Light is required for proper female mate choice between winged and wingless males in *Drosophila*

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In many animal species, females choose potential mating partners according to their own preferences. Thus, female preference-based mate choice affects intra-specific mating success and prevents interspecific mating. To clarify the neuronal basis of female mate choice, it is essential to identify the important relevant sensory cues. In the fruitfly *Drosophila melanogaster*, the courtship song of males promotes female sexual receptivity. When wild-type virgin females can freely choose one of two types of courting males (winged or wingless males), they prefer to mate with winged males. Here, we report a crucial sensory cue relevant to this female mate choice. In a female choice test, female receptivity toward winged and wingless males was markedly reduced when females had auditory impairments, although females with visual or olfactory impairments showed normal receptivity similar to wild-type females. However, females with visual impairments did not show clear mate preference toward winged males. Thus, these findings suggest that females utilize visual cues in mate choice between winged and wingless males in *Drosophila*.

**Key words:** mate choice, female mate preference, courtship song, *Drosophila*

Female mate preference is an important factor for intra-specific mating success, sexual selection and reproductive isolation (Ptacek, 2000; Andersson and Simmons, 2006; Laturney and Moehring, 2012). If females are courted by multiple conspecific males simultaneously, they must recognize characteristics of each courting male through sensory inputs and finally decide on the best partner to accept. Thus, it is considered that complex brain functions, such as recognition of sensory information, integration of multiple sensory inputs, and individual recognition, are associated with the female decision.

In the fruitfly *Drosophila melanogaster*, both sexes utilize several sensory cues for mating success (Hall, 1994). The courtship song of males, which is produced by male wing vibration during courtship, plays an especially important role in mating success and is detected by the females' aristae, which are bristles extending from the third antennal segment (Manning, 1967). Virgin females frequently accept winged males, whereas the female acceptance of courting males whose wings are surgically removed (wingless males) is markedly reduced (Tomaru et al., 2000). Similarly, in comparison with normal intact females, females whose aristae are surgically removed (aristaless females) mate infrequently with winged males (Tomaru et al., 2000). Thus, receiving a courtship song promotes sexual receptivity and mating success in *Drosophila* virgin females. When wild-type virgin females can freely choose one of two types of courting males (winged or wingless males), they actively mate with winged males (Tomaru and Oguma, 2000). Thus, in this female choice test, it is considered that females determine which males are winged on the basis of sensory information from males. Here, we examined whether females utilize auditory, olfactory and visual cues to determine the suitable mating partner between winged and wingless males.

First, we performed a no-choice test to confirm that the male courtship song actively promotes female sexual receptivity, as previously reported (Tomaru et al., 2000). The wild-type *D. melanogaster* Canton-S (CS) strain was used in the experiments. Flies were raised on glucose-yeast-cornmeal medium at 25.0 ± 0.5 °C in a 12-h light:12-h dark (LD) cycle. Virgin male and female flies were collected without anesthesia within 8 h of eclosion and maintained in food vials until the experiments. All the experiments were conducted during the daytime between Zeitgeber time (ZT) 0 and ZT6 at 25.0 ± 0.5 °C and 50–70% relative humidity. A male fly and a female
fly (4- to 6-day-old virgins) were placed in a circular acrylic plastic observation chamber (15 mm diameter × 3 mm depth). Using hard disk video recorders (HDR-CX590V, Sony, Japan), we recorded their mating behavior for 20–25 min in a temperature- and humidity-controlled cabinet (temperature, 24.5 ± 0.5 °C; humidity, 50 ± 10%; illuminance, 180 ± 50 lux). After recording, we observed mating behavior until copulation occurred or until 20 min had elapsed by video playback. We measured mating frequency, which was defined as the percentage of pairs that copulated during the 20-min period. We also observed male courtship behavior for 10 min or until copulation occurred, and calculated the courtship index (CI), an indicator of male courtship activity that is defined as the percentage of time spent courting within a given observation period. In the no-choice test, wild-type females did not accept wingless males, although winged and wingless males courted the females at similar levels (Fig. 1A and 1C). Wild-type females with intact aristae accepted winged males more frequently than aristaless females did, although aristaless females elicited male courtship as vigorously as intact females (Fig. 1B and 1D).

Next, we conducted the female-choice test using wild-type flies. A female, a winged male and a wingless male (4- to 6-day-old virgins) were introduced into an acrylic plastic observation chamber. For analysis of female mate choice under illumination, mating behavior was recorded for 20–25 min. After recording, we observed mating behavior until copulation occurred or until 20 min had elapsed. Subsequently, we determined the number of females that mated with a winged male \( N_{\text{winged}} \) or a wingless male \( N_{\text{wingless}} \). Referring to the isolation index reported by Stalker (1942), we calculated the female choice index (FCI) as follows: 

\[
\text{FCI} (%) = \left( \frac{N_{\text{winged}} - N_{\text{wingless}}}{N_{\text{winged}} + N_{\text{wingless}}} \right) \times 100
\]

