

SPIRACLE CONTROL IN ADULT DRAGONFLIES (ODONATA)

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INTRODUCTION

Hoyle (1960) has recently shown that carbon dioxide has a direct action on the closer muscle of spiracle 2 of the desert locust, *Schistocerca gregaria* Forskål, reducing the electrical responses and the tension developed. Nevertheless, except in flight, the valve movements are nearly always synchronized with ventilation, and it seems unlikely that this action of carbon dioxide plays much part in the normal regulation of the spiracle, except in the presence of high concentrations of carbon dioxide (Miller, 1960*b*). To a considerable extent the action appears to be over-ridden in the interests of ventilation synchronization. When, however, the mesothoracic ganglion is uncoupled from the ventilatory rhythm by section of the cord between it and the metathoracic ganglion, the frequency of motor impulses in the nerve to spiracle 2 falls and this has the effect of increasing the sensitivity of the spiracle muscle, the valve now opening in 1-2% carbon dioxide. The peripheral action of carbon dioxide may provide an unimportant means of spiracle control in the locust, but in an insect, such as the adult dragonfly, whose spiracles are not usually synchronized with ventilation, it may have a more significant part to play.

To investigate this hypothesis the control of spiracle 2 in the adult dragonfly has been examined. Four species were chosen for study, each showing slight differences in spiracle behaviour. It is shown here that the spiracle is dually controlled, at the spiracle muscle by the action of carbon dioxide and from the central nervous system as a result of partial anoxia and other stimuli. Differences of spiracle behaviour in the four selected species are shown to correspond firstly to small differences in the morphology of the tracheal system and secondly to differences in the behaviour and habitat of the adult insects.

A neuro-muscular system, in which the responsiveness of the muscle to excitation of the motor nerve is variable, necessitates the monitoring of muscle movements at all times with recordings from the motor nerve. Only by doing this will the contribution of the peripheral mechanism to spiracle control be distinguishable from that of the central nervous system. For this reason the relation between spiracle movements and nerve impulses may seem at times to be unduly laboured in the following account.

While there are numerous accounts of experimental investigations of larval respiration in Odonata (summarized by Corbet, 1962), respiration in the adult has received little attention. Detailed anatomical studies of the adult spiracles and tracheal system have been made by Landois & Thelen (1867), Calvert (1893), Tillyard (1917), Wolf

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(1935) and other authors; attention has been paid to the respiratory system at the final emergence (Müller, 1924; Straub, 1943; Needham, 1944); observations on ventilation in resting and excited dragonflies are recorded by Barlow (1855); and ventilation and spiracle behaviour have been examined in *Libellula quadrimaculata* in flight by Fraenkel (1932a).

MATERIAL, METHODS AND TERMINOLOGY

Material. Investigations were started in England on *Aeshna juncea* L., *Libellula quadrimaculata* L. and *Sympetrum striolatum* Charp.

In Uganda preliminary observations have been made on 35 species (Anisoptera), and four species have been studied in detail:

(1) Aeshnidae: *Acanthagyna (Gynacantha) villosa* Gruenberg. This is a large crepuscular dragonfly common in forests near Kampala. It was caught near forest margins during the evening flight period. Twenty-three specimens examined.

(2) Gomphidae: *Ictinogomphus ferox* Ramb. This is a large and abundant species, active for periods throughout the day. Mature specimens were taken near Lake Victoria and teneral individuals were obtained by collecting pharate larvae as they emerged from the lake prior to eclosion about 1 hr. after sunset. Thirty-five specimens examined.

(3) Libellulidae, Libellulinae: *Hadrothemis defecta pseudodeflecta* Pinhey. A common medium-sized dragonfly of which mature and teneral individuals were captured in forests near Lake Victoria. Twenty-seven specimens examined.

(4) Libellulidae, Zyxommatainae: *Pantala flavescens* Fabricius. This dragonfly is a seasonal migrant which normally appears in large numbers in Uganda during the two annual wet seasons. Mature specimens were caught over open ground near Kampala, and teneral individuals obtained by breeding out larvae in the laboratory. About seventy examined.

