

## MOTOR PATTERNS DURING FLIGHT AND WARM-UP IN LEPIDOPTERA

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### INTRODUCTION

One method of analysing the mechanisms by which the central nervous system produces patterned motor output involves describing the motor patterns underlying simple behavioural acts. On the basis of the described patterns, models of the central nervous interactions can be formulated. Further understanding of the central mechanisms controlling a group of muscles can be obtained by comparing the motor patterns of the same set of muscles during the performance of different behavioural acts. For example, the thoracic muscles of the Orthoptera, which have been extensively studied, are employed in flying, walking and singing (Wilson & Weis-Fogh, 1962; Wilson, 1962; Bentley & Kutsch, 1966; Elsner, 1967). In many insects the flight muscles are involved in another behavioural act, a pre-flight warm-up. During warm-up heat is produced by activating the flight muscles without, in many cases, any flapping of wings, and the thoracic temperature is raised to the value observed during flight. In some insects, such as the relatively large moths of the families Sphingidae and Saturniidae, the difference between the thoracic and the ambient temperatures may be more than 10° C. (Bachmetjew, 1901; Dotterweich, 1928; Bodenheimer, 1934; Oosthuizen, 1939; Dorsett, 1962; Adams & Heath, 1964; Heath & Adams, 1965). In the present study the motor patterns of warm-up and flight in several species of Lepidoptera are compared in order to develop a model of the central nervous interactions which generate the behaviour.

Behaviour preparatory for flight has been studied previously by a number of workers. Warm-up behaviour has been observed in both nocturnal and diurnal insects (Dotterweich, 1928), although the latter may also elevate their body temperature by absorbing solar radiation (Vielmetter, 1958; Clench, 1966). Warm-up occurs both in insects with an asynchronous flight system, such as Coleoptera and Hymenoptera, and in insects with a synchronous flight system, such as Lepidoptera. During the warm-up of insects with an asynchronous type of flight, wing movements are not visible, although the vibrations of the thorax may be audible (Krogh & Zeuthen, 1941; Esch, 1964; Leston, Pringle & White, 1965). The increase in temperature during the warm-up is associated with electrical activity in the flight muscles, and in bees the frequency of muscle potentials during the warm-up is similar to that of flight (Esch, 1964). The absence of wing movements cannot be due to phase changes between antagonistic muscles, as is possible in insects with synchronous flight. To

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explain the occurrence of flight-muscle activity without wing movement, Leston *et al.* (1965) suggest that in the beetle *Acilius sulcatus* the folded wings are uncoupled from the muscles because of the configuration of sclerites at the base of the wings. During the pre-flight behaviour of insects with a synchronous type of flight, the wings are usually held in a position similar to the resting position and vibrated slightly (Bachmetjew, 1901; Dotterweich, 1928; Dorsett, 1962). This behaviour, analogous to shivering in mammals, will be called 'wing-vibrating' in the present paper. Until recently no information was available about the motor patterns underlying wing-vibrating. In 1966, Moran & Ewer described the patterns of action potentials recorded from a wing depressor muscle of a hawk moth and a saturniid and compared this activity with the patterns recorded during flight in the same species. In their study no observations were made on the relative timing of the excitation of antagonistic muscles. In the present study I examined the phase relationships between motor units during warm-up and compared these patterns with the patterns of muscular activity during flight. In order to illustrate the effect of the warm-up behaviour on thoracic temperature, the results are introduced with a summary of the available data on thermoregulation in the animals studied. A model describing central nervous mechanisms which could produce the observed motor patterns is proposed. An abstract summarizing some of the work has been published (Kammer, 1965).

#### MATERIALS AND METHODS

Phase relationships between motor units during warm-up were studied most extensively in the lime hawk moth, *Mimas tiliae* L. These animals were obtained commercially from England as pupae; after a period of storage in the cold, the pupae developed at room temperature. A smaller number of observations have been made on another hawk moth, *Celerio lineata* Fabricius, on a skipper, *Hylephila phylaeus* (Drury), and on several saturniids, including *Antheraea polyphemus* (Cramer), *Hyalophora cecropia* (L.), *H. promethea* (Drury) and *Samia cynthia* (Drury). *Celerio* and *Hylephila* were collected locally, and the saturniids were obtained as pupae from several sources in the United States. Muscle potentials were recorded from tethered animals by means of 50 or 100  $\mu$  copper wire, insulated except at the tip and inserted into mesothoracic muscles. [More details on methods and muscle nomenclature are provided in an earlier paper (Kammer, 1967).] The impulses were either observed and photographed directly with a Grass C-4 camera or recorded on magnetic tape with an Ampex SP 300 tape recorder and photographed later. The term *period* will be used to designate the interval from the beginning of one burst of impulses to the beginning of the next burst. In a flying animal this interval corresponds to one cycle of up and down movements of the wings. The term *burst length* will mean the number of muscle potentials per burst.

In a few experiments thoracic temperatures were measured to the nearest degree by means of a Tri-R electronic thermometer, which consists of a Wheatstone bridge and a meter, and a small-bead thermistor which was inserted into the haemocoel underneath the mesoscutellum. The thermistor and electronic thermometer were calibrated by comparison with a mercury thermometer graduated in 0.5° C. The thermistor leads were short, but no special precautions were taken to reduce the loss

of heat via the leads. Unless other temperatures are given, the room temperature during the experiments was 22–24° C.

RESULTS

A. *Hylephila phylaeus*

In the field the skipper *Hylephila phylaeus* adjusts its body temperature by orienting to the sun with its wings partially spread and by wing-vibrating, but the extent of temperature regulation is not known. In the laboratory the thoracic temperature of both inactive and flying animals increases as the ambient temperature increases. The temperature of quiescent animals is approximately equal to room temperature. During flight the maximum temperature attained was 2–3° C. higher than the ambient temperature over a range of 18–30° C. (Fig. 1). An increase of 2° C. is small in absolute terms, but for an animal weighing approximately 75 mg., with little insulation and a relatively large ratio of surface to volume, it represents a substantial production of heat.

During flight each motor unit is active once per wingbeat period, which lasts 20–25 msec. at room temperature (Fig. 2A). Synergistic muscles are active approximately synchronously, and antagonists are active in antiphase. During wing-vibrating the period is the same as in flight, but some of the phase relationships are altered. The

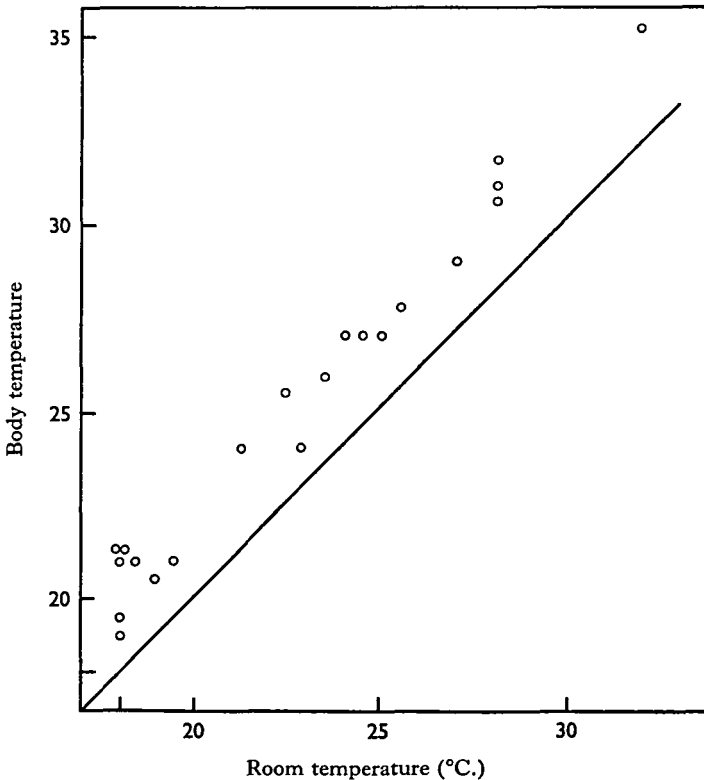


Fig. 1. *Hylephila phylaeus*, maximum thoracic temperature attained during flight at different room temperatures. The diagonal line represents equality. The data were taken from ten animals.

