



Female preference for fly song: playback experiments confirm the targets of sexual selection

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(Received 19 September 1997; initial acceptance 1 December 1997;
final acceptance 14 February 1998; MS. number: 5651)

ABSTRACT

The courtship song of *Drosophila* is thought to be involved in sexual selection and species recognition. Because of the mating system of flies, however, directly demonstrating that song influences female preference is difficult. The majority of previous studies have used an experimental design that potentially confounds male and female reactions to song. In *D. montana*, correlational evidence has suggested that males that produce short sound pulses consisting of a high number of sound cycles (i.e. a high carrier frequency) have a higher mating success than other males. In this study, we played synthetic song that varied in pulse length and carrier frequency to individual females in the laboratory, both alone and in the presence of mute males. We scored female preference via an acceptance posture, 'wing spreading', which the females of this species usually display prior to mounting by a male. Females responded to synthetic song in the absence of males. The presence of mute males significantly increased their overall responsiveness, but the relative effectiveness of the songs did not change, eliminating male reaction to song as a possible confounding factor in the results. The interaction between pulse length and carrier frequency determined the discrimination between song types, with females responding most readily to song consisting of short pulses with a high carrier frequency. Thus, direct examination of female preferences supports the previous studies of male mating success, and confirms female song preference as a likely determinant of male mating success.

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Studies of the courtship songs of fruit flies have most often concentrated on their potential role in speciation (e.g. Bennet-Clark & Ewing 1969; Kyriacou & Hall 1982; Ewing & Miyan 1986; Tomaru & Oguma 1994a; Tomaru et al. 1995) or on their genetic control (Cowling 1980; Kyriacou & Hall 1986; Hoikkala & Lumme 1987; Tomaru & Oguma 1994b; Pugh & Ritchie 1996; Ritchie & Kyriacou 1996). These songs, however, will also play an important role in sexual selection within the species if the female preference discriminates between male songs found within a population. Analysing female preferences for songs is notoriously difficult in *Drosophila*, as the mating system of fruit flies does not easily lend itself to acoustic playback experiments (Crossley & Bennet-Clark 1993). Some experiments have suggested that differences

in song of the magnitude seen between species influence female mating speed (Bennet-Clark & Ewing 1969; Kyriacou & Hall 1982; van den Berg 1988; Greenacre et al. 1993; Tomaru et al. 1995). These experiments have typically been carried out by confining a group of females with mute males and recording the mating speed of the flies while playing different songs through a loudspeaker. One serious drawback in mass mating trials is that one can measure only average group mating speeds. These averages are confounded by marked day-to-day variation in mating speed, requiring large numbers of replicates. These experiments have usually implied that female preferences are broadly rather than narrowly tuned (e.g. van den Berg 1988). An interesting exception is Tomaru et al.'s (1995) demonstration that heterospecific song inhibits mating in *D. biauraria*. It is, however, difficult to distinguish whether the female preferences are really broad, or whether the preferences cannot be precisely determined with the method used. An additional problem is that most of these experiments also fail to distinguish between the effect of song on females and

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males. von Schilcher (1976a) and Crossley et al. (1995) carried out playback experiments with single-sex groups and found that song stimulated greater activity in males (this effect is lessened, but not removed, if their arista are amputated: von Schilcher 1976a). If this effect was species specific, increased mating speed in mixed-sex groups might reflect the stimulatory effect of song on males rather than female song preferences. von Schilcher (1976b) and Kyriacou & Hall (1982) have shown that playback can increase mating speed in mixed-sex groups after removal of the males' arista, but there is some question concerning whether such males are completely 'deaf' (Burnet et al. 1977).

Indirect evidence has suggested that fly song may be subject to considerable sexual selection (Ewing & Miyayama 1986; Ritchie & Gleason 1995). In many species, including *D. melanogaster*, male song is not necessary for the flies to copulate, but hearing the song lowers the amount of courtship the female requires from a male (Ewing 1964; Burnet et al. 1971). In *D. montana*, song is an obligatory component of courtship, and the females hardly ever accept courtship without this signal (Liimatainen et al. 1992). Aspi & Hoikkala (1995) carried out a correlational study of song and mating success in *D. montana* and *D. littoralis* in the wild. Comparisons between the songs of males caught in copula with average songs of the males of the same population showed that successful males had shorter sound pulses and a higher carrier frequency (evidenced by a greater number of cycles within a pulse). A. Hoikkala & L. Suvanto (unpublished data) also showed that *D. montana* males with a high-frequency song obtain more matings in choice experiments in the laboratory, partly because of the tendency of these males to begin courtship early, and partly because of active female choice. *Drosophila montana* females obtain an indirect benefit (fitter progeny) when mating with males with preferred song characters (Hoikkala et al. 1998). The question of whether females really choose males on the basis of male song characters and not a correlated character remains unanswered, however. Independent verification that females prefer particular song traits would demonstrate that there is substantial sexual selection operating on male songs and female mating preferences in *D. montana*.

