

Acoustic communication in *Drosophila melanogaster* courtship: Are pulse- and sine-song frequencies important for courtship success?

Fanny Rybak, Thierry Aubin, Bruno Moulin, and Jean-Marc Jallon

Abstract: During courtship, the male *Drosophila melanogaster* (Diptera, Drosophilidae) emits two kinds of acoustic signals by means of wing vibrations oriented towards the female: pulse song and sine song. To examine the roles of these two song types, different experimental signals were played back to single pairs of flies (with the males made mute by wing removal). To preserve the natural pattern of sound emission, all the experimental signals were constructed from a representative complete song that included both the pulse and the sine components, recorded during a successful courtship. The first experiment showed that playback of a natural complete song restored to wingless males courtship success comparable to that of normal winged males. In the second experiment, in which we distinguished between the effects of pulse song and sine song, pulse song alone was sufficient to stimulate matings between wingless males and virgin females; sine song had a less stimulating effect. In a third experiment we examined the consequences of modifying the carrier frequency of either signal. This had no particular effect on responses to either pulse or sine song. These results suggest that for both these signals, frequency is not involved in the mating success of *D. melanogaster*.

Résumé : Pendant la parade sexuelle, le mâle de *Drosophila melanogaster* (Diptera, Drosophilidae) produit deux types de signaux acoustiques par vibration alaire en direction de la femelle, le chant pulsé et le chant sinusoïdal. Afin de rechercher le rôle de ces deux chants, différents signaux expérimentaux ont été diffusés à des couples isolés de mouches (dont les mâles avaient été rendus muets par ablation des ailes). Afin de préserver le rythme naturel d'émission sonore, tous les signaux expérimentaux ont été construits à partir d'un chant complet représentatif incluant les composantes pulsée et sinusoïdale, enregistré au cours d'une parade qui a conduit à l'accouplement. Une première expérience a montré que la diffusion d'un chant complet naturel restaure aux mâles aptères un succès de parade comparable à celui de mâles ailés normaux. Dans une seconde expérience, nous avons distingué les effets du chant pulsé et du chant sinusoïdal; le chant pulsé seul est suffisant pour stimuler les accouplements entre mâles aptères et femelles vierges. Le chant sinusoïdal a un effet stimulateur moindre. Dans une troisième expérience, nous avons examiné les conséquences de la modification de la fréquence de chacun des deux types de signaux. La valeur de la fréquence n'a aucun effet particulier sur les réponses, ni pour le chant pulsé ni pour le chant sinusoïdal. Cela semble indiquer que la fréquence de ces signaux acoustiques n'est pas impliquée dans le succès de la parade sexuelle de *D. melanogaster*.

Introduction

During *Drosophila* courtship, the two sexual partners exchange information through several channels: chemical, tactile, visual, and acoustic (Ewing 1983). The male produces two kinds of acoustic signals by means of wing vibration: pulse song and sine song. The sounds are perceived through the arista, a branched organ on the third antennal segment

(Petit 1958; Uga and Kuwabara 1965; Manning 1967; Burnet et al. 1971; Eberl et al. 1997), which acts as a transducer, transmitting the vibrations to the chordotonal sensilla of Johnston's organ in the second antennal segment.

Several studies have described the acoustic parameters of the signals emitted by male *Drosophila melanogaster*. The pulse song (PS) consists of intermittent trains of sound pulses, each pulse consisting of one or two cycles (Bennet-Clark and Ewing 1970; Kulkarni and Hall 1987). The sine song (SS) is a sinusoidal hum (Schilcher 1976a). Most reported analyses based on selected bouts of courtship song have revealed the carrier frequencies of both signals: the mean frequency of the PS has been estimated to be between 200 and 280 Hz (Cowling and Burnet 1981; Wheeler et al. 1988) and the frequency of SS around 160–170 Hz at 25°C (Schilcher 1976a; Kyriacou et al. 1978; Cowling and Burnet 1981). Furthermore, the interpulse interval (IPI) has been found to have a modal value of 30–35 ms at 25°C (Bennet-Clark and Ewing 1968; Cowling and Burnet 1981; Wheeler et al. 1988; Ritchie et al. 1994), depending on the population studied. A rhythmic modulation in the production of the IPI, with a period of approximately 50–65 s, has been also reported by

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Kyriacou and Hall (1980, 1989) and Alt et al. (1998). Recently, our analysis of complete courtships, i.e., until copulation occurred, has revealed stability in the pattern of PS, with 90% of bursts containing fewer than 12 pulses (Aubin et al. 2000).

By analysing songs, one can extract the potential acoustic parameters involved in courtship success; however, the only way to understand coding systems is to play back experimental signals to flies. Observation and quantification of behavioural responses allow the assessment of which acoustic features carry information.