dorsal longitudinal muscles and the subalar muscles, synergistic depressors of the wings in flight, are excited in antiphase during warm-up (Fig. 2B). Some elevator muscles maintain the flight phase with respect to the dorsal longitudinal muscle and thereby become active at the same time as the subalar muscle. Other elevator muscles shift out of phase with their synergists of flight and become synchronous with the dorsal longitudinal muscle. The identity of the elevator muscles was not determined.

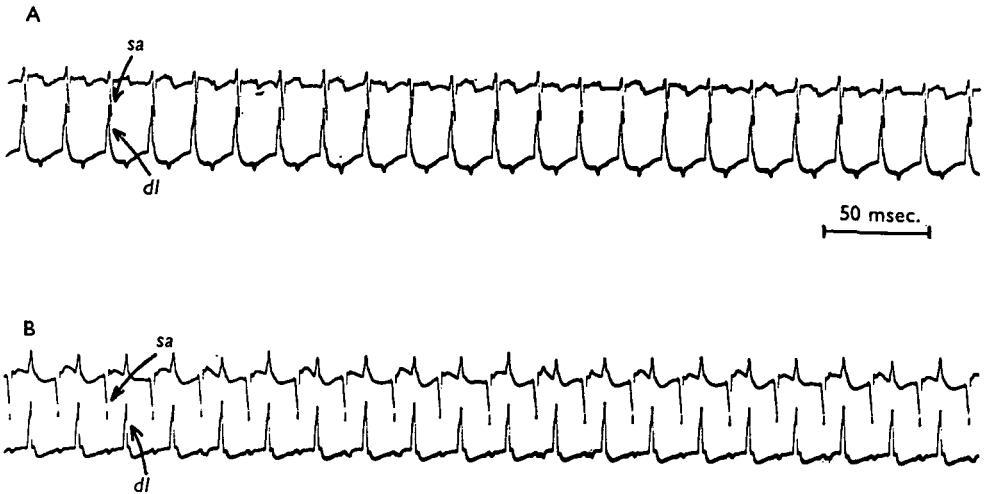


Fig. 2. Muscle potentials from the skipper *Hylephila phylaeus*, during flight (A) and wing-vibrating (B). The potentials identified were from the subalar (*sa*) and the dorsal longitudinal (*dl*) muscles. During flight these two muscles are active simultaneously, whereas during wing-vibrating they are active alternately.

It appears that during wing-vibrating some units maintain the phase relationships characteristic of flight, while other units shift about  $180^\circ$  in phase. During warm-up the wings are held vertically and moved slightly. Wing movements with an amplitude intermediate between those of wing-vibrating and flight were not observed, suggesting that intermediate phase patterns of muscle excitation do not occur. The transition between wing-vibrating and flight appears to be abrupt, but no recordings of muscle activity during the transition were made.

### B. Saturniids

Saturniid moths are large (the specimens used weighed 250–1500 mg., depending on species and sex) and well insulated with a dense coat of scales. [The furry coats of moths from other families have been shown to be effective in reducing heat loss (Church, 1960).] Saturniids are known to produce elevated thoracic temperatures when active (Bachmetjew, 1901; Oosthuizen, 1939). As an example, in the present study an animal flying at an ambient temperature of  $22^\circ\text{C}$ . had a thoracic temperature of  $32^\circ\text{C}$ . The same animal attained a temperature of  $32^\circ\text{C}$ . by means of wing-vibrating prior to flight.

The patterns of muscle activity during flight in saturniids have been discussed in a previous paper (Kammer, 1967), and some additional examples are given in Fig. 3. Each motor unit may be excited several times per wingbeat, and the burst length is

positively correlated with wingbeat period at any given amplitude of stroke. During wing-vibrating the wings are held horizontally and slightly more anteriorly than in a quiescent animal. The period is short, one-third to one-half as long as the wingbeat period during flight, and the burst consists of one or two impulses instead of four to six. Similar results have been reported for *Nudaurelia cytherea capensis* Stoll by Moran & Ewer (1966). The occurrence of short periods and small burst lengths is consistent with the positive correlation observed between wingbeat period and burst length during flight. During wing-vibrating the dorsal longitudinal and the subalar muscles retain the synchrony seen in flight or shift in phase only slightly (Fig. 3 B<sub>2</sub>). On the other hand, some elevator muscles (not identified further) have been observed to shift in phase with respect to the depressor muscles so that both groups of muscles are excited at the same time (Fig. 3 A<sub>2</sub>). Although the data are incomplete, such



Fig. 3. *Samia cynthia*. (A) Potentials from a wing depressor muscle (upper traces, dorsal longitudinal muscle) and an elevator muscle (lower traces). (A<sub>1</sub>) During flight the activity of the two antagonists alternates. (A<sub>2</sub>) The transition between flight (left side of record) and wing-vibrating (right) occurs abruptly, and burst length and period are reduced. (B) Potentials from two different depressor muscles (upper traces, dorsal longitudinal muscle; lower traces, subalar muscle). Both muscles are active at about the same time during flight (B<sub>1</sub>) and wing-vibrating (B<sub>2</sub>).

observations, when considered together, suggest that during wing-vibrating all the motor units are excited approximately synchronously.

#### C. *Celerio lineata*

In the hawk moth *Celerio lineata*, Heath & Adams (1965) found that body temperature during flight was maintained at 32°–40° C. over a range of ambient temperatures from 10° to 35° C. The mean thoracic temperature at the end of the warm-up prior to flight was 38° C., i.e. within the range of temperatures observed during flight (Adams & Heath, 1964). Like the saturniids, this species of hawk moth is relatively large (weight approximately 1 g.) and well insulated. However, its motor pattern

