IS THE 'CLICK' MECHANISM OF DIPTERAN FLIGHT AN ARTEFACT OF CCl₄ ANAESTHESIA?

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SUMMARY

1. A frame-by-frame analysis of the wing movements of a fly shows characteristics which are incompatible with the accepted model for dipteran flight.

2. Higher amplitude wing beats result in greater velocities on the upstroke.

3. Detailed examination of the wing base articulation has uncovered a wing stop and DVM stretch mechanism.

4. It is argued that there is elastic energy storage during the downstroke which is released on the upstroke.

5. A novel mechanical model for wing movements is proposed which incorporates all the new findings and argues against a 'click' mechanism.

INTRODUCTION

The currently accepted model of how dipterans move their wings in flight is based upon the work of Boettiger & Furshpan published between 1950 and 1952 and is now entrenched in the literature. Their observations were made upon the flesh fly *Sarcophaga bullata* after anaesthetization with carbon tetrachloride (CCl₄). This substance causes tonic contractions in many thoracic muscles including the pleuro-sternal muscles that tension a system of levers controlling wing movements. Although Boettiger & Furshpan (1950, 1952) do not state the time course of events, they do describe a progression in the effects of CCl₄. Initially, these resemble the effects of other well-known anaesthetics in producing anaesthetic flight (e.g. ether, carbon dioxide). Wingbeat frequency steadily increases until the wings suddenly stop in either the fully elevated, or depressed position. If the fly is now removed from the CCl₄ and the wings moved towards the mid-point, they will suddenly accelerate and flight oscillations again begin. Longer exposures produce a lasting effect in which moving the wings results in them 'clicking' from one extreme position to the other. It is this latter condition that appears to have been used in the analyses.

The mechanical system described by Boettiger & Furshpan (1952) fitted well with the physiological properties of insect fibrillar muscles (Pringle, 1949, 1957; Boettiger,

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1957) which produce the power for flight. Within the skeletal framework, these muscles do not contract as a direct response to neural stimulation (but see Boettiger, 1957); rather they respond to fast stretches by rapid increases in tension. Thus, the 'click' system of the accepted model appeared to be an ideal mechanism to provide the requisite fast stretches. However, in recent studies of the wing base articulations in several dipterans (Miyan & Ewing, 1985) we have found a structural impediment to the 'click' on the downstroke; this has led us to re-examine the flight system and present a novel model with an elastic recoil mechanism acting at the bottom of the downstroke which has some of the properties found in the upstroke of the locust (Weis-Fogh, 1961).

MATERIALS AND METHODS

Specimens of *Musca domestica, Drosophila melanogaster* and *D. virilis* were taken from our own stocks and those of the Parasitology Sub-Department of Edinburgh University.

For high speed filming of flight, individual flies were glued by their scutum to tungsten wire. This was held in a pin vice positioned to place the fly in front of the ×2·75 objective of a modified Cooke, Troughton & Simms monocular microscope. This had had its base removed and was mounted on a heavy optical bench such that the 75 mm objective lens of a Fastax, type WF-17 camera, also mounted on the optical bench, could be manoeuvred over the ×6 eyepiece. The Fastax camera has a revolving prism shutter and is capable of taking exposures up to 8000 frames per second (f.p.s.). We used a speed of 4000 f.p.s. together with Ilford H.P.S. film and high intensity illumination from two, 100-W special reflector lamps. Flight was initiated with a gentle puff of air and continued without further stimulation throughout filming. A time marker recorded intervals of 1 ms on one edge of the film.

Flies were filmed along their longitudinal axes (head-on) and from the side. A frame-by-frame examination was completed on a Lytax film analyser. Tracings were taken of the position of the radial wing vein and wing base together with outlines of the thorax, allowing analysis of the wing movements in tethered flight.

For morphological studies, specimens were fixed in alcoholic Bouin (after Pantin, 1946) and dissected from a number of different approaches. Thoraxes were bisected along a longitudinal axis, or across the transverse axis at the level of the wing bases. A half thorax was then pinned onto Sylgard (Dow Corning) and examined under a Wild M5 stereo microscope. Drawings were made using a *camera lucida* attachment.