Female wing spreading is virtually always associated with successful copulation in several *Drosophila* species (Ikeda et al. 1981; Oguma et al. 1996), including *D. montana* (Vuoristo et al. 1996). It indicates copulatory readiness of the females, provoking males to mount. *Drosophila mercatorum* females will show the response to males they cannot come into contact with, probably in response to their song (Ikeda et al. 1981). We examined the wing-spreading response of individual *D. montana* females to synthetic song in the presence and absence of mute males. We varied the length and the carrier frequency of the sound pulses over the range found within the species. Our results indicate that the wing-spreading gesture can be used as a measure of the preference females have for song, and confirm that males producing short, high-frequency pulses will be favoured by sexual selection.

METHODS

Stocks

We used flies collected from three lines originating from Kemi and Oulanka, Finland, which had been cultured for several years in the laboratory. The flies were maintained on malt medium (Lakovaara 1969) in constant light between 20 and 25°C. This regime resembles natural conditions during the breeding season in northern Finland. The flies were sexed within 2 days of their emergence and the males and females were maintained in separate malt-medium vials (9.5 × 1.5 cm diameter), containing four flies per vial. Virgin females were tested at the age of 3–5 weeks, when the flies are sexually mature in these culturing conditions (A. Hoikkala, unpublished data).

Song Synthesis

Features of the shape of pulses of song (pulse length and spectral properties) are thought to influence the preference of *D. montana* females. In the songs of wild-caught males, the pulse length varies from 6 to 25 ms at a temperature of 19 ± 1°C. The carrier frequency varies from less than 200 to nearly 400 Hz, and rises about 10 Hz per 1°C (Hoikkala 1985; Aspi 1992; Aspi & Hoikkala 1995; Hoikkala & Isoherranen 1997). The pulse length is shorter (approximately 14 versus 16 ms) and the frequency higher (approximately 320 versus 280 Hz) in mated flies compared to the mean of the population (Aspi 1992; Aspi & Hoikkala 1995). These two traits (and the number of cycles in a pulse) covary in songs, making it difficult to determine which of the characters are important in mate choice. In our study, we changed the pulse length and carrier frequency independently.

We synthesized song using the 'Signal' system (Electronic Design) with an A/D rate of 4 kHz. We used pulse lengths of 8, 13 and 18 ms, and carrier frequencies of 200, 300 and 400 Hz (Fig. 1). Pulse envelopes took 3 ms each to reach and decline from maximum amplitude. The interpulse interval was 25 ms and song was patterned into bursts of 1 s followed by 1 s of silence. This is typical for a sustained bout of courtship, when wing spreading and mounting usually occur. Songs were filtered between 150 and 1500 Hz and we recorded them on to TDK EC-1M cassette loops using a Marantz CP430 cassette recorder. Playback was via Sony SRS-38 loudspeakers and sound pressure levels were adjusted to 80 dB within the mating chamber, monitored with a 'Realistic' sound level meter (33–2050 on 'fast' setting).

Virgin females were individually confined in a transparent plastic mating chamber (1 × 4.5 × 4.5 cm) with nylon gauze floor and ceiling, held around 20 cm above the loudspeaker. Playback was continued for 10 min or until the female raised her wings for approximately 2 s (Vuoristo et al. 1996). We tested 12–18 females (average 15, total 268) with each song for each condition (with/without a male). When we used males, we made them mute by removing their wings under anaesthetic in advance of the experiment. These flies

