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Female Choice, Female Reluctance to Mate and Sexual Selection on Body Size in the Dung Fly *Sepsis cynipsea*

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Abstract

We investigated the mechanisms of sexual selection in the common dung fly Sepsis cynipsea and how these affect selection on body size at the population level. Because of the presumed costs associated with mating, we predicted that there would be a decrease in the general reluctance of females to mate with any particular male at higher male densities at the mating site, a fresh cow pat, resulting in indirect female choice and a decrease in the strength of sexual selection. In contrast, classical direct female choice and male-male competition should result in increased selection intensities because more opportunities for choice and competition exist at higher densities. Female reluctance to mate and female assessment of males are expressed in prominent female behaviour to repel mates in several insect species, including S. cynipsea. Laboratory pair-wise choice experiments showed that large males were more likely to obtain copulations, which also ensued more promptly, suggesting female assessment of male quality (direct female choice). There was a basic influence of male activity but little further effect of male scramble competition on the outcome of mating. Another laboratory experiment showed a decrease in female shaking duration per male, associated with an asymptote in the shaking duration per female, as male density and harassment increased, but did not show the increase in mating frequency predicted by the female reluctance hypothesis. A study estimating sexual selection differentials in the field showed that directional selection for larger males was present overall and was negatively related to seasonally mediated variation in male density. Our study suggests that direct female choice in combination with indirect female choice (due to an interaction of female reluctance to mate and male persistence) is most consistent with the behavioural and selection patterns observed in S. cynipsea, but male effects cannot be definitively excluded.

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Introduction

Population density and the operational sex ratio have long been identified as major ecological variables affecting the evolution of mating systems and mating behaviour (Emlen & Oring 1977; Thornhill & Alcock 1983). Both male-male competition and female choice, the 'classical' mechanisms of sexual selection (Darwin 1871), predict a positive relationship between the strength of sexual selection and local population density. This is because, at least in theory, there is more opportunity for competition and choice when more competitors and more potential mates, respectively, are present (Sutherland 1987; Wilkinson 1987). On the other hand, local population density at the mating site has also been shown to be negatively associated with the strength of sexual selection on morphological characters (Arnqvist 1992a). This outcome is behaviourally based on the female reluctance hypothesis: when male mating attempts are frequent, females decrease their general reluctance to mate with any particular male to reduce the costs of male harassment, mate choice and mate rejection such as increased predation or reduced foraging efficiency (e.g. Wilcox 1984; Fairbairn 1993; Rowe 1994). This results in lower levels of mate rejection behaviour at higher population (male) densities and/or male-biased operational sex ratios (Arnqvist 1992b; Rowe et al. 1994; Weigensberg & Fairbairn 1994; Vepsäläinen & Savolainen 1995), and ultimately in smaller sexual selection differentials because inferior phenotypes are more likely to obtain matings (Arnqvist 1992a). This phenomenon has been termed 'convenience polyandry' (Thornhill & Alcock 1983; Rowe 1992). In its strictest interpretation, female reluctance thus results in 'indirect', as opposed to 'direct' (or active), female choice (as defined by Wiley & Poston 1996), and sexual selection becomes a side-effect of natural (viability) selection (Rowe 1994; Rowe et al. 1994).

Direct female choice and female reluctance resulting in indirect female choice are not mutually exclusive mechanisms of sexual selection: they can co-occur in any particular mating system and are generally difficult to separate from each other as well as from male influences on the outcome of mating (Rowe et al. 1994; Wiley & Poston 1996). For example, overt female shaking behaviour may indicate her unwillingness to mate, or may be her means of identifying males of superior quality, or may result from the male's persistence in mating (Arnqvist 1989; Ward et al. 1992; Rowe et al. 1994; Weigensberg & Fairbairn 1994). The intersexual conflict underlying female reluctance behaviour has been intensively studied in water striders (Arnqvist 1989, 1992b; Rowe 1992, 1994; Rowe et al. 1994; Weigensberg & Fairbairn 1994; Jablonski & Vepsäläinen 1995; Vepsäläinen & Savolainen 1995), but not so much in other species (but see Conner 1989; McLain 1982, 1992; Crean & Gilburn 1998). Furthermore, the link between the behavioural mechanism (female reluctance to mate) and its population consequences in terms of sexual selection has, to our knowledge, been directly demonstrated only twice in the same species, the water striders *Gerris odontogaster* (Arnqvist 1992a, b) and *Aquarius remigis* (Weigensberg & Fairbairn 1994; Fairbairn & Preziosi 1996). Wilkinson (1987) investigated the effect of the operational sex ratio on sexual selection in a laboratory study of *Drosophila melanogaster*, and found no relationship. Here we attempt to link sexual selection on body size at the behavioural and population levels in *Sepsis cynipsea*, a small (<4 mm in body length) and abundant European dung fly of ant-like appearance.