RESULTS

Fig. 1 shows the results of a frame-by-frame analysis of wing position during the wingbeat. Four cycles are shown in which the wing attained a different amplitude in each. It can be seen that the velocity of the upstroke always exceeds that of the downstroke, that there is a dramatic acceleration at the start of the upstroke, and that the downstroke is completed in a set time interval, regardless of wingbeat

amplitude. The inset in this figure shows an idealized plot of wing movements, on the same axes as the main figure, based upon the dynamics of the 'click' mechanism (Boettiger & Furshpan, 1952). It is clear that the observed wing movements do not fit the accepted model.

Plotting the angular changes in wing position between successive frames against frame number gives the 'velocity' histograms shown in Fig. 2A–C. These are plotted for three wingbeat amplitudes of (A) 142°, (B) 158° and (C) 180°. These plots confirm the points made for Fig. 1 in showing a lack of any bistability in either half of the wingbeat cycle. It is also clear that there is a marked difference between the two halves of the wingbeat and that both show changes in characteristics with varying amplitudes of wing movements. At low amplitudes the downstroke histogram is 'skewed' to the right while at higher amplitudes this is reversed with a marked skew to the left. The upstroke follows essentially the same pattern, although it becomes skewed at much lower amplitudes than the downstroke. At high amplitudes, the initial acceleration and velocity of the upstroke increase to give a significant discontinuity in the plot.

In looking for an explanation for the observed wing movements, we carried out a detailed examination of the wing base articulation in a number of dipterans. The results of these studies are reported fully elsewhere (Miyan & Ewing, 1985) but some of the important details are illustrated in Fig. 3. We have demonstrated a wing
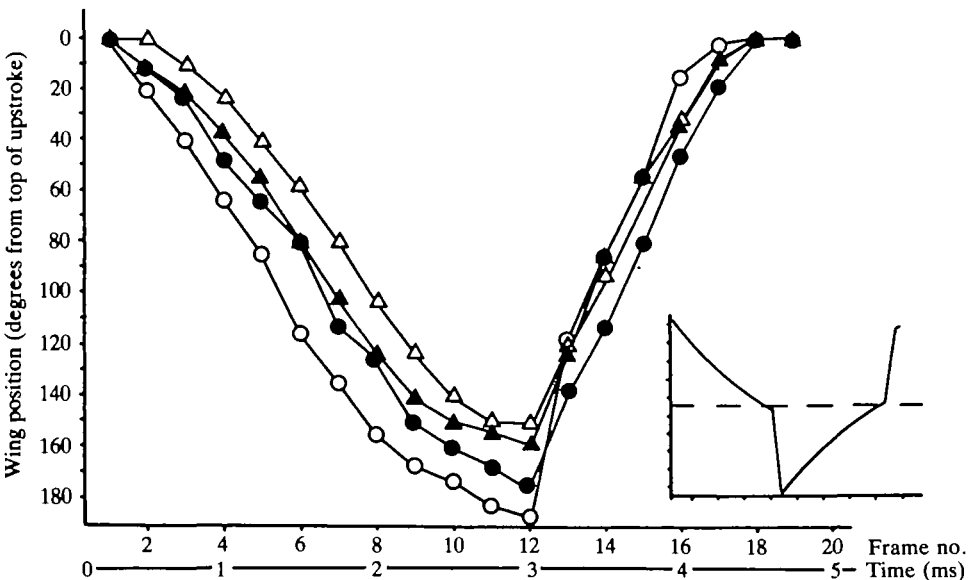

