

## THE WING MECHANISM INVOLVED IN THE COURTSHIP OF *DROSOPHILA*

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### INTRODUCTION

Many species of *Drosophila* have courtship displays in which the male vibrates one or both wings after approaching the female. In *Drosophila melanogaster* the major part of this display occurs with the wing nearer the female extended to nearly 90° from the long axis, in which position the wing is vibrated (Spieth, 1952). It has been shown that this vibration is accompanied by a train of sound pulses (Shorey, 1962) and that such sound pulses provide stimuli which render the females sexually receptive (Bennet-Clark & Ewing, 1967).

Several different species of *Drosophila* have been studied and we have shown that many of these have a characteristic courtship song (Ewing & Bennet-Clark, 1968) where there is, within any species, close control over the interval between sound pulses, the frequency of the sound within the pulse and the duration of each pulse.

The simplest song, acoustically, is a train of single cycles of pressure change each of 3 msec. duration produced at 34 msec. intervals by *Drosophila melanogaster*. *D. persimilis* produces trains of 3-7 cycles of a frequency of 525 cyc./sec. at 55 msec. intervals. *D. bipectinata* produces single cycles of 2 msec. duration, repeated at 8.4 msec. intervals. In each species the wings can be seen to vibrate while this sound is produced (Ewing & Bennet-Clark, 1968).

This paper describes the probable nature of the aerodynamic events causing these sounds and examines the wing mechanism involved, relating this to normal flight and flight initiation in *Drosophila* and other Diptera.

### MATERIALS

*D. melanogaster* and *D. persimilis* were reared on standard yeast and molasses medium in half-pint milk bottles. Adult males and females were separated on the first day after eclosion and brought together for courtship experiments from the 2nd to the 5th day.

*Chrysomyia putoria* was also used. The adults were maintained on meat and sugar and the larvae were fed on meat.

### METHODS

#### I. *Photographic*

An Exa IIb 35 mm. reflex with extension tubes and bellows was used in conjunction with a 5 cm. lens and shutter turned so that the original front of the lens faced the film: this arrangement gives better resolution where the object to be photographed is very small. Photographs were taken at a real aperture of F/60 to F/80 at a lens-to-film

distance of 15–20 cm. At this aperture the ommatidia of the compound eyes are just resolved and the depth of focus is about 1 mm.

For the majority of photographs Ilford mark v film was used and developed in Paterson's Acuspeed. FP3 was used where higher quality was required and was developed in Microphen.

Illumination was provided by an E.M.I. No. 6 Strobe flash lamp. Normally, this lamp gives continuous flashes at up to 1000 per second, at which speed the energy in each flash is 0.04 joules. By special triggering from a gated sine-wave generator, the lamp will give bursts of 2 or more flashes, each of up to 2 joules, at intervals down to 1 msec. As the lamp has two separate xenon tubes which flash alternately, one of these can be obscured so as to give a train of alternately dim and bright flashes.

For flight photographs flies were attached by the head and anterior part of the notum to entomological pins using cellulose paint as adhesive. The camera shutter, on opening, triggered a train of 3 to 5 flashes of 1 msec. interval. The fly was placed in front of the lamp and a multiple silhouette of wing position was obtained.

For courtship photographs *Drosophila melanogaster* was confined in a Perspex cell 12 mm.  $\times$  9 mm. and 3 mm. deep. *D. persimilis* has longer wings and will not court unless the cell is 6 mm. deep. A variety of exposures was given, ranging from 3 flashes at 1 msec. intervals to 5 at 5 msec. intervals. The firing of these very bright flashes did not appear to disturb the course of courtship.

## II. Acoustic recording

The methods used for recording courtship sounds have already been described (Ewing & Bennet-Clark, 1968).

Flight sounds were observed by placing the tethered fly 5 mm. in front of the diaphragm of a Foster D.F.I. microphone. The time delay thus introduced is less than 0.1 msec. and has been ignored. The microphone output was amplified and recorded on an Ampex SP 300 tape recorder with a band width of 0–1.5 kcyc. The microphone output was also used to trigger the E.M.I. No. 6 Strobe lamp to allow wing position to be observed.

