COMPLEX COURTSHIP SONGS IN THE *DROSOPHILA FUNEBRIS* SPECIES GROUP: ESCAPE FROM AN EVOLUTIONARY BOTTLENECK

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Abstract. Due to properties of the neuromuscular system involved in courtship song production in *Drosophila* there is a minimum song inter-pulse interval of approximately 10 ms. Species within the *funebris* species group all produce songs which contain such pulses. It is suggested that this has tended to produce an evolutionary bottleneck with regard to song pattern and, in order to facilitate species recognition, the following trends have occurred within this group of species. (1) The minimum inter-pulse interval songs are arranged into bursts with species specific inter-burst intervals (all species except *D. subfunebris*). (2) Distinct secondary songs which do not contain minimum inter-pulse interval bursts have been added to the song repertoire (all except *D. funebris* and *D. subfunebris*). (3) The original minimum inter-pulse interval bursts have been incorporated into the secondary song (*D. subfunebris*).

Introduction

There are between one and two thousand species in the genus *Drosophila* and in closely related genera such as *Zaprionus* and *Scaptomyza*. To date almost all the species examined have been shown to produce songs during courtship (Ewing & Bennet-Clark 1968; Ewing 1970). There is both direct and circumstantial evidence to show that the songs are involved in the maintenance of sexual isolation (Bennet-Clark & Ewing 1969). In the majority of cases where the ecology of *Drosophila* species has been examined, more than one species has been found to occupy similar niches and there is thus a high probability that some interspecific courtship occurs (Parsons 1973). It is therefore important that the songs are different, particularly in closely related species, where there is a possibility of interbreeding. So far this has been shown to be true. Although song pattern is not the sole sexual isolating mechanism, it is usually unambiguous and will allow rapid species identification (Spieth 1974; Ewing 1977a). It is of adaptive advantage that a female rejects an unsuitable mate as early as possible in courtship even although, due to other isolating mechanisms, there is no ultimate likelihood of miscegenation.

However, there may be limits to the number of discrete songs that can evolve. These will be set, first, by the properties of the receptor system and its ability to discriminate different sounds and, second, by the mechanics of sound production and attributes of the neuromuscular system. Thus, for example, the songs are perceived by Johnston's Organ on the antenna which has an upper frequency limit of about 500 Hz (Ewing 1978). Songs composed of frequencies above this limit will not be discriminated. Also, the antenna can not differentiate between single cycle pulses of different frequencies (Ewing 1978) and this reinforces earlier behavioural experiments by Bennet-Clark & Ewing (1969), who demonstrated that songs made up from such pulses are separated solely on the basis of inter-pulse interval.

There are also constraints on song pattern due to the mechanics of song production and its neuromuscular basis. One important song characteristic is the pulse repetition rate. The maximum rate for pulsed song is controlled by the requirement of a neurogenic muscle (the pulse timer muscle) to undergo cyclical contraction and relaxation, and an upper figure for this would be approximately 100/second, i.e. an inter-pulse interval (i.p.i.) of 10 ms (Pringle 1974; Ewing 1977b). It is therefore interesting to note that the shortest i.p.i.'s reported so far are of this order and are found in three unrelated *Drosophila* species. Thus they are likely to have evolved independently. The species are *D. athabasca* with an i.p.i. of 11·7 ms, *D. bipectinata*, 8·4 ms and *D. funebris*, 8·3 ms (Ewing & Bennet Clark 1968; Ewing 1978).

The song of *D. funebris* has a complex structure. Between 5 and 6 single cycle pulses are grouped into bursts with a relatively constant inter-burst interval. Songs of this type are commonplace in Orthoptera (Alexander 1962), but have not been described for *Drosophila*. Such patterning of songs would permit a much greater range of song types and might partially overcome the constraints described above. It thus seemed possible that species closely related to *D. funebris* might also have songs with minimum i.p.i.'s but with different inter-burst

intervals, this latter characteristic becoming important in species recognition. I therefore examined the songs of species within the *funebris* species group.