After capture in the wild, adults were placed in dark containers and on return to the laboratory they were kept in dark moist boxes at 15° C. If not used during the first 2 days after capture they were fed on small insects twice a week. Identifications were made with the key constructed by Pinhey (1961) and with the generous help of Dr P. S. Corbet.

Methods. Spiracles on the dragonfly thorax occupy exposed positions while those on the abdomen are partly concealed by the terga. Three methods have been used to record spiracle valve movements:

(1) Two observers with microscopes made simultaneous records of valve movements and ventilation in one insect by using tapping keys which wrote on a kymograph (Shazli, 1957).

(2) More accurate records of valve activity were obtained by manually following the valve movements under the microscope with a small pointer connected to a lever which wrote on a kymograph. The method is based on that described by Case (1956).

(3) In order to display valve movements alongside records from the spiracle motor nerve on the oscilloscope, a small piece of tungsten wire was attached to the stylus of a gramophone pick-up which was connected to the oscilloscope pre-amplifiers. The wire was made to touch the valve in such a way that it was moved during valve opening and closing (Case, 1957; Hoyle, 1959).

Nerve impulses were recorded extracellularly with two hooked platinum wire electrodes (0.05 mm. in diameter), insulated to near the tip with 'Araldite' and mounted in Singer manipulators. They were displayed using equipment already described (Miller, 1960*a*), but for the work in Uganda a Telequipment double-beam oscilloscope (D31) was used in place of the Tektronix model (532).

The physiological saline used in most experiments was that employed in cockroach experiments by Kerkut & Ridge (1961). Experiments were carried out at room temperatures (23–27° C. in Uganda).

Impulses in the transverse nerve from the mesothoracic ganglion to spiracle 2 have been recorded close to the spiracle and also centrally near the ganglion. For the first site the cuticle is cut round the spiracle on three sides and the spiracle folded anteriorly to expose the closer muscle and its motor nerve. Damage to air-sacs lying close to the spiracle is hard to avoid and such damage usually alters the activity of the muscle. In most experiments, therefore, the central recording site was used (Fig. 2). The insect is fixed ventral side up, the legs are removed and a small amount of cuticle round the coxal cavity of one mesothoracic leg is cut away. In smaller species, the sternal apophysis, which passes close to the mesothoracic ganglion, must be cut through and removed; but in Aeshnidae it can be left. The transverse nerve is then located running across the dorsal side of the coxal cavity. It occupies a similar position in all the species studied. A small drop of paraffin oil is run into the cavity and, when ready for recording, the nerve is lifted up on the electrodes into the oil, to be re-lowered into the saline between records. Records have been taken intermittently from such preparations for several hours and spiracle activity has continued apparently without change. No differences between the records from the peripheral and central recording sites can be detected.

To record abdominal ventilation a simple mechanical method, already described (Miller, 1960*a*), was employed.

Terminology. The spiracles are numbered from 1 to 10 on each side of the insect. Nos. 1 and 2 are situated on the thorax and nos. 3–10 on the abdomen. Nos. 1–3 supply large tracheae to the flight muscles. Nos. 4–10 lie concealed under the ventral extensions of the abdominal terga; no. 10 is considerably larger than the remainder.

Muscles are named according to the scheme used by Clark (1940) as modified by Neville (1960).

GENERAL OBSERVATIONS ON VENTILATION

Active pumping movements by the abdomen are brought about by the alternate contraction and relaxation of the posterior dorso-ventral muscles (expiratory stroke) and the anterior dorso-ventral muscles (inspiratory stroke) in each segment (Calvert, 1893; Whedon, 1918). During weak ventilation expiration alone is due to active muscular contraction, inspiration being caused by the elastic return of the tergites. There are no auxiliary ventilating mechanisms such as occur in the locust (Miller, 1960*a*).

In the Libellulidae, many of which have broad abdomens, several authors have noted that the amplitude and frequency of ventilation are greater than in other families. Pumping movements are, however, always detectable in all dragonflies examined, except for a brief cessation at the start of flight or struggling. They may be weak and infrequent in teneral insects or in species with slender abdomens (e.g.

