Light-affected male following behavior is involved in light-dependent mating in *Drosophila melanogaster*

Takaomi Sakai¹, Kunio Isono², Masatoshi Tomaru¹, and Yuzuru Oguma^{1,*}

¹*Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, and*

²*Graduate School of Information Sciences, Tohoku University, Sendai 980-77, Japan*

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Because the males of the *Drosophila melanogaster* species subgroup contributed to the light dependency of mating success and this was not due to the light-dependent nature of general activity, we have suggested that the light dependency of mating is caused by the light-dependent mechanism of male-specific sexual behavior. Courtship behavior of the wild type males of *D. melanogaster* was investigated in light and dark with a female partner with a severe *norpA* mutation. Total duration, frequency and bout length of all courtship elements, except for bout length of orientation and attempted copulation, were significantly lower in the dark than in light. Particularly, male following behavior was strongly affected by light. The sequence analysis of courtship behavior revealed that the courtship transition from orientation to following was severely disturbed in the dark. An unique transition from orientation to wing vibration, skipping the following phase was frequently observed in the dark. Under dim red light, single males showed a similar high locomotor activity in the dark as in light during the period over which male courtship behavior was investigated and analysed. It was suggested that the male following behavior requires visual cues and that the following is the major behavioral element involved in the light dependency of mating in *Drosophila*.

INTRODUCTION

The mating success in many species of *Drosophila* is light-dependent (e.g., Grossfield, 1971). Earlier we reported that males predominantly contributed to the light dependency of mating in the *D. melanogaster* species subgroup and suggested that the light dependency of mating success depended on the male-specific sexual behavior because the locomotor activity of the flies was not light-dependent (Sakai et al., 1997). Thus the male courtship behavior in *Drosophila* may be affected by light.

Mating of a fruit fly, *Drosophila*, consists of sequential elements of characteristic behavior; the sequence of this behavior has been extensively studied, particularly in *D. melanogaster* (e.g., Bastock and Manning, 1955; Cobb et al., 1986; Welbergen et al., 1987). The male courtship behavior involves several courtship elements (Fig. 1); the male orients himself toward the female partner (orientation), follows her when she moves (following), spreads one of the wings and vibrates (wing vibration), licks her genitalia, and attempts to copulate (attempted copulation). Finally, the female accepts the courting male (copulation).

Spieth and Hsu (1950), Manning (1959), and Robertson (1983) reported that *D. melanogaster* did not show a reduc-

tion in the frequency of mating success in the dark like other species. However, Markow (1975) and Cobb and Ferveur (1996) reported that *D. melanogaster* showed a reduction in the mating frequency in the dark as in many other species. Because of the contrary results, it is not yet clear how important a role light and/or visual stimuli play in *D. melanogaster* mating success. Male visual mutants of *D. melanogaster* are known to show an increased rate of courtship breaks, extended courtship latency and also reduced courtship duration (Crossley and Zuill, 1970; Markow and Manning, 1980; Tompkins, 1984). Based on the abnormal courtship in *D. melanogaster* visual mutant males, it is expected that light and/or visual stimuli affect male courtship in this species. It is not yet clear, however, whether the abnormal courtship arises from only the visual defect of the mutation in the male or from an altered male-female interaction induced primarily by the mutation of the males. Wild type males may show abnormality of some specific courtship behavior sequence in the dark, and these abnormalities may induce the light dependency of mating in this species.

Throughout the observation of courtship behavior in this report we used a wild type male and a *no receptor potential* *A*^{P24} (*norpA*^{P24}, Lindsley and Zimm, 1992), a practically null mutation in *norpA*, strain female. The phospholipase C (PLC) encoded by *norpA* gene is known to be essential in

* Corresponding author.

visual transduction cascades (Pearn et al., 1996). This male-female combination enables us to analyze the normal response to light in males without considering the female visual response to males or the visual environment.

We first compared the mating frequency of *D. melanogaster* in light and the dark for mating experiments of short (20-min) and long (1-h) duration. The male courtship sequences were then analyzed and compared between light and dark. From the analysis of courtship sequence, we revealed that male following behavior was strongly affected by light.