Fig. 1. Plots of wing position measured on successive frames of a film taken at 4000 f.p.s. (time equivalent = 0·25 ms frame^{-1}). The symbols refer to four sequences where the wing reached a different amplitude in each. 0° is taken as the position of the wing at the beginning of the downstroke. Notice the marked difference in the two halves of the cycle and that there are none of the characteristics predicted from a bistable model. The insert shows a predicted plot based on a bistable model under maximum tension.
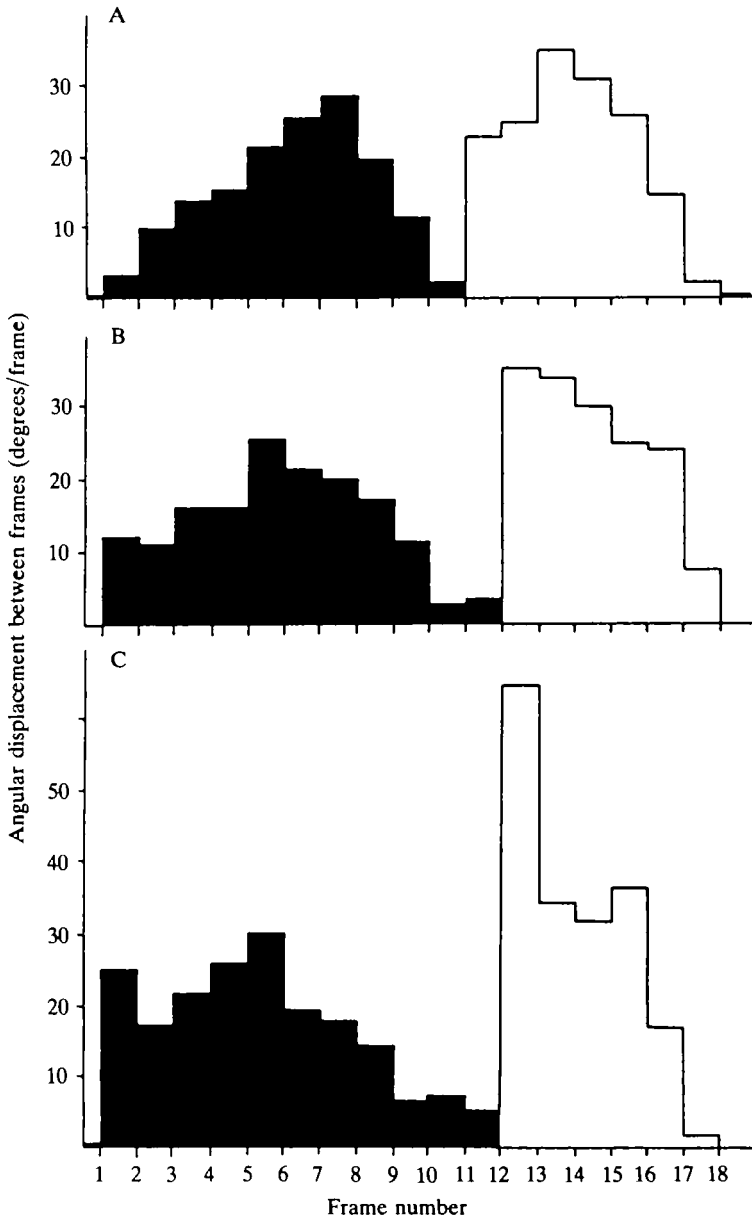
Fig. 2. Histograms of wing displacement measured between successive frames. These give an indication of the average velocity of the wing within the period covered by the inter-frame interval. The shaded parts are for the downstroke, while the unshaded parts refer to the upstroke phase of the wingbeat. There is an inherent bias in the histograms due to the two-dimensional nature of the measurements taken from the films. The normal wing movement traces a tight elliptical path such that at the top and bottom of the wingbeat, the measurements will not give an accurate description of the movement. However, the large proportion of the beat covers an essentially linear path allowing a fair assessment of wing movements. Wingbeat amplitude: A, 142°; B, 158°; C, 180°.

base stop mechanism that operates a few degrees below the horizontal position on the downstroke. Also, we have found that during the downstroke there is a locking mechanism of the first axillary sclerite onto the parascutal shelf that results in lifting of the scutum as the wing continues down. This action results in direct stretching of the dorsoventral muscles. Finally, we have identified the third axillary muscles as one of the controlling elements for the downstroke amplitude. These muscles exert a downward force that acts beyond the stop on the lower half of the wingbeat cycle and therefore pulls the wings down.

