The effect of transient food stress on female mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni*

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The effect of transient nutritional stress (sucrose culture medium) on female mate preference was investigated in the stalk-eyed fly *Cyrtodiopsis dalmanni*. Two experimental treatments were run in parallel. In the first, female preference was initially tested on corn (standard medium) and subsequently on sucrose (novel medium). In the second, female preference was tested in the reverse order: sucrose then corn. Females fed on corn had stronger preferences for large-eyespan males than females fed on sucrose. Female age, experience or the order of exposure to culture media had no effects on the strength of preference. An additional experiment demonstrated that the sucrose diet reduced the number of developing and mature eggs. We discuss the adaptive significance of changes in female preference due to transient nutritional stress, and the effect of changes in female preference on the strength of sexual selection.

Keywords: condition dependent; handicap; mate preference; nutritional stress; sexual selection; stalk-eyed fly

1. INTRODUCTION

Until recently, little was known or understood about variation in female mating preferences, despite the great attention paid to sexual selection over the last decade (Andersson 1994). There is some evidence for genetic variation in female preference, but preference genes have not yet been mapped (Bakker & Pomiankowski 1995). There is more evidence for environmental factors affecting preference: for example, several studies have measured variation in mate preference against predation risk, parasitism, copying, age and experience (Jennions & Petrie 1997). Two experimental studies have demonstrated that female morphological phenotype affects preference. Jennions *et al.* (1995) found that larger female African painted reed frogs (*Hyperolius marmoratus*) were more likely to choose lower-frequency sounds than smaller counterparts when given a choice between two artificial male calls. In addition, Hingle *et al.* (2000) showed in the stalk-eyed fly species *Cyrtodiopsis dalmanni* that females with larger eyespan were better at distinguishing between males. When the difference between males was large, both large- and small-eyespan females preferred the larger males, but when differences between males were slight only the large-eyespan females preferred the larger males.

These morphological phenotypes may be closely related to female condition. Traditionally, condition has been thought of as the nutritional state of an individual (Andersson 1982; Nur & Hasson 1984). More recently, condition has been redefined more broadly as the pool of resources that can be allocated to different traits (Rowe & Houle 1996). As such, condition is likely to be closely linked to major fitness components such as fecundity and body size. This has led some authors to suggest that mate choice will often show condition dependence (Jennions & Petrie 1997; Bakker 1999), since it is a costly behaviour in many species (Pomiankowski 1987). There is some recent,

indirect evidence for this hypothesis. Bakker *et al.* (1999) found that female sticklebacks (*Gasterosteus aculeatus*) from high-condition families preferred red-bellied males. In contrast, females from low-condition families preferred orange-bellied males. López (1999) found that unparasitized female guppies (*Poecilia reticulata*) showed stronger preferences for the attractive male. Infected females swam less frequently between males than control females, and signalled acceptance of mates more frequently. Although not measured directly, it is likely that parasite infection reduced female condition.

Here, we investigated how a component of condition affects mate preference in the stalk-eyed fly *C. dalmanni*. A sucrose diet was used to cause nutritional stress, as sucrose has been shown to reduce egg laying in other insect species, such as *Drosophila melanogaster* (Ashburner 1989). This effect of sucrose is reversible because females quickly recover their egg-laying ability when they are moved back to normal food (Ashburner 1989). Using a transient stress allowed us to test the same females when in high and low condition.

We also used a stress that affects egg laying because this is likely to be relevant to conditions experienced in the wild. In *D. melanogaster*, females show a wide range of variation in egg production under natural conditions. Indeed, as many as 70% of females in the wild show no ovarian activity (Boulétreau 1978). This is probably due to nutritional stress, and is usually abolished in females reared in the laboratory with food provided *ad libitum* (Boulétreau 1978). Similar variation in ovarian activity has been found amongst wild-caught *C. dalmanni* (Wilkinson & Reillo 1994).

In the first experiment, we investigated the effect of transient nutritional stress on female preference in *C. dalmanni*. A second experiment was carried out to assess the effect of the transient nutritional stress on the number of mature and developing eggs. This experiment allowed us to confirm that a sucrose diet reduced female fecundity and that this effect was reversible.