To compare the FCI between different conditions, we used the test of indepen-

![Fig. 1. No-choice test using wild-type flies. (A and B) Cumulative mating frequency (%). The observation period was 20 min, and 40 pairs were observed. ***, \( P < 0.001 \). (A) The chi-square test with Yates' correction was used for comparison of mating frequency. (B) The G-test with Williams' correction was used for comparison of mating frequency. (C and D) CI (%). In each box plot, the box encompasses the interquartile range, a line is drawn at the median, and the vertical bars extend to the 5th and 95th percentiles. Each square within a box represents the mean. The observation period was 10 min. \( N = 20 \) in each experiment. In most cases, CI was not distributed normally. Thus, we used a nonparametric test (Mann-Whitney \( U \) test). NS, not significant. (A and C) A pair of female and winged or wingless male was used in this experiment. (B and D) A pair of winged male and intact or aristaless female was used.](image-url)
Light is required for female mate choice

dence of contingency table for statistical analysis (G-test with Williams' correction). When females accept winged and wingless males equally, the FCI is 0%. Among 50 mated females, 40 mated with winged males [Table 1, WT (Intact)], and the mean FCI was 60% [Fig. 2A, WT (Intact)]. However, the CI was not significantly different between winged and wingless males [Fig. 2D, WT (Intact)]. In contrast to intact wild-type females, aristaleless females did not often mate with males [Table 1, WT (Aristaless)]. Winged and wingless males courted aristaleless females at similar levels [Fig. 2D, WT (Aristaless)]. The FCI of mated females was not significantly different between intact and aristaleless females (Fig. 2A).

To examine whether females utilize visual cues for choosing winged males, the female-choice test was performed in darkness. Acrylic plastic observation chambers were placed in a light-tight box for 20 min, and subsequently we determined the number of females that mated with a winged male or a wingless male by direct observation. The FCI of wild-type females in darkness was significantly lower than that in a lighted room (Fig. 2B). Moreover, we used blind mutant females [no receptor potential Ap24 (norpA24)] (Pearn et al., 1996) and performed the female-choice test in the lighted room. The FCI of norpA24 females was also significantly lower than that of wild-type females (Fig. 2C). The reduced FCI of norpA24 females was evidently not due to a lower sexual attractiveness of these females because they were courted as vigorously as wild-type females (Fig. 2E). We cannot completely exclude the possibility that the genetic background of norpA24 females contributes to the reduced FCI. However, since the FCI was reduced in wild-type females in darkness and norpA24 females in light, our results indicate that light is required for choosing winged males in the female-choice test.

We next used mutant females without a sense of smell [Odorant receptor co-receptor1 (Orco1)] (Larsson et al., 2004) because it is possible that by vibrating a wing during courtship, winged males scatter a male-specific volatile pheromone (Kurtovic et al., 2007) more widely than wingless males do, and that females choose winged males via olfaction. Orco1 flies were backcrossed for six generations to white flies having the CS genetic background. The FCI of Orco1 females was intermediate between those of wild-type and norpA24 females (Fig. 2C). Thus, although our results did not provide clear evidence that Drosophila females utilize olfactory cues for proper mate choice, it is possible that olfactory cues are somewhat effective for proper female mate choice.

In this study, we showed that proper mate choice is disturbed in females lacking light information. The role of vision in females has not been clarified in the Drosophila mating system. However, it has recently been reported that males with vivid wings are more attractive to females than those with dull wings; wing color information therefore affects mate choice by Drosophila females (Katayama et al., 2014). Thus, it is possible that the attractiveness of wingless males to females decreases because such males lack wing color information. Alternatively, females may visually recognize male wing vibration, but not wing color. Although the specific visual information responsible for proper female mate choice remains elusive, our findings also suggest that female vision plays an important role in Drosophila mate choice.

Aristaleless females showed an extremely low mating frequency in the female choice test, whereas the lack of visual or olfactory cues did not affect female sexual receptivity. In addition, light is required for choosing winged males in the female choice test. Taking these observations together, it is possible to explain this female mate choice as follows. The courtship song produced by a winged male facilitates female sexual receptivity. However, auditory information alone is insufficient for females to determine which male is winged; females finally choose a winged male on the basis of mainly visual cues from males with or without wings. In the female choice test,

<table>
<thead>
<tr>
<th>Female type</th>
<th>Number of observations</th>
<th>Number of mated females</th>
<th>Total mating frequency (%)</th>
<th>G-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (Intact)</td>
<td>70</td>
<td>40</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>WT (Aristaless)</td>
<td>257</td>
<td>35</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>WT (Light)</td>
<td>135</td>
<td>83</td>
<td>17</td>
<td>100</td>
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<td>WT (Dark)</td>
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<td>WT</td>
<td>166</td>
<td>85</td>
<td>25</td>
<td>110</td>
</tr>
<tr>
<td>norpA24</td>
<td>162</td>
<td>66</td>
<td>44</td>
<td>110</td>
</tr>
<tr>
<td>Orco1</td>
<td>152</td>
<td>75</td>
<td>35</td>
<td>110</td>
</tr>
</tbody>
</table>

The G-test with Williams' correction was used for statistical analysis of total mating frequency. WT, wild-type; *** P < 0.001; NS, not significant.
about 20% of aristaless females mated with winged or wingless males (Table 1) and the FCI of aristaless wild-type females is similar to that of the intact wild-type females (Fig. 2A). von Schilcher (1976) reported that an artificial courtship song activates male locomotion and that the sound intensity threshold of the effect of the song is markedly lower on aristaless males than on intact males. Nevertheless, aristaless males can respond to artificial song with a high sound intensity (von Schilcher, 1976). Considering these results, it is possible that aristaless females are not completely deaf and that some aristaless females become sexually excited upon hearing the courtship song and finally choose winged males on the basis of visual cues from courting males.

In *Drosophila*, behavioral assay based on female mate choice has been extensively used in studies of sexual selection or speciation (Hoikkala and Aspi, 1993; Bakker and Pomiankowski, 1995; Wu et al., 1995; Carson, 2002). Besides, the behavioral assay of female mate choice between winged and wingless males can also be used for clarifying the brain function relevant to integration of auditory and visual cues in *Drosophila*. Thus, further studies of the neural mechanisms underlying female mate choice will provide new insights into higher brain functions.

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Light is required for female mate choice.

REFERENCES