From an analysis of the high speed film, we have found that the wing bends beyond the stop on the downstroke; at higher amplitudes the wing base, distal but close to the stop, is forced further down suggesting a bending of stiff elastic elements in the
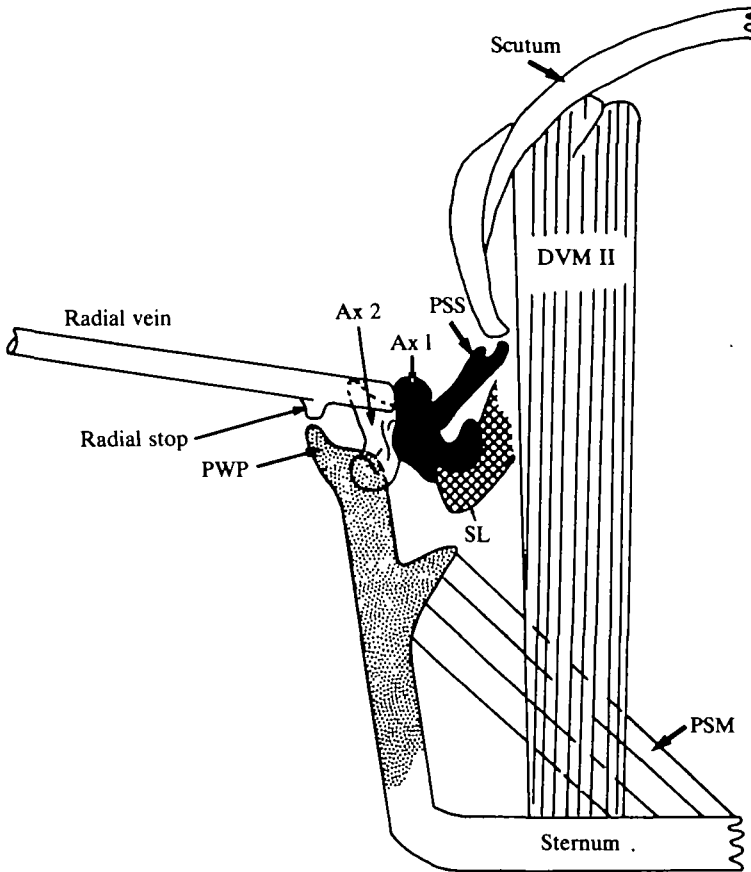

Fig. 3. Semi-diagrammatic drawing of the wing base anatomy showing the relationship between the radial vein of the wing, the first and second axillary sclerites (Ax 1 and Ax 2), the parascutal shelf (PSS), the pleural wing process (PWP), scutum and scutellar lever (SL). Also included are the pleurosternal muscle (PSM) which tensions the wing base system, and the medial dorsoventral muscle (DVM II) which attaches on the scutum adjacent to the wing base. Note the radial stop and its relationship to the PWP, and the medially projecting arm of the first axillary sclerite which locks onto the PSS during the downstroke.

thorax. This bending lags behind the movement of the distal wing areas and never attains the same amplitude. In contrast, at low amplitudes, there is little observable bending of the wing. However, within the natural range of wing movements, there is always a greater acceleration on the upstroke than on the downstroke (see Fig. 1), which suggests that there might well be some bending of elastic wing base elements even when there is no detectable strain (i.e. bending) in the wing. The relationship between the amplitude of the wingbeat and its up- and downstroke 'velocities' is illustrated in Fig. 4. Within the limits of the data, there is a clear difference between the two half-cycles with a significantly greater correlation (of the fitted line) between the upstroke velocity and wingbeat amplitude than with the downstroke.