Movements of the notum were observed by attaching the pin tethering the fly to a gramophone pickup. Any fly produced a wide range of signals depending on the orientation of the fly with respect to the pickup and the length and thickness of the supporting pin. Flies were oriented so that vertical movements of the notum were recorded. The thoracic click mechanism excited a resonance of the whole pickup assembly at about 2 kcyc.

All electrical signals were monitored on a C.R.O. and could be photographed while occurring or from tape recordings.

## RESULTS

### I. Comparison of flight and courtship sounds in *Drosophila melanogaster*

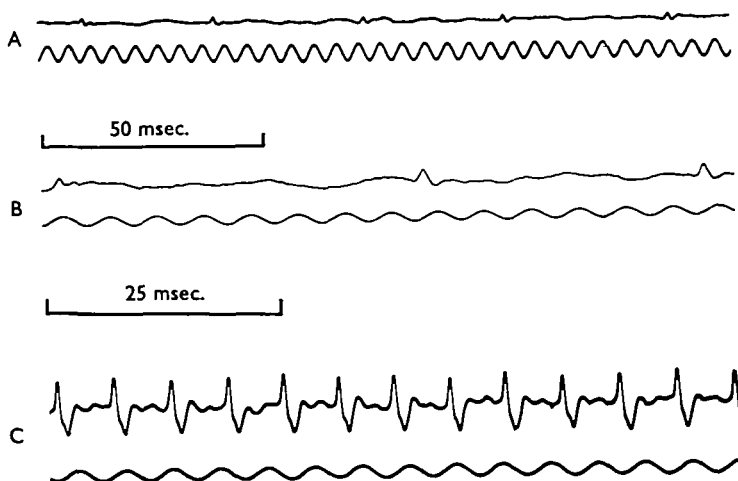
Sound recordings of courtship of *D. melanogaster* showed a component consisting of a single cycle of pressure change of 3 msec. duration which, at 25° C., was repeated every 33 msec. (Text-fig. 1A, B).

Recordings of normal *D. melanogaster* during tethered flight taken from behind the

fly showed that during each wing beat lasting 5.5 msec. there was a fast component of 2.5 msec. duration during which the pressure rose sharply and then fell. During the remainder of the wing beat the pressure behind the fly changed either very little or very slowly (Text-fig. 1 C).

The general form and time course of the courtship sound pulse and fast component of flight sound were closely similar.

Different sounds were recorded if the fly was oriented differently with respect to the microphone. While these contained fast components they did not appear to resemble courtship sound and so are not considered further here.



Text-fig. 1. Records of sounds produced by *D. melanogaster*. A and B, courtship sound pulses of 3 msec. duration with, below, 200 cyc./sec. time marker. C, flight tone recorded from behind the fly with fast component of 2.5 msec. duration. Pressure increases cause upward movement of the trace. Below, 200 cyc./sec. time marker.

## II. *The time course of wing position during the normal wing beat*

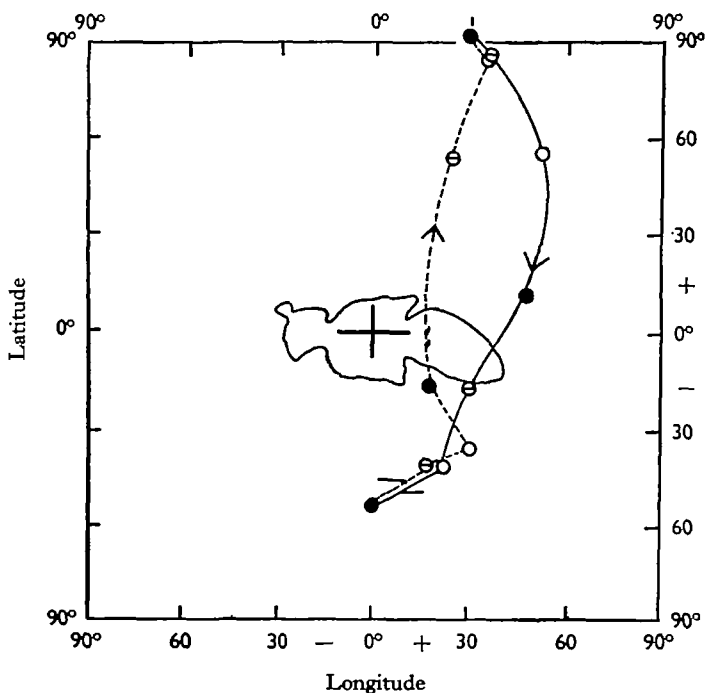
Photographs taken during tethered flight at 1 msec. intervals allowed a plot to be made of wing position at these intervals through one cycle. By interpolation several photographs were combined to resolve wing position to  $\frac{1}{3}$  msec.