D. funebris Species Group

Five species have been described within this species group: *D. funebris*, *D. trispina*, *D. subfunebris*, *D. multispina* and *D. macrospina*. The last named has been split into three sub-species, *D. m. macrospina*, *D. m. limpiensis* and *D. m. ohioensis*. I was able to record the songs of all of these except for *D. trispina* and *D. m. ohioensis* which were not obtainable. The stocks used are listed in Table I.

D. funebris is a cosmopolitan species, *D. multispina* is confined to Japan and the remaining species have mainly allopatric distributions in North America. Hybridization experiments between the species which occur within North America suggest that *D. funebris* is the most distinct species as it does not hybridize with the remaining species but some degree of inter-

breeding is possible between some strains of the *D. macrospina* sub-species, *D. subfunebris* and *D. trispina* (Patterson & Stone 1952; Okada 1955).

The distribution of the species in conjunction with crossing experiments suggests that *D. funebris* is the more primitive species from which *D. multispina* and the North American species have independently evolved. The *D. macrospina* complex is still undergoing speciation.

Table I. D. funebris Group Species Used

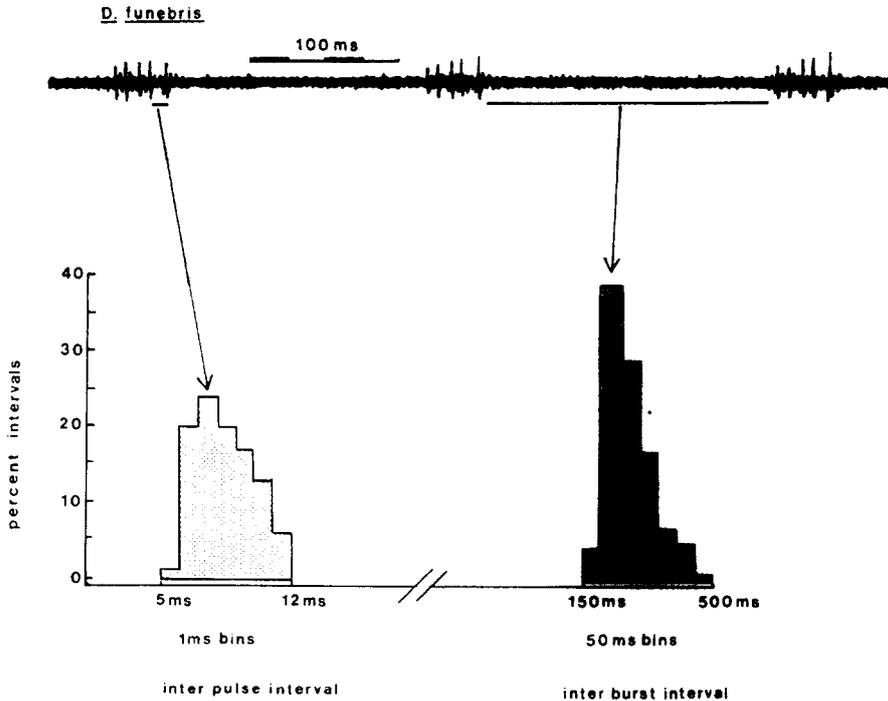| Species	Locality
D. funebris	Toronto, Canada
D. subfunebris	Pasadena, California
D. multispina	Sapporo Hokkaido, Japan
D. m. macrospina	Albuquerque, New Mexico
D. m. limpiensis	Patagonia, Arizona

Fig. 1. *D. funebris*. At the top an oscillogram shows an excerpt from a song with three bursts each consisting of 5 single cycle pulses. Bars under the oscillogram and the arrows leading from them relate the interval histograms to the song characteristics with inter-pulse intervals within bursts on the left and between bursts (inter-burst intervals) on the right.