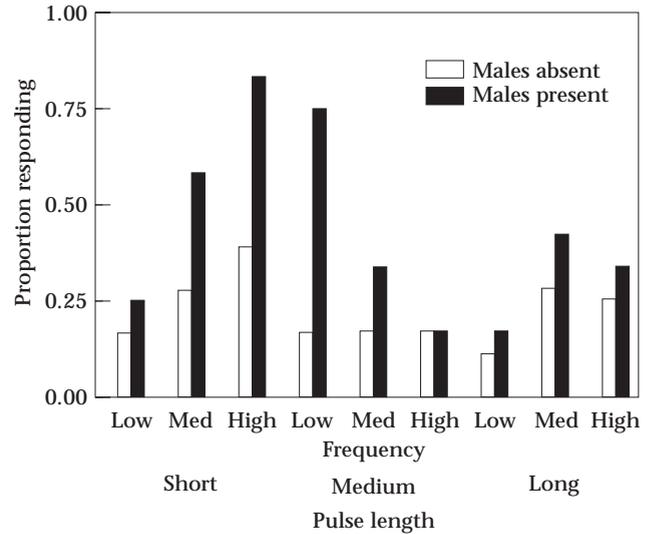
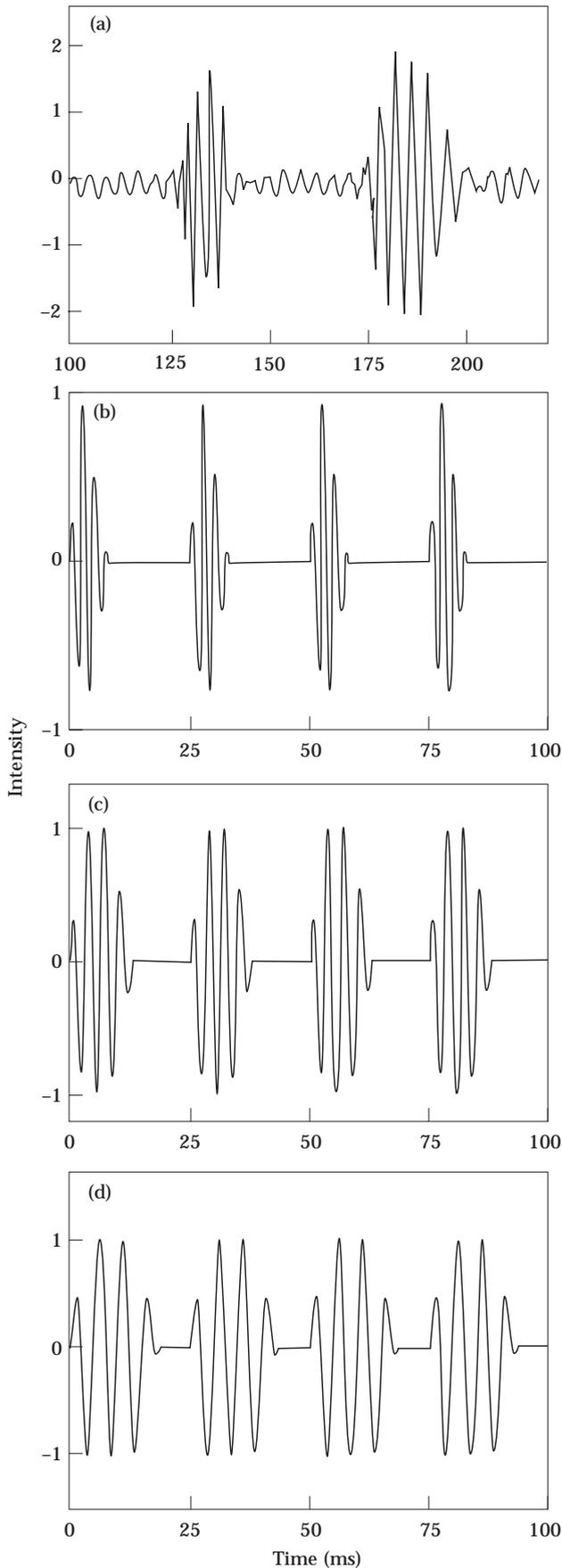


Figure 2. The proportion of females showing wing spreading to the synthetic song models, which varied in both pulse length and carrier frequency. Trials were carried out in both the presence and absence of mute males.

appeared to behave normally. Playback took place in a sound-attenuated room, at ambient temperatures (20–25°C).

To examine the incidence of wing spreading we carried out an analysis of deviance of a generalized logistic linear model of the proportion of females responding. The presence or absence of males, the three levels of pulse length and carrier frequency and the interaction terms were used as predictors. Changes in deviance from the models with or without these predictors can be analysed for significance using chi-square tests (Genstat Committee 1993). Analysis of the latencies to respond (with nonresponders given a maximal score) gave essentially identical results.

RESULTS

Figure 2 shows the proportion of females responding to each song model and Table 1 the analysis of deviance. Around 20% of females responded to synthetic song in the absence of any additional stimuli from courting males, although response levels were higher (about 40% overall) when the female was accompanied by a mute male. These males will provide additional stimuli, including pheromones and genital licking. Female response rate differed between the song models. The most effective song was that consisting of short, high-frequency pulses (more than 60% of females responded to this song) with the next effective songs being those consisting of short, medium-frequency pulses and medium length,

Figure 1. Pulses of *D. montana* song. (a) Song from a real male; (b–d) are examples of synthetic songs used in playback experiments. (b) Short pulse length and high carrier frequency; (c) medium pulse length and carrier frequency; (d) long pulse length and low carrier frequency. The Y axis has arbitrary intensity units.