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2. MATERIAL AND METHODS

(a) *Flies and rearing*

Flies were taken from a laboratory population descended from a field collection made in 1993 by A.P. in Ulu Gombak, Malaysia. The population has been maintained at a large population size in cage culture at 25 °C on a 12 L:12 D cycle. Eggs were collected over a two-week period. Damp paper towels were placed on the floor of stock cages for 24 h. Eggs laid on the paper were transferred to plastic containers lined with moist cotton wool and filter paper covered with a thin layer of puréed sweetcorn. After 15 days, pupae were removed from the filter paper and placed in cages to eclose. Adult flies were fed on puréed sweetcorn that was changed twice per week, until the flies reached sexual maturity (at six weeks). Two weeks after eclosion, flies were separated into virgin male and female groups to prevent mating.

(b) *Experiment 1: female preference*

Sexually mature females (six to eight weeks old) were collected over ice, and their eyespans were measured using a digital camera attached to a monocular microscope and computer. Any females with eyespans of less than 6 mm were discarded, as a previous study had showed that they have weaker mate preference (Hingle *et al.* 2000). The remainder were assigned to 24 cages (14 cm × 14 cm × 30 cm), until each contained six females.

Cages of females were divided equally between two treatments. One treatment group was fed a sucrose solution twice weekly. Sucrose was administered as a solution of 65 g of sucrose dissolved in 260 ml of boiled water. The solution was allowed to cool and then placed in small weighing-boats. The second treatment group was fed puréed sweetcorn twice weekly in similar weighing-boats. Flies were kept on this initial food regime for 30 days. Feeding regimes were then reversed: sucrose-fed females were given sweetcorn, and sweetcorn-fed females were given sucrose. Flies were kept for a further 30 days on the new food regime. Each 30-day period was split into two parts. For the first 14 days, flies were allowed to acclimatize to the food regime. For the following 16 days, female preference was measured.

A total of 20 cages was observed: ten sucrose–corn cages and ten corn–sucrose cages. When females from experimental cages died, they were replaced using females from replacement cages that followed exactly the same food regime but were not observed. Twenty-one replacement females were required for sucrose–corn cages (13 when fed corn medium and eight when fed sucrose medium). Fourteen replacement females were required for corn–sucrose cages (nine when fed corn medium and five when fed sucrose medium).

Sexually mature males (six to eight weeks old) were collected at the same time, and their eyespans were measured using the same procedure. They were divided into two groups of 30. One group had large eyespans (greater than 9.0 mm, mean $\pm$ s.d. 9.13 mm $\pm$ 0.03 mm) and the other small eyespans (less than 7.5 mm, 6.67 $\pm$ 0.08 mm). In addition, a further 20 large- and small-eyespan males were held separately and used to replace dead males, so as to maintain a constant density. Males were maintained throughout the experiment on a corn diet (except when they were being used to evaluate female mate preference).

Female mate preference was measured on a daily basis. At dusk (defined as the last half-hour before lights were turned off) on the first day, large-eyespan males were taken from their

holding cage and put into five cages from the sucrose–corn treatment and five cages from the corn–sucrose treatment. Similarly, small-eyespan males were taken from their holding cage and put into the remaining five sucrose–corn cages and five corn–sucrose cages. Mating behaviour was observed from dawn on the following day, for 1.5 h. In *C. dalmanni*, very little mating occurs at other times of the day (Wilkinson & Reillo 1994). During this period, the number of copulations by each male was recorded. We defined copulation as the mounting of a female by a male, and engagement of their genitalia. All observations were made by A.H. After this period, males were removed and returned to the large and small holding cages. At dusk on the second day, female cages that had been tested with large-eyespan males were tested with small-eyespan males, and vice versa. This procedure was repeated for a total of 16 observational days in each stage of the experiment.

Males could not be left in cages all day, as this may have led to variation in male condition when exposed to the different food media. We could not add both large- and small-eyespan males to the same cage at dusk, as this would have led to male-to-male competition and so obscured the effects of female mate choice. Given these constraints, we decided that the best solution was to place large- and small-eyespan males in experimental cages on alternate days.