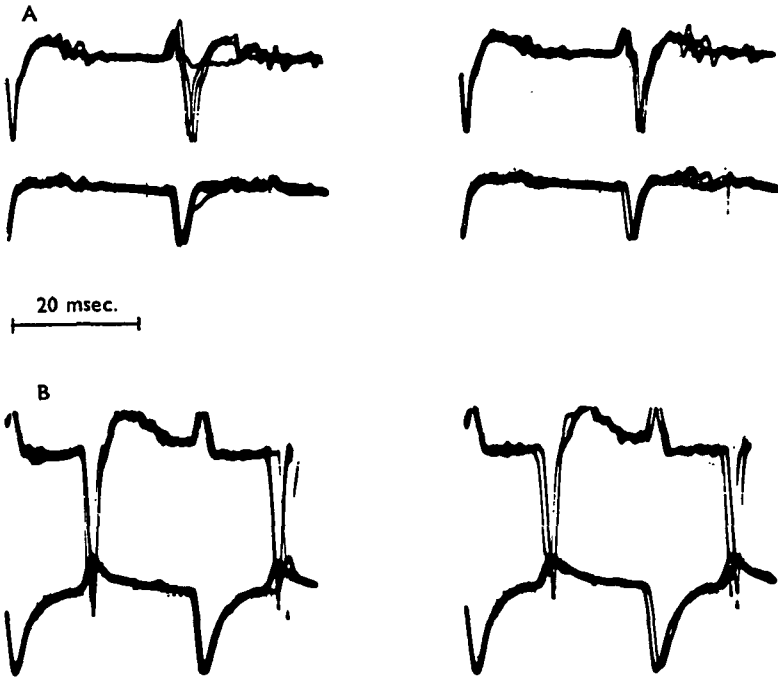


Fig. 4. *Celerio lineata*, superimposed sweeps showing muscle potentials from two depressor muscles, the dorsal longitudinal (upper traces) and the subalar (lower traces). (A) During flight the depressors fire synchronously. (B) During wing-vibrating the depressors fire alternately.

during flight resembles that of skippers rather than saturniids. Wingbeat periods of 25–35 msec. have been observed, in comparison with 20–25 msec. in skippers and 100–150 msec. in saturniids. Each motor unit is usually activated only once per wing-stroke; pairs of impulses are uncommon (Fig. 4A). During wing-vibrating the subalar and dorsal longitudinal muscles, which are synergists in flight, are out of phase (Fig. 4B). In this respect, also, the motor pattern resembles that of the skipper.

#### D. *Mimas tiliae*

Because previous investigators (Dotterweich, 1928; Bodenheimer, 1934; Dorsett, 1962; Adams & Heath, 1964; Heath & Adams, 1965) had documented the ability of several species of hawk moths to elevate their body temperatures, I did not monitor

temperatures in the lime hawk moth, *Mimas tiliae*. However, three temperature measurements made at the end of bouts of activity indicated that this species also has a thoracic temperature greater than ambient; the temperatures observed were 31°, 32°, and 36° C. at a room temperature of 24° C.

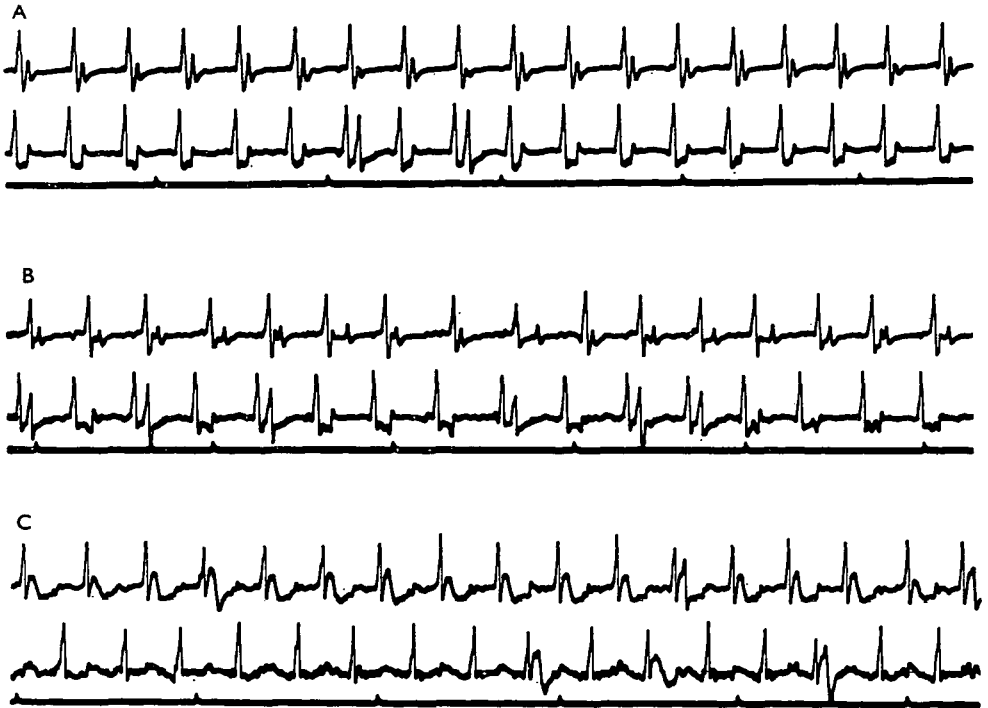


Fig. 5. *Mimas tiliae*, muscle potentials recorded during two stages of warm-up and flight (upper traces, elevator, possibly tergo-sternal muscle; lower traces, subalar muscle). Time mark 100 msec. (A) Wing-vibrating. (B) Later in warm-up; note phase shift. (C) Flight.

During flight the pattern of muscle activity in the lime hawk moth (Fig. 5 C) is similar to the pattern described above for the skipper *Hylephila* and the hawk moth *Celerio*, and also to the pattern previously reported for the locust *Schistocerca gregaria* (Wilson & Weis-Fogh, 1962). In each motor unit there are one or two closely spaced muscle potentials during each wingbeat period of 24–30 msec. During steady flight wingbeat frequency and amplitude vary only slightly, in contrast to the marked variability of these parameters in saturniids (Kammer, 1967). In hawk moths which had assumed the full flight posture, as indicated by the position of the hind legs, the phase of elevator impulses with respect to muscle potentials of the dorsal longitudinal muscle was 0.4 or 0.5. The subalar muscle, a direct depressor, was commonly active synchronously with the dorsal longitudinal muscle, but sometimes the subalar lagged or led the dorsal longitudinal by a few milliseconds. Between synergists, therefore, small changes in phase are possible during flight. It is possible that, as in the locust, these changes are associated with the mechanisms controlling lift (Gettrup & Wilson, 1964) or turning (Waldron, 1967).

The warm-up behaviour in the lime hawk moth resembles the behaviour described by Dorsett (1962) for *Deilephila nerii* and other hawk moths. In order to describe warm-up in *Mimas tiliae*, I estimated the amplitudes of wing movements visually. In a few cases the estimates were checked by measuring photographs of the experimental animal. Wing-vibrating, as described above for other species, constitutes the first stage of the warm-up behaviour. Wing-vibrating in *Mimas* is characterized by