I. ferox and in *Zygoptera*); in other species they may reach high frequencies (up to 200 cyc./min.) after flight or after capture, when general excitation seems to induce frequencies which are not experimentally obtainable by treatment with carbon dioxide or oxygen lack at various temperatures.

After decapitation ventilation movements gradually decrease and struggling or treatment with carbon dioxide produce a much smaller response than in the intact insect. Huber (1960) has demonstrated the importance of centres in the suboesophageal ganglion of the cricket, which with electrical stimulation produce either an increase or an inhibition of ventilation, and it seems likely that similar control from the head may exist in the dragonfly. According to Matula (1911) decapitation of the larval dragonfly produces hyperventilation, and he has demonstrated the presence of a respiratory centre in the prothoracic ganglion which stimulates ventilation in response to oxygen lack. In the adult, however, the evidence does not suggest that there is one dominant centre located in a particular part of the central nervous system. The effect of the cephalic and thoracic ganglia on ventilation seems to be similar to their effect in the locust (Miller, 1960*a*), but the thorax of the dragonfly does not contain a rhythmically discharging centre, as will be shown below.

MORPHOLOGY AND ACTION OF SPIRACLE 2

All the spiracles of the adult dragonfly possess valves which are closed by a short triangular muscle (the closer) and opened by a cuticular spring on relaxation of the muscle. They thus resemble spiracle 2 of the locust in which a single muscle controls opening and closing, but the dragonfly is unusual among more primitive insects, whose abdominal spiracles usually possess separate muscles for opening and closing (Maki, 1938).

Most of the experimental work to be described here has been carried out on spiracle 2 which is similar in all the species studied (Fig. 1). It lies in an elevated ring of cuticle on the metepisternum just posterior to the intersegmental suture. The movable valve, which closes in a ventral direction, is joined to the peritreme by a fold of corrugated cuticle. The wide base of the triangular closer muscle arises on the intersegmental suture and its truncated apex is inserted on an L-shaped manubrium, formed from a continuation of the hardened free margin of the valve. Contraction of the closer pulls the valve down, partly unfolding the corrugated cuticle, and the valve then pivots about the posterior end of its hardened margin to complete the movement. It closes against a pad of soft cuticle inside the elevated ring. On relaxation of the muscle the valve springs open very rapidly. In most species spiracle 2 remains permanently closed when the dragonfly is inactive; there is, however, no locking device and closure depends on a maintained tetanus in the muscle brought about by continuous excitation of the motor nerve. Slight differences in the morphology of spiracle 2 in *I. ferox* are shown in Fig. 1.

Permanent stickiness or greasiness of the valve margin can readily be seen in spiracles 1 and 2; it probably brings about a better seal when the valve is closed and may possibly be caused by continuous secretion of a wax solvent.

In *I. ferox* sections of the closer muscle have been stained with Heidenhein's iron haematoxylin after fixation in Baker's formaldehyde-calcium. They show the presence

of tubular muscle fibres from 2 to 8 μ in diameter, the thinner fibres occupying the centre and outer side of the muscle. There is no clear division into two sizes of fibre as occurs in the locust (Hoyle, 1959), and the inter-Z distance in all is 3–6 μ . The whole muscle is 270–300 μ long.

Innervation. In the Aeshnidae the three thoracic ganglia are well separated, but in the Gomphidae and Libellulidae there is considerable fusion of the meso- with the metathoracic ganglion. The closer muscle of spiracle 2 is innervated, as in other