DISCUSSION

Fig. 5 illustrates the features of the 'click' mechanism. As reviewed by Pringle (1957), this mechanism consists of four basic elements: the dorsal roof, or scutum of
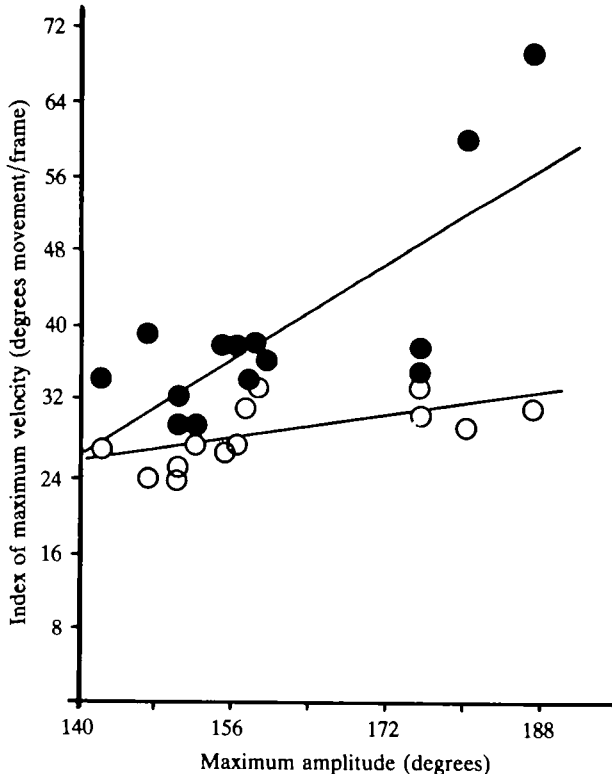

Fig. 4. Relationship between the maximum velocity (as measured in Fig. 2) achieved on the upstroke (filled circles) and the downstroke (open circles) and the amplitude of the wing movement. It is clear that the upstroke always has a greater velocity than the downstroke and that at higher amplitudes the difference is very marked. The correlation coefficients for the fitted lines are: for the downstroke, 0·53 ($P > 0·05$) and for the upstroke, 0·75 ($P < 0·01$). The difference between the two half-cycles argues against a bistable model which predicts a symmetrical relationship between them.

the thoracic box (1) which acts like a leaf spring producing lateral force; the parascutal shelf (2), a length of cuticle hinged medially to the scutum and articulating laterally with the axillary sclerites (3) which, for simplicity, can be grouped together as a unit that rotates about a pivot on the pleural wing process (4). This latter process is part of the ventral thorax and produces a force opposing that of the scutal spring (1) by the action of the pleurosternal muscles (see Fig. 5). The point of articulation between the axillary sclerites and the parascutal shelf is also the point at which the scutellar lever acts (Boettiger & Furshpan, 1952; Fig. 3). It is this joint which is moved by the indirect, fibrillar muscles acting upon the lever. The 'click' mechanism requires that as the lever is depressed, it encounters an increasing resistance as its position approaches the mid-point due to the resultant of the forces from the scutal spring (1) and the pleural wing process (4). This opposing (negative) force is maximal at the
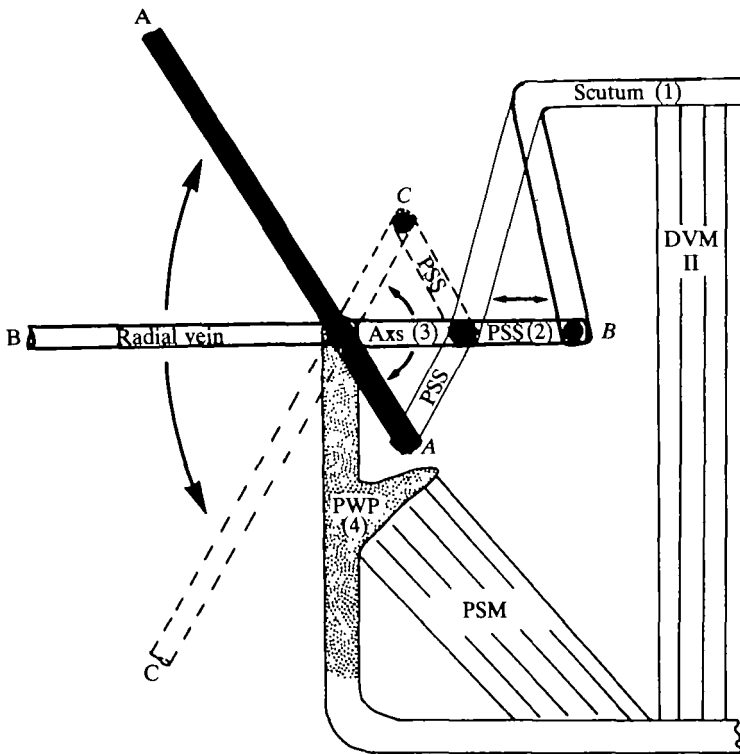

