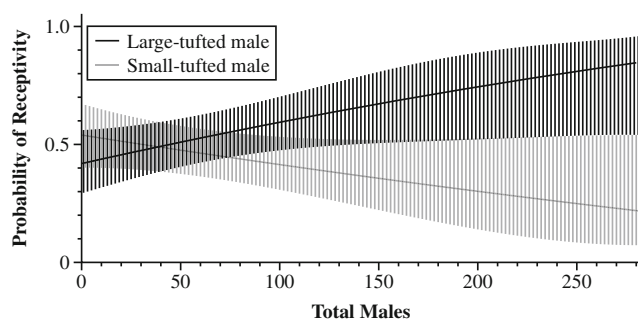
Fig. 5 Logistic regressions of the total number of males that penultimate female *Schizocosa ocreata* saw and the probability of them demonstrating receptivity to small-tufted males (*gray line*) or large-tufted males (*black line*) as an adult. There is a significantly increased likelihood for adult females to be receptive to large-tufted males if they see more cumulative males during their penultimate stage (logistic regression $Z=2.126$, $P=0.0335$). While the relationship between total males and the likelihood for females to be receptive to males with small tufts is negative, it is not significant (logistic regression $Z=1.586$, $P=0.1130$). *Vertical lines* indicate the standard error of the predicted probability of the logistic regression fit

This paper highlights the importance of timing and context in SCAP. As with other studies in *Schizocosa* spp. (Hebets 2003, 2007; Hebets and Vink 2007; Rutledge et al. 2010; Rutledge and Uetz 2014), we focused on effects of experience during the penultimate stage. In nature, female *S. ocreata* would likely encounter males during their penultimate stage and their adult stage, but few studies on any invertebrates to date have compared the effects of juvenile versus adult experience on mate preferences. That such plasticity can occur in adulthood based on juvenile experience alone demonstrates the importance of the penultimate stage in *S. ocreata*. However, these results were only seen in the context of two-choice (simultaneous presentation) trials and not no-choice (single presentation) trials. In this study, such a result might imply that female *S. ocreata* need to be able to visually compare potential mates in order to demonstrate plasticity (but see Hebets 2003 and Rutledge et al. 2010 where female *Schizocosa* spp. demonstrated experience effects in no-choice designs using live males). Additionally, in a meta-analysis on 40 species, mating preferences were significantly stronger in two-choice experimental designs than no-choice experimental designs (Dougherty and Shuker 2014). This may be due to the costs of rejecting potential mates when presented a single male at a time (see Dougherty and Shuker 2014 for detailed discussion), possibly explaining the lack of a significant effect of experience observed in no-choice presentations in this particular context.

In several taxa, females become less selective as they age (Gray 1999; Kodric-Brown and Nicoletto 2001; Uetz and Norton 2007), presumably in order to ensure that they do not miss out on mating opportunities. In *S. ocreata*, females at 2 weeks post-maturity prefer males with large tufts, but females at 5 weeks post-maturity no longer exhibit these preferences (Uetz and Norton 2007). This study demonstrates that the correlation between the cumulative number of males observed during the penultimate stage and adult female selectivity is retained during the fifth week post-maturity. This suggests that female preferences are repeatable despite female aging, presumably due to experience effects, which were not tested in Uetz and Norton (2007). However, females in other taxa do not always maintain their mating decisions at a later age, as interactions between age and social experience can lead to females with relaxed preferences (e.g., Tinghitella et al. 2013). While the repeatability values for female selectivity reported in this paper appear to be low, the repeatability of mating preferences is often lower in comparison to other behaviors (see review in Bell et al. 2009).

Increasing evidence that conditions in an individual's social environment impact mating decisions (e.g., Rosenqvist and Houde 1997; Hebets 2003; Walling et al. 2008; Rutledge et al. 2010; Fowler-Finn and Rodriguez 2012) suggests that there may be selection for plasticity (i.e., instead of rigid preferences). That such plasticity is demonstrated in an

invertebrate species adds to current research that demonstrates that invertebrate behaviors are not rigid in their mating preferences (butterflies: Westerman et al. 2012, 2014; crickets: Bailey and Zuk 2008; Bailey 2011; Kasumovic et al. 2012; Bailey and Macleod 2014; Atwell and Wagner 2014; fruit flies: Dukas 2005; katydids: Fowler-Finn and Rodriguez 2012; wolf spiders: Hebets 2003; Hebets and Vink 2007; Hebets 2007; Rutledge et al. 2010). Studies on the effects of the experience with the presence vs absence of experience with male courtship signals in some of these studies are biologically relevant (e.g., Bailey and Zuk 2008; Bailey 2011; Bailey and Macleod 2014). However, future studies on SCAP in all animal taxa should be sure to investigate the variation in the levels of experience (as opposed to presence vs absence), with consideration for the appropriate experimental design (e.g., experience with simultaneous vs subsequent male encounters and/or variation in encounter rate) to manipulate the perceived availability of potential mates.

Interestingly, the results from this study were based on social experience in the visual modality alone. However, male *S. ocreata* use both visual and vibratory courtship signals, leading to questions as to whether females demonstrate plasticity in mate preferences in response to variation in the availability of potential mates using vibratory signals as well. Vibratory signals are hypothesized to be plesiomorphic (Stratton 2005) and dominant (Hebets et al. 2013). However, within *Schizocosa*, courtship of male *S. ocreata* has a particularly high visual complexity score and has been shown to be equivalent to vibratory signals in eliciting female responses (see Uetz et al. 2009 and Hebets et al. 2013 for further discussion). The results of this study further emphasize the importance of visual signals in *S. ocreata* by demonstrating plasticity in female mate preferences in response to visual signals alone and add to the growing body of literature that female selectivity can be impacted by an individual's social environment.

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