The mating system of *S. cynipsea* is as follows. Large numbers of males wait for females coming to lay eggs on and around fresh cow pats (Parker 1972a). Operational sex ratios are typically extremely male-biased (Parker 1972a, b; Blanckenhorn et al. 1999a). As soon as a female arrives, males try to seize and guard her during oviposition, and subsequently attempt to copulate with her away from the dung (Parker 1972a, b; Ward 1983). Direct aggressive or territorial interactions among males are rare; males mainly scramble for access to a female (Ward et al. 1992). In response to male mating attempts, females show characteristic shaking behaviour during all phases of pairing, indicating reluctance to mate and/or some sort of male assessment. Thus there is apparent sexual conflict over mating (Ward et al. 1992; Rowe et al. 1994). Only about 40% of the pairs formed in the field eventually copulate (Parker 1972b; Ward 1983; Ward et al. 1992). Ward (1983) showed in one English population of *S. cynipsea* that large males have a mating advantage, indicating that sexual selection operates, but he did not quantify it.

In a first experiment (mate choice experiment), we tried to corroborate Ward's (1983) field results by investigating whether large males also have a mating advantage in pair-wise laboratory choice situations. As in nature, the competing males were not isolated from each other, so any mating advantage of large phenotypes in this experiment could be a consequence of male-male (scramble) competition and/or female choice. This experiment further served to identify any other male and female attributes that correlate with, or predict, mating success and shaking duration. In a second laboratory experiment (male density experiment), we investigated female reluctance to mate in situations where the number of available males (or the operational sex ratio) was manipulated. This experiment featured one treatment where a female only faced one male, thus excluding male-male interactions, and two more with three and six males. The female reluctance hypothesis predicts that female rejection rates should decrease and mating frequency and/or duration should consequently increase in response to increased harassment by males; the former prediction is not compatible with direct female choice (Rowe et al. 1994). The female reluctance hypothesis further predicts that more successful (high-quality; here larger) males should achieve copulation after longer struggles (because females may not want to copulate to avoid presumed costs of mating, but better males are more persistent and eventually succeed), whereas they should achieve copulation after shorter struggles when direct female choice occurs (because females more readily accept desirable males; Crean & Gilburn 1998). On the basis of the findings of our two laboratory experiments, we predicted that the strength W. U. Blanckenhorn et al.

of sexual selection in the field should be inversely related to male density if female reluctance is the primary mechanism of selection, whereas it should show a positive relationship if direct female choice and/or male-male competition is primarily operating (Arnqvist 1992a, b; see above). We used data from a field study of sexual selection to test this prediction (Blanckenhorn et al. 1999a), which is to our knowledge the most extensive data set available for testing the population consequences of female reluctance to mate.

Materials and Methods

The individuals used for the laboratory experiments were F1 or F2 offspring from laboratory cultures of a population in Fehraltorf, near Zürich (N47°23', E8°44'). Our general laboratory rearing procedures are described in Reusch & Blanckenhorn (1998) and Blanckenhorn et al. (1998). All animals had continuous access to ad libitum sugar and pollen (for food), water, and fresh dung (for food and oviposition) in 3.5-1 plastic containers. They were kept in groups in a climate chamber at 25–30°C (range), $\approx 30-40\%$ relative humidity, and a photoperiod of 16 h. However, all behavioural observations were conducted at room temperature.

Mate Choice Experiment

Males and females were separated within 24 h of emergence to guarantee their virginity, and were then separated by sex, family and age group and kept with the above nutrients in 3.5-1 rearing containers. For the experiment, two males, one large and one small but otherwise randomly chosen, were isolated into a 50-ml glass vial containing a smear of dung on filter paper. The size difference between the males was large enough to be detected by eye, so no marking was necessary, and the head width (including eyes) of all test individuals was determined after the experiment using a binocular microscope (mean head widths were 0.83 + 0.009 mm for small males and 1.01 ± 0.005 mm for large males, with a minimum difference of 0.15 mm). Head width accurately reflects overall body size in this species (Reusch & Blanckenhorn 1998; Blanckenhorn et al. 1999a). At least 30 min after the males had entered the experimental vial, one of the previously isolated females with a swollen abdomen (indicating eggs ready to be laid) was added and observations began at once. To avoid brother-sister pairings the males came from a different rearing container from the female. Females were used between one and five times to investigate their willingness to mate repeatedly. About 10% of the original sample of 120 small males were used more than once (due to low numbers of very small males), but in new combinations of females and competing males. At the time of their first experimental pairing (henceforth termed 'trial'), adult females were 7-17 d old, and they were a maximum of 34 d old in the subsequent trials. Males were 7–38 d old.