Methods

The methods for recording courtship songs have been adequately described elsewhere (Ewing & Bennet-Clark 1968; Bennet-Clark 1972). Briefly, virgin flies (usually one male and one female) of between 3 and 6 days of age were introduced into gauze cells with a Perspex roof and the cell placed over the ribbon of a Grampian GR2 microphone. Some of the species examined were reluctant to court, possibly due to the small size of the cells (18 × 10 × 5 mm) which it was necessary to use in order to obtain satisfactory song records. These flies were often very inactive and, in order to counteract this tendency, two or three females were provided for a single male. Sounds picked up by the microphone were amplified and recorded on a Tandberg 3000X tape recorder. Courtship was simultaneously observed with a low power binocular microscope and the sounds produced by the flies monitored using head-phones. The intervals between pulses are temperature dependent and therefore all observations were carried out in a constant temperature room at 25·5 ± 0·5 C (Shorey 1962). The song records were subsequently filmed from an oscilloscope display (Tektronix 502) and the film analysed by manual measurement. An examination of Figs. 1 to 4 demonstrates the measures employed. It should be noted that an inter-pulse interval (i.p.i.) is measured from the beginning of one pulse to the beginning of the next whether the pulses are mono- or polycyclic. I have also used the term inter-burst interval (i.b.i) which is self-explanatory although it should be noted that an i.b.i. is also an i.p.i. as the former is measured from the beginning of the last pulse in a burst to the beginning of the first pulse of the subsequent burst. However no ambiguity arises with regard to the species described in this paper as the distributions of i.p.i.'s and i.b.i.'s do not overlap.
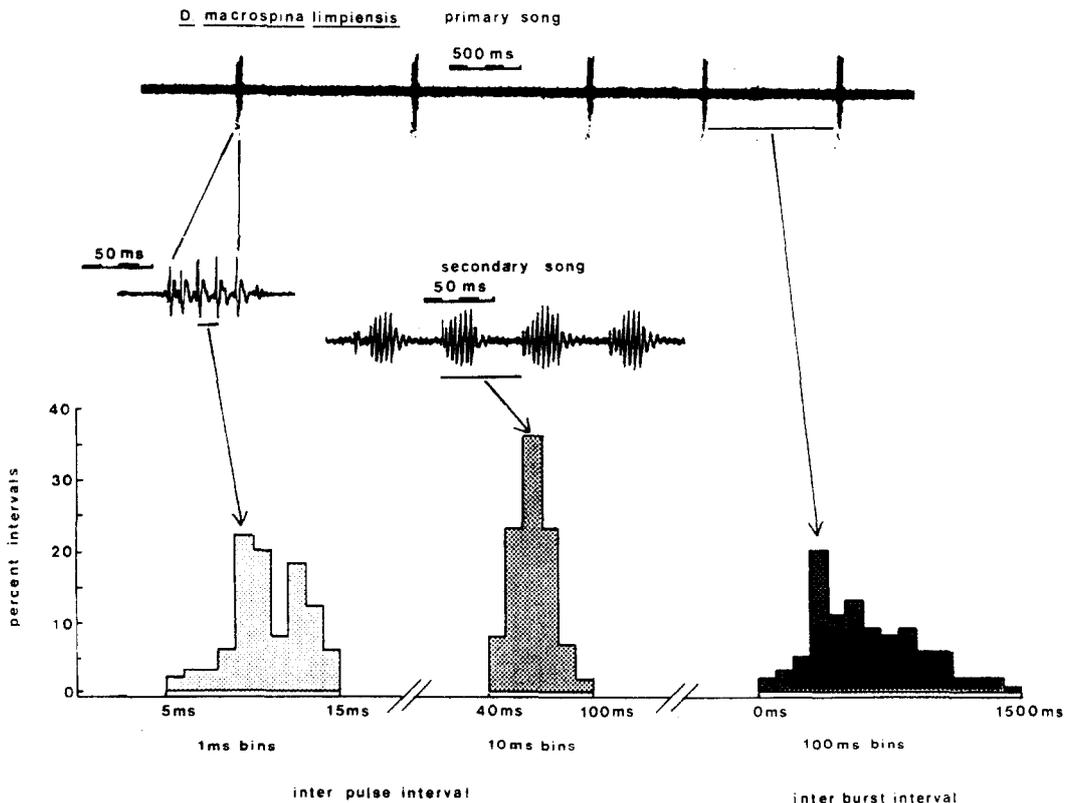

