



Female swordtail fish use chemical cues to select well-fed mates

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Selection favours females that attend to reliable information about male health or genetic quality. Male nutritional state can be an important indicator of mate quality since poor nutrition can be related to reduced sperm quality, low sperm quantity and sexually transmitted diseases. Here we show that female swordtail fish, *Xiphophorus birchmanni*, preferred the chemical cues of males that were well fed over those that had been food deprived. However, focal females did not discriminate among the odour of other females with respect to nutritional state, suggesting that they were responding to a sex-specific chemical cue, not simply to food excretions. It is therefore likely that nutritional condition is related to the production of sexual pheromones in males. Our results suggest that chemical cues convey information about male nutritional state, and that females attend to these cues during mate choice. By doing so, females are able to discriminate among males that may differ in important aspects of quality.

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Even in species where females do not receive direct benefits from males, it pays females to attend to male traits that indicate male health and/or genetic quality (Andersson 1994; Kokko et al. 2003). First, selection will favour females making decisions that minimize the cost of mating. Females should thus prefer to mate with males that are less likely to carry disease and more likely to have viable sperm. Second, females may prefer traits that are honest indicators of heritable variation in male genetic quality. Nutritional condition may be a particularly important indicator of male health or genetic quality. Poor nutrition in males can indicate inability to contend with a fluctuating environment (Plath et al. 2005) and has been associated with reduced sperm count (Dunn & Moss 1992), low sperm quality (Izquierdo et al. 2001) and susceptibility to disease (Moret & Schmid-Hempel 2000), so it benefits females to attend to cues that provide information about male nutritional state. In the house finch, *Carpodacus mexicanus*, males in good condition show increased growth rate and brighter, more conspicuous plumage (Hill & Montgomerie 1994), which females prefer (Hill 1990). The notion that females use signals to select mates reared on a particular diet has also been tested in poeciliid fish. For example, in guppies, *Poecilia reticulata*, diets high in carotenoids lead to brighter red and

orange spots on males, which females prefer (Kodric-Brown 1985, 1989), and in cave mollies, *Poecilia mexicana*, females prefer to associate with well-nourished males over starved males and discriminate between males using visual and nonvisual (likely mechanosensory) cues (Plath et al. 2005).

Chemical cues may be especially reliable indicators of male nutritional state since the metabolic energy can be converted directly into the cue (Bradbury & Vehrencamp 1998), and females may attend to excretions carrying cues of preferred prey items. For example, in the beetle, *Neopyrochroa flabellata*, females sample pheromones from the male's cephalic gland and mate preferentially with males fed on cantharidin (Spanish fly, *Lytta vesicatoria*; Eisner et al. 1996). Specific nutrients can also attract potential mates to the cue. Ferkin et al. (1997) showed that both male and female meadow voles, *Microtus pennsylvanicus*, are attracted to the odour of opposite-sex conspecifics on a high-protein diet; however, this study did not exclude the possibility that individuals were attracted to protein by-products, such as volatile amino acids in urine (Nation 2002). Furthermore, diet can affect olfactory-related behaviours, as in the Mediterranean fruit fly, *Ceratitidis capitata*, where protein increases a male's probability of releasing pheromones in a lek (Yuval et al. 2002).

We tested the hypothesis that male nutritional state directly affects the sexual attractiveness of chemical cues in the swordtail fish, *Xiphophorus birchmanni*, controlling for female preference for dietary excretions. Chemical communication is an important method of species

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recognition in both female (Fisher et al. 2006) and male (Wong et al. 2005) *X. birchmanni*, as well as females in other swordtail species (Crapon de Caprona & Ryan 1990; McLennan & Ryan 1997, 1999; Hankison & Morris 2003). We suggest that the widespread usage of chemosignals may reflect their value as indicators of male quality.