During each trial, we recorded all relevant behaviour that occurred from the time we added the female: (a) the frequency of each male's mating attempts; (b) the time each male spent riding on the female's back (defined as 'pairing'); (c) the

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total duration of female shaking when paired to a particular male; (d) whether, when and with whom the female copulated and oviposited. A trial ended after 90 min or, if the female copulated, with the termination of copulation, as typically there was very little pairing activity thereafter.

Male Density Experiment

Randomly chosen males from the rearing containers were marked with one of six model aeroplane colours and then transferred singly into 100-ml glass bottles with miniature dishes of the above nutrients. Females (unmarked) were treated similarly. Individuals of both sexes had been isolated for at least 2 d prior to the experiments to increase their willingness to mate. At the time of the experiment, females and males were 7–26 d old. Presumably, these individuals had already mated in the rearing containers, as mating activity starts soon (2–3 d) after emergence (Schulz 1989). The use in this experiment of non-virgins from the outset differed from the situation in the mate choice experiment and is likely to correspond better to the typical natural situation.

For the experiment, one, three or six individually identifiable males spanning a range of sizes were added to the bottle of a particular female (again avoiding brother–sister matings) and observations began at once. The sizes of the males used were not known until we measured their head widths after the experiments. We recorded the behaviour elements described above for the mate choice experiment. Again, the trial was terminated after 90 min or when the female had copulated (i.e. had genital contact) with one of the males for at least 10 min.

Field Estimates of Sexual Selection

To investigate the population consequences of mating behaviour in the field, we used data from Blanckenhorn et al. (1999a). This study was conducted with the same Fehraltorf population as described here. We took samples throughout the 1995 season (mid-May to late Sep.), on each occasion from two fresh (<60-minold) cow pats, usually on dry days between 10:00 and 17:00 h. At each sampling, we first estimated local male density on and within \approx 30 cm around the pat by counting the number of single and paired males. We then collected at least 20 pairs and 20 single males and measured their head width as above.

Univariate linear selection coefficients were calculated using standard methods (Arnold & Wade 1984a, b; Blanckenhorn et al. 1999a, b). For each sample (dung pat), we first produced standardized z-scores (z_i) for body size (head width) by subtracting the population mean [mean(x)] from each value (x_i) and dividing by the standard deviation (s_x): $z_i = [x_i - mean(x)]/s_x$. The instantaneous fitness, $w_i = 1$ for paired and $w_i = 0$ for unpaired males, was transformed to relative fitness, $w_i' = w_i/mean(w)$, where mean fitness was the proportion of paired males estimated just prior to sampling (Brodie & Janzen 1996; Blanckenhorn et al. 1999b). As already indicated, this number is equivalent to the operational sex ratio (number of females/number of males) because unpaired females rarely occur at the pat. We produced body size z-scores by weighting the means and variances using our

population count of paired and unpaired males. This was necessary because we did not always sample both mating categories in proportion to their actual occurrence. Our procedure corrects for this bias in our samples (Blanckenhorn et al. 1999b). The resulting regression coefficients of relative fitness on the standardized trait value are the selection differentials, which in the case of univariate measures are identical to selection intensities, reflecting the combined effects of direct and indirect selection coefficients using linear regression is common practice despite the fact that binary characters are not normally distributed, because coefficients derived from logistic regression 'are not yet interpretable in the context of equations for evolutionary change' (Brodie et al. 1995; Brodie & Janzen 1996; Blanckenhorn et al. 1999b). However, we derived the significance levels for our individual data points reported here from logistic regression (Blanckenhorn et al. 1999a, b). Our study yielded 41 independent sexual selection differentials (one per dung pat), which we then plotted against the estimated local male density.

Results

Mate Choice Experiment

We performed a total of 120 trials with 52 different females. Sixteen of the 52 females did not copulate at all; of these, nine females did not copulate with either male during two trials and seven females died unmated after their first trial. These females were excluded from subsequent analyses. Of the remaining 36 females, only three (8.3%) copulated twice, and none copulated more often, even though some had up to five opportunities. Thus 39 of the remaining 95 trials (41.1%) resulted in copulations of the female with one of the males. Twenty-eight (77.8%) virgin females copulated at their first opportunity, and eight females first copulated at their second or even third opportunity. These results indicate that even virgin females do not readily engage in matings.