Fig. 2. *D. macrospina limpiensis* song. As for Fig. 1 but one burst is shown on an expanded scale (on the left) and the secondary song consisting of polycyclic pulses is illustrated in the middle.

A minimum of five individuals from each species was examined.

Results

The songs within the *funebris* species group are among the most complex described in any insects. They are illustrated in graphic form in Figs. 1 to 4 and Table II lists the statistical data. I.p.i.'s and i.b.i.'s were tested for heterogeneity between individuals of the same species and, as no significant differences were found, the data for each species were pooled.

Two types of song pulse are performed: monocyclic pulses produced by a single up-and-down wing movement and polycyclic pulses which are due to small amplitude wing vibrations (Bennet-Clark & Ewing 1968). The former have a pulse duration of approximately 3 ms in all the species and are thus similar to the pulses found in many other *Drosophila* species (Ewing 1977a). These pulses are difficult to characterize as they are asymmetrical in form. Pulse length rather than intra-pulse frequency is thus a more valid measure. Also it is known that the frequency within single cycle pulses is not discriminated by the flies over quite wide limits (Bennet-Clark & Ewing 1969; Ewing 1978), and therefore an accurate measure of this song parameter is relatively unimportant. The polycyclic pulses are almost symmetrical and their description in terms of intra-pulse frequency is thus valid: the polycyclic pulses of *D. macrospina* have a frequency of 264 ± 27 Hz ($N = 18$) and those of *D. subfunebris* 355 ± 33 Hz ($N = 24$). All the species except *D. subfunebris* produce bursts of single cycle pulses near the minimum i.p.i. and the bursts are separated by intervals whose means differ significantly. I call these primary songs. *D. subfunebris* does produce similar minimum i.p.i. bursts but interspersed
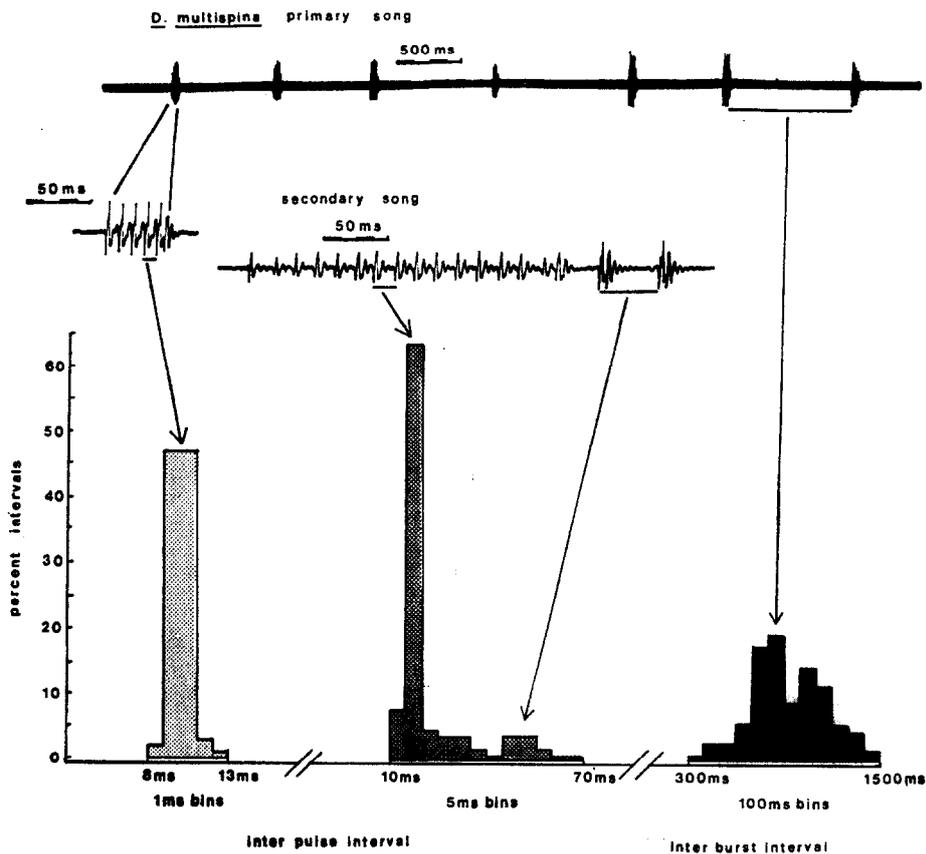