Xiphophorus birchmanni live in lowland, freshwater streams in the Río Pánuco basin in eastern Mexico (Rauchenberger et al. 1990). These streams are subject to seasonal flooding, drought and significant anthropogenic disturbance (Fisher et al. 2006), which are likely to influence resource availability. *Xiphophorus birchmanni* live in groups with intense male–male competition (personal observation; cf. *X. nigrensis*, Morris et al. 1992), thus physical condition is likely to vary considerably among males (Maynard Smith 1982). As expected, females in the genus attend to intraspecific variation in a number of male traits (Rosenthal et al. 1996, 2001; Kingston et al. 2003), and *X. birchmanni* females discriminate among males based on body size and dorsal fin size (H. S. Fisher & G. G. Rosenthal, unpublished data). Here we test two hypotheses: (1) females attend to chemical cues indicating nutritional condition of males, and (2) these cues are sex specific and are used in mate choice.

METHODS

All subjects were wild-caught adults from the Río Garces, Hidalgo, Mexico (20°57'22N, 098°16'48W). In captivity, animals were housed in large (200-litre) aquaria, maintained on a 12:12 h light:dark cycle, and fed twice a day on TetraMin flake food (Tetra, Melle, Germany) ad libitum and supplemented with live brine shrimp (*Artemia* sp.).

Female Preference Tests

We randomly selected 30 females and 10 males from the population. The males were divided into two groups (A and B) of five and each group was placed into its own well-planted 40-litre aquarium; females were housed separately in a 200-litre aquarium. In experiment 1, during the testing period, group A males (well fed) were given 0.1 g of TetraMin at 0900, 1100, 1300 and 1500 hours (wild swordtails are most active and feed during this midday period; personal observation), the focal females were fed 0.5 g TetraMin at the same intervals, and group B males (food deprived) were given no food for five consecutive days. Withholding food for this amount of time and longer is not detrimental to the health of the fish and has been used in other nutritional studies of poeciliid fish (Plath et al. 2005). After this 5-day period, we conducted preference tests (see below) using 15 females, then returned all animals to a normal feeding schedule. After 10 days we performed experiment 2, which involved switching group A males to group B conditions and vice versa. We tested a new group of 15 focal females with these males. During the study, all animals were closely monitored, and no disease or deaths occurred.

Stimulus preparation and preference tests closely followed published methods (McLennan & Ryan 1997,

1999). We prepared stimulus water for female mate choice experiments by placing five males into a single 20-litre collection aquarium adjacent to a 20-litre aquarium containing five conspecific females (to provide visual stimulation). Males remained in the collection aquarium for 3 h. Preference tests were conducted in an aquarium (length \times width \times height = 75 \times 30 \times 30 cm) divided lengthwise into three equal sections by lines drawn on the sides of the tank, and filled with 40 litres of water. Each test tank had two stimulus delivery systems located at either end of the tank carrying stimulus water from well-fed and food-deprived males. Each stimulus delivery system consisted of 2.5-mm silicone tubing fixed to the bottom of a collection aquarium with a suction cup on one end, and 1 cm above the waterline of the test tank on the other end. The collection aquarium was raised above the test tank to aid the flow of stimulus water into the test tank, and a valve controlled the flow to a rate of 5 ml/min.

Each female was acclimatized for 30 min in the test tank containing testing water; stimulus flow was then initiated. A preference test began once the female had passed into both side compartments, and continued for 300 s. If the female did not visit both compartments within 300 s, the trial was ended. Variables scored were (1) latency, the time from initiation of stimulus flow until the female came within 25 cm of each stimulus outflow, and (2) association time, the total time spent within 25 cm of stimulus outflow. In cases in which the female failed to respond to both stimuli within 300 s, latency was scored as 300 s for the stimulus not visited, and association time was scored as zero. Association time is widely used in studies of mate choice in fish, and is the standard measure of mating preferences in poeciliids, including swordtails (see Wong et al. 2005 and references therein). In a closely related species, *X. nigrensis*, association time in laboratory trials is a strong predictor of association in open-field trials and of observed mate choices in the wild (Ryan et al. 1992). Association time is thus a meaningful estimate of mating preference.

To determine whether the females were attending to food cues contained in the male excretion, or sexual pheromones, we repeated the experiment but used conspecific females to produce the stimuli. Methods followed those stated above, except that we selected new females for the 'well-fed' and 'food-deprived' groups. In this part of the experiment, females were less responsive, so we tested 30 females to obtain sufficient data for statistical analysis.