Several playback experiments have already been performed with *D. melanogaster*, and the intensity and the rhythms of the songs produced by the male are the two parameters that have been most intensively studied with regard to their role in coding systems. Eberl et al. (1997) found that the amplitude thresholds necessary to elicit a response varied between 72 and 95 dB (sound pressure level; SPL) and Crossley et al. (1995) found that they varied between 78 and 108 dB (particle velocity). As the amplitude shape of such signals is strongly modified during propagation, even at a short field range (Bennet-Clark 1971; Wiley and Richards 1982; Michelsen et al. 1987), this parameter is less likely to be implicated in the coding-decoding process of pulse and sine songs. For pulse song, the IPI has been found to be species-specific (Bennet-Clark and Ewing 1969; Schilcher 1976b). The rhythmicity of the IPI is also thought to be involved in species recognition (Kyriacou and Hall 1982, 1986; Greenacre et al. 1993; Ritchie et al. 1999) and is presumed to enhance the receptivity of females (Kyriacou and Hall 1984). Both these parameters act as targets for species-specific recognition of a male by a female. Sine song seems to have priming effects on the receptivity of both the female and the male (Schilcher 1976a, 1976b), although results varied among studies (Kyriacou and Hall 1982; Crossley et al. 1995; Eberl et al. 1997).

All previous playback experiments (Bennet-Clark and Ewing 1969; Schilcher 1976a, 1976b; Kyriacou and Hall 1982, 1984, 1986; Crossley and Bennet-Clark 1993; Greenacre et al. 1993; Crossley et al. 1995) shared two features. First, the signals played back were always artificial signals, constructed using particular acoustic parameters obtained by analysis. Second, apart from a few recent studies (Tomaru et al. 1995; Ritchie et al. 1998; Isoherranen et al. 1999), signals were played back to groups of flies (males and females or several flies of the same sex). Such protocols are not particularly artificial, since, in the wild, courtship occurs on food resources, and when a male courts a female there are often numerous flies in the same place. Nevertheless, such a situation leads to numerous interactions between individuals, particularly males.

In our study we observed the behaviour of single pairs of flies when natural or modified songs were played back. Measuring the responses to song of flies in such an experimental situation allows the sound stimuli tested to be separated from other stimuli resulting from the presence of other flies. The first step was to assess the importance of the role of acoustic signals in the sexual behaviour of *D. melanogaster*. The second step was to study the relative influence of pulse song and sine song. As the importance of frequency parameters has been less studied than temporal and intensity parameters, a third step was to vary the frequency of pulse and

sine songs progressively without changing the natural intensity and rhythms of production.

Materials and methods

Flies

We used an old Canton S strain of *D. melanogaster* that has been kept in the laboratory since 1978. The stock was raised on cornmeal-agar-molasses-yeast medium in controlled temperature and light conditions: 25°C with a 12 h light : 12 h dark cycle with lights on at 09:00.

Virgin males and females were sexed within 4 h of eclosion under light CO₂ anaesthesia and placed in vials with fresh medium. Females were placed into groups of 5, while males were housed alone.

Wings were removed from males at the time of sexing, using fine scissors and forceps: the wing was spread from the thorax using the forceps and then cut at the level of the humeral cross vein (see cut A in Ewing 1964). As the arista are involved in sound perception and hygrosensation (Sayeed and Benzer 1996), males' arista were kept intact so as not to disturb any sensation or courtship interactions between male and female, and in particular to allow the perception of acoustic signals emitted by the female (Paillette et al. 1991).

Flies were 3–4 days old when tested.

Breeding conditions and experimental procedures followed Canadian Council of Animal Care guidelines.

Recording method

The original signal (termed full song; FS) was recorded in an insulated anechoic room belonging to the Institut National de la Recherche Agronomique at Jouy en Josas. Flies were put in a cylindrical chamber 10 mm in internal diameter, the same diameter as the microphone grid. The floor of the chamber was the microphone itself, protected by a fine nylon mesh. A 4176 BRUEL and KJAER ½ in. prepolarized condenser microphone coupled to a ZC 0020 BRUEL and KJAER preamplifier (frequency range 6.5 Hz and 12.5 kHz ± 1 dB) was used. Recording was then done on one channel of a SONY TCD 3 D.A.T. recorder (the frequency response curve was flat within 1 dB between 20 Hz and 20 kHz) with a sampling frequency of 48 kHz. The signal recorded corresponded to 16 bits of digital data. These data were then transferred to a PC by means of an AUDIOMEDIA III PCI acquisition card interface and finally stored as a file on the computer hard disk for all subsequent processing.

Playback-experiment design

We used two mating chambers (7 mm inside diameter × 3 mm high) with transparent nylon net floors. The chambers were included in a Plexiglas floor wheel placed on nylon mesh 5 mm above a SOSHIN loudspeaker (50 mm diameter, 8 Ω). A roof wheel with a small hole was superposed on the floor wheel; it could rotate on a central axis. The flies were introduced through the hole. This apparatus was similar to the one used by Tomaru et al. (1995) except that the loudspeaker was smaller and there were only two mating chambers. Experimental signals were played back using a SONY TCD 3 D.A.T. recorder and transmitted to the loudspeaker via a 10-W power amplifier built in the laboratory. Experimental signals were played at a mean SPL of 80 dB mea-

Table 1. Frequency and temporal parameters of the full song (FS) used as the original signal and the ranges of average population values of songs ($n = 22$) from which the FS was selected.