Fig. 3. *D. multispina* song. As for Fig. 2.

in the intervals between them are found 3 to 5 polycyclic pulses (4·0 ± 0·7). In addition, *D. multispina* and the *D. macrospina* subspecies perform a secondary song which, in the latter consists of polycyclic pulses at a repetition rate distinct from and intermediate between the i.p.i. and i.b.i. of the primary songs. The data for *D. m. macrospina* is deficient, particularly, for
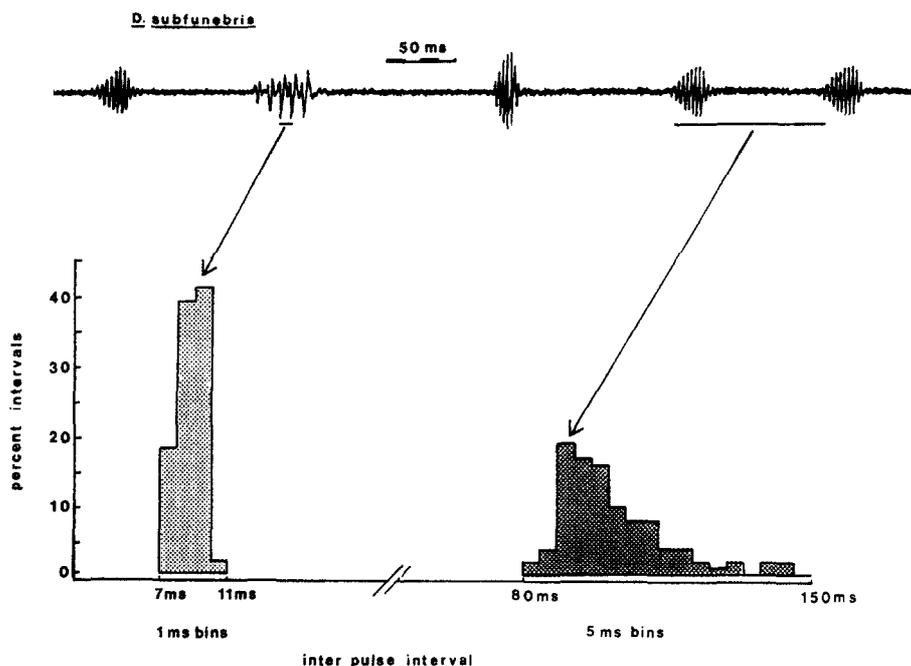

Fig. 4. *D. subfunebris* song. The primary song present in the preceding species is absent. The song consists of polycyclic pulses interspersed with bursts of single cycle pulses.