Statistical Analysis

We used a chi-square test to compare the number of responsive females in the trials conducted on male and female chemical stimuli. To assess female preference for well-fed and food-deprived stimuli within each set of trials, we analysed the association time data using paired, two-tailed *t* tests. To compare between sets of trials, we used the net association time (association time with well-fed stimuli – time with food-deprived stimuli) and performed unpaired, two-tailed *t* tests. We then used

paired, two-tailed t tests to compare female latency to each stimulus.

RESULTS

Females showed a significant preference for the cues of well-fed males over those of food-deprived males in all trials combined (paired t test: $t_{26} = 3.92$, $P = 0.0005$; Fig. 1). This preference was expressed in the first experiment (paired t test: $t_{13} = 3.00$, $P = 0.01$) and, more importantly, in the second experiment, when the previously preferred well-fed males were assigned to the food-deprived group and vice versa (paired t test: $t_{12} = 2.47$, $P = 0.03$). Correspondingly, there was no significant difference between experiments in females' net preference (unpaired t test: $t_{12} = 0.038$, $P = 0.97$; Fig. 2).

Focal females showed no preference for cues of well-fed or food-deprived females (paired t test: $t_{17} = 0.410$, $P = 0.68$; Fig. 3). Significantly fewer females responded to cues from both well-fed and food-deprived females (30 females tested, 18 responded) than to the same cues from males (30 females tested, 27 responded; chi-square test: $\chi^2_1 = 7.2$, $P = 0.0073$).

There was no significant difference in female latency to respond to both stimuli among trial types (paired t tests: male cue trials: $t_{26} = 0.32$, $P = 0.75$; female cue trials: $t_{17} = 1.02$, $P = 0.32$).

DISCUSSION

Our findings suggest that female *X. birchmanni* discriminate between well-fed and food-deprived males via chemical cues and use this information to associate more frequently with well-fed males (Fig. 1). Our study further revealed that this association is not explained by a general attraction to well-fed individuals, or to individuals fed a similar diet (Ward et al. 2004), because focal females were not attracted to cues from well-fed females (Fig. 3). Selection should favour food-deprived males that maintain their attractiveness to conspecific females, as is the case in meadow voles (Pierce et al. 2005); however, in a fluctuating

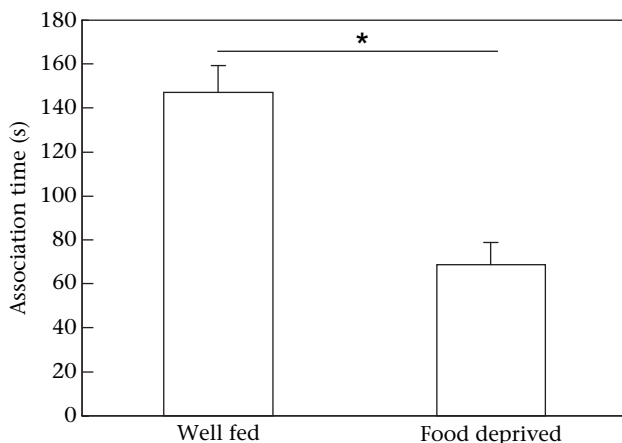


Figure 1. Mean + SE time that focal females associated with chemical cues from well-fed and food-deprived males. * $P = 0.0005$.

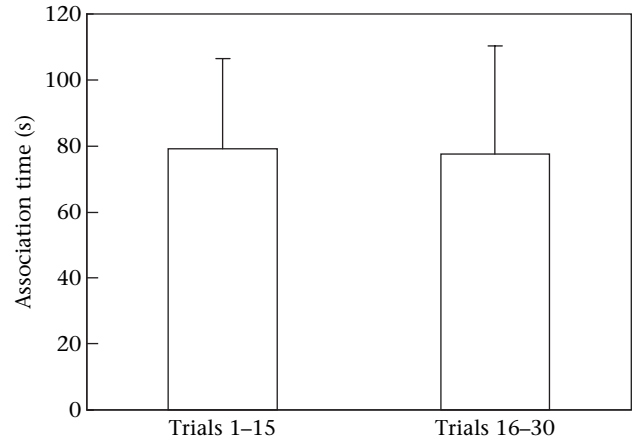


Figure 2. Net mean + SE time that focal females associated with chemical cues from well-fed and food-deprived males (time spent with chemical cues from well-fed males – time spent with chemical cues from food-deprived males). Trials 1–15: group A = well-fed males; group B = food-deprived males. Trials 16–30: male roles reversed.