Female mate preference was calculated at the level of the cage, using the total number of copulations with large and small males. Copulation preference was measured as $CP = C_L - C_S$, where C_L is the number of copulations by the large males, and C_S is the number of copulations by the small males, over the full 16 days of observation.

A number of further checks on the data were carried out. Our measure of preference, CP, could be subject to a bias in favour of cages in which more copulations occurred. For example, if two female cages had the same ratio of small-eyespan male to large-eyespan male copulations, the cage with the greater total of copulations would be assigned a stronger preference. So we tested for any dependence of CP on the total number of copulations. We also looked for changes in the number of copulations through time, and differences in the total number of copulations of flies on sucrose and corn diets. Finally, as a precaution, we re-analysed all CP tests using a relative preference measure $CP_R = (C_L - C_S)/(C_L + C_S)$.

(c) *Experiment 2: egg production*

Female reproductive potential was measured by counting the number of eggs in female ovaries. Six cages were set up, each containing 30 sexually mature females and five sexually mature males. Two of the cages were assigned to each of three treatments: sucrose–corn, corn–sucrose and a control. Sucrose–corn flies were fed sucrose from day 1 to day 8, and then switched to corn until day 22. Corn–sucrose flies were fed corn from day 1 to day 8, and then switched to sucrose until the end of the experiment (day 22). Control treatment flies were fed corn throughout the experiment.

Starting on day 8, and then on every second day (i.e. days 10, 12, etc., until day 22), three females were randomly sampled from each cage and placed on ice. Their ovaries were dissected and placed on a microscope slide. Digitized images of the ovaries were recorded and the numbers of mature and developing eggs counted. Mature eggs were defined as stages 12–14 and developing eggs as stages 8–11 using King's standard stages of oogenesis (King 1970; Ashburner 1989). All ovaries were dissected and scored by A.H.

(d) Statistical procedures

CP distributions were normally distributed using the Shapiro–Wilks' W -test (Zar 1984). Distributions of CP were tested for deviations from random mating (mean of zero) and differences between treatments using t -tests. Paired t -tests were used to compare the effect of food within the same cage. Differences in copulation frequency between food media and experimental stages were analysed in a model I two-way ANOVA with experimental stage and food as fixed factors. The dependency of CP on copulation number was analysed using least-squares regression for sucrose-fed flies separately for each treatment and for corn-fed flies separately for each treatment. The numbers of mature and developing eggs in the ovaries were compared between treatments and the control using t -tests for all days on which dissections were made (days 8, 10, . . . , 22). A sequential Bonferroni correction (Sokal & Rohlf 1995) was used to adjust for multiple comparisons, and is reported where significant. All t -tests were two-tailed.

3. RESULTS

(a) Experiment 1: female preference

Pooling over both treatments (sucrose–corn and corn–sucrose), females preferred to copulate with large-eyespan males (mean $\pm$ s.d. CP = 26.65 ± 13.24 ; t -test, $t_{19} = 9.002$, $p < 0.001$). Previous studies have shown that there is a similar preference for larger-eyespan males when females are given a binary choice between small- and large-eyespan males (Wilkinson *et al.* 1998; Hingle *et al.* 2000). Our method differs from these previous studies as small- and large-eyespan males were presented on alternate days.

Similar mate preference was seen when analysing each treatment separately. Sucrose–corn females (CP pooled across food media) preferred large-eyespan males (CP = 26.50 ± 14.96 , $t_9 = 5.601$, $p < 0.001$), as did corn–sucrose females (CP = 26.80 ± 12.09 , $t_9 = 7.010$, $p < 0.001$), and there was no difference in preference due to the order in which females were fed sucrose and corn diets ($t_{18} = 0.346$, $p = 0.564$).