MATERIALS AND METHODS

Flies and rearing condition. Males were collected from Canton-S strain, a wild type *D. melanogaster* stock. Females were from a mutant stock *no receptor potential* A^{P24} , one of the severest alleles of *norpA* mutations that almost completely abolish the ERG response and eliminates the transcripts and the *norpA* proteins from all tissues (Pearn et al., 1996). Females were thus considered to be completely visually inactive. They were maintained separately for 7-8 days until the experiment. Rearing conditions were the same as described in Sakai et al. (1997).

Mating experiments. Males and females were allowed to mate for twenty min or one h (20-min and 1-h experiment, respectively). Each of the mating experiments was carried out 19-20 times (20-min experiment) or 12 times (1-h experiment). Further details about the mating experiment are given by Sakai et al. (1997). The chi-square test with Yate's correction was used for comparisons of the mating frequency between light and dark.

Observation and analysis of courtship behavior. We observed courtship behavior in single pairs of wild type Canton-S males and blind mutant females (*norpA*^{P24}), to avoid female effects between light and dark, under two light conditions (light and dark). A male and a female were introduced into an acrylic plastic chamber (10 mm diameter $\times$ 6 mm depth) with an aspirator. Observation in light was performed under a microscope until copulation occurred, or until five min had passed. We recorded male behavior using a program of an event recorder on a MS-DOS computer. Male courtship behavior was classified into five courtship elements (see Fig. 1); no courtship (a male did not court the female), orientation, following, wing vibration, and attempted copulation. Changes in the behavior of males were recorded manually by pressing keys of the computer and the event and the time were automatically stored by the program for later analysis. In the dark experiment, an infrared camera (C2400-02, Hamamatsu Photonics Inc.) was set up above the observation chamber and the chamber was illuminated by an infrared light source (> 860 nm) from an oblique angle (Oguma et al., 1996). Fly

Fig. 1. Courtship behavior of *D. melanogaster*. orientation, a male orients toward the female; following, a male follows a female when she moves; wing vibration, a male spreads one of the wings and vibrates; attempted copulation, a male attempts to copulate; copulation, a female accepts a male's attempted copulation.

behaviors were videotaped (PVM-171, Sony Inc.) for five min, then played back, and male courtship was analysed as described above.

Courtship latency was defined as the time spent by flies from introduction into the chamber until the first courtship occurred. Percentage of total duration in each male was calculated as follows: (the total time spent in each behavior elements) $\times 100$ / (the observation time). Frequency of each behavior element during observation was counted for each pair. Bout length (s) of each behavior element was calculated as the mean time spent in one act in one observation period. The means and standard errors of the percentage of total durations and the frequency of each behavior element of 50 pairs were calculated for each the two light conditions. In the bout length, the means and standard errors were calculated for each element of male performance. The Mann-Whitney *U* test was used for each comparison in light and the dark.

Courtship sequence analysis. The analysis of male courtship responses was based on a first order Markov

chain. Behavioral sequence data from observation of 50 pairs were organized into a five by five preceding-following transition matrix with five behavior categories. Each cell in the matrix (N_{ij}) was the number of times behavior i was preceded by behavior j . In the present analysis, the observed transition frequencies of the 5×5 matrix were compared with a random model by the chi-square test. In our study, a behavioral event cannot be preceded by itself. Consequently, the diagonal cells of the transition matrix were considered as structural zeros (Bishop et al., 1975; Hoikkala and Welbergen, 1995). Although we tried to avoid cells with sampling zero by using a large sample size, some occurred in this transition matrix. Thus, we used the method reported by Fienberg (1969) to determine the size of the constant to be added to each cell frequency to remove sampling zeros. Expected transition frequencies were calculated after Bishop et al. (1975). Two criteria as described by Hoikkala and Welbergen (1995) were applied to analyze the contingency tables: (1) at most 20% of the cells were allowed to contain expected values of fewer than 5 and (2) the matrix contained at least 125 ($5 N^2$) transitions.

Adjusted residuals were calculated to identify the cells of a contingency table responsible for a significant overall chi-square value (Everitt, 1992). The positive values of the adjusted residuals were compared with 5% standard normal deviates. We defined a significant transition when the cells exceeded 5% standard normal deviates. The significant transitions are shown in the kinematic diagram. We computed the frequency of specific transitions that were significant in both light and dark as a conditional probability (Wood et al., 1980). For example, the transition frequency of orientation to following was calculated by dividing the number of transitions from orientation to following by the number of transitions from orientation to any other behavior (N). The specific transitions were compared between the two light conditions by chi-square test of 2×2 tables (Wayadande and Nault, 1996).