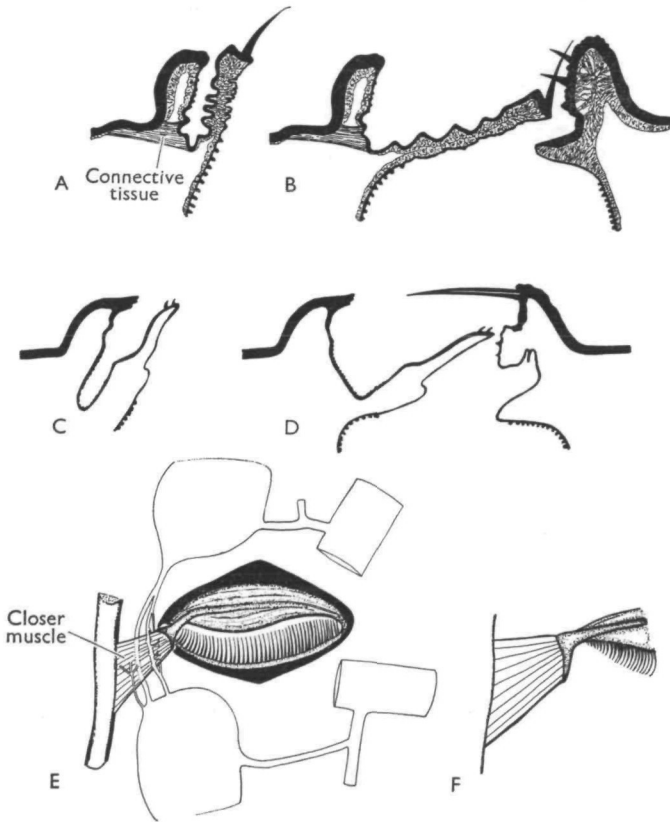


Fig. 1. A and B, *Aeshna juncea*. Transverse sections of spiracle 2, with the valve in the open position (A) and closed position (B). C and D, *Ictinogomphus ferox* (as in A and B). E, *Ictinogomphus ferox*. Spiracle 2, inner view, showing the tracheal supply to the closer muscle. F, *Aeshna juncea*. Closer muscle of spiracle 2.

insects, by the median nerve of the mesothoracic ganglion. After leaving the mid-dorsal surface of this ganglion the median nerve immediately divides into right and left transverse nerves which travel laterally to their respective spiracles, giving off branches to nerves IIN₃ and IIN₂ on the way (Fig. 2). Two motor axons, each 5–6 μ in diameter, run in the transverse nerve and supply the muscle.

Simultaneous records from the two transverse nerves show an identical pattern of impulses in each and, as in the dragonfly larva (Zawarzin, 1924) and the locust (Miller, 1960b), the axons in the median nerve probably divide to run down each transverse nerve.

Hair sensilla on the valve margin and on the peritreme are innervated by sensory nerves which run into the main nerve trunks IIN₃ and IIIN₂ (Fig. 2). Unilateral stimulation of these hairs produces a reflex burst of motor impulses in the transverse nerves and closing of both spiracles (Fig. 4D).

After section of the transverse nerve at any point the valve activity is completely altered, whereas damage to neighbouring structures or section of other nerves has no similar effect. Valve movements do not seem to be modified as a result of sensory input from the vicinity of the spiracle (except for the hair sensilla already mentioned), a conclusion reached by Case (1957) for the cockroach.