Table II. Characteristics of the Songs of the Species with the *Drosophila funebris* Species Group

Species	Primary song						Secondary songs			
	Inter-pulse interval			Inter-burst interval			Inter-pulse interval			
	Mean	SD	N	Mean	SD	N	Mean	SD	N	
D. funebris	8·4	1·4	134	279·6	57·5	190	—	—	—	
D. macrospina macrospina	12·3	1·5	60	446·2	42·4	42	75·0	19·8	8	
D. macrospina limpiensis	10·7	2·1	149	609·2	322·0	142	64·4	11·8	121	
D. multispina	9·9	0·6	164	923·1	225·0	115	26·7	11·8	13	(first interval)[2]
							17·1	3·7	138	(intermediate intervals)
							41·6	10·5	13	(penultimate interval)
							52·6	7·5	13	(last interval)
D. subfunebris	8·6	0·8	431[1]	—	—	—	138·8	33·6	189	

Note 1. These bursts are incorporated into the secondary songs of *D. subfunebris*, the primary song being absent in this species.
2. Figure 3 illustrates the intervals that are referred to.

the secondary song, as this sub-species, although courting persistently, produced rather little song. *D. multispina*'s secondary song has a complex structure and consists of a burst of 12 to 20 ($15 \cdot 1 \pm 2 \cdot 8$) pulses of which the first, penultimate and ultimate intervals are longer than the intervening ones. The pulses themselves are monocyclic except for the last two which are usually of two cycles and these are always louder.

Discussion

It is clear from the song descriptions that there are several distinct characteristics which could be used in discriminating the songs of individual species. In the absence of direct experimental evidence it is difficult to know which of these are used by females or indeed what the function of the different songs is. In all the species with two song types there was a marked tendency for the secondary song to occur later in courtship, the primary song inevitably being performed first. This is similar to the situation found in some species of the *D. affinis* sub-group where low repetition rate songs tend to occur early in courtship and high repetition rate songs just before intromission (Chang & Miller in preparation).

In *D. melanogaster* the inter-pulse interval appears to be important in species identification and a second song, sine song, lowers the female's threshold for mating (Bennet-Clark & Ewing 1967, 1969; von Schilcher 1976). These songs are, however, relatively simple and to dissect out the separate components of the *funebris* species group songs in a similar manner would be difficult.

There are differences between some of the species in the mean inter-pulse intervals within bursts (see Table II). However the significance of these differences is dependent on sample size. The mean inter-burst interval varies, between species, from 280 to 923 ms. Observation of flies within stock bottles or population cages, which constitutes a much more natural situation than the small ($18 \times 10 \times 5$ mm) recording cells, suggests that females seldom receive more than a very few seconds courtship from any individual male and therefore will sample only a few bursts of pulses. Figure 5 shows means and 95% confidence limits for the i.p.i.'s within five bursts, taken at random, from males of the four species that produce this type of song. From this it can be seen that it is unlikely that i.p.i. within bursts would be adequate for species identification.

The same rationale applies to inter-burst interval in those species in which this interval is both long and irregular. Thus while *D. funebris* probably does use inter-burst interval, and indeed in the absence of an alternative song there is no other auditory characteristic that it can use, the acquisition of secondary songs in the remaining species suggests that they use other song clues either as an alternative or in addition to i.b.i. *D. subfunebris* has followed this evolutionary trend to a point where the primary song has disappeared and traces of it occur as a relic in the secondary song in the form of isolated single cycle bursts. Another way of considering the song *D. subfunebris* is as an amalgam of the two song types. Thus the primary song in *D. funebris* and the secondary ones in *D. subfunebris* *D. macrospina* and *D. multispina* contain species specific information which can be utilized by females. Song probably is not used in discriminating between the *D. macrospina* sub-species and previous studies on the *D. paulistorum* semi-species does indicate that song is a conservative character and is not one of the initial isolating mechanisms to evolve (Ewing 1970). It has been suggested that pheromones are more likely to be useful in this role (Ewing & Manning 1967) and it may be relevant that it was difficult to record song from *D. m. macrospina*: this sub-species may place more reliance on clues other than auditory ones.