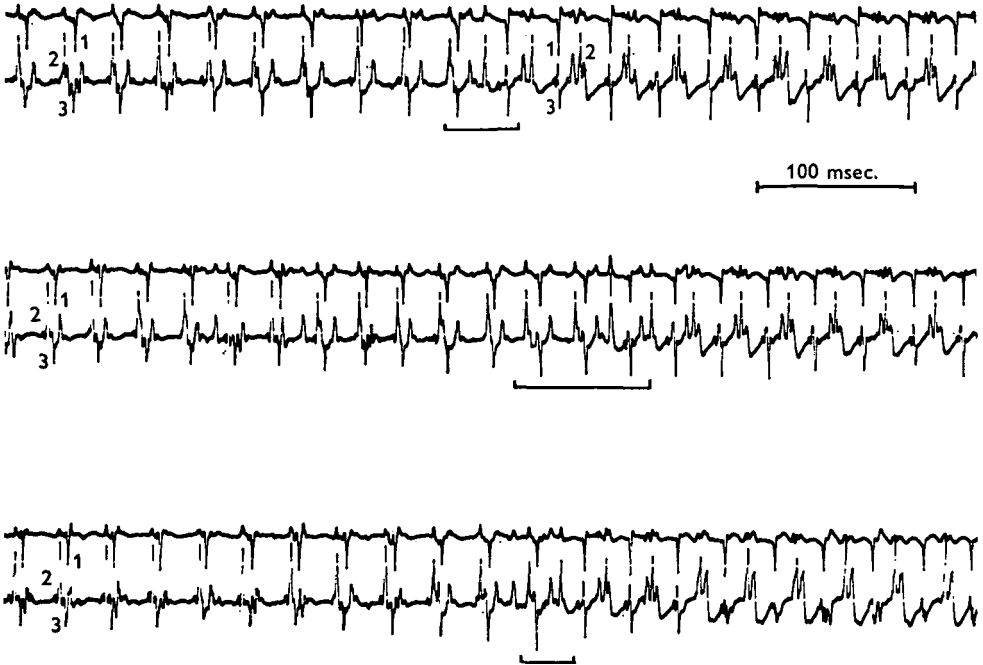


Fig. 6. *Mimas tiliae*, three examples of an abrupt shift from warm-up (left side of each record) to flight (right). The three samples were taken from the same experiment. The transition in each case is indicated by a bracket. Note that the period during warm-up is the same as the wing-beat period of flight. (Muscles: 1, subalar; 2, elevator, possibly the posterior tergo-coxal; 3, dorsal longitudinal.)

wing movements of minimal amplitude, about  $2-3^\circ$  of arc, with the wings held at an angle slightly higher than at rest. In later stages of warm-up the amplitude gradually increases until the flight amplitude is achieved. In some cases warm-up did not begin with wing-vibrating, but instead commenced with wing movements of somewhat larger amplitude ( $5-10^\circ$ ). The transition from the final stage of warm-up to flight, as inferred from the patterns of muscle potentials, occurs rapidly. In several species of hawk moths Dorsett (1962) observed that during warm-up the thoracic temperature rises and the period between muscle potentials shortens. In *Mimas tiliae* also there is a decrease in period, and at the end of warm-up the period is approximately equal to the wingbeat period which occurs during flight. This equality is most easily seen in records of transitions from warm-up to flight (Fig. 6).

During wing-vibrating all the motor units from which recordings were obtained were active nearly synchronously. Synchrony within 3 msec. has been observed between the dorsal longitudinal and subalar muscles and between these depressors and



the tergo-sternal, anterior tergo-coxal, posterior tergo-coxal, and the dorsal oblique elevator muscles. Mesothoracic muscles which have not been sampled include the basalar muscle, several small muscles which run from the pleuron to the axillary sclerites, and the tergo-trochanteral, the only muscle which can function in moving both wing and leg. Although only three or four units were observed at any one time, the observations taken together suggest that during wing-vibrating many and possibly all units are synchronous. Compared to the flight pattern, this synchrony entails a  $180^\circ$  phase shift between antagonists and continued synchrony between synergists. As in saturniids but not in the skipper *Hylephila* nor the hawk moth *Celerio lineata*, the dorsal longitudinal and the subalar muscles are excited at the same time.

During intermediate stages of warm-up, when the wing movements cover an angle of  $10-25^\circ$  of arc, the activity of antagonistic muscles still overlaps, but incompletely. Muscle potentials of antagonists may occur as much as 10 msec. apart (Fig. 5 B), in comparison to an interval of 15 msec. between these impulses during flight (Fig. 5 C). The gradual increase in stroke amplitude during the final stages of warm-up may be produced by a gradual shift in the phase of the elevator muscles with respect to the depressors, a shift from synchrony to antiphase (Figs. 5, 7 middle). The resulting smooth conversion from wing-vibrating through later stages of warm-up to flight is probably the normal sequence. However, the transition from warm-up to flight can be more abrupt (Fig. 6). Such transitions show at least a superficial resemblance to cases of phase multistability which Wyman (1966) described for some species of flies. The fact that the phase can change in one cycle indicates that the coupling of at least some of the units into the flight pattern does not depend on a temporal summation of small effects. In addition, it is important to note that the assumption of phases characteristic of flight can occur at different times for different units (Fig. 7 middle; compare the phases of units 1 and 3 with respect to unit 2). The warm-up pattern does not change into the flight pattern in a single step.

Occasionally during pre-flight behaviour strokes of large amplitude were produced by unusual patterns of excitation. During some extended bouts of warm-up (possibly the result of experimental treatment), the subalar muscle and the dorsal longitudinal muscle became asynchronous, and there was an interval of 7-10 msec. between these two depressor muscles, while the period was 22-24 msec. (Fig. 8). At the same time, excitation of the subalar and an elevator muscle in some cases coincided, but in other cases the elevator was activated in the middle of the interval between the depressor impulses. The most interesting feature of this pattern is the fact that, in contrast to the wing-vibrating and flight patterns of *Mimas*, the two depressors were not in phase.

In another of these unusual patterns of excitation an elevator unit fired at a frequency higher than the flight frequency and higher than that of other units active concurrently (Figs. 7 top, 9, 10). Conversion to the flight pattern involved a reduction in the firing frequency of this unit as well as synchronization into the proper phase. This reduction in frequency suggests that coupling of neurons into the flight-pattern generator may involve inhibitory interactions. In one of these cases (Fig. 9) reduction in frequency and production of the flight pattern occurred when an antagonistic muscle, the dorsal longitudinal, began to fire, suggesting that the activity of antagonists may be necessary for the generation of the period observed during flight. However, since the activity of all other antagonistic units was not recorded, this conclusion

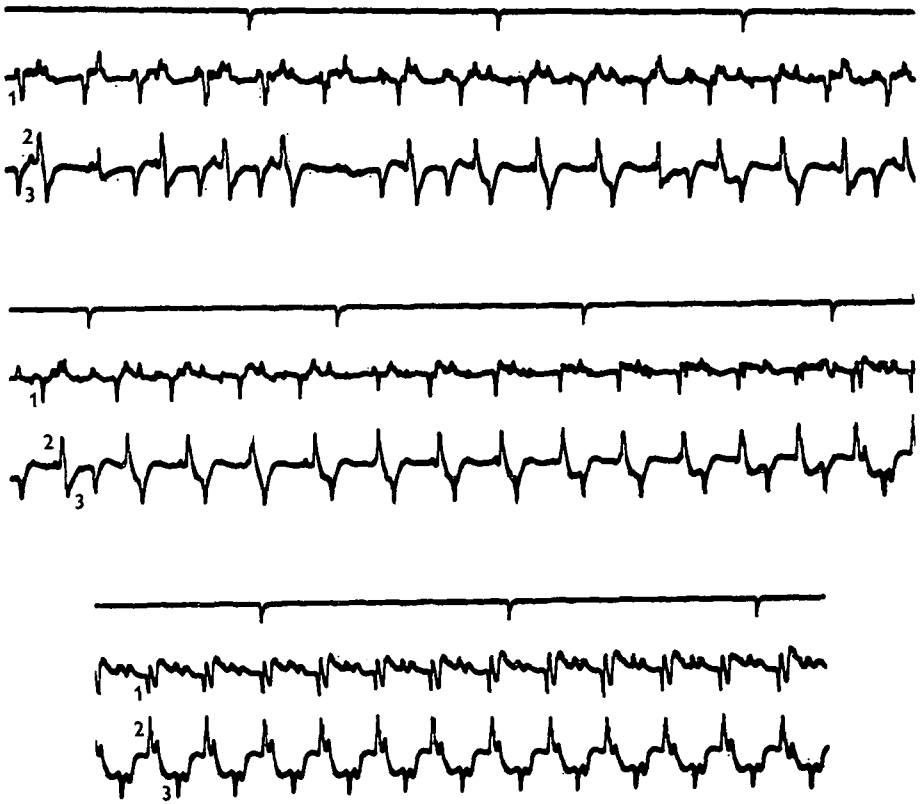


Fig. 7. *Mimas tiliae*, continuous record showing a late stage of warm-up (top record) and a gradual phase shift (middle) to flight (bottom). The warm-up pattern is unusual in that the elevator muscle (3, tergo-sternal) is firing at a higher frequency than the depressor muscles (1, subalar; 2, dorsal longitudinal). Time mark, 100 msec.