Measurement of locomotor activity. We measured the locomotor activity in *D. melanogaster* Canton-S strain. We used the same method as described in detail by Sakai et al. (1997). Thirty-five males were observed both in the dark and light. The Mann-Whitney U test was used for comparisons of locomotor activity between in light and the dark.

RESULTS

Mating experiments. Mating frequency for 20-min or 1-h was compared in light and the dark (Table 1). In the 1-h experiment more than 95% of the females were inseminated both in light and the dark. There was no significant difference in mating frequency between two light conditions as reported by others (Spieth and Hsu, 1950; Manning, 1959). In the 20-min experiment, however, mating

Table 1. Mating frequency (%) of the 20-min and 1-h experiment in light and dark

Mating period	Mating frequency (%)				χ^2
	Light	(n)	Dark	(n)	
20-min	76.5	(200)	66.8	(190)	4.02 *
1-h	97.5	(120)	95.0	(120)	0.46

n, number of dissected females; χ^2 , chi-square value with Yate's correction; *, $p < 0.05$.

frequency was 67% in the dark and 77% in light. This small but significant difference confirmed the results by Markow (1975) and Cobb and Ferveur (1996). Therefore we conclude that *D. melanogaster* shows a weak, but significant light dependency of mating during shorter periods of mating.

Observation of courtship behavior in the dark. In the dark, flies randomly walked around the mating chamber from the time of introduction into the chamber until when the first courtship occurred. Mean courtship latency in the dark (58.5 ± 31.2 s) was significantly longer than that in light (30.1 ± 22.7 s, $U = 65$, $p < 0.001$). Orientation was observed as frequently in the dark as in light. After orientation males usually shift the behavior to following towards the female in light. In the dark, however, following after orientation was rare. They sometimes showed wing vibration immediately after they oriented to the female. Attempted copulation was also rare in the dark and many males misdirected (e.g., to the female's head or thorax). When males performed attempted copulation from the wrong direction, females usually decamped. Similar abnormal courtship in attempted copulation was also mentioned in *D. auraria* and *D. triauraria* (Oguma et al., 1996). All courtship elements in the dark were usually observed when the pairs were in a close proximity, although it was not always so in light. Surprisingly, males in the dark occasionally showed wing vibration or attempted copulation without the presence of a female partners, which were never observed under the light.

Analysis of male courtship behavior. In order to compare the male courtship behavior in light and the dark, we carried out statistical comparisons of the mean frequency, the mean bout length, or the total duration of the four courtship elements. The frequency of each behavior element during observation was shown in Figure 2. All of the frequencies were higher in light than in the dark. In light, the frequency of following (27.4 times) was higher than that of any other behavior element (Fig. 2). In the dark, however, the following occurred at low frequency (2.7 times in Fig. 2). The bout length of one act in each behavior element was shown in Figure 3. The bout length of following and wing vibration were longer in light than in the dark (Fig. 3). There was no significant difference between the

Fig. 2. Mean total frequency of each courtship behavior elements in light (open bars) and the dark (solid bars). Error bars show standard error. W. V., wing vibration; A. C., attempted copulation. $n = 50$; *, $p < 0.05$; ***, $p < 0.001$.

Fig. 3. Mean bout length (s) of one act in each courtship behavior element in light (open bars) and the dark (solid bars). $n = 50$ except for following in light ($n = 47$), A. C. in light ($n = 36$) and A. C. in the dark ($n = 23$). For further explanation see Fig. 2.

two light conditions in bout lengths of orientation and attempted copulation (Fig. 3). Figure 4 shows mean total duration in each courtship behavior element. Duration of each behavior element in the light was significantly higher than that in the dark. Males in light spent 38.2% for following and this value was the highest of all the elements, while males in the dark spent almost no time following (0.5% in Fig. 4). The results of total duration, frequency and bout length in light confirmed those of Welbergen et al. (1987). We concluded that among the male courtship elements, following is the most severely disturbed behavioral element when it is performed in the dark.

Courtship sequences. The results of courtship sequence analysis in light and dark are shown in Figure 5. Males

Fig. 4. Mean total duration of each courtship behavior elements in light (open bars) and the dark (solid bars). **, $p < 0.01$; ***, $p < 0.001$. For further explanation see Fig. 2.