	Mean \pm SE ^a	Population range
Pulse song		
Frequency (Hz)	238.2 \pm 2.2 (1003)	224.5–254.1
Interpulse interval (IPI) (ms)	31.14 \pm 0.46 (828)	30.2–41.93
No. of pulses per burst	5.6 \pm 0.3 (179)	3.64–12.36
Duration of pulse-song burst (ms)	145.59 \pm 11.33 (179)	107.67–417.48
Sine song		
Frequency (Hz)	167.5 \pm 1.8 (104)	161.7–171.8
Duration of sine-song bout (ms)	463.17 \pm 0.47 (104)	164.22–518.66

^aNumbers in parentheses show the number of values.

sured with a 4176 BRUEL and KJAER ½ in. prepolarized condenser microphone at the level of the floor of the observation cell.

Modified songs

Eleven signals were used for the playback experiments, and each signal tested was played back for 30 min.

The first signal (“full song”; FS) was a natural song, including both pulse and sine songs. Based on previous courtship-song analysis (Aubin et al. 2000) we selected one song that was representative of the species. It was produced by a mature male *D. melanogaster* during a successful courtship of a mature virgin female. The acoustic parameters of the FS were analysed and the results are summarized in Table 1. All these parameters fall in the natural ranges for *D. melanogaster*. For measuring brief sound impulses in the frequency domain, a fast Fourier transform (FFT) analysis is not adapted (Randall and Tech 1987) and so frequency analysis was carried out using the zero-crossing method. The second signal (pulse song with a natural frequency of 240 Hz; PS240) was obtained by filtering the FS (bandpass 170–600 Hz), which removes the bouts of sine song. The third signal (sine song with a natural frequency of 170 Hz; SS170) was obtained using another bandpass filtration (bandpass 100–170 Hz), which removes the pulses. Bandpass filtration was done by applying optimal filtering with a FFT (Press et al. 1988; Mbu-Nyamsi et al. 1994). The window size of the FFT was 4096. The other signals were songs with a natural temporal and intensity pattern of emission but with frequencies modified. Signals with modified frequencies were obtained by means of frequency shifts up and down applied to the two previous signals described above: PS240 and SS170. The values tested were 200 Hz (PS200), 280 Hz (PS280), 320 Hz (PS320), and 360 Hz (PS360) for the pulse song and 130 Hz (SS130), 150 Hz (SS150), 190 Hz (SS190), and 210 Hz (SS210) for the sine song. All these songs had the same duration as the FS (5 min).

The frequency-shift values for pulse song were higher than those for sine song because the natural frequency of pulse song showed greater variations than the natural frequency of sine song (Wheeler et al. 1988). The range of frequency values tested was larger than the range of frequency values naturally produced by male *D. melanogaster*. Thus, 320 and 360 Hz are frequencies that are almost never produced in natural pulse song, and 130, 190, and 210 Hz are frequencies that are never produced in natural sine song. All

these frequency-shift values fall within the hearing sensitivity of the arista (Göpfert and Robert 2001).

Modification of frequencies was carried out by picking the data record through a square window and applying a brief overlapping (50%) FFT followed by a linear shift in values of each spectrum and a short-term inverse FFT (Randall and Tech 1987). The window size was 4096 points ($\Delta f = 11.72$ Hz). With a frequency shift up or down, the resulting envelopes of pulse song and sine song were shortened or lengthened but the overall AM shape (i.e., the relative amplitude variations versus time) remained unchanged. In the same way, occurrences of pulses and sine bouts were not modified.

Experimental conditions

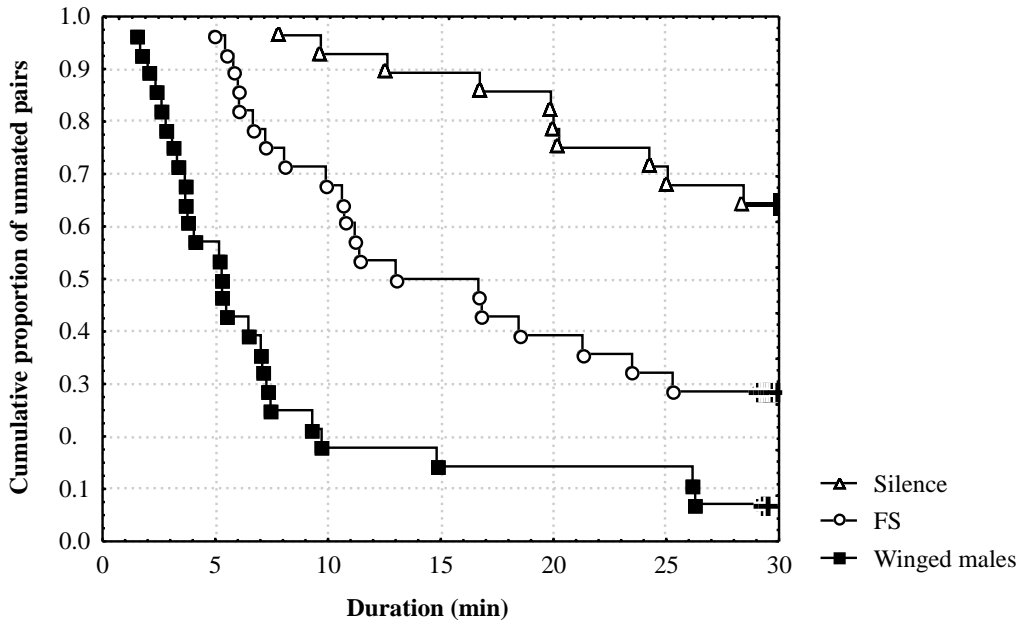
All experiments were performed between 09:00 and 13:00 at a temperature of $25 \pm 1^\circ\text{C}$ (measured with a digital thermometer) controlled by a heating/cooling system.