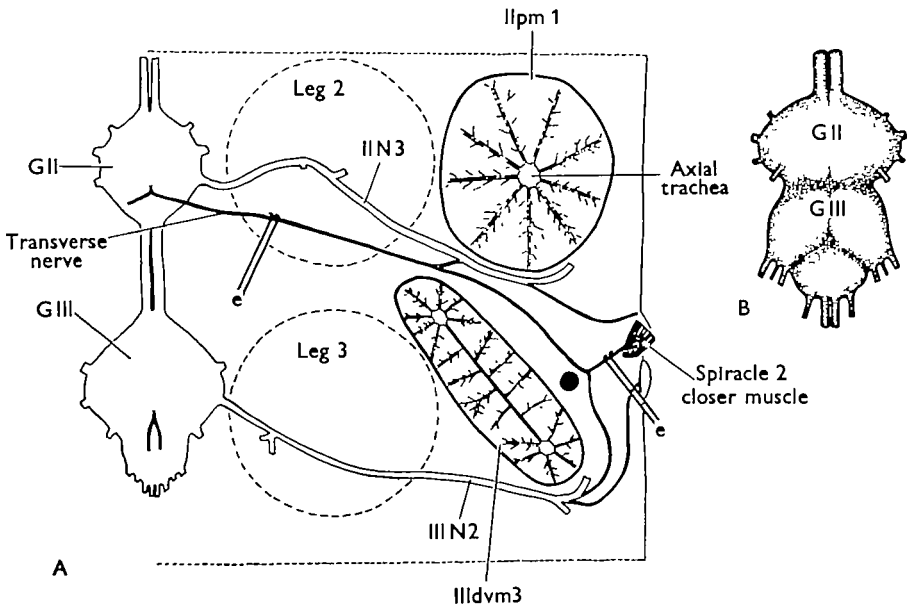


Fig. 2. A, *Aeshna juncea*. Dorsal view of the innervation of the right spiracle 2. *e*, pairs of recording electrodes; GII and GIII, meso- and metathoracic ganglia; IIpm₁, pleuroradial muscle; IIIIdvm₃, first subalar depressor. B, *Pantala flavescens*. Diagram illustrating the degree of fusion between meso- and metathoracic ganglia.

SPIRACLE BEHAVIOUR IN THE INTACT DRAGONFLY

This will be considered when the dragonfly is at rest, in flight and during recovery from flight.

At rest. About 5 min. after a bout of struggling or flight the spiracles resume a pattern of behaviour which usually continues with little change for several hours, provided the dragonfly remains undisturbed; this is termed the resting spiracle behaviour. In *A. villosa*, *I. ferox* and *H. defecta* at rest only spiracles 1 and 10 are functional (Fig. 3). In the first two species spiracle 1 makes continual fluttering movements, normally between 10 and 50% open. In *H. defecta* the behaviour is similar, but irregular fluttering is replaced by a partial closing movement during each expiratory stroke of the abdomen. In all three species spiracle 10 opens briefly towards the end of expiration, but is otherwise closed. The remaining spiracles may remain closed or, in mature insects and particularly in gravid females, spiracles 4-9

may open briefly during inspiration. In *P. flavescens* spiracle behaviour is similar to that in *H. defecta*, but in addition spiracles 2 and 3 usually make a quick opening movement at the end of expiration (Fig. 3). In teneral insects, or in mature individuals examined at 15° C., these spiracles normally remain closed as in the other species.

In flight. Dragonflies were glued ventrally to a rigid post and flown in front of a wind tunnel in a wind speed of 3 m./sec. as described by Neville (1960). Flight was initiated by loss of tarsal contact and by pinching the abdomen. Most dragonflies flew intermittently in short bursts of a few seconds duration, and the best performances were obtained from mature adults recently caught in the field. One uninterrupted flight lasting over an hour was performed by *H. defecta*.

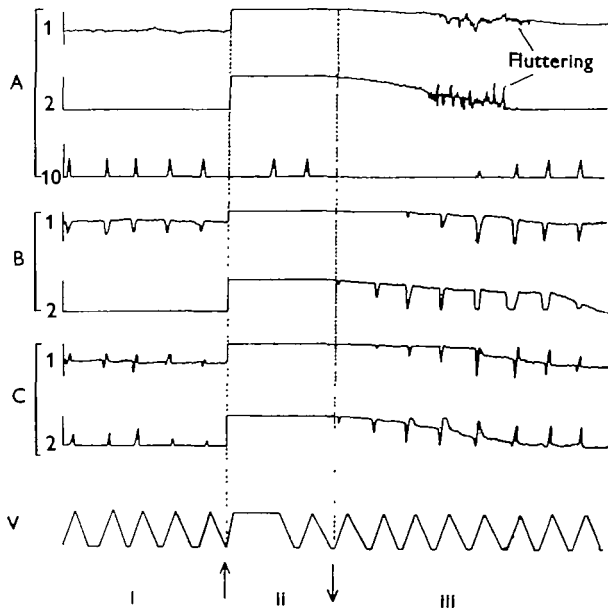


Fig. 3. Spiracle activity (i) at rest, (ii) in flight, and (iii) during recovery from flight. A, *Acanthagyna villosa*; B, *Hadrothemis defecta*; C, *Pantala flavescens*; V, ventilation (upstroke = expiration; downstroke = inspiration); 1, 2 and 10, spiracles 1, 2 and 10 (in each, opening is upwards); first arrow, flight starts; second arrow, flight ends.