Fig. 5. Diagrammatic representation of the 'click' mechanism. Notice that the fully up and down positions of the wing, represented by the radial vein, coincide with equally stable positions of the wing base articulation. The mid-point of the movement corresponds to a bistable position, where there is maximum lateral force from the scutum, at which the wing can 'click' up or down. The model adequately explained the observed wing movements under the influence of CCl₄ and fitted well with the physiological requirements of the fibrillar, indirect muscles. Notice the free hinge between the parascutal shelf (PSS) and the axillary sclerites (Axs). The numbers correspond to the numbers of the elements in the text. The letters (*A, B, C*) correspond to positions of the wing and basal elements during the stroke. PSM, pleurosternal muscle; DVM II, medial dorsoventral muscle; PWP, pleural wing process.

'unstable' mid-point beyond which it is converted to a positive force aiding the powerstroke. A similar sequence is followed on the upstroke (see inset, Fig. 1).

The results which we have presented in this paper are incompatible with a bistable system as there is no indication of any deceleration towards the middle of either stroke and certainly no rapid acceleration immediately after it, i.e. at the 'click' points in the up- and downstrokes. Our studies have demonstrated that during the downstroke, the 'click' to positive force does not occur due to a locking mechanism of the first axillary sclerite onto the parascutal shelf. The shelf-sclerite system effectively becomes a single unit for the lower part of the wingbeat which lifts the dorsal scutum and results in direct stretching of the dorsoventral muscles, an essential feature not explained by the original model (Fig. 6).

We propose that there is an input of energy into an elastic system during the downstroke in addition to a stretching of the dorsoventral, indirect wing elevators. This stored energy must necessarily increase as the amplitude rises, producing a greater force on the upstroke. This is clearly seen in the present results (Figs 1, 2, 4).
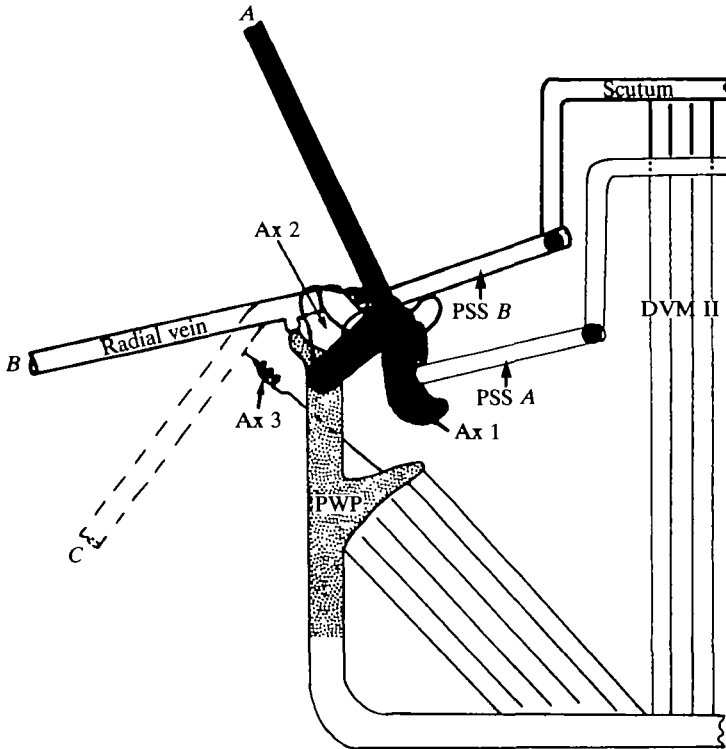