We next pooled females by diet (corn or sucrose). Corn-fed females showed a strong preference for large-eyespan males (CP = 22.05 ± 9.34 , $t_{19} = 10.553$, $p < 0.001$). This preference was exhibited by both the sucrose–corn females (CP = 20.80 ± 7.05 , $t_9 = 9.327$, $p < 0.001$) and corn–sucrose females (CP = 23.30 ± 11.45 , $t_9 = 6.435$, $p < 0.001$; figure 1). In contrast, sucrose-fed females showed a much weaker preference for large-eyespan males (CP = 4.60 ± 7.37 , $t_{19} = 2.790$, $p = 0.012$). This weaker preference for large-eyespan males shown by females on sucrose was lost when treatments were analysed separately, as both sucrose–corn females (CP = 6.00 ± 7.97 , $t_9 = 2.38$, $p = 0.041$; n.s. after sequential Bonferroni adjustment with $k = 9$ tests for preference) and corn–sucrose females (CP = 3.20 ± 6.84 , $t_9 = 1.479$, $p = 0.173$; figure 1) showed no evidence of mate preference on sucrose.

A more exact test for differences in preference on the two food sources was possible because the same cages of females were fed sequentially corn then sucrose (or vice versa), allowing a direct comparison of preference of the same females on different food. Females had stronger preferences for large-eyespan males when fed corn rather than sucrose (paired t -test: $t_{19} = 7.508$, $p < 0.001$).

Figure 1. Mean preference of each treatment at each stage of the experiment (i.e. when fed sucrose or corn); error bars indicate standard errors. Tests for difference from random mating, after sequential Bonferroni adjustment, are indicated by n.s., $p > 0.05$ and *** $p < 0.001$. Sucrose–corn and corn–sucrose flies did not differ in preference when fed the same diet.

Sucrose–corn and corn–sucrose females showed similar effects when analysed separately (sucrose–corn, $t_9 = 5.530$, $p < 0.001$; corn–sucrose, $t_9 = 5.220$, $p < 0.001$; figure 1), which indicates that the reduction in strength of preference from corn to sucrose was not affected by the order in which food was changed.

Further tests were carried out to test for confounding factors relating to changes in the total number of copulations in different groups. First, we tested for dependence of the copulation preference (CP = $C_L - C_S$) on the total number of copulations ($C_L + C_S$). CP did not increase with the total number of copulations for corn-fed or sucrose-fed flies in either treatment (all $p > 0.05$).

Second, we checked for changes in the number of copulations through time. There was no difference between the number of copulations measured in the first and second 30 days ($F_{1,36} = 2.356$, $p = 0.134$). There was also no affect of food on the copulation rate ($F_{1,36} = 3.379$, $p = 0.074$). The interaction term was also non-significant ($F_{1,36} = 2.241$, $p = 0.143$), showing that the age of females did not affect their copulation rate differently on corn and sucrose. We also checked for changes in preference through time. The corn–sucrose females were 30 days older than the sucrose–corn females when their mate preference was tested on corn, and 30 days younger when their mate preference was tested on sucrose. In neither case did these differences in age and experience make a detectable difference to the strength of female preference on a particular food source (corn, $t_{19} = 0.346$, $p = 0.564$; sucrose, $t_{19} = 0.710$, $p = 0.411$).

Finally, all CP analyses presented above were repeated using CP_R, which controls for variation in copulation number between cages. The same results were found as with the CP measure of preference.

Figure 2. Paired ovaries taken from females fed for eight days on (a) corn or (b) sucrose, on the same scale.

(b) *Experiment 2: egg production*

The ovaries of females fed on sucrose were shrunken in comparison with those of females fed on corn (figure 2). Pooled dissection counts from six females through time are shown in figure 3. The mean number of mature eggs in the ovaries of sucrose–corn females on day 8 was fewer than that in the control group ($t_{10}=3.796$, $p=0.004$), but did not differ between the corn–sucrose and control groups ($t_{10}=1.210$, $p=0.254$; figure 3a). A similar pattern was seen for the number of developing eggs: sucrose–corn females had significantly fewer developing eggs on day 8 than control females ($t_{10}=5.053$, $p<0.001$), but the corn–sucrose and control females did not differ ($t_{10}=0.065$, $p=0.950$; figure 3b).

Food was changed on day 8 in the sucrose–corn and corn–sucrose treatments. An increase in ovarian activity in response to the new corn diet was seen in the sucrose–corn females relative to control females by day 18 for mature eggs ($t_{10}=0.072$, $p=0.944$; figure 2a) and by day 16 for developing eggs ($t_{10}=1.736$, $p=0.113$; figure 3b). The new sucrose diet of corn–sucrose females caused a reduction in the number of mature and developing eggs relative to control females, which became significant by day 22 for mature eggs ($t_{10}=2.373$, $p=0.039$) and by day 18 for developing eggs ($t_{10}=3.282$, $p=0.008$).