Text-fig. 2 shows the position of the distal end of vein  $R_{4+5}$  (throughout this paper, the nomenclature follows that of Miller, 1950) of a male fly which flew particularly regularly at 255 beats.sec.<sup>-1</sup>. The plot is made on Mercator's projection using similar co-ordinates to those of Vogel (1967) and indeed the extremes of wing position correspond closely to one of the stroke planes described by him.

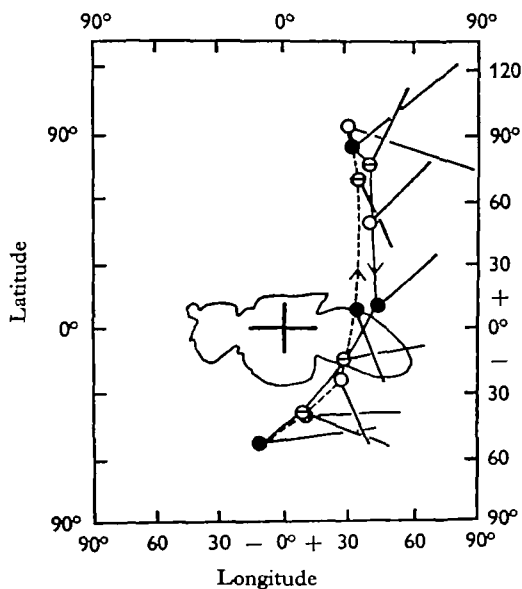
It will be seen that, while wing speed is more or less constant through the down-stroke, the upstroke starts slowly, then the wing moves rapidly upwards, and then dwells for about 1 msec. at the top of the stroke, vertically above the abdomen.

Text-fig. 3 shows the position of the wing chord at  $\frac{2}{3}$  of the wing span for the same fly at the same times. As the plot is made on Mercator's projection, the wing chord appears larger at high latitudes.

It will be seen that the wing has a small negative angle of inclination to the horizontal



Text-fig. 2. Plot, on Mercator's projection, of the time course of movement of the tip of the wing at vein  $R_{4+5}$  in *D. melanogaster* during flight at 255 beats. $\text{sec}^{-1}$ . Downstroke continuous line, upstroke dashes. Time markers: ●, ○ msec.; ⊖,  $\frac{1}{3}$  msec.; ⊕,  $\frac{1}{3}$  msec.; ●, 1 msec.; etc.