Fig. 8. *Mimas tiliae*, continuous record showing muscle potentials during a final stage of warm-up (upper) and flight (lower). In this warm-up the dorsal longitudinal (1) and subalar (3) muscles are out of phase, and the elevator (2, tergo-coxal muscle) fires in the interval between the two depressors. In flight, the dorsal longitudinal and subalar muscles are in phase. Time mark, 100 msec.

is not well founded. Observations made simultaneously on a greater number of units would be required to determine whether or not the production of activity at flight frequency depends on the participation of specific antagonistic pairs.

A pattern which was observed in only one animal but which persisted for several minutes is shown in Figure 11. The upper line represents potentials recorded with a pair of electrodes, one in the dorsal longitudinal muscle and the other in an elevator, probably the tergo-sternal muscle. The lower line represents potentials recorded with a single electrode in the subalar region. All four units were on the same side of the mesothorax. Between the units in each line the phase relationships were those of flight, but between units of different lines all possible phases occurred. In order to determine whether in the latter case one particular phase relationship was preferred, a sequence of phases was measured on a continuous record (Fig. 12). The sampling

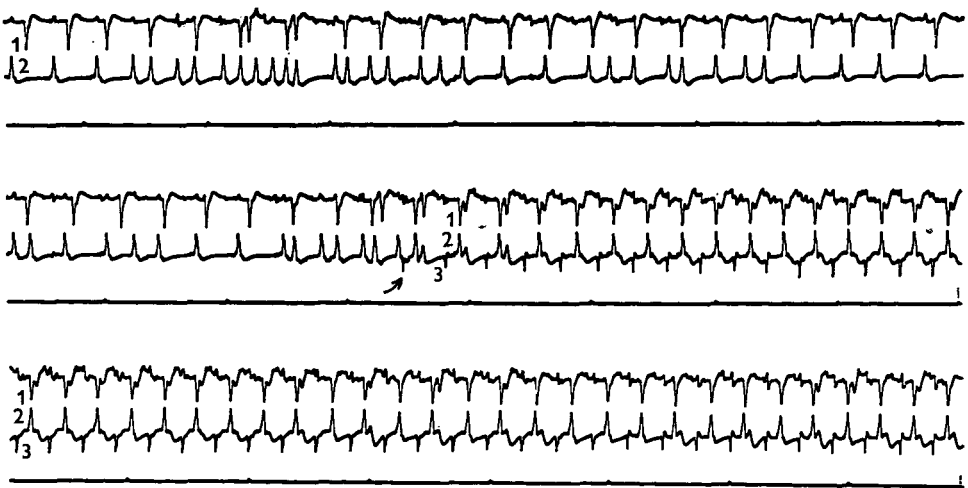


Fig. 9. *Mimas tiliae*, continuous record of an unusual warm-up pattern and transition to flight. The flight pattern appears when the depressor unit becomes active (arrow). (Muscles: 1, posterior tergo-coxal, motor unit 1; 2, posterior tergo-coxal, motor unit 2; 3, dorsal longitudinal). Time mark, 100 msec.

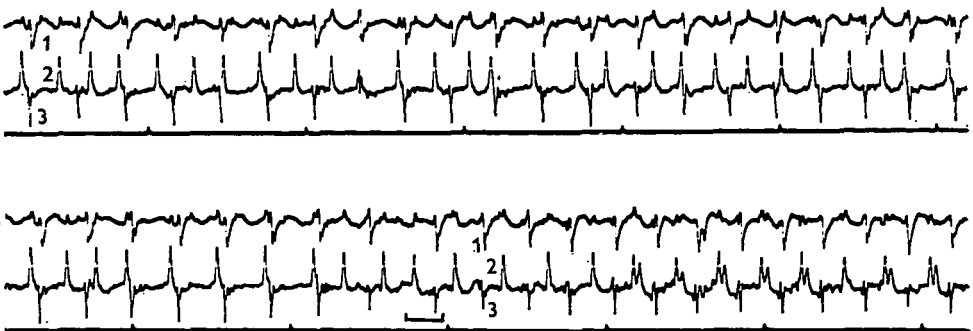


Fig. 10. *Mimas tiliae*, continuous record of warm-up and, at bracket, transition to flight. In most of the warm-up pattern shown the elevator (2, probably the posterior tergo-coxal muscle) is firing at a higher frequency than the depressors (1, subalar muscle; 3, dorsal longitudinal muscle). Time mark, 100 msec.

was frequent enough to approximate local maxima and minima, and between the points shown the phase changed smoothly. A large number of phase relationships were stable for short periods of time, but the preferred phase was about 1.0. That is, most commonly there was approximate synchrony between the units being measured. The presence of this preferred phase is more clearly shown in a histogram of the same data (Fig. 13). Phase relationships in which there is drifting interspersed with stability

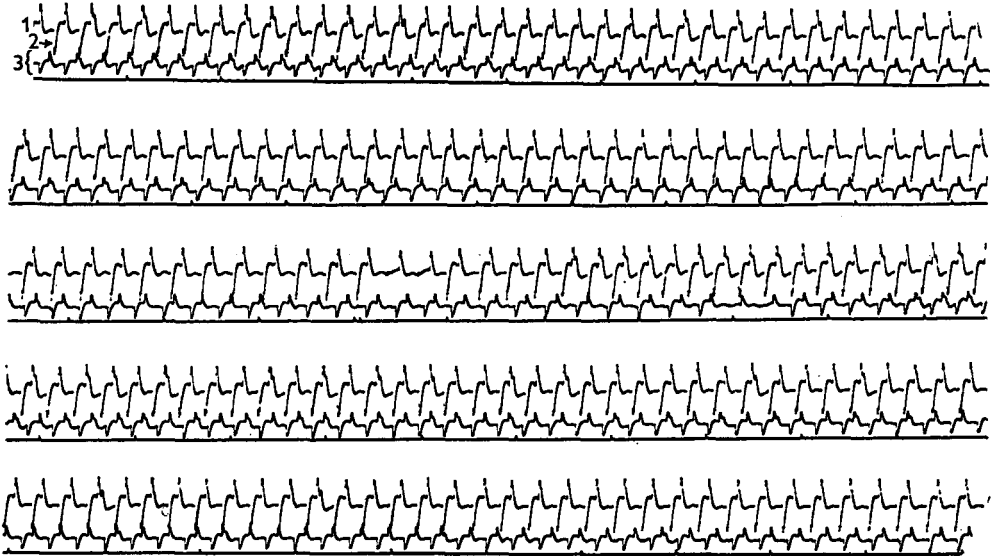


Fig. 11. *Mimas tiliae*, continuous record showing changes in phase between some units, while other units retain the phases characteristic of flight. (1, elevator, possibly the tergo-sternal muscle; 2, dorsal longitudinal muscle; 3, potentials recorded with a single electrode in the vicinity of the subalar muscle.) Time mark, 100 msec.

at a particular phase characterize 'relative co-ordination' (von Holst, 1935, 1939). This phenomenon was first described by von Holst for the interactions between the oscillations of two fish fins. Recently the same phenomenon was observed during walking in insects (Wendler, 1964*a, b*). The present example represents relative co-ordination between the motor units which move one appendage rather than relative co-ordination between appendages.