One male was introduced into each chamber with an aspirator via a hole in the ceiling partition. After a 2-min period during which the males habituated to their new environment, one female was introduced into each chamber and the playback session began. Courtship behaviours of two pairs were observed simultaneously for 30 min. Then the mating speed and the courtship success of single pairs subjected to various conditions were analysed.

Four separate experiments were performed. The first tested the reliability of the playback modalities. The courtship success of intact winged males with females was compared with that of wingless males with females under two sets of conditions: first with wingless males and FS playback and secondly with wingless males in silence. The second experiment aimed to distinguish the effects of pulse song and sine song on the courtship success of single pairs of flies. Three experimental conditions were compared: wingless males in silence, wingless males given PS240 playback (sine song removed), and wingless males given SS170 playback (pulse song removed). Finally, in experiments 3 and 4 we examined the effects of modifying the frequencies of pulse and sine songs. For pulse songs, frequencies of 200, 240, 280, 320, and 360 Hz were tested, corresponding to PS200, PS240, PS280, PS320, and PS360. For sine songs, frequencies of 130, 150, 170, 190, and 210 Hz were tested, corresponding to SS130, SS150, SS170, SS190, and SS210.

For the first two experiments, the control situation was the

Fig. 1. Comparison between survival curves for *Drosophila melanogaster* in the unmated state in courtship tests with wingless males with and without song playback and with winged males ($n = 28$ in each experimental situation). A “+” sign between 28 and 30 min indicates censored data. The distributions are significantly different ($\chi^2 = 37.22491$, $p < 0.001$).



observation of courtship by a wingless male in the absence of broadcast signal.

As all the modified signals used in our playback experiments were built from the same natural song (FS), the problem of pseudoreplication should have occurred (McGregor et al. 1992). Nevertheless, for each playback experiment, natural song and a modified song were presented in association. For each reconstruction, the FS was modified along only one dimension: a frequency parameter. When a simple dimension is used as an independent variable, the levels of the variable could be under the direct control of the experimenters, and thereby less likely to be affected by external variables (Hopp and Morton 1998). The use of artificially modified or built stimuli gave us control over the differences between our stimuli, greatly minimizing pseudoreplication (McGregor et al. 1992).

Observation of courtship: the parameters measured

To quantify the effects of the different signals played back, we have taken into account the following parameters: courtship latency, copulation latency, and courtship duration. Courtship latency corresponds to the time (in seconds) elapsed between the introduction of the female into the chamber and the first orientation of the male toward her. Copulation latency corresponds to the time (in seconds) elapsed between the introduction of the female into the chamber and copulation. Courtship duration is the time (in seconds) spent by each pair of flies in the unmated state. It has been calculated, for all pairs, as copulation latency minus courtship latency. As the observation period was limited to 30 min, when a male and a female did not mate within this limited time, their copulation latency was scored as equal to the maximum time (30 min) and their courtship duration was assigned to be censored observation (Hald 1949).

Finally, the proportion of matings was also scored for each experimental condition.

Statistical analysis

First, the data were tested for normality using Lilliefors and Shapiro–Wilk tests. As the data were usually not normally distributed, non-parametric analyses were used.

For each experiment, courtship latencies were compared between groups using a Kruskal–Wallis test for K independent samples. The distributions of courtship durations, i.e., the periods during which the male and the female remain unmated, were compared using a survival-analysis approach. For each experimental condition, a survival function can be defined as the probability that an individual courtship duration T is longer than a given time t : $S(t) = P(T > t)$.

Graphs of $S(t)$ (cumulative proportion of unmated pairs) against t (duration) were plotted using the Kaplan and Meier (1958) product-limit estimator. Such plots are known as survival curves. Then several survivorship distributions in a given experiment were compared using the Gehan–Wilcoxon test. Briefly the procedure is as follows: a score is attributed to each duration using Mantel’s procedure (Mantel 1967), then a χ^2 value is calculated on the basis of the sums of this score (in each experimental situation).

The numbers of copulations that occurred in each experimental condition were compared using a G test (Sokal and Rohlf 1981).

All statistic computations were carried out with Statistica version 5 software.

Results

Importance of courtship song

Twenty-eight pairs of flies were tested for each experimental condition. Figure 1 compares survival functions of the unmated state in each experimental condition. There was a significant difference between the three distributions ($p < 0.001$). The durations in the unmated state were, on average, shorter when males were winged and when a FS was played

Fig. 2. Comparison between survival curves for *D. melanogaster* in the unmated state in courtship tests with wingless males, using either pulse- or sine-song playback and silence ($n = 28$ in each experimental situation). The distributions are significantly different ($\chi^2_2 = 21.83021, p < 0.001$). A “+” sign between 28 and 30 min indicates censored data.