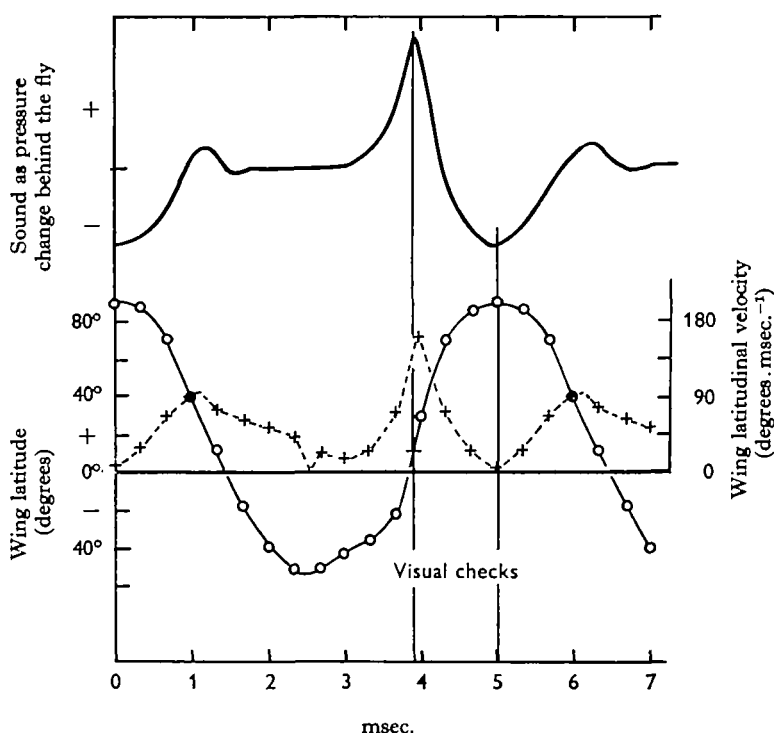
Text-fig. 3. Plot, on Mercator's projection, of successive positions of the wing chord at  $\frac{1}{3}$  wing span in *D. melanogaster* from the same photographs as were used for Text-fig. 2. The course of the leading edge is shown by a continuous line in the downstroke and by dashes in the upstroke. Same time markers as Text-fig. 2.

during the major part of the downstroke. After  $\frac{1}{3}$  msec. of upstroke, the wing starts to rotate to a positive inclination of  $60-70^\circ$ . Towards the top of the rapid upstroke the angle of inclination decreases, becomes markedly negative then decreases to the small negative angle found during the downstroke.

The wing appears to rebound considerably at both the top and the bottom of the stroke.

### III. *The relation between wing position and the pressures produced*

By using the pressure peak or trough of the flight sound to trigger the strobe lamp the wing position at these points in flight was observed. The pressure peak occurs when the wing is in the early part of the upstroke, at a latitude of  $-10^\circ$  to  $+10^\circ$  and the trough occurs with the wing vertically above the body at an angle of inclination from  $+20^\circ$  to  $-20^\circ$ .



Text-fig. 4. Plot of sound, wing latitude and wing latitudinal velocity against time for a 5 msec. wing beat. Top trace, sound. Upward movement of the trace indicates pressure rise behind the fly. Lower traces; wing latitude, circles and continuous line; wing latitudinal velocity, crosses and dashes. The vertical lines at 3.8 msec. and 5 msec. show the visual checks described in the text.

The results of this section and sections I and II are summarized in Text-fig. 4, where a plot of the sound produced behind the fly is drawn on the same time scale as a plot of wing latitude during the wing beat. The plot of wing latitude against time is differentiated to give a plot of the vertical velocity of the wing against time.

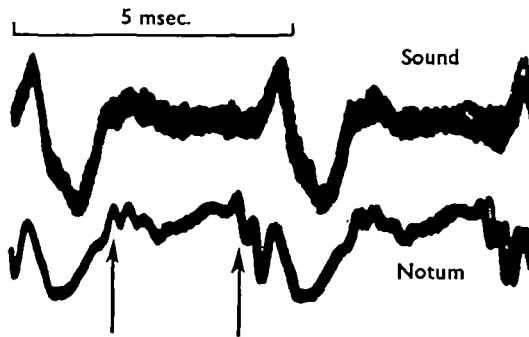
The plot of sound-pressure change follows a similar time course to that of vertical velocity.

For the purposes of this graph a wing-beat cycle of 5 msec. has been taken and the

time scale of Text-fig. 1C has been compressed and that of Text-fig. 2 expanded. This is justified as we have found that flies with different wing-beat frequencies produce similar proportions of fast and slow sound components.

#### IV. *The relation between the thoracic click mechanism and the flight sounds*

A simultaneous recording from the notum via a gramophone pick up and from a microphone placed behind the fly showed the relation between thoracic click movements and the sound produced during the wing beat. Text-fig. 5 shows that the clicks occur at the beginning and end of the fast component of the flight sound; these points are indicated by arrows on the lower trace of the record.