#### DISCUSSION

During warm-up in *Hylephila*, *Celerio* and *Mimas*, the period is equal to the wing-beat period of flight at the same thoracic temperature. In some species (*Hylephila* and probably *Celerio*) some of the phase relationships between antagonistic motor units are the same as those of flight. These results suggest that the central nervous mechanisms which control muscle activity during warm-up are related to, or derived from, the mechanisms which produce the patterned motor output of flight. Assuming that these mechanisms are related, I compared the patterns observed during warm-up and flight and used these data to develop a model of the pattern-generating mechanisms underlying both behavioural acts. The main purpose of the following discussion is presentation of this model.

Besides the similarities between the motor patterns of warm-up and flight, the following results are important for the construction of the model:

(1) During wing-vibrating several units were active at the period and burst length observed during flight, but the phase relationships between some or all of the units were different from those of flight (all cases examined). Production of the wingbeat period appears to depend on concurrent activity in a number of motor units, perhaps including antagonists, but not on the alternating activity of these units (*Mimas*).

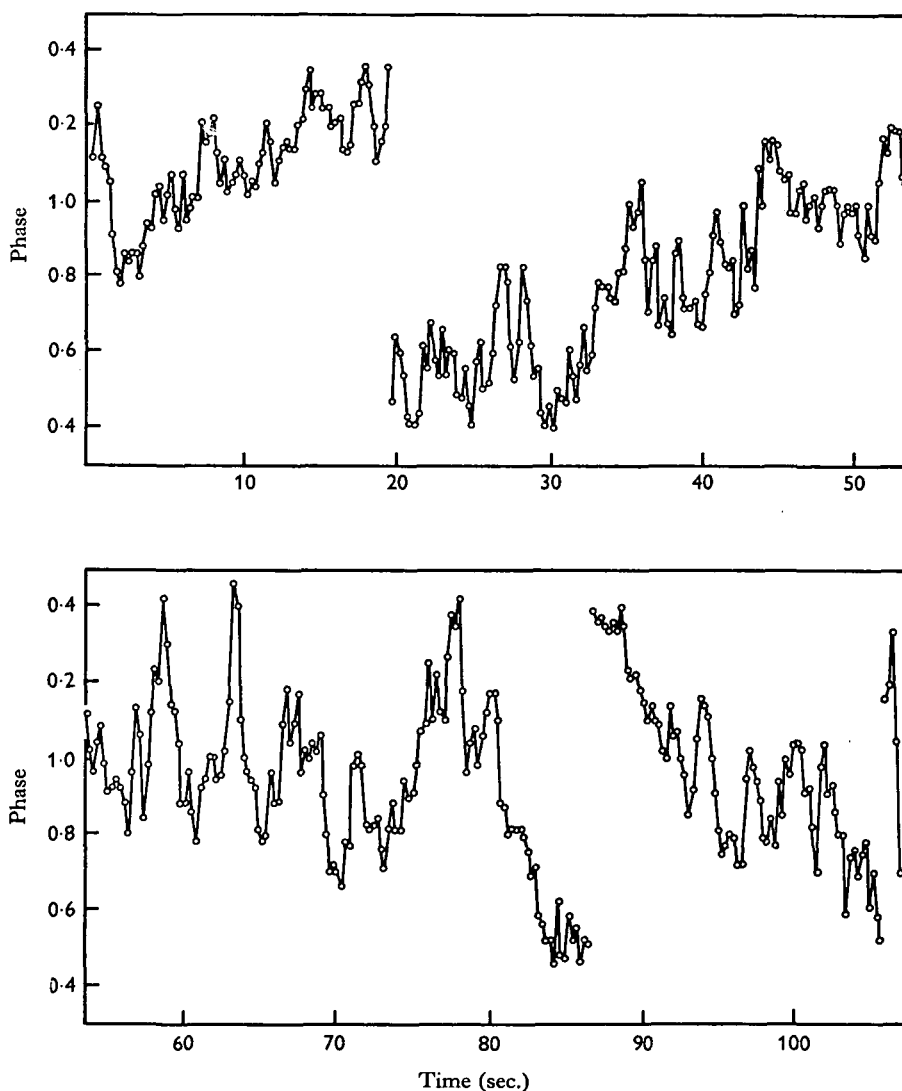


Fig. 12. Relative co-ordination between two motor units in *Mimas tiliae*. The phase of an unidentified unit (upward deflexion on line 3 in figure 11) was measured with respect to the impulses of the dorsal longitudinal muscle (unit 2 in figure 11). Individual measurements were made every 250 msec. For clarity, the phase is plotted around 1.0 instead of from 0.0 to 1.0. The lower plot is a continuation of the upper. It is clear that the phase changed repeatedly and all possible phases occurred. There were short periods of stability at various phases but an overall preference for a phase of approximately 1.0 (compare with figure 13).

(2) Several units displayed the flight pattern while other units fired at a different frequency (unusual warm-up pattern in *Mimas*).

(3) Some groups of motor units were active in the flight pattern while other units fired in different phase patterns. Muscles which are synergists in flight were  $180^\circ$  out of phase in *Hylephila* and *Celerio*. In one unusual pattern in *Mimas* some pairs of units fired alternately as in flight, but the two sets of alternating units were only relatively co-ordinated.

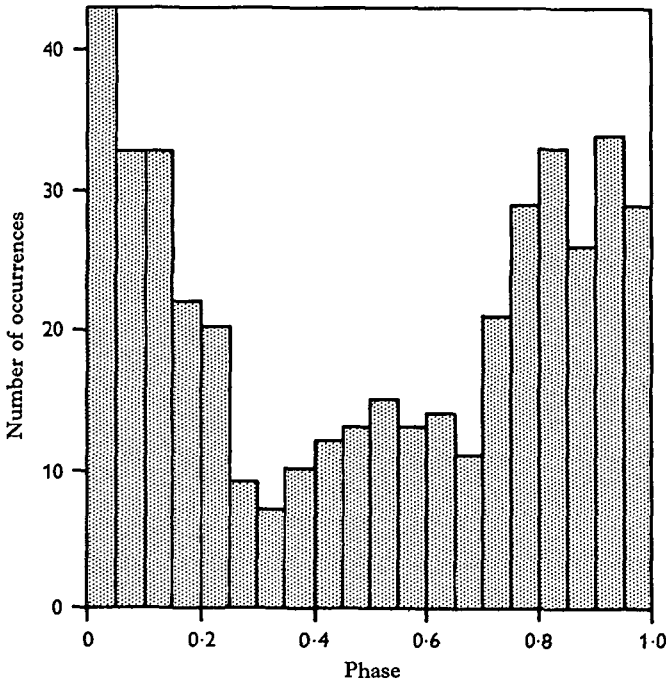


Fig. 13. Histogram of the data presented in figure 12. Phases around 0.0 (which is equivalent to 1.0) occurred more frequently than phases of 0.5. The observed motor units were co-ordinated, but weakly.