The small male performed no mating attempt, or had no chance to perform one before copulation ensued, in 13 of the 39 trials leading to copulation, while this was the case for the large male in only five trials (binomial test: p = 0.096); in all other trials both males attempted to mate at least once. Note that, in all 13 of the cases in which the small male did not attempt to mate, only one mating attempt by the large male occurred before copulation ensued, whereas, in three of the five cases in which the large male did not attempt to mate, more than one mating attempt of the small male was necessary to achieve copulation. In 19 of 27 trials, the male that performed more mating attempts eventually achieved copulation (binomial test: p = 0.052; this includes the 18 cases where one of the males performed no attempt); in all other trials, the two males had the same number of mating attempts. In the 21 trials during which both males attempted matings, the individual first on the female's back was not the one that eventually copulated in seven cases (binomial test: p = 0.190), and in six of these the large male eventually copulated (binomial test: p = 0.125). These results indicate that, in a very basic sense, male (in)activity influences the outcome of mating.

We next asked whether shaking duration is related to the female's willingness to copulate. For all the 32 females that copulated at least once and underwent at least two trials (four of the 36 females died before their second opportunity), we computed the average total shaking duration with the small and the large males, separately for the trials that led to copulation and those that did not. We then performed doubly repeated-measures ANOVAS of mean shaking duration and number of mating attempts (log-transformed) with copulation (yes or no) and male size (small or large) as the repeated factors and female size (head width) as the covariate. Female shaking lasted longer when copulation did not ensue $(F_{1,31} = 7.50, p = 0.011)$ and showed a tendency to increase with female size $(F_{1,31} = 3.72, p = 0.063, partial r = +0.36)$, but shaking duration did not differ according to male size ($F_{1,31} = 0.59$, p > 0.2), nor was the interaction significant $(F_{1,31} = 1.23, p > 0.2, Table 1)$. Also, the number of mating attempts was significantly higher when copulation did not ensue, but there were no other significant effects (effect of copulation: $F_{1,31} = 24.82$, p > 0.001, all other effects p > 0.2, Table 1), while shaking duration per mating attempt was affected only by female size, increasing as female size increased, as above ($F_{1,31} = 3.68$, p = 0.064; all other effects p > 0.2, Table 1). For both shaking duration and male mating attempts, the effect of copulation disappeared when trial duration was entered as a covariate, thus accounting for the fact that trials ended sooner when copulation occurred

Table 1: Values ($\mathbf{x} \pm \mathbf{SE}$) for female and male behaviour in the mate choice experiment
when copulation ensued or did not $(n = 32 \text{ females})$. Female shaking duration and male
mating attempts are given in absolute as well as relative terms, the latter accounting for the
different trial duration when copulation occurred

	Copulation		
	No	Yes	
Shaking duration per trial (s)			
with small male	1048 ± 204	505 ± 147	
with large male	1407 ± 250	488 ± 115	
Time spent shaking (%)			
with small male	19.41 ± 3.79	18.66 ± 4.33	
with large male	25.77 ± 4.90	27.26 ± 5.51	
No. of mating attempts per trial			
from small male	3.26 ± 0.49	1.40 ± 0.28	
from large male	4.09 ± 0.68	1.28 ± 0.20	
No. of mating attempts per hour			
from small male	2.17 ± 0.33	4.12 ± 1.30	
from large male	2.72 ± 0.46	4.74 ± 1.15	
Shaking duration/attempt (s)			
with small male	397 <u>+</u> 93	515 ± 162	
with large male	532 ± 233	424 ± 107	

(Table 1, all factors p > 0.1). This analysis indicates that females unwilling to copulate simply keep on shaking for longer at approximately the same rate in the face of constant male attacks.

Overall, large males had a mating advantage: they obtained 26 of 39 matings (binomial test: p = 0.053). We next tried to identify variables that predict copulation success in the 36 trials of different females that lead to copulation. Whether a female mated with the small or the large male was regressed on a number of independent variables in a stepwise multiple logistic regression. The final model only included trial duration and the shaking durations with the small and the large males (Table 2; all independent variables log-transformed). Trial duration was shorter when the female copulated with the large male (Table 2). Furthermore, shaking lasted longer with the male the female eventually copulated with, so shaking durations with both males (but not the interaction between the two)

Table 2: Values ($\bar{x} \pm SE$) for male and female behavioural and body size traits used in the logistic regression analysis to identify variables that predict copulation success of small and large males in the mate choice experiment (n = 36 females)