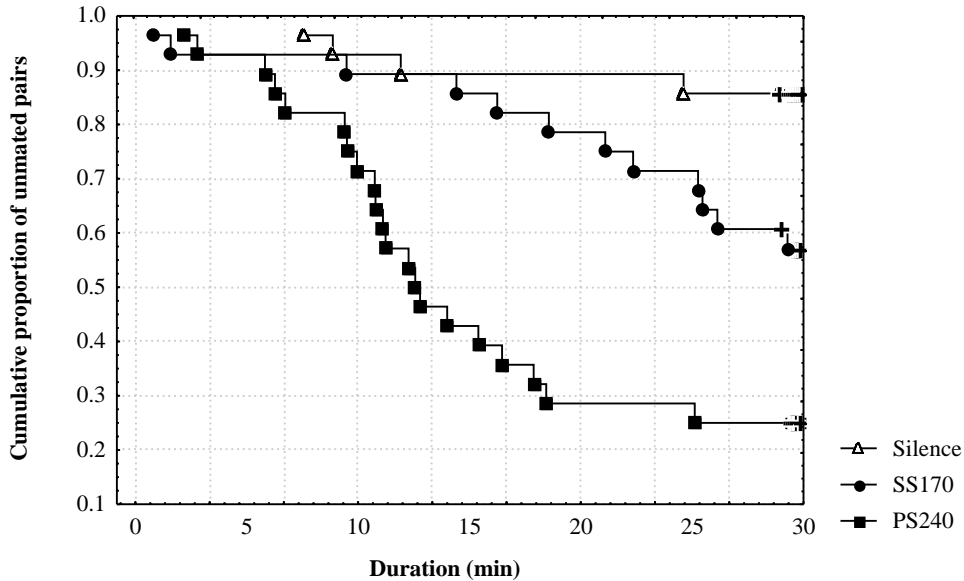


Table 2. Proportions of matings in the different experimental conditions.

	Proportion of matings
Expt. 1	
Wingless males + silence	10/28
Wingless males + FS	20/28 ^a
Winged males	26/28 ^a
Expt. 2	
Wingless males + silence	4/28
Wingless males + SS170	12/28 ^a
Wingless males + PS240	21/28 ^a
Expt. 3	
Wingless males + PS200	23/30
Wingless males + PS240	23/30
Wingless males + PS280	18/30
Wingless males + PS320	20/30
Wingless males + PS360	13/30 ^b
Expt. 4	
Wingless males + SS130	14/36
Wingless males + SS150	15/36
Wingless males + SS170	17/36
Wingless males + SS190	15/35
Wingless males + SS210	20/36

^aSignificant difference between the wingless males + silence condition and the other conditions (expts. 1 and 2).

^bSignificant difference between the wingless males + PS240 and the wingless males + PS360 conditions (expt. 3).

back to wingless males than in the control situation (wingless males + silence) ($W = 3.312082, p < 0.001$, and $W = 5.53072, p < 0.001$, respectively). There was also a significant difference between the survival curves obtained when males were winged and wingless but a FS was played back ($W = 3.902894, p < 0.001$). The median courtship duration was much longer when a FS was played back to wingless males than when males were winged and sang their own

song (890 versus 317 s). Finally, after 30 min the number of matings was significantly higher for winged males than for wingless males with FS ($G = 4.41, p < 0.05$), and significantly higher for wingless males with FS than for wingless males in silence ($G = 7.15, p < 0.01$) (Table 2).

The respective importance of pulse and sine song

Twenty-eight pairs of flies were tested for each experimental condition. The distributions of the cumulative proportions of nonmated pairs differed significantly among the three experimental situations ($p < 0.001$) (Fig. 2). As is shown by the comparison of survival curves, playback of PS240 to wingless males led to significantly more matings in a shorter time compared with the control condition (wingless males + silence) ($W = 4.27513, p < 0.001$). Playback of SS170 had also an effect on the proportion of matings: wingless males + SS170 mated three times more than wingless males in silence (Table 2), and the survivorship functions describing the cumulative proportions of the pairs that remained unmated versus time in these two conditions were significantly different ($W = 2.10602, p < 0.05$). The comparison of the survival curves obtained in the two playback conditions also showed a significant difference ($W = 3.105285, p < 0.005$). There were more matings (75 versus 42.9%) when a pulse song (PS240) was played back than when a sine song (SS170) was played back. In addition, the duration in the unmated state was shorter when PS240 was played back than when SS170 was played back (median courtship durations were 761 and 1771 s, respectively).

The importance of frequency values in the pulse song

Thirty pairs of flies were tested for each experimental condition. As Fig. 3 shows, there was a significant difference among the distributions of the cumulative proportions of unmated pairs obtained for each experimental condition ($p < 0.02$). All the pulse songs played back, except PS360, had a similar effect on the courtship success of wingless

Fig. 3. Comparison between survival curves for *D. melanogaster* in the unmated state as a function of the frequency of the pulse song played back ($n = 30$ in each experimental situation). The distributions are significantly different ($\chi^2_4 = 11.73$; $p < 0.02$). A “+” sign between 28 and 30 min indicates censored data.