(4) The phase relationships shifted abruptly from those of warm-up to flight (saturniids, *Mimas*, probably *Hylephila*) or the transition was more gradual (typical pattern in *Mimas*). Not all units were synchronized into the flight pattern at the same time (*Mimas*).

It is clear that some features of the flight pattern can appear in the absence of other features. This result suggests that several separable mechanisms participate in the generation of the flight pattern. In particular, because repetitive activation of a motor unit at the wingbeat period is not dependent on coupling with antagonists into the phase relationships of flight, the mechanism generating wingbeat period can be separated from the mechanism generating phase relationships.

#### *Generation of the rhythm—Is there a pacemaking centre?*

In any rhythmic activity, whether it be the movement of a wing or the activity cycle of a whole animal, a basic problem is the origin of the rhythm. Is the cycling dependent

on peripheral or external cues, or is it endogenous to the central nervous system? In locust flight the timing of the muscle excitations is not determined by sensory feedback but is a product of the central nervous system (Wilson, 1961, 1964). The same is true of flies, because there are no phase relationships between wing movement and muscle excitations (Wilson & Wyman, 1963). Although rigorous proof is lacking for the Lepidoptera, a central origin for the flight pattern would be expected on the basis of homology. This expectation is supported by two facts. First, because wing movement is slight during wing-vibrating any sensory input is very likely to be different from the input during flight; nevertheless, the wingbeat period and some of the flight phasic patterns still occur. Secondly, saturniids with denervated wings no longer receive input from receptors in and on the wings but are still able to fly (Kammer, 1967).

If the central origin of the patterned rhythmicity is accepted, the question which next arises concerns the mechanism of rhythm production. One possibility is that a pacemaking 'centre' drives follower cells at the wingbeat frequency. According to this hypothesis, the rhythm is inherent in pacemaker neurons, although they may require input for excitation. An alternative hypothesis suggests that the wingbeat period is generated by interactions among a few or many neurons which by themselves are not rhythmically active. In this view, the output reflects oscillatory properties of a network rather than those of individual cells.

Arguments against the hypothesis of a single pacemaking centre can be constructed from observations on the warm-up behaviour, as follows:

(1) If the excitation of motor units is timed by a pacemaker, the production of alternating activity in synergists (as during wing-vibrating in skippers and *Celerio*) would require excitation of the group of synergists by a pacemaker firing at twice wingbeat frequency. If the pacemaker does fire at twice wingbeat frequency, a mechanism would be required to ensure that individual output units fire at only half the pacemaker frequency, and it is reasonable to think that the production of the period observed during warm-up and flight is accomplished by the same mechanism which produces the alternating activity of antagonists (as during wing-vibrating in skippers and flight in all species). When there is no alternating activity in antagonists (as during wing-vibrating in *Mimas*), motor unit activity at twice wingbeat frequency would be expected. However, this expectation is not fulfilled. Although it is possible to formulate other interpretations than the one just presented, it is difficult to derive the wing-vibrating patterns from a single pacemaker.

(2) During the transition from warm-up to flight in *Mimas* various units became co-ordinated into the flight pattern at different times, indicating that the mechanism which couples units into the flight pattern operates separately on the various units. During the transition there occurred a variety of phase relationships intermediate between those of wing-vibrating and flight. This complexity in the phase relationships and in the time of co-ordination of units into flight is difficult to explain in a simple way with a single-pacemaker hypothesis. As a minimum it would be necessary to postulate that there are many variable connexions between pacemaker and motor neurons and among motor neurons or their antecedents.

(3) The most convincing piece of evidence against the hypothesis of a single pacemaking centre is the instance of relative co-ordination seen in *Mimas*. Here,

although two pairs of units were each co-ordinated into the flight pattern, there was usually a slight difference between the frequencies of the two pairs, and all possible phase relationships occurred. Because of this frequency difference, there cannot be a common pacemaker for the two pairs of motor units. A redundancy of pacemakers, each with its set of follower neurons, all 'absolutely co-ordinated' during flight, is possible. However, with this system, as with the single pacemaker, it is difficult to postulate simple mechanisms for the production of all observed patterns of warm-up and flight.

In summary, the hypothesis of a single pacemaking centre can be excluded on the basis of the observations on warm-up patterns, especially those of *Mimas*. However, there is at present no unambiguous evidence which rules out the hypothesis of multiple pacemakers. A model developed from the latter hypothesis and capable of producing the observed variety of patterns would be complex and would require a hierarchical arrangement of a relatively large number of neurons. An alternative model, derived from the observations summarized above, and based on the alternative hypothesis that the motor output is generated by interactions within a network, is here proposed.

#### *A model of the flight-pattern generator*

From studies on locusts, flies, and electronic and computer models, Wilson (1966*b*) has devised a model which explains, in part, the production of the flight pattern in *Schistocerca gregaria*. He postulates that there are small groups of neurons coupled by mutual excitation, which causes the units to fire in approximate synchrony, while the whole system is interconnected by mutual inhibition, which produces the alternation between antagonists. This hypothesis thus postulates a multiplicity of interactions between a number of units, a proposal which can also be made on the basis of the data on Lepidoptera. In Wilson's analysis no special rhythmic properties for single cells are assumed; the output rhythm is the property of the entire network. A similar model, with some additional details, can account for flight and warm-up patterns of motor-unit activity in Lepidoptera.

The main features of this model are summarized in the following postulates:

(1) The output of the flight-pattern generator reflects the oscillatory properties of a network of neurons rather than oscillatory properties of pacemaker neurons.

(2) The most elementary oscillator consists of a small group of synergistic neurons (i.e. those neurons which control the activity of muscles synergistic in flight; they may be motor neurons or antecedents of the motor neurons). Positive feedback among these neurons, which also receive unpatterned excitation, results in the production of bursts of output pulses at the wingbeat period. Bursts are terminated because of accumulating refractoriness in the neurons and a concomitant decline in the amount of excitation which any unit receives from other neurons in the group.

(3) The alternating activity of antagonistic neurons results from negative coupling between a few elementary oscillators. The resulting network, a subset of the neurons involved in co-ordinating the thoracic muscles, can produce the flight pattern of activity in the muscles which it controls.

(4) Several similar subsets coupled together constitute the flight-pattern generator.

According to this scheme the most elementary oscillator consists of a small number



of like units, either elevator or depressor motor neurons or their respective antecedents, firing rhythmically at the wingbeat period. The group fires in approximate synchrony because of excitatory coupling among the units. The neurons are not spontaneously active but receive excitatory, unpatterned input from other neurons; a burst is initiated when the level of excitation in a unit exceeds threshold. The output burst could consist of one, two, or several pulses, as discussed in a previous paper (Kammer, 1967). The burst may be terminated by refractoriness of the neurons (Wilson, 1966*b*), a decline in the level of excitation, inhibitory feedback from synergists via a pathway with a delay, or by any combination of these mechanisms. Several of these elementary oscillators would be required to supply all the mesothoracic muscles on one side of the animal (bilateral interactions are ignored in the present analysis).