Text-fig. 5. Simultaneous record of flight sound of *Drosophila* recorded from behind the fly and movements of the notum recorded by gramophone pickup. Arrows indicate the clicks of the thoracic mechanism.

Thus it appears that, in terms of sound measured behind the fly, the normal upstroke of flight is noisy and the downstroke quiet, that thrust rises sharply at the start of the upstroke then falls away and may even reverse towards the top of the upstroke, and that the thrust produced in the downstroke is either very small or changes very little.

These conclusions are substantially similar to those of Nachtigall (1966) though he studied *Phormia* which appears to be capable of modifying the stroke to a greater extent than *Drosophila*.

#### V. *Wing movements during courtship of Drosophila melanogaster*

Courting male *D. melanogaster* stand behind or to one side of the female and extend one wing, normally the one nearer the female. The wing is brought out quickly and is held slightly above horizontal and at slightly less than  $90^\circ$  from the longitudinal axis. The angle of inclination to the horizontal is positive and from  $20^\circ$  to  $50^\circ$ .

From this starting position the wing is vibrated rapidly. It is not possible to see details of this, but it is clear that the wing is raised and not lowered from the resting position. Sometimes the wing is scooped forward to an angle of inclination of  $+70^\circ$  and is depressed slightly below horizontal.

Photographs were taken of courting pairs of flies. In one series a double flash was used, the first of which was dimmer and occurred 2 msec. before the other. Plate 1a



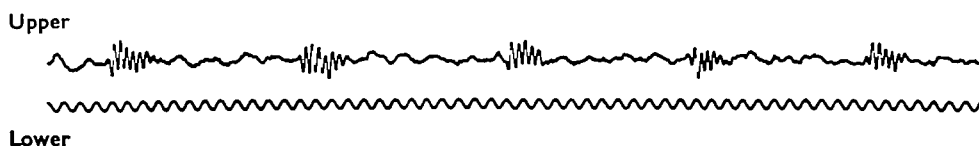
A further series of four exposures at 1 msec. intervals was made to see whether there was any change in angle of inclination through the stroke. There is little change in the angle of inclination in the course of the upstroke, but the angle of inclination appears to be  $5^{\circ}$  to  $10^{\circ}$  less during the downstroke. This is difficult to measure with certainty but it is clear that the wing beat is not symmetrical (see Plate 1, figs. 1-3).

In most cases where the extended wing vibrates a similar but far smaller vibration of the folded wing can be seen. This occurs in synchrony with the beat of the extended wing but does not usually exceed  $4^{\circ}$  in amplitude.

As a working hypothesis it may be suggested that the courtship sound of *D. melanogaster* is produced by one half of a single wing beat of  $30^{\circ}$  amplitude and 7.5 msec. total duration. The stroke plane is not normal either to the long axis of the fly or to the wing surface and so thrust is produced differently in the upstroke and the downstroke; the courtship wing beat has these features in common with the flight wing beat.

#### VI. *Wing movements during courtship of Drosophila persimilis*

The courtship looks very similar to that of *D. melanogaster* but the sound produced is entirely different (Waldron, 1964; Ewing & Bennet-Clark, 1968) being a series of trains of up to seven cycles of 525 cyc./sec. sound repeated every 55 msec. (Text-fig. 7).



Text-fig. 7. Upper trace, record of sound produced by *D. persimilis*;  
lower trace, 200 cyc./sec. sine wave.

This was photographed using 5 flashes, alternately bright and dim, at 2 msec. intervals. Plate 1 fig. 6 shows that the wing is raised in about 2 msec. and then lowered in the same time and this has been repeated for  $2\frac{1}{2}$  wing beat cycles. The angle of inclination of the wing to the horizontal, unlike that in *D. melanogaster*, is very small and rarely exceeds  $10^{\circ}$ . The amplitude of vertical movement is far smaller and does not exceed  $15^{\circ}$ . The beat is thus aerodynamically symmetrical, in contrast to the beat of *D. melanogaster*, and a cycle of compression and rarefaction is produced on both the upstroke and the downstroke.