At the start of flight, in all the species examined, spiracles 1, 2 and 3 open instantly and remain open until after flight has ceased (Fig. 3). When the flight performance is poor and frequently interrupted, spiracle closing may follow the cessation of wing beats almost immediately, an occurrence which has been observed most often in small species.

Abdominal pumping movements continue during flight, usually with only a small increase in frequency and amplitude. Spiracles 4-9 are synchronized with pumping, opening during inspiration.

Pinching the abdomen sometimes fails to initiate flight, but spiracles 1, 2 and 3 may still open instantly to re-close after 1-2 sec. Alternatively, an abrupt movement in front of the head sometimes produces immediate spiracle opening without subsequent flight. It is clear, therefore, that immediate spiracle opening can occur in the absence

of wing beats and at times in the absence of any other visible movement. Opening at the start of flight anticipates the demands for increased respiration, and is normally released by the same stimuli which give rise to flight. Similar anticipatory opening in other insects has been noted by du Buisson (1924), Fraenkel (1932*a*) and Bursell (1957). Vigorous struggling by the legs is also accompanied by immediate spiracle opening in the dragonfly.

After flight. In all four species there is a gradual closing of the spiracles after flight. Closing is slower in large species. The spiracles close in the order, 2-3-1, spiracle 1 returning to its resting behaviour (Fig. 3).

In *A. villosa* the spiracles close with characteristic fluttering movements. Spiracle 10 (and probably 4-9) remain shut throughout the first 8-12 ventilatory strokes after flight. In general *I. ferox* the behaviour of spiracles 1, 2 and 3 is similar. In mature *I. ferox*, however, synchronized movements temporarily appear, with slight (spiracle 1) or complete (spiracles 2 and 3) closing movements during expiration. Synchronized movements may persist indefinitely in spiracle 1, but more often there is a return to the resting behaviour already described (Fig. 3).

In *H. defecta* spiracles 1, 2 and 3 all make large amplitude movements during recovery from flight, closing just after the start of the expiratory stroke and re-opening at or just before its completion. As recovery proceeds the amount of opening during inspiration decreases until spiracles 2 and 3 remain closed. In *P. flavescens* three successive stages can be recognized during recovery from flight (Fig. 3). In the first the spiracles make brief closing movements during expiration but are otherwise wide open. In the second the closing movements become larger; they are followed by full opening before the end of expiration; then as inspiration begins the spiracles close about half-way, to re-close fully at the start of the next cycle. In the third stage the closure during inspiration becomes greater until it is complete, the spiracles then flicking open rapidly at the end of expiration but otherwise remaining closed as at rest.

While the sequence of events during the return of the spiracles to their resting behaviour does not change, the speed of recovery may vary greatly in different individuals of the same species. (Fig. 3 represents an unusually rapid recovery in all three species, chosen for convenience.) For example in *H. defecta* full spiracle closing may occur almost immediately after flight, or synchronized movements may continue for 1-2 min. in spiracles 1, 2 and 3. It will be shown elsewhere (Miller, 1962) that different rates of closing of the spiracles after flight and variations in the spiracle sensitivity to carbon dioxide probably depend on the water balance of the insect.

To summarize, in most resting dragonflies only spiracles 1 and 10 are active (in *P. flavescens* spiracles 2 and 3 may be active as well). After flight other spiracles are temporarily open as equilibrium is restored. The abdominal spiracles when active are synchronized with ventilation. The thoracic spiracles of *A. villosa* never are, whereas those of *I. ferox* are sometimes synchronized and those of *H. defecta* and *P. flavescens* are always synchronized when active. Synchronization of the thoracic spiracles apparently always occurs in the Libellulidae, Gomphidae and Corduliidae (33 species examined), but in Aeshnidae its occurrence is more variable; of eleven species examined, it has been noted in three.