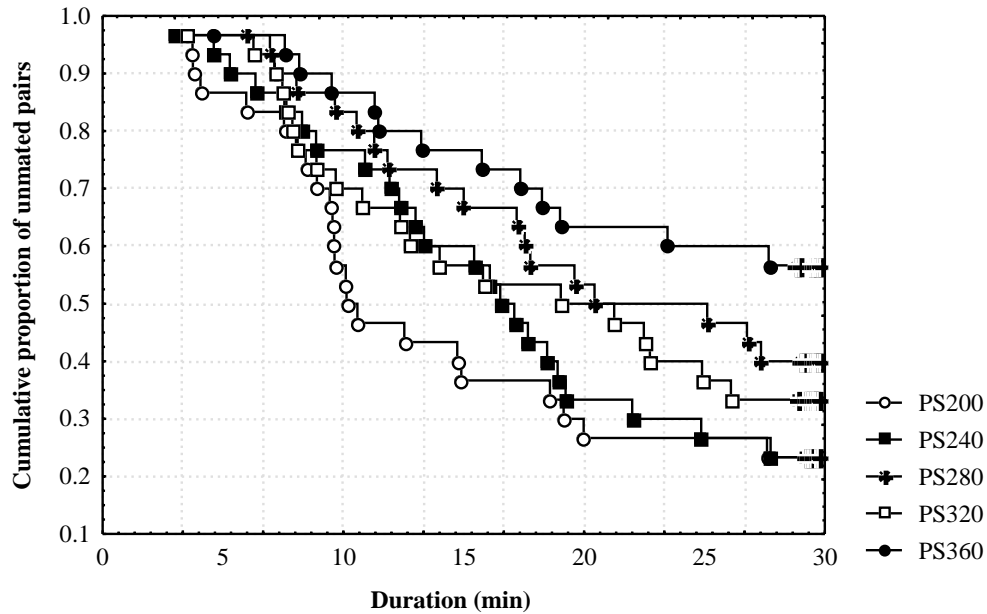
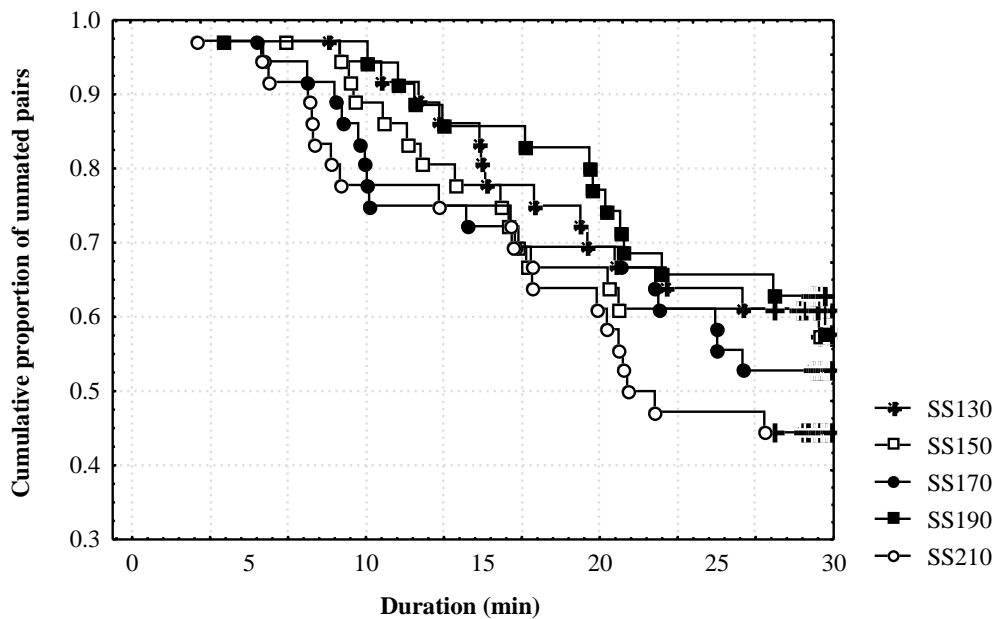


Fig. 4. Comparison between survival curves for *D. melanogaster* in the unmated state as a function of the frequency of the sine song played back ($n = 36$, except for SS190 playback, where $n = 35$). A “+” sign between 28 and 30 min indicates censored data.



males. Actually, individual comparisons showed no difference between the distributions of cumulative proportions of unmated pairs obtained using playback of a pulse song with a natural frequency (PS240) and each distribution obtained using playback of a pulse song with modified frequencies of either 200, 280, or 320 Hz. Likewise, the number of matings after 30 min did not differ among PS200, PS240, PS280, and PS320 conditions ($G = 2.83$, $p > 0.05$). However, there was a significant difference between the PS240 and PS360 conditions in both the distributions of the cumulative pro-

portions of unmated pairs ($W = 2.357057$, $p < 0.02$) and the proportions of matings (Table 2).