Fig. 5. Kinematic diagram of courtship sequence in light (a) and the dark (b). Numbers inside squares are total frequencies of each behavior. Arrows represent positive significant transitions. Numbers beside arrows are total transition frequencies.

Table 2. Comparisons of significant transition in light and dark

Significant transition	Conditional probability (%)				χ^2
	Light	(n)	Dark	(n)	
N. C. to Orientation	76.70	(476)	88.79	(651)	28.62 ***
Orientation to Following	63.74	(1186)	17.45	(659)	362.41 ***
Following to N. C.	20.62	(1353)	74.44	(133)	181.88 ***
W. V. to A. C.	7.99	(814)	11.32	(265)	2.37

n, see Materials and methods. χ^2 , chi-square value with Yate's correction; N. C., no courtship; W. V., wing vibration; A. C., attempted copulation; ***, $p < 0.001$.

Table 3. Mean locomotor activity levels in light and the dark for male at two or twenty minutes after introduction into an observation chamber

After introduction	Mean locomotor activity		U
	Light	Dark	
2-min	35.49 $\pm$ 3.51 [†]	30.40 $\pm$ 3.37	520
20-min	11.74 $\pm$ 2.22	4.00 $\pm$ 1.62	395.5 *

The Mann-Whitney U test was used for comparisons, light vs. dark. n = 35. U, Mann-Whitney's U; [†], standard error; *, $p < 0.05$.

began courtship with orientation both in light and the dark. In light, the transition from orientation to following was then almost always observed (Fig. 5a). On the other hand, the males in the dark showed the transition from orientation not only to following but also to wing vibration (Fig. 5b). Males in light showed the major transition from following to wing vibration or, less frequently, stopped courtship (Fig. 5a). In the dark, however, males almost all failed to further proceed with courtship and the transition from following was exclusively to no courtship (Fig. 5b). Males in light also frequently went back to following after they vibrated wings (Fig. 5a) while males in the dark never showed a significant transition from wing vibration to following (Fig. 5b).

The results of the comparisons of specific transitions between light and dark are shown in Table 2. The transitions from no courtship to orientation and from following to no courtship were more frequent in the dark than in light. The transition from orientation to following was drastically reduced in the dark. There was no significant difference between the two light conditions in the transition from wing vibration to attempted copulation.

Locomotor activity. Since the major activity of male following behavior involves walking, we then investigated the effect of light upon the single male locomotor activity under similar conditions in which the mating experiments were carried out (Table 3). Although there was no significant difference between the two light conditions in the 2-min experiment, males became significantly more inactive in the dark than in the light in the 20-min experiment. Therefore, we concluded that the severe reduction of the

male following activity in courtship in the dark cannot be simply explained by change in the locomotor activity.

DISCUSSION

While the activities of most courtship elements in the dark were significantly lower than those in light, following was most strongly affected by light for the mean frequency, the mean bout length and the mean total duration (Figs. 2, 3, and 4). The total time spent by males for following in the dark was only one hundredth of that in the light. Sequence analysis revealed that following in the dark often resulted in the cessation of the courtship transition to wing vibration while the transition was usually seen for males in light (Fig. 5). Instead, a shortcut transition from orientation to wing vibration by-passing following was specifically observed in the dark (Fig. 5). Males in light also frequently went back to following after they vibrated wings while males in the dark did not show the significant transition from wing vibration to following. We conclude that males predominantly show difficulty in performing and to maintain following behavior in the dark.

It has been believed until recently that the mating of *D. melanogaster* was light independent (they could mate in the dark as well as in light) from experiments where males and females were mated for long mating periods (Spieth and Hsu, 1950; Manning, 1959; Robertson, 1983). In the present study, *D. melanogaster* showed mating frequency as high in the dark as that in light in the 1-h experiment. However the mating frequency of *D. melanogaster* in the dark was significantly lower than that in light in the 20-min mass mating experiment. Cobb and Ferveur (1996) reported essentially the same result for the 40-min pair mating experiment. It is clear that the past studies did not detect the light dependency of mating in *D. melanogaster* simply because the mating period was too long.