	Copulation with:				
	small male (n = 12) large male (n = 24)		Wald χ^2 ; p^1		
Variables in the final model					
Trial duration (s)	3124 ± 557	1390 ± 294	7.76; 0.005		
Shaking duration per trial (s)					
with small male	1072 ± 397	199 ± 113	11.48; 0.001		
with large male	355 <u>+</u> 182	603 ± 187	4.72; 0.030		
Variables not in the final model					
from small male	2.17 ± 0.55	0.71 ± 0.21	1.23: 0.267		
from large male	1.42 ± 0.47	1.17 ± 0.12	1.29; 0.237		
Copula duration (s)	1666 + 156	1734 + 129	0.15: 0.700		
Female head width (mm)	1.03 ± 0.03	1.04 ± 0.03	0.12; 0.733		
Accessory variables Time spent shaking (%)					
with small male	31.03 ± 7.59	16.67 ± 7.95	_		
with large male	10.73 ± 4.24	39.78 ± 7.13	_		
No. of mating attempts per h					
from small male	3.64 ± 0.82	4.19 <u>+</u> 1.67			
from large male	3.41 ± 1.74	6.98 ± 1.30	—		
Shaking duration/attempt (s)					
with small male	828 <u>+</u> 321	188 ± 113	—		
with large male	232 ± 129	588 ± 187			

¹ Stepwise logistic regression results on log-transformed data.

entered the model (Table 2). This reflects a combination of direct or indirect female choice with male persistence (or the lack thereof), since shaking duration can be regarded as a female trait as well as a male trait (his ability to hold on). However, large males did not achieve copulation after shorter struggles with the female than did small males (603 s vs. 1072 s, planned comparison: $t_{34} = 1.24$, p > 0.2, cf. Table 2), which would be expected if shaking duration reflects direct female assessment of her mate. On the other hand, the converse did not occur either (i.e. large males did not achieve copulation after longer struggles with the female), which would have indicated indirect female choice resulting from an interaction of female reluctance and male persistence. Note that the multiple regression approach taken controls for the dependence of shaking duration (and any other variable) on trial duration in a manner equivalent to the computed ratios also presented in Table 2, but that covariance analysis as performed here is generally to be preferred over the analysis of ratios for statistical reasons (e.g. Ranta et al. 1994). Note also that it appears from both Tables 1 and 2 that larger males performed more mating attempts per unit time, but that this effect was never significant when controlling for the other variables.

Male Density Experiment

For this analysis, we excluded all trials where no pairing activity (male mounting, female shaking, oviposition or copulation) occurred. As in the mate choice experiment, the female (i.e. the group) is the experimental unit, as individual males within a group cannot be treated as independent. Repeated-measures ANOVA could not be applied here because the number of repeats varied among treatments (male density). Therefore, analyses were performed on group means (log-transformed), whereby we excluded all inactive males within any group (i.e. those that did not attempt to mate at all).

The female reluctance hypothesis predicts that mating frequency should increase in response to increased harassment by males. The probability that the female copulated did not change with male density (logistic regression: Wald $\chi^2 = 0.688$, p > 0.2), as copulation occurred in 17 of 42 (40.5%), 15 of 38 (39.5%), and 6 of 20 (30%) groups of densities 1, 3 and 6, respectively.

The female reluctance hypothesis further predicts that female rejection behaviour (i.e. shaking) should decrease in response to increased harassment by males. Female shaking occurred during all phases of the pairing process, but most of it occurred before the females started to lay eggs. For those groups where the female eventually copulated, we calculated the percentage of female shaking before, during and after oviposition (i.e. immediately before copulation): this was 71.0, 6.6 and 22.4% for density 1 (n = 17), 82.9, 16.8 and 0.3% for density 3 (n = 15), and 84.4, 4.7 and 10.9% for density 6 (n = 6). Subsequently we only analysed the total amount of shaking during all three phases. The total number of male mating attempts per group (i.e. male harassment) indeed increased with male density (twoway ANOVA: $F_{2,94} = 61.88$, p < 0.001), and was also greater when no copulation ensued ($F_{1,94} = 11.98$, p = 0.001, interaction p > 0.2), while the number of attempts