As in *D. melanogaster* the folded wing also moves, but through less than  $2^{\circ}$ .

#### VII. *Walking movements during courtship*

Many of the exposures showed leg movements. While most vibration occurred with the fly standing still, it was also seen when the fly was walking. There did not appear to be any dependence of wing vibration on leg movement or vice versa.

#### VIII. *The effect of leg amputations on courtship and flight*

As the courtship wing beat shows many similarities to the normal flight wing beat, it seems probable that initiation is similar in both. In flight it has been suggested that the beat is initiated by the tergo-trochanteral muscle of the meso-thorax—Miller's



(1950) extra-coxal depressor—and this has been tested experimentally by Boettiger & Furshpan (1952) and Nachtigall & Wilson (1967).

Ideally, this problem should be approached simultaneously from mechanical and electrophysiological standpoints but owing to the small size of *Drosophila* this is not possible and a purely mechanical and surgical approach has been used.

Amputation at the proximal end of the coxa cuts the trochanteral insertion of the extra-coxal depressor of the trochanter (Miller's extra-coxal depressor No. 66 in the mesothorax and No. 86 in the meta-thorax).

Males with the middle leg amputated bilaterally half way up the coxa were very active, jumped upwards about 5 mm. and courted females, producing normal courtship sounds as they did so.

As a control, males having their legs amputated beyond the trochanter also vibrated their wings normally.

Males with the hind legs amputated, either above or below the trochanter, jumped rather better than those with the middle legs amputated and courted and vibrated their wings successfully.

All flies with leg amputations flew readily, though walking performance was impaired.

An operation that destroyed both jumping and flight performance was a rather deep incision made between the coxae of legs 2 and 3 to cut muscles 48*a* and *b* (nomenclature follows Miller, 1950). These flies walked normally with the first two pairs of legs but the wings were held in a lowered position.

### IX. *Experiments with Chrysomyia*

This large fly jumps to a height of about  $1\frac{1}{2}$  cm. and flies steadily when tethered.

Tethered specimens flew steadily with about 180 beats.sec.<sup>-1</sup>. Several specimens were observed to fly with both wings, then to fold one wing and fly at the same wing-beat frequency, then to continue, using both wings, still at the original frequency.

Amputations were performed as in *Drosophila*. Flies with one pair of legs amputated above or below the coxa jumped with the remaining legs and started flying. In general, they jumped higher using the middle legs than using the hind legs. Incisions made along the front of the hind coxae destroyed the flies' ability to jump and to start flying, though in many cases these flies buzzed in anaesthetized flight. When walking, such flies held the wings depressed below the normal rest position.

### DISCUSSION

The photographic and acoustic records suggest that the courtship sounds of *D. melanogaster* are produced by isolated wing beats whose character is similar to those of normal flight.

There are various noteworthy differences. The amplitude of the courtship wing beat is about one quarter of the normal flight wing beat and, not surprisingly, the sound level produced is very much less as, from such acoustic sources, sound level increases as the square of the amplitude of vibration.

The frequency of the sounds produced in the two situations is similar, with a cycle

length of 3 msec. for courtship and 2.5 msec. for flight. In courtship, however, only one wing is extended and so the thoracic loading would be less than that in flight. Other flies control the wing-beat frequency by the activity of the pleurosternal muscle (Miller's No. 59), which tenses the resonant thoracic box (Nachtigall & Wilson, 1967). and are able to maintain the typical oscillation frequency when the inertial loading is changed markedly by the folding of one wing.

In courtship the angle of incidence of the wing does not change as markedly as during normal flight. This is probably because, to maintain the normal oscillation frequency, the tension in the pleurosternal muscle must be greatly reduced and so rotational effects of the click mechanism are also reduced. *Chrysomyia*, flying with one wing folded, shows less pronation and supination than when flying normally and the wing-beat amplitude is much reduced. A similar finding is reported for *Muscina* (Nachtigall & Wilson, 1967).