In the present behavioral study, we used the *norpa*^{P24} female, a virtually blind mutant with no electroretinograms of the compound eyes and ocelli. Thus, the break of male following behavior in the dark could not be due to female effects. Tompkins et al. (1982) suggested that *D. melanogaster* males must perceive female movements to perform normal courtship and copulation based on results from experiments using immobilized mutant female

(*shibire*, rapid paralysis at high temperature) or visual mutant male (*optomotor blind*, little or no response to certain horizontally moving stimuli). Therefore one of the visual cues for courting males may include detection of female movements.

High locomotor activities in males lead to shorter courtship latencies and/or higher levels of courtship activity in the *D. melanogaster* species subgroup (Cobb et al., 1987). Therefore if the locomotor activity of males in light is higher than that in the dark, shorter latencies and higher levels of courtship activity in light would be expected. However, males showed a similar locomotor activity measured during a one min period two min after the introduction of the males. This corresponds to the middle minute of the courtship observation period (five min). Therefore, we suggest that low male courtship activities including following cannot be simply explained by the light dependency of the locomotor activity and that visual cues like moving females are involved in the courtship of *D. melanogaster*.

In *norpA* and *ora* (*outer rhabdomere absent*, lack of six retinula cells) mutant males, similarly, their courtship latency was abnormally long in light (Markow and Manning, 1980), suggesting that the males perceive visual cues associated with females to initiate courtship quickly (Tompkins, 1984). In the present study, courtship latency in the dark was longer than that in light (30.1 ± 22.7 s in light; 58.5 ± 31.2 s in the dark; $U = 65$, $p < 0.001$). The frequency of orientation in the dark was lower than that in the light (Fig. 2). It is therefore suggested that visual cues also facilitate initiation of orientation as speculated by Ewing (1983). On the other hand, there was no significant difference in the bout length of orientation between light and the dark (Fig. 3). Therefore non-visual cues, such as the female contact pheromone (Jallon, 1984) or the volatile pheromone (Tompkins et al., 1980) may be required to maintain orientation.

Changes by light in the courtship elements other than following may also contribute to modify the mating frequency of *D. melanogaster*. Among them wing vibration is known to play an important role in sexual excitement for female *D. melanogaster* (Bennet-Clark and Ewing, 1969; von Schilcher, 1976; Kyriacou and Hall, 1982). Frequent vibration in light led to more transition from orientation to following (Table 2) and significant transition from following to wing vibration (Fig. 5). In the dark, total duration of wing vibration was very short (Fig. 4) because of the male's lower frequency and shorter bout length of wing vibration (Figs. 2 and 3). One possibility is that the decrease of following in the dark leads to a quantitative decrease of wing vibration in the dark and that the decrease of the mating frequency in the dark depends on a quantitative decrease of male wing vibration in the dark, which leads to low female receptivity. Alternatively the lower locomotor activity level observed in the dark in the 20-min experiments may contribute to the light dependency of the mat-

ing success, as lower locomotor activity may directly reduce the chance to approach females to initiate and maintain courtship.

In *D. simulans* and *D. sechellia*, belonging to the *D. melanogaster* species subgroup, the levels of light dependency of mating differ between the two species and males predominantly contribute to the low frequency of mating success in the dark (Sakai et al., 1997). Further genetic studies and inter-specific comparisons of the light dependency of male courtship behavior may provide information on species-specific differentiation of male courtship behavior in *Drosophila*.