per active male did not change with male density (Table 3). The effect of male density remained ($F_{2,93} = 54.10$, p < 0.001), while that of copulation disappeared $(F_{1,93} = 0.12, p = 0.727)$, when trial duration was entered as a covariate because there were more mating attempts during the longer trials when no copulation ensued (effect of trial duration: $F_{1,93} = 5.52$, p = 0.021, r = +0.26, cf. Table 3). The total shaking duration per female first increased with male density and then levelled off ($F_{2.94} = 8.06$, p = 0.001), and was lower when copulation occurred $(F_{1.94} = 12.80, p = 0.001, interaction p > 0.2, Fig. 1b)$. At the same time, female shaking per active male tended to decrease with male density ($F_{2.94} = 2.79$, p = 0.066) and was also less when copulation occurred ($F_{1.94} = 11.93$, p = 0.001; interaction p > 0.2, Fig. 1a). Again, for both variables the effect of male density remained (total shaking duration per female: $F_{2,93} = 4.55$, p = 0.013, and female shaking per active male: $F_{2,93} = 5.40$, p = 0.006), while that of copulation disappeared ($F_{1.93} = 1.39$, p = 0.242 and $F_{1.93} = 1.68$, p = 0.198, respectively), when trial duration was entered as a covariate (effect of trial duration: $F_{1,93} = 21.25$, p < 0.001, r = +0.66 and $F_{1.93} = 21.72$, p < 0.001, r = +0.69, respectively).

Larger males had a mating advantage in this experiment too. Males were on average larger in those groups where copulation with any male ensued than in those where it did not (copulation: $F_{1,94} = 11.59$, p = 0.001, density: $F_{2,94} = 5.75$, p = 0.004, interaction p > 0.2, Fig. 2). When comparing the size of the male with whom the female copulated with the mean size of his competitors in the same

	Male density		
	1 male	3 males	6 males
Trial duration (s) not copulated copulated	5400 (n = 25) 1219 \pm 331 (n = 17)	$5400 (n = 23) 2229 \pm 440 (n = 15)$	$5400 (n = 14) 1680 \pm 324 (n = 6)$
No. of mating attempts per active male not copulated copulated Total no. of mating attempts not copulated copulated Total no. of mating attempts per h not copulated copulated	$\begin{array}{c} 2.88 \pm 0.32 \\ 1.71 \pm 0.22 \\ 2.88 \pm 0.32 \\ 1.71 \pm 0.22 \\ 1.92 \pm 0.22 \\ 7.95 \pm 1.17 \end{array}$	$\begin{array}{c} 2.61 \pm 0.21 \\ 1.73 \pm 0.18 \\ 6.13 \pm 0.45 \\ 3.87 \pm 0.39 \\ 4.09 \pm 0.30 \\ 14.76 \pm 4.33 \end{array}$	$\begin{array}{c} 2.00 \pm 0.21 \\ 2.17 \pm 0.31 \\ \\ 8.86 \pm 1.04 \\ 7.50 \pm 1.02 \\ \\ 5.91 \pm 0.69 \\ 17.77 \pm 2.56 \end{array}$

Table 3: Values ($x \pm SE$) for trial durations and male mating attempts in the male density experiment



Fig. 1: Values $(\bar{x} \pm SE)$ for (a) female shaking duration per active male and (b) total female shaking duration as a function of male density in the mate choice experiment for the females which eventually copulated with one of the males (filled circles) and those that did not (open circles). The dotted line denotes the combined shaking duration for both categories. The numbers show the percentage of time the female spent shaking (a) per active male and (b) per group

group for those groups of densities 3 and 6 where copulation ensued (repeatedmeasures ANOVA: $F_{1,19} = 2.50$, p = 0.131), and when considering the size difference between successful and unsuccessful males for density 1 only ($t_{40} = 2.50$, p = 0.172), the same trend was apparent but was not significant (Fig. 2). Lastly, when analysing female shaking duration as a function of male size for density 1, where interactions among males were excluded, we found a significant interaction between size and copulation ($F_{1,38} = 4.18$, p = 0.047, main effects p > 0.1): when no copulation ensued shaking duration increased with male size (r = +0.45),



Fig. 2: Values ($\bar{x} \pm SE$) for head width of all males of those groups where copulation with any male ensued (circles) compared to those where copulation did not ensue (open squares). For the groups where copulation ensued, the mean head width of those males which achieved copulation with the female (filled circles) is additionally compared to the group mean of those which did not (open circles)

suggesting greater persistence of larger males, but when copulation ensued shaking duration decreased with male size (r = -0.34), suggesting earlier female choice of larger males.