The importance of frequency in the sine song

Thirty-six pairs of flies (only 35 pairs for SS190) were tested for each experimental condition. Figure 4 gathers the survival functions of the unmated state as a function of the frequency of the sine song played back. A comparison of the distributions of the cumulative proportions of the unmated pairs as a function of time in the five modified song

conditions (SS130, SS150, SS170, SS190, and SS210) indicated no difference ($\chi^2_4 = 4.135428$, $p = 0.38801$). Likewise, the proportions of matings obtained at the end of the experiment did not differ between conditions (Table 2).

Discussion

This study focuses on the courtship success and mating speed of single pairs of *D. melanogaster* when synthetic signals are played back. A single pair of flies is the simplest experimental model for studying the effects of the signals on the interactions between a male and a female during courtship. Indeed, female *D. melanogaster* show no obvious behavioural responses, such as wing spreading or flicking, when they hear acoustic stimuli, unlike other species such as *Drosophila montana* or *Drosophila mercatorum* (Ritchie et al. 1998; Ikeda et al. 1981). So it is practically impossible to measure the sexually stimulatory effects of playbacks on isolated females. A major advantage of measuring the responses of a single pair of flies to song is that it enables the tested sound stimuli to be separated from other stimuli produced by other flies. Thus, group effects can be avoided, such as summation of stimuli provided by several males, aggressive encounters between males, etc. Nevertheless, a disadvantage of testing single pairs of flies is that the females are unable to make simultaneous comparisons between different courting males.

All signals played back to flies were produced using the natural FS as a temporal template. This procedure allowed us to analyse only the effects of the signal category (pulse song or sine song) or of modification of the frequency of either type of signal.

The results obtained in the first experiment show that such an experimental protocol was suitable for studying the coding process of acoustic signals. A complete natural song, which carried both a natural pattern of emission and natural frequencies, played to wingless males restored to them a high level of courtship success, close to that of winged males. For wingless males without the help of played-back song, courtship success was considerably reduced, as already reported (Sturtevant 1915; Rendel 1951; Ewing 1964). Hence, the acoustic signals produced by males seem to play a major role in sexual stimulation. However, normal winged males mated more often and faster than wingless males when a complete natural song was played back. Four explanations of this result are possible. First, as demonstrated by Bennet-Clark and Ewing (1967), when a male vibrates his wings, the tonic component of the stimulation acts as a stimulus. Wingless males like those used in our experiments cannot produce air currents, and the playback provides only the phasic component of the acoustic stimuli. It may also be that the directionality of the stimulus is unnatural, as it comes from below in the playback experiments and from the same horizontal plane with a winged male. Third, the playback of signals and the behaviour of the wingless male, which performs a vigorous courtship (including tapping, licking, and attempted copulation) despite lacking wings, could interfere with each other. Hence, the female could be sometimes hampered and confounded by hearing acoustic signals that do not correspond to the behaviour of the male. A fourth explanation is that wing vibrations could also play a part in spread-

ing male cuticular hydrocarbons, which are thought to enhance female receptivity (Jallon 1984; Scott 1994; Rybak et al. 2002).

Only Crossley et al. (1995) played back a signal that included both pulse and sine components, but flies of either sex were used in groups. Those authors showed that such a signal leads to a decrease in the locomotor activity of females and an increase in the courtship activity of males towards other males. The authors concluded that the combination of the two effects during male–female interactions would facilitate mating. By showing that a complete song of *D. melanogaster* obviously facilitates the mating of a wingless male, our results with single pairs of flies support the interpretations of Crossley et al. (1995).

By separately analysing the effects of sine and pulse songs, we emphasized their distinct effects. When pulse song alone was played back to a female associated with a wingless male, it had a beneficial effect on mating success (experiment 2), as did the complete song, including the sine component (experiment 1). Thus, pulse song alone seems to be sufficient to restore high courtship success to wingless males.

The effect of sine-song playback on the mating success of single pairs of flies is less important than that of pulse-song playback. Our results indicate that the playback of sine song has positive effects on the mating success of single pairs of flies. Nevertheless, when only a sine song is played back to wingless males, they do not attain a proportion of matings comparable to that of winged males. It can be added that the results obtained with sine-song playback (experiment 2) are close to those obtained using silence in experiment 1. Sine song has been found to have a noticeable effect on the locomotor activity of groups of either female (Schilcher 1976a) or male (Crossley et al. 1995) flies. The effect of sine song on the locomotor activity of the males described by Crossley et al. (1995) is similar to that which we found on the mating success of pairs of flies, in that it is less pronounced than when a pulse + sine song is played back. So, in different experimental conditions, with flies grouped or only in pairs, sine song has intermediate effects on both locomotor activity and courtship success. Schilcher (1976b) and Kyriacou and Hall (1984) also demonstrated that the playback of sine song to females before they were placed with males prestimulated them sexually. This signal should lead the female to a receptivity threshold that triggers her acceptance of the courting male. All these results strongly suggest that sine song plays a role in mate recognition and (or) sexual stimulation, but may act synergistically with parameters of the pulse song such as IPI values or burst organization.