4. DISCUSSION

Our experimental design allowed us to investigate the effect of a transient food stress on female mate preference in *C. dalmanni*. Sucrose-fed females showed a weak or non-significant preference for large-eyespan males. The same females had a significantly stronger preference for large-eyespan males when fed on corn. This result provides strong evidence that diet alters the strength of female preference. The experiment was designed to test for a number of other factors, but none of these could account for the data recorded. Females were switched from

sucrose to corn, or from corn to sucrose. The ordering of the food stress had no effect on female preference: the change in preference was the same, though in the opposite direction, for flies moved from sucrose to corn and vice versa. This further showed that food stress did not cause any permanent change in female preference. There was also no detectable change in female preference that could be explained by age or experience. Nor was there any evidence of changes in copulation rate due to the different diets.

To further elucidate the observed change in female preference, female egg production on sucrose and corn media was examined (figure 2). Variation in the numbers of developing and mature eggs was large. However, it was clear that females on sucrose tended to have reduced numbers of mature and developing eggs. This reduction was apparent after 8–14 days on the sucrose diet. However, the effect of sucrose on reducing egg production was reversible: once put back on a corn diet, egg counts returned to the level seen in control flies.

Two types of interpretation are consistent with the results obtained in our experiments. The first is that females reduced the effort invested in mate preference when they had no eggs to fertilize. This makes adaptive sense, as females are unlikely to benefit from choosing between males when they are not laying fertile eggs. Such an explanation makes a number of assumptions, in particular that females gain fitness benefits from mating with large-eyespan males and avoid fitness costs when they fail to exert mate preference. There is evidence that male eyespan is a sensitive indicator of male genetic quality in stalk-eyed flies (David *et al.* 1998, 2000; Wilkinson & Taper 1999), but there are no direct or indirect measures of costs associated with female mate choice. In this species, the male spermatophore is small and unlikely to provide any nutritional benefits to the female (Kotrba 1996). Mating is quick and usually completed within 50 s (Baker *et al.* 2001). In the laboratory, males frequently

Figure 3. Mean number of (a) mature and (b) developing eggs in the ovaries of six females from one of three treatments: sucrose-corn, corn-sucrose and control (always corn). Error bars indicate the standard error of the mean. Food type was changed on day 8.

approach females for copulations that females can refuse by walking or flying away. These observations suggest that if there are costs of choice, they are very minor.

The second explanation is that sucrose caused an overall change in female behaviour, of which female preference is just one aspect. Against this hypothesis, females did not change their copulation rate as a result of sucrose exposure. Likewise, females fed on sucrose did not appear to be any less active or show any obviously abnormal behaviour. There was also no increase in mortality when flies were kept on the sucrose diet in experiment 1. So we have no evidence for an overall change in female behaviour. It would be illuminating to study other interventions that alter egg production, to see whether there is a direct association of this with changes in female preference.

In the wild, there is substantial variation in egg number among female stalk-eyed flies. Wilkinson & Reillo (1994) reported that mature egg numbers ranged from zero to 35 per female. The reduction of ovarian activity in females fed sucrose therefore mimics the range of ovarian activity observed in the wild. This suggests that the changes in female preference observed in this study are relevant to female behaviour in the wild. The effect of transient nutritional stress in the wild will be to weaken female mating preference. However, the net effect on male eyespan may be either neutral, or

even an increase in selective pressure. Stressed females mated randomly but produced few offspring, whereas unstressed females had strong preferences and produced many eggs. So productive females tend to be those that mate with preferred males.

Our previous study of phenotype-dependent female preference in stalk-eyed flies showed that females with wider eyespans had stronger preferences when the difference between potential mates was relatively slight (Hingle *et al.* 2000). The present study shows that transient stresses that affect fitness components (and hence condition) over short periods of time can also alter female preference. If condition-dependent mate preference is a general phenomenon, and high-condition females also tend to be the most fecund, the strength of sexual selection may be increased.

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