*Drosophila persimilis* produces a short burst of small-amplitude beats when courting. This occurs at a wing-beat frequency that is considerably higher, 250 beats.sec.<sup>-1</sup>, than normal flight and suggests that here there may be some tensing of the pleurosternal muscles or a different activation of the indirect flight muscles from that found in *D. melanogaster*. The former suggestion is more acceptable as it accounts for the failure of the wing to oscillate in *D. melanogaster*.

The courtship wing beat in both species presents problems similar to those of flight initiation. The mesothoracic extra-coxal trochanter depressor muscle has been suggested as a flight starter, most recently by Nachtigall & Wilson, but they find, as do we, that amputation does not prevent the start of flight and also that the metathoracic legs are involved in the starting jump.

*Drosophila* courts without movement of the leg below the coxa and jumps, flies and courts readily without the meso-thoracic leg. If courtship wing vibration is a modification of flight, it certainly is not initiated in the manner that has been postulated for flight. There are serious drawbacks to the mechanism that has previously been put forward; if contraction of the extra-coxal depressor of the trochanter were to pull the wing upwards from its stable lower position, the wing would then click upwards at a rate faster than the muscle could contract and this could not cause a jump. However, on the ensuing downstroke, the upward movement of the notum would be transmitted through the tensed depressor muscle. An analogous situation has been described in the flea, which transmits stored energy through a muscle which is too small to provide instantaneously the very considerable power required for the jump (Bennet-Clark & Lucey, 1967). The present explanation accounts for the ability of many species of fly to jump using the hind legs alone. Many Diptera have no extra-coxal depressor of the mesothoracic trochanter (Smart, 1959); and while many of these do not jump, many of them are active and successful fliers. (*Glossina* is unusual in lacking this muscle, but in having a starting jump).

The present contention is that one must look elsewhere for a flight starter; and that, to satisfy the requirements, it must be a wing levator. Of the available muscles Miller's 48a and 48b are attractive, being tergal remoters of the mid-coxa modified as indirect flight muscles; cutting these destroys the flight initiation and the jump of *Chrysomyia* and *Drosophila*, and also *Drosophila*'s ability to produce courtship vibration. This suggestion does not appear to conflict with the experimental findings of

Nachtigall & Wilson (1967) and overcomes the anomalies that accompany the suggestion that flight-starting is performed by the extra-coxal depressor of the mid-trochanter.

In turn, the present suggestion that muscle 48 is both the flight starter and the sound-producing muscle requires experimental and histological examination, and it must be demonstrated that this muscle is capable of producing useful changes in tension within 8 msec., this being the interval between sound pulses in one of the species we have examined, *D. bipectinata*.

#### SUMMARY

1. The sounds produced during courtship in *Drosophila melanogaster* are similar to a component of the flight tone.

2. The normal flight wing beat has a rapid upstroke and a slower downstroke. By analysis of the form of the wing beat and its velocity it is shown that the sound that is heard from behind the fly is produced by a rapid increase of thrust in the early part of the upstroke followed by decrease in thrust, or even thrust reversal, during the period which the wing is rotating at the top of the stroke. The downstroke is relatively quiet.

3. The courtship wing vibration consists of a series of single wing beats of about 7.5 msec. duration. Courtship sounds consist of single cycles of pressure change of 3 msec. duration produced at 34 msec. intervals at 25° C. These figures are compatible with the suggestion that courtship sound pulses are produced by one half of an asymmetric wing beat.

4. *Drosophila persimilis* produces short trains of pure notes of 1.9 msec. period. Here, the wing vibrates at about 250 beats per second and the wing beat is symmetrical.

5. The differences between these two species is discussed in terms of inertial loading of the thorax and the tension in the muscle activating the click mechanism.

6. The courtship wing beat occurs when the extra-coxal depressor of the trochanter of either the mesothoracic or the metathoracic legs is cut. This suggests that this muscle is not responsible for the initiation of flight, but instead, acts as a transmitter of thoracic movement causing the starting jump.