The next stage of the model, the coupling of elementary oscillators into subsets each of which can generate the flight pattern of alternating activity in some antagonistic muscles, can be achieved by inhibitory interconnexions between elementary oscillators. Alternation could also be produced by additional excitation via a delay circuit (Pavlidis, 1965). In either case, the result is a network with oscillatory properties different from those of the elementary oscillator.

The final stage of the model, the coupling of several of these networks into the flight-pattern generator, may involve excitatory interactions among synergists in different subsets. (However, as in previous interconnexions, inhibition between antagonists is also possible.) The redundancy of subsets generating the flight pattern and the large number of interactions involved in the flight-pattern generator would lend stability to the whole. Redundancy can explain the persistence of the flight pattern in some units while another motor neuron is firing at a higher frequency or is silent. Postulation of at least two subsets generating the flight pattern is necessary because of the case of relative co-ordination which was observed between pairs of antagonists in *Mimas tiliae* (Fig. 11). This pattern could be produced by weakly coupled subsets oscillating at slightly different frequencies. It is characteristic of coupled oscillators in general (Wever, 1965; Wilson, 1966*a*) that, when interactions between oscillators are weaker than those which produce phase-locking, certain phase relationships may be relatively stable and persist for different lengths of time, depending on the strength of the coupling and the difference between the frequencies of the oscillators. Between stable phase relationships, the phase shifts smoothly and relatively rapidly. These characteristics were observed in the instance of relative co-ordination in one specimen of *Mimas*.

Although the proposed model can account for the occurrence of relative co-ordination, an alternative explanation of this pattern is possible. In this explanation emphasis is placed on the fact that both alternating activity of antagonistic neurons and firing at the wingbeat period can result from reciprocal inhibition between antagonists (as discussed by Wilson, 1966*b*). That is, a single pair of antagonistic units can produce the flight pattern. In the case of relative co-ordination, two such pairs of antagonists could have been active but only weakly coupled. Although this explanation of the relative co-ordination of units cannot be excluded, a network in which generation of the wingbeat period is dependent on the alternating activity of antagonists could not produce the wing-vibrating pattern in *Mimas*.

*Derivation of the warm-up patterns from the model*

In order to explain the warm-up patterns in a manner consistent with the model of the flight-pattern generator it is necessary to postulate different interactions among certain components of the model. The skipper and *Celerio* patterns, in which some units alternate as in flight but shift  $180^\circ$  in phase with respect to other units, could result if subsets of the pattern-generating mechanism continue to produce the flight pattern, while coupling between the subsets is altered. If between some antagonists in different subsets there is an excitatory coupling which is stronger than the coupling between synergists in different subsets, these antagonistic units will fire simultaneously. The result could be a  $180^\circ$  phase shift between the output of subsets. In other words, the difference between wing-vibrating and flight patterns in these animals could result from alterations in the relative strength of the excitatory coupling between antagonists and synergists of different subsets. In order to explain the synchrony of elevator and depressor muscles during wing-vibrating in *Mimas* and saturniids, a different modification in the interactions between units is required. Synchrony would result if the elementary oscillators were positively coupled and if the connexions which produce alternation during flight were inactive or overpowered. Phase relationships intermediate between wing-vibrating and flight, as observed in *Mimas*, could be produced if the interconnexions which produce alternation are active but are too weak to overcome completely the influences toward synchrony. According to the model, the period during wing-vibrating is the same as that of flight (as in *Mimas*, *Celerio* and *Hylephila*) because the wingbeat period is generated by the elementary oscillators. The short period during wing-vibrating in saturniids can be explained by postulating that, in these animals with a low and variable wingbeat frequency during flight, the elementary oscillator is able to generate a wide range of frequencies. The short periods and small bursts during wing-vibrating would then reflect a property of the elementary oscillator.

*Transition between warm-up and flight*

According to the model presented above, the transition from warm-up to flight involves alterations in the coupling among the units of the pattern-generating system. In *Mimas* and saturniids the interactions which produce alternation become effective. In *Hylephila* and *Celerio* the relative effectiveness of the excitatory couplings between antagonists and synergists are reversed, so that only synergists fire synchronously. In both cases, the changes which cause the shift in the output pattern from warm-up to flight would be expected to alter the period between the firings of any one unit. Altered interactions may influence period in saturniids, but in the other species examined the periods at the end of warm-up and the beginning of flight are approximately the same. At least three explanations for the absence of a change in period can be proposed. Possibly the altered interconnexions are balanced in terms of the excitation and inhibition supplied to an elementary oscillator, and they compensate for each other. Perhaps each elementary oscillator is so stable that its output frequency is not altered by changes in the input which it receives from other neurons in the system. Or possibly additional input with no phasic effect compensates for the inhibition or excitation which produces the phase relationships of flight.

The preceding discussion has assumed that couplings or interactions between

neurons can be altered to produce the changes in pattern which occur when an animal stops wing-vibrating and begins to fly. However, the means for regulating strength of coupling are unknown. It is possible that the change from warm-up to flight is controlled by command fibres similar to those which are known to occur in crayfish (Wiersma & Ikeda, 1964; Kennedy, Evoy & Hanawalt, 1966; Atwood & Wiersma, 1967). Command fibres could be activated by sensory input from a puff of air, a moving object, or a tactile stimulus, any of which can initiate the transition from warm-up to flight. In an undisturbed animal such inputs are not present, but the transition may nevertheless occur. Two mechanisms which may be involved in bringing about a spontaneous transition can be suggested. First, within the thorax there may be receptors which monitor the body temperature. When the temperature is high enough, the input from these receptors could cause the transition. Although internal temperature receptors have not been demonstrated in insects, their presence is suggested by the fact that many insects regulate their body temperature, either metabolically or behaviourally. Secondly, the pattern generator itself could be temperature-sensitive. The configuration which yields the wing-vibrating pattern may be more stable at low body temperatures (although excitatory input such as that from a strong external stimulus could alter the preferred mode of oscillation, resulting in flight at low body temperatures). When the thoracic temperature is high enough, the flight-generating configuration would be more stable, and the transition would occur automatically.

#### SUMMARY

1. The patterns of muscle activity during warm-up were compared to those of flight. In the skipper *Hylephila phylaeus* and in the hawk moths *Celerio lineata* and *Mimas tiliae* the intervals between bursts of muscle potentials are the same as the wingbeat periods of flight at the same thoracic temperature, and the burst length is the same as in flight. In saturniids the period and burst length are both shorter during wing-vibrating than during flight.

2. During wing-vibrating the amplitude of the wing movement is small, and some of the muscles which are antagonists in flight are active simultaneously. In *Hylephila phylaeus* and *Celerio lineata* there is a phase change between some synergistic muscles, while some antagonistic pairs retain the phase relationships of flight. During wing-vibrating in *Mimas tiliae* and in saturniids all the motor units sampled were active at the same time.

3. In *M. tiliae* a variety of phase relationships intermediate between those of wing-vibrating and flight were observed, including a case of 'relative co-ordination' between motor units in the mesothorax. The results exclude the possibility that a single pace-making centre drives the motor neurons in the flight pattern.

4. A model of the central nervous interactions which generate the observed motor patterns is proposed. It is postulated that a small group of positively coupled neurons produces bursts of impulses at the wingbeat frequency and that these groups interact to generate the phase relationships seen during warm-up and flight.

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