Field Estimates of Sexual Selection

Sexual selection differentials per cow pat varied seasonally but tended to be positive and were often individually significant (Blanckenhorn et al. 1999a; Fig. 3). Local competitor density, most probably reflecting population density, affected the strength of sexual selection in the field (Fig. 3). Sexual selection differentials showed a negative relationship with male density (Spearman rank correlation $r_s = -0.39$, n = 41, p = 0.011, as the data are heteroscedastic). However, this correlation



Fig. 3: Standardized sexual selection differentials as a function of male density per cow pat during 1995 data set. Filled circles denote individually significant selection differentials

disappeared when the effect of season was removed, although a negative tendency remained ($r_s = -0.24$, p = 0.132).

Discussion

In *S. cynipsea*, larger males have a mating advantage in the field and in the laboratory (Ward 1983; this study). In principle, this may be due to direct female choice (i.e. assessment and rejection of undesirable males), indirect female choice (i.e. decreased female reluctance to mate due to factors other than mate quality), and/or greater persistence of larger males in scramble competition (Parker 1972a, b; Arnqvist 1992b; Rowe 1992; Ward et al. 1992; Rowe et al. 1994; Wiley & Poston 1996). Both female assessment of males and female reluctance to mate are expressed in prominent female behaviour to repel mates in several insect species including *S. cynipsea* (Parker 1972a; McCauley 1981; Thornhill & Alcock 1983; Ward 1983; Ward et al. 1992; Rowe et al. 1994). Our data indicate that all three mechanisms are operating in *S. cynipsea*, but to varying degrees.

Mating behaviour always results from an interaction between the mating partners. Discriminating between male-male competition and female choice, the two principal mechanisms of sexual selection, is therefore inherently difficult, particularly if the two processes result in the same outcome (e.g. if the trait chosen by the female is also the one that gives the male a competitive edge; Andersson 1994; Wiley & Poston 1996; Birkhead 1998). What we thus can hope for at best is qualitatively, and perhaps quantitatively, to attribute the variance in the outcome of mating to male and female effects. In our mate choice experiment one female faced two potentially interacting males. To obtain a mating, males had to be actively performing mating attempts, and in this sense they obviously (but trivially) influenced the outcome of mating. However, while total shaking duration when no mating occurred was longer due to longer trials, neither shaking duration nor the number of male mating attempts differed according to male size; instead, shaking lasted longer with the male with whom the female eventually copulated (Table 2). These results suggest that longer cumulative shaking durations primarily reflect the female's unwillingness to mate given persistent (i.e. constant) male mating attempts and, conversely, that shorter trial durations and less shaking indicate her willingness (or need) to copulate with a particular male. Ward et al. (1992) also found that females shook less when they had just laid many eggs and therefore diminished their sperm reserves. Results from the male density experiment support this interpretation. When females were confronted with only one male, thus excluding potential effects of male scramble competition, shaking duration decreased with male size when copulation ensued, suggesting female preference for larger males, whereas when no copulation ensued shaking duration increased with male size, reflecting greater persistence of larger males. This trend was also apparent, but non-significant, in the mate choice experiment (Table 1). Overall, therefore, we rank the influence of the female on the outcome of the pairing, i.e. female choice of some sort, higher than that of the male (see also Ward et al. 1992). This might be expected in a species such as S. cynipsea, where males are smaller than females and apparently cannot force copulations (Parker 1972b). However, we acknowledge the possibility that some male trait other than his persistence in mating may explain the better success of large males, as shaking duration can also be interpreted as a male trait (his ability to hold on), ultimately rendering it impossible to disentangle male and female effects definitively.

Differentiating between direct female choice and indirect female choice due to female reluctance to mate is equally difficult, and our results are equivocal in that they yielded evidence for both. As just argued, some sort of female assessment of male quality (e.g. his body weight or his ability to hold on), i.e. direct female choice, is suggested by earlier copulations (in the mate choice experiment) and less female shaking (in the male density experiment) with the larger, preferred males. At the same time, evidence for female reluctance to mate is also seen in (i) the high frequency of mate refusals even by virgin females and the low number of re-matings by non-virgins in the mate choice experiment, (ii) the decrease in shaking duration per male, and the asymptotic shaking duration per female, in response to increasing male density and harassment in the male density experiment, and (iii) the negative relationship of sexual selection differentials and male density found in the field (discussed below). Counter-evidence is (iv) the lack of an increase in mating frequency in response to male harassment in the male density experiment, and (v) a negative (in the male density experiment) or no (in the mate choice experiment) relationship, as opposed to the predicted positive relationship, between shaking duration and male body size when mating occurs. The last result is more consistent with direct female choice.