environment influenced by seasonal and stochastic events that reduce resource availability, the ability to forage consistently enough to maintain energy reserves should also reflect successful competition for food resources. In this study, males were either well fed or food deprived, and females consistently preferred the well-fed group. Therefore, our results suggest that, in swordtails, (1) male chemical cues function as a dynamic indicator of male quality (Rosenthal et al. 1996), providing females with information about current male condition, and (2) that females use this information for optimizing their mating behaviour.

There are several plausible explanations for how females are able to differentiate among males based on nutritional status, and why they are attracted to cues produced by well-fed males. First, nutritional state may impose a mechanistic constraint on the production of sexual pheromones. Regular feeding may be required by male swordtails to produce, or produce enough, cue to attract females.

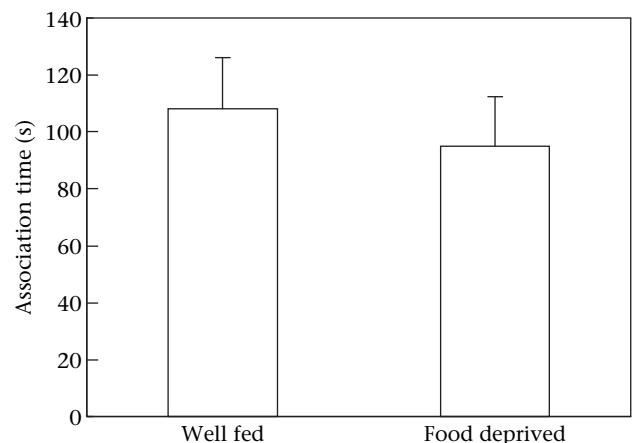


Figure 3. Mean + SE time that focal females associated with chemical cues from well-fed and food-deprived females.

However, in Hawaiian *Drosophila*, there is no relation between feeding regime and the quantity of pheromone that males deposit (Jones & Widemo 2005). Second, female swordtails may be attending to a direct, dietary by-product, but only in combination with a sex-specific signal. Third, females may be attracted to a competitive signal used in male–male interaction, and only well-fed males compete. All of these explanations are consistent with the notion that the chemical signal produced by well-fed males is a sexually selected trait used by females in determining ‘high-quality’ mates.

Overall, females associated significantly more with the well-fed male stimulus. All females, however, did at least inspect the food-deprived male cue, and some females even associated with this cue during the trial. Thus, we hypothesize that females may show variation in their preference for well-fed males depending on their own nutritional state (Fisher & Rosenthal, *in press*), or that they may be less attentive to male cues when they are exploring their environment, possibly in search for food.

Nutritional condition can contribute significantly to individual odour cues (Brown *et al.* 1996; Olsén *et al.* 2003) and encompasses most of the important aspects of male quality. For example, poor nutrition has been associated with inability to contend with environmental fluctuations (Plath *et al.* 2005), reduced sperm count (Dunn & Moss 1992), poor sperm quality (Izquierdo *et al.* 2001) and increased susceptibility to disease (Moret & Schmid-Hempel 2000). Swordtail females would therefore benefit by selecting the highest-quality male they can find. The importance of making the most informed and accurate mating decision possible is reinforced by the observation that mating can be costly for females, often because of reduced foraging time and exposure to disease. Costs are particularly high in viviparous fish like *Xiphophorus* with internal fertilization. Viviparous females pay high physiological costs and are exposed to increased risk of predation when gravid. Moreover, the male intromittent organ, the gonopodium, in poeciliids is armed with hooks, spines and claws; it attaches onto the female’s genitalia and is only freed after vigorous shaking (Constantz 1989).

Even in species where males provide no direct benefits, nutritional condition can provide critical information for mate choice. Chemical signals, either directly through control of pheromone production, or indirectly as excreted metabolites, are likely to be an important source of information about nutritional condition for mate choice.

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