Fig. 6. Semi-diagrammatic representation of the model as seen by the authors. Notice the locking of the first axillary sclerite (Ax 1) onto the parascutal shelf (PSS), the action of the radial stop, the actual pivot for wing movement which switches from the base of axillary 2 (Ax 2) to the crest of the pleural wing process (PWP), and the action of the third axillary muscles (Ax 3) which pull the wing distal to the stop thereby controlling the amplitude of the downstroke. The locking of Ax 1 onto PSS causes a direct stretching of the dorsoventral muscles (DVM II) which is left to an indirect mechanism in the 'click' model. There will also be a more direct effect on the dorsolateral muscles by DVM contractions *via* this articulation.

Both halves of the wingbeat are affected in similar ways by amplitude changes (Fig. 2); it could be argued that the forces required for these changes are produced by elements that are not part of the indirect flight motor and which act throughout the cycle by tonic effects. Such elements would include the 'direct', non-fibrillar muscles (e.g. the third axillary muscles).

But what of the apparent demonstration of a bistable 'click' mechanism under CCl_4 anaesthesia? CCl_4 anaesthesia forces the system into an abnormal state by excessive tension produced by tonic contractions of the pleurosternal muscles. There is an abnormal inward movement of the pleural wing process resulting in a disengaged stop mechanism that allows the wings to 'click' fully down under the influence of large forces in the wing base (Fig. 7). The fully up position is a stable point in both models.

One final point that is pertinent to the present hypothesis concerns the frequent observation that the amplitude of wingbeats is controlled by varying the limits of the downstroke. This is clearly seen in turning reactions of flies where the amplitude, *via* the downstroke limit, is controlled independently on each side of the animal (Heide, 1971, 1983; Nachtigall, 1968; Nachtigall & Wilson, 1967; Vogel, 1967). This is consistent with the present model since we believe that the degree of downstroke can be controlled by means of the non-fibrillar muscles (Miyan & Ewing, 1985).
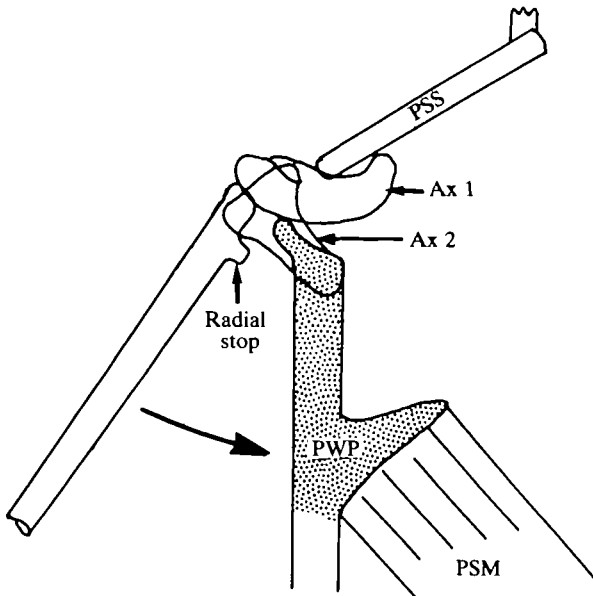

Fig. 7. Diagram of the possible effects of CCl_4 on the wing base articulation. Due to the superstimulation of the pleurosternal muscles (PSM), there is a greater inward movement of the pleural wing process (PWP) than would normally occur. This results in the stop mechanism being disengaged which allows the wings to 'click' fully down. It is possible that the third axillary muscles will be under tetanus from CCl_4 adding to the downward pull on the wing. The wings will automatically 'click' up when moved past the midpoint due to the wing base anatomy. PSS, parascutal shelf; Ax 1, Ax 2, first and second axillary sclerites.

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