Our extensive field data set revealed a negative relationship between local male density at the mating site (the cow pat) and the intensity of sexual selection, as predicted by the female reluctance hypothesis (Arnqvist 1992a). Sexual selection can thus show negative density dependence, which by itself may stall evolutionary change. In the field we only measured the pattern, but as just argued we could establish the probable process or underlying mechanism in the laboratory: increased convenience polyandry (Thornhill & Alcock 1983; Arnqvist 1992b; Rowe 1992). The operational sex ratios used are well within those observed in the field, which range up to eight males per female (Blanckenhorn et al. 1999a). An alternative explanation of our field results relates to the fact that sexual selection intensity covaries seasonally with population density and temperature-mediated adult body size in S. cynipsea (Blanckenhorn et al. 1999a) as well as in some other species (Conner 1989; McLain 1992; McLain et al. 1993; Nishida 1994). Population density is typically low at the beginning and the end and high in the middle of the season, while the converse is true for body size (Blanckenhorn et al. 1999a). Lower sexual selection intensities in summer could be explained by direct female choice alone if females had an absolute (or even relative) weight threshold below which they would reject a male (the simplest selection mechanism possible): females then would have difficulties choosing amongst large numbers of invariably small males in summer, but not in spring and autumn when fewer males of a wider range of size are available. The correlation of our sexual selection differentials with male density shown in Fig.3 indeed disappears when using residuals with seasonal temperature variation removed, though a negative trend remains. Thus, our field data may be interpreted as supporting neither the positive relationship between the strength of sexual selection and population density predicted by direct female choice (Sutherland 1987; Wilkinson 1987) nor the negative relationship predicted by the female reluctance hypothesis (Arnqvist 1992a, b), but rather the net result of a combination of both (i.e. no relationship).

Several behavioural studies of water striders support the female reluctance hypothesis in that female frequency and/or the duration of mating increased (Arnqvist 1992b; Rowe 1992; Weigensberg & Fairbairn 1994; Jablonski & Vepsäläinen 1995; Vepsäläinen & Savolainen 1995), and female struggling decreased (Arnqvist 1992b; but not always: Rowe 1992; Weigensberg & Fairbairn 1994), with increasing male harassment. Female reluctance to mate is expected if there are costs associated with mating (Thornhill & Alcock 1983; Rowe et al. 1994). Such costs can be multiple: reduced foraging success (e.g. Wilcox 1984), increased predation (e.g. Fairbairn 1993; Rowe 1994), or internal (e.g. genital; Kummer 1960) or external (e.g. wing; Cartar 1992) injuries which may decrease longevity. Several of these costs are likely to apply in S. cynipsea, and some are currently under investigation. For example, males have species-characteristic spines (setae) on their fore femora, which clasp the female's wing bases (Hennig 1949; Pont 1979) and may cause wing injuries (Mühlhäuser 1998). The males' genitals also feature hooks and spines which could potentially injure females internally (Ward et al. 1992). At the same time it is clear that indirect female choice due to female reluctance to mate can only evolve if the cost of avoiding matings (i.e. the costs of rejecting mates by struggling; Rowe 1994) does not exceed the cost of mating, otherwise females would try to offset one cost with another. Similarly, direct female choice of desirable phenotypes is always in the interest of the female and is expected to evolve unless the costs of choice exceed the benefits (Andersson 1994; Alatalo et al. 1998). In S. cynipsea the cost of rejecting the male and the cost of assessing the male may be the same if both depend on shaking, and this may be the case in many species. Direct and indirect female choice due to female reluctance to mate are therefore likely to co-occur, as they mark the ends of a continuum of two sexual selection mechanisms that are difficult to separate, and the relative importance of which depends on the precise manifestation of their cost and benefits in any given mating system and environment (Rowe et al. 1994; Wiley & Poston 1996; this study). It is thus not surprising that the degree to which direct female choice has been invoked varies in the various water strider studies investigating the female reluctance hypothesis (Arnqvist 1992a, b; Rowe 1992; Rowe et al. 1994; Weigensberg & Fairbairn 1994; Vepsäläinen & Savolainen 1995).

In summary, our study suggests that direct female choice in combination with indirect female choice due to female reluctance to mate is most consistent with the behavioural processes and selection patterns observed in *S. cynipsea*. The effect of male scramble competition appears to be minor but cannot be excluded entirely. To disentangle the effects of direct and indirect female choice in any species it is necessary to assess the costs of mating, such as internal injuries or a greater risk of predation (Kummer 1960; Ward et al. 1992; Fairbairn 1993; Rowe 1994), relative to the costs

of choice and the costs of avoiding matings, such as external (e.g. wing) injuries or a greater risk of predation (Alatalo et al. 1998; Mühlhäuser 1998).

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