One outstanding question concerns the complex patterning of the secondary song of *D. multispina*. This appears to contain surplus information for species identification, particularly as the only closely related species with which
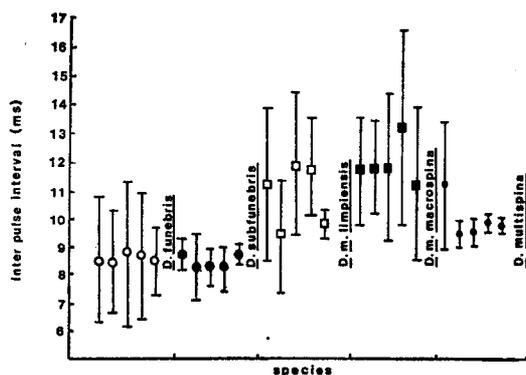

Fig. 5. Mean inter-pulse intervals and 95% confidence limits are illustrated for five individual bursts taken at random from the songs of each of the species.

it is sympatric is *D. funebris*. However these secondary song bursts are rigidly structured and the receptor system is capable of coding the different characteristics of interval, intensity and number of cycles. If *Drosophila* can utilize such complex information then there is no limit to the number of discrete song types that could evolve.

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REFERENCES

Alexander, R. D. 1962. Evolutionary change in cricket acoustical communication. *Evolution*, **14**, 443–467.

Bennet-Clark, H. C. 1972. Microphone and pre-amplifier for recording courtship song of *Drosophila*. *D.I.S.*, **49**, 127–128.

Bennet-Clark, H. C. & Ewing, A. W. 1967. Stimuli provided by courtship of male *Drosophila melanogaster*. *Nature Lond.*, **215**, 669–671.

Bennet-Clark, H. C. & Ewing, A. W. 1968. The wing mechanism involved in the courtship of *Drosophila*. *J. exp. Biol.*, **49**, 117–128.

Bennet-Clark, H. C. & Ewing, A. W. 1969. Pulse interval as a critical parameter in the courtship song of *Drosophila melanogaster*. *Anim. Behav.*, **17**, 755–759.

Chang, H.-C. & Miller, D. D. Courtship and mating sounds in species of the *Drosophila affinis* subgroup. In preparation.

Ewing, A. W. 1970. The evolution of courtship songs in *Drosophila*. *Rev. comp. Animal*, **4**(4), 3–8.

Ewing, A. W. 1977a. Communication in Diptera. In: *How Animals Communicate* (Ed. by T. A. Sebeok), pp. 403–417. Indiana University Press.

Ewing, A. W. 1977b. The neuromuscular basis of courtship song in *Drosophila*: the role of the indirect flight muscles. *J. comp. Physiol.*, **119**, 249–265.

Ewing, A. W. 1978. The antenna of *Drosophila* as a 'love song' receptor. *Physiol. Entomol.*, **3**, 33–36.

Ewing, A. W. & Bennet-Clark, H. C. 1968. The courtship songs of *Drosophila*. *Behaviour*, **31**, 287–301.

Ewing, A. W. & Manning, A. 1967. The evolution and genetics of insect behaviour. *Ann. Rev. Entomol.*, **12**, 471–494.

Okada, T. 1955. *Systematic Study of Drosophilidae and Allied Families of Japan*. Tokyo: Gihodo.

Parsons, P. A. 1973. *Behavioural and Ecological Genetics*. Oxford: Clarendon Press.

Patterson, J. T. & Stone, W. S. 1952. *Evolution in the Genus Drosophila*. New York: Macmillan.

Pringle, J. W. S. 1974. Locomotion: flight. In: *The Physiology of Insecta* (Ed. by M. Rockstein), 2nd Edn, Vol. III, pp. 433–476. New York: Academic Press.

Schilcher, F. von 1976. The function of pulse song and sine song in the courtship of *Drosophila melanogaster*. *Anim. Behav.*, **24**, 622–625.

Shorey, H. H. 1962. Nature of the sound produced by *Drosophila melanogaster* during courtship. *Science, N.Y.*, **137**, 677–678.

Spieth, H. T. 1974. Courtship behavior in *Drosophila*. *Ann. Rev. Entomol.*, **19**, 385–405.