7. Cutting one of the dorso-ventral indirect flight muscles stops courtship vibration, the initiation of flight and jumping, and it is suggested that this muscle is more likely to be involved in flight-starting and courtship vibration.

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#### REFERENCES

- BENNET-CLARK, H. C. & EWING, A. W. (1967). Stimuli provided by courtship of male *Drosophila melanogaster*. *Nature, Lond.* **215**, 669-71.  
BENNET-CLARK, H. C. & LUCEY, E. C. A. (1967). The jump of the flea: a study of the energetics and a model of the mechanism. *J. exp. Biol.* **47**, 59-76.  
BOETTIGER, E. G. & FURSHPAN, E. (1952). The mechanics of flight movements in Diptera. *Biol. Bull. mar. biol. lab. Woods Hole* **102**, 200-11.  
EWING, A. W. & BENNET-CLARK, H. C. (1968). The courtship songs of *Drosophila*. *Behaviour* (in the Press).  
MILLER, A. (1950). The internal anatomy and histology of the imago of *Drosophila melanogaster*. In Demerec, M. *Biology of Drosophila*, pp. 420-534. New York and London: Wiley.  
NACHTIGALL, W. (1966). Die Kinematik der Schlagflügelbewegungen von Dipteren. Methodische und analytische Grundlagen zur Biophysik des Insektenflugs. *Z. vergl. Physiol.* **52**, 155-211

- NACHTIGALL, W. & WILSON, D. M. (1967). Neuromuscular control of dipteran flight. *J. exp. Biol.* **47**, 77-97.
- SHOREY, H. H. (1962). Nature of the sound produced by *Drosophila melanogaster* during courtship. *Science, N.Y.* **137**, 677-8.
- SMART, J. (1959). Notes on the mesothoracic musculature of Diptera. *Smithson, misc. Collns* **137**, 331-364.
- SPIETH, H. T. (1952). Mating behaviour within the genus *Drosophila* (Diptera). *Bull. Am. Mus. Nat. Hist.* **99**, 401-74.
- VOGEL, S. (1967). Flight in *Drosophila*. II. Variations in stroke parameters and wing contour. *J. exp. Biol.* **46**, 383-92.
- WALDRON, I. (1964). Courtship sound production in two sympatric sibling *Drosophila* species. *Science, N.Y.* **144**, 191-193.

## EXPLANATION OF PLATE I

Fig. 1. Male *Drosophila melanogaster*, in the centre, courting female, at left. Two flashes, 2 msec. apart, 1st dimmer. Upward stroke; initial position lat.  $+20^\circ$ , long.  $+15^\circ$ , inclination  $+30^\circ$ ; final position lat.  $+37^\circ$ , long.  $+35^\circ$ , inclination  $+42^\circ$ .

Fig. 2. Male *D. melanogaster* courting, standing  $20^\circ$  head down,  $30^\circ$  right of camera. Exposure as Fig. 1. Downward stroke; initial position lat.  $+40^\circ$ , long.  $+35^\circ$ , inclination  $+20^\circ$ ; final position lat.  $+20^\circ$ , long.  $+15^\circ$ , inclination  $+20^\circ$ .

Fig. 3. Male *D. melanogaster* courting. Three flashes, at 3 msec. intervals, the first and last dimmer than the middle one. One of the dim exposures finds the wing raised. The folded wing also moves.

Fig. 4. Male *D. melanogaster* courting. Exposure as in Fig. 3 but with the middle wing position above that of the other two exposures.

Fig. 5. As Fig 4 but from in front. The inclination of the wing to the horizontal is about  $+40^\circ$ , the fly standing  $20^\circ$  head up and  $15^\circ$  left of the camera.

Fig. 6. Male *D. persimilis* courting. Five flashes at 2 msec. intervals, the first bright, then dim bright alternately. The angle of incidence is less than  $10^\circ$ . The extreme wing positions are lat.  $27^\circ$  by long.  $33^\circ$  and lat.  $18^\circ$  by long.  $20^\circ$  and the wing appears three times in the raised position, at the first, middle and last exposure and twice in the lower position, exposures 2 and 4.