Modifying the frequency of pulses did not seem to affect mating success significantly. It means that for pulse song, the information is not carried by the frequency but by other parameters such as the temporal pattern of emission of pulses (IPI) and bursts. All pulse songs with artificially modified frequencies, except one, have the same effect as a natural pulse song. Only the pulse song with a frequency of 360 Hz lowers mating success. In the past, Bennet-Clark and Ewing (1969) played back to groups of flies artificial pulse songs with three frequencies: 166, 333, and 666 Hz. The observed responses to these three frequencies were similar. Why did 360 Hz reduced mating in our study but the higher frequency, 666 Hz, used by Bennet-Clark and Ewing (1969)

did not? One possible explanation might be linked to the sensitivity of the arista, the receptor organ for acoustic signals. This organ has a sensibility threshold around 450 Hz (Ewing 1978) and a resonant frequency between 180 and 220 Hz (Bennet-Clark and Ewing 1967). It should vibrate with maximum amplitude at the resonant frequency and if the frequency of the stimulating signal rises, the amplitude of the vibration should decrease. So it is possible that PS360, which is close to the sensitivity threshold, is perceived by flies weakly or not at all. In Bennet-Clark and Ewing's (1969) experiment, the pulse frequency of 666 Hz, even though it is higher, might still be perceived because it is emitted at a pressure level considerably higher (a peak-to-peak SPL of 122 dB) than in our study (a peak-to-peak SPL of 80 dB). To explain why different pulse song frequencies do not affect mating success, we propose the following hypothesis: the pulse duration (around 3 ms) is probably too short to allow the fly to analyse and decode the frequency. According to the theory of spectral analysis (Pimonow 1962), the ability to measure the frequency of a continuous periodic event (such as a tone) is constrained by the time interval over which the animal can measure it. That is, uncertainty in measuring frequency decreases when the duration of analysis increases (see also Bregman 1978; Dooling 1982; Beecher 1988). In vertebrates, for example, the time threshold necessary for discriminating modulation up to 333 Hz is approximately 3 ms for the sedge warbler, *Acrocephalus schoenobaenus* (Brackenbury 1978). Similarly, in auditory processes used for analysing speech, the minimal time over which differences in waveforms can be distinguished (auditory temporal acuity) is estimated as 2 ms (Green 1985). In insects (Tettigoniidae), Rössler et al. (1990) have shown that at the level of the auditory neuropile in the tympanal organs, single short impulses (200 μ s) of varying carrier frequencies (10, 20, and 50 kHz) are discriminated. But the neuropile is the first location for the processing of auditory information. In the current state of knowledge of the physiological basis of the auditory integration process (from the first acoustic-signal receptors to the integrative centres in the brain), one cannot know whether insects have faster systems of spectral analysis than vertebrates. So we can only suppose that, as for vertebrates, a minimal time period is necessary for analysing a frequency, and this period should be at least 3 ms, i.e., longer than the pulse duration.

Concerning the sine song, there is no significant difference between the five frequency-modified signals. As for pulse song, it does not seem that different sine-song frequencies affect mating much. This result seems surprising. First, flies are likely to be able to analyse the frequency of sine-song bouts, which, in contrast to pulses, provide a time period long enough to allow accurate frequency analysis. Second, as sine songs are produced at different frequencies among the closely related species of the *D. melanogaster* complex (*D. melanogaster*, *Drosophila simulans*, *Drosophila mauritiana*, and *Drosophila sechellia*) (Cowling and Burnet 1981), these parameters seem adequate to play a role in species-specific recognition. Nevertheless we did not observe any differences between the effects of a sine song with a *D. simulans*-like frequency (SS190) and a natural *D. melanogaster* sine song on *D. melanogaster*. Moreover, Tomaru and Oguma (2000) demonstrated recently in choice tests that female *D. melano-*

gaster preferred winged male *D. sechellia* to wingless males *D. melanogaster*, while male *D. sechellia* produced no sine songs at all (Cobb et al. 1989). It seems, then, that the frequency parameter in the sine song is not taken into account by female *D. melanogaster* for species recognition, which could be one explanation of the relatively broad female mate recognition demonstrated by Tomaru et al. (2000). Nevertheless the sine song has an effect on the mating behaviour. So if sine-song frequency is not taken into account, which other acoustic parameters are likely to be analysed, especially with regard to intraspecific female choice? Another parameter that could be used by the female to make a choice between several males of the same species could be amplitude, as the SPL of the signal depends on the size of the sound-producing structure (the larger the wing, the louder the sound produced). The amplitude could then be used by the female to estimate the size of the male, and consequently could be an indicator of his fitness, as has been argued for Caribbean fruit flies (Burk and Webb 1983) and for *D. melanogaster* (Partridge et al. 1987). An alternative explanation is that the sine signal would be analysed not in terms of quality (frequency, duration, amplitude) but in terms of quantity. The male would provide the female with a stream of sine songs whose effect should summate and finally reach a critical level at which she accepts copulation. This concept of sexual stimulation of the female by summation of acoustic stimuli has already been suggested by Schilcher (1976b).

To conclude, we have demonstrated that song is an important factor in the courtship of single pairs of flies, particularly pulse song, the effect of sine song being more subtle. Our tests have also suggested that the frequencies of pulse and sine songs do not seem to affect mating success in the species *D. melanogaster*.

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