Table 1. Analysis of deviance of the proportion of females showing the wing-spreading response to the song models

Source of deviance	df	Deviance	P
Males	1	12.71	<0.001
Frequency	2	3.56	NS
Pulse length	2	3.93	NS
Frequency*Pulse length	4	16.23	0.003
Males*Frequency*Pulse length	7	7.16	NS
Residual	1	0.23	—

low-frequency pulses (both around 45% effective). The combination of pulse length and carrier frequency was more important than either parameter alone in determining the effectiveness of the song (Table 1). The pattern of variation was the same in both the presence and absence of males (indicated by the absence of a significant three-way interaction in Table 1), which indicates that variation in response was not due to the effect of the song models on male courtship intensity.

DISCUSSION

Variation in the male courtship song of *D. montana* is due to the condition of the male, environmental conditions (particularly temperature) and genetic variation (Aspi & Hoikkala 1993). Males producing short sound pulses with a high number of sound cycles (a combination that leads to high carrier frequency) have a higher mating success both in the wild (Aspi & Hoikkala 1995) and in laboratory experiments (A. Hoikkala & L. Suvanto, unpublished data). Our playback experiments have shown that females respond selectively to male songs with different pulse characters, preferring songs with short pulses and a high carrier frequency. By removing other factors that could influence mating success, we have demonstrated that *D. montana* females can exercise selection solely on the basis of male song characters.

Aspi (1992) and Aspi & Hoikkala (1995) found that mated *D. montana* and *D. littoralis* males collected in the wild had significantly shorter and higher-frequency sound pulses than average. The synthetic songs used in our playback experiments covered the range of song variation found in the wild. Female preferences within this range are directional for shorter pulse length (overall, song with short pulses produced a response in over 40% of playbacks, medium and long pulses were both less than 30% effective). The most efficient songs had the shortest pulse length (8 ms) and highest frequency (400 Hz). The next most efficient songs had, however, low or medium carrier frequency (Fig. 2). The combinations of pulse width and carrier frequency meant that these preferred songs had a similar number of cycles per pulse, perhaps implicating this trait in the female preferences. Only playbacks varying pulse width, frequency and cycles per pulse independently could disentangle the contribution of these interrelated traits.

In most *Drosophila* species that have been studied, male courtship songs have been found to be an important, but

not obligatory, part of courtship (Ewing 1964; Burnet et al. 1971). Only in three species, *D. montana*, *D. ezoana* (Hoikkala 1988; Liimatainen et al. 1992) and *D. busckii* (Bixler et al. 1992) have females been found to refuse to mate in the absence of song. Most other studies of fly song have emphasized its role in species recognition, perhaps because female preferences seem to be broadly rather than narrowly tuned to male song parameters. However, this may arise from the low resolution of playback experiments rather than reflect the preferences females have (see Introduction). Our results confirm the importance of male song characters to mating success in *D. montana*. Our experiment might even underestimate the extent of discrimination for song parameters in species such as *D. montana*, where female song preference is relative rather than absolute (Hoikkala & Aspi 1993). In playback experiments females can hear only one song, whereas in the field there is potential for comparison between the songs of multiple simultaneous or sequentially courting males.

With *D. montana*, there is also strong evidence that characteristics of the song determine sexual selection. Pulse traits are very sensitive to environmental effects in a way that would give the female information about the condition of a male when courting (Hoikkala & Isoherranen 1997). Such condition dependency is characteristic of traits evolving under sexual selection (Andersson 1994; Johnstone 1995; Rowe & Houle 1996), which potentially enables the females to choose a high-quality mating partner on the basis of his song characters. There is evidence that females will obtain an indirect benefit from song-determined partner choice: egg-to-adult survival rate is positively correlated with the carrier frequency of the father's song (Hoikkala et al. 1998). In *D. melanogaster*, the heritability of the interpulse interval of song within a population is asymmetric, with less heritability for shorter intervals (Ritchie & Kyriacou 1996), suggesting directional selection on the trait. Differences between strains are additive (Cowling 1980; Ritchie et al. 1994). In *D. montana*, the number of pulses in a pulse train (which is not a target of female choice) shows significant levels of additive genetic variation both in the laboratory and in the wild (Aspi & Hoikkala 1993). In other song traits, high environmental variation in the wild seems to mask genetic variation, reducing the heritability (Aspi & Hoikkala 1993; Hoikkala & Isoherranen 1997).

The success of playback experiments with individual females potentially opens up several new aspects for the study of mating preferences in *Drosophila*, including the effects of temperature on female preferences and the repeatability and heritability of female preferences, assessed independently of mating. The fact that females respond to songs in the total absence of males also confirms the assumption of many previous playback experiments, that differences in mating speed are not confounded by the effects of song on male courtship.

Acknowledgments

M.G.R. was funded by the NERC (U.K.), A.H. by the Academy of Finland. Jenny Gleason and two referees gave very helpful comments on the manuscript.

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