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REFERENCES

- Bastock, M., and Manning, A. (1955) The courtship of *Drosophila melanogaster*. *Behaviour* **8**, 85–111.
- Bennet-Clark, H. C., and Ewing, A. W. (1969) Pulse interval as a critical parameter in the courtship song of *Drosophila melanogaster* Anim. Behav. **17**, 755–759.
- Bishop, Y. M. M., Fienberg, S., and Holland, P. W. (1975) Discrete multivariate analysis: theory and practice. MIT Press, Cambridge.
- Cobb, M., Burnet, B., and Connolly, K. (1986) The structure of courtship in the *Drosophila melanogaster* species sub-group. *Behaviour* **97**, 182–211.
- Cobb, M., Connolly, K., and Burnet, B. (1987) The relationship between locomotor activity and courtship in the *melanogaster* species sub-group of *Drosophila*. Anim. Behav. **35**, 705–713.
- Cobb, M., and Ferveur, J.-F. (1996) Evolution and genetic control of mate recognition and stimulation in *Drosophila*. *Behav. Proc.* **35**, 35–54.
- Crossley, S., and Zuill, E. (1970) Courtship behaviour of some *Drosophila melanogaster* mutants. *Nature* **225**, 1064–1065.
- Ewing, A. W. (1983) Functional aspects of *Drosophila* courtship. *Biol. Rev.* **58**, 275–292.
- Everitt, B. S. (1992) The Analysis of Contingency Tables, 2nd ed. Chapman & Hall, London.
- Fienberg, S. (1969) Preliminary graphical analysis and quasi-independence for two-way contingency tables. *Appl. Stat.* **18**, 153–168.
- Grossfield, J. (1971) Geographic distribution and light-dependent behavior in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **68**, 2669–2673.
- Hoikkala, A., and Welbergen, P. (1995) Signals and responses of females and males in successful and unsuccessful courtships of three Hawaiian lek-mating *Drosophila* species. *Anim. Behav.* **50**, 177–190.
- Jallon, J.-M. (1984) A few chemical words exchange by *Drosophila* during courtship and mating. *Behav. Genet.* **14**, 441–478.
- Kyriacou, C. P., and Hall, J. C. (1982) The function of courtship song rhythms in *Drosophila*. *Anim. Behav.* **30**, 794–801.
- Lindsley, D. L., and Zimm, G. G. (1992) The Genome of *Drosophila melanogaster*. Academic Press, New York.
- Manning, A. (1959) The sexual behaviour of two sibling *Drosophila* species. *Behaviour* **15**, 123–145.
- Markow, T. A. (1975) Effect of light on egg-laying rate and mating speed in phototactic strains of *Drosophila*. *Nature* **258**, 712–714.
- Markow, T. A., and Manning, M. (1980) Mating success of photore-

- ceptor mutants of *Drosophila melanogaster*. Behav. Neural Biol. **29**, 276–280.
- Oguma, Y., Jallon, J.-M., Tomaru, M., and Matsubayashi, H. (1996) Courtship behavior and sexual isolation between *Drosophila auraria* and *D. triauraria* in darkness and light. J. Evol. Biol. **9**, 803–815.
- Pearn, M. T., Randall, L. L., Shortridge, R. D., Burg, M. G., and Pak, W. L. (1996) Molecular, biochemical, and electrophysiological characterization of *Drosophila norpA* mutants. J. Biol. Chem. **271**, 4937–4945.
- Robertson, H. M. (1983) Mating behavior and evolution of *Drosophila mauritiana*. Evolution **37**, 1283–1293.
- Sakai, T., Isono, K., Tomaru, M., and Oguma, Y. (1997) Contribution by males to intraspecific variation of the light dependency of mating in the *Drosophila melanogaster* species subgroup. Genes Genet. Syst. **72**, 269–274.
- Spieth, H. T., and Hsu, T. (1950) The influence of light on the mating behavior of seven species of the *Drosophila melanogaster* species group. Evolution **4**, 316–325.
- Tompkins, L., Hall, J. C., and Hall, L. M. (1980) Courtship-stimulating volatile compounds from normal and mutant *Drosophila*. J. Insect Physiol. **26**, 689–697.
- Tompkins, L., Gross, A. C., Hall, J. C., Gailey, D. A., and Siegel, R. W. (1982) The role of female movement in the sexual behavior of *Drosophila melanogaster*. Behav. Genet. **12**, 295–307.
- Tompkins, L. (1984) Genetic analysis of sex appeal in *Drosophila*. Behav. Genet. **14**, 411–440.
- von Schilcher, F. (1976) The function of pulse song and sine song in the courtship of *Drosophila melanogaster*. Anim. Behav. **24**, 622–625.
- Wayadande, A. C., and Nault, L. R. (1996) Leafhoppers on leaves: An analysis of feeding behavior using conditional probabilities. J. Insect Behav. **9**, 3–22.
- Welbergen, P., van Dijken, F. R., and Scharloo, W. (1987) Collation of the courtship behaviour of the sympatric species *Drosophila melanogaster* and *Drosophila simulans*. Behaviour **101**, 253–274.
- Wood, D., Ringo, J. M., and Johnson, L. L. (1980) Analysis of courtship sequences of the hybrids between *Drosophila melanogaster* and *Drosophila simulans*. Behav. Genet. **10**, 459–466.