PERIPHERAL CONTROL OF THE SPIRACLES

Records from the motor nerve to spiracle 2. In all four species at rest there is a more or less continuous discharge of impulses in the transverse nerve to the spiracle. The discharge is unaffected when the nerve is crushed distal to the electrodes, but ceases when it is crushed proximally. Complete denervation of the meso- and metathoracic ganglia, except for the median nerves and anterior and posterior connectives, has no effect on the discharge. An apparently rhythmical change in frequency occurring several times a second is sometimes recorded (Fig. 4A); it is due to the two axons

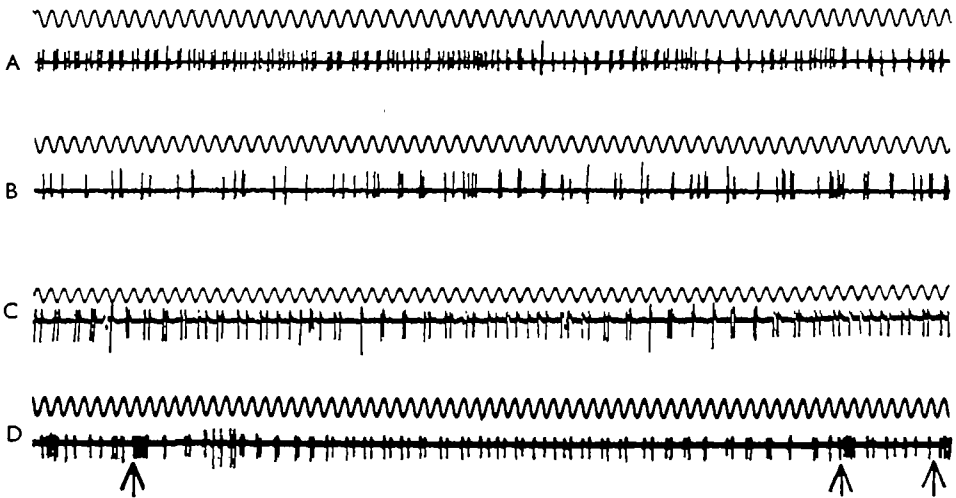


Fig. 4. Traced oscilloscope records from the transverse nerve to spiracle 2 of *Acanthagyna villosa* (A and B) and *Ictinogomphus ferox* (C and D). A, At rest; B, during recovery from flight; C, at rest, D, at rest; arrows mark moments when hairs on peritreme of spiracle were touched. Time marker, 50 cyc./sec.

firing at slightly different frequencies, which produces beats. A similar occurrence was noted in the locust (Miller, 1960*b*). Often the impulses are paired, as was pointed out by Hoyle (1959) in the locust, each axon providing one member of the pair. The term 'flight' below refers to bursts of wing beats at 20–25 strokes/sec. lasting 1–5 sec. It is initiated by tapping the abdomen and is performed with the pterothorax rigidly fixed.

In *A. villosa* (results from 5 insects) there is a constant stream of impulses at 120–140 per sec. (60–70 per sec. in each axon) when the spiracle is closed (Fig. 4A). During flight the nerve is silent and the spiracle wide open. After flight, as the spiracle flutters, there is an irregular pattern of impulses at reduced frequency (commonly about 60–80 per sec.), with momentary pauses of about 40 msec. duration, occurring 10–15 per sec. (Fig. 4B).

In *I. ferox* (12 insects) when spiracle 2 is closed records show a pattern similar to that in *A. villosa* but the frequency is lower (60–76 per sec. in the two axons, Fig. 4C). When synchronized movements appear after flight, closing corresponds to an increase in frequency of up to 250 per sec., while during opening a value of about 60 per sec. is maintained.

In *H. defecta* (6 insects) all records show a well-marked rhythmic alteration in frequency corresponding to ventilation. During expiration there is a high-frequency burst of impulses at 220–250 per sec. (2 axons), while for the remainder of the cycle the impulses maintain a steady value of 80–90 per sec. (Fig. 5 A). Even when the spiracles remain closed the rhythm persists in the transverse nerve. During flight the nerve is silent, and afterwards there is an immediate resumption of rhythmic firing usually at reduced frequencies, the corresponding values being about 60–90 per sec. and 20–40 per sec. Gradually as the spiracles re-close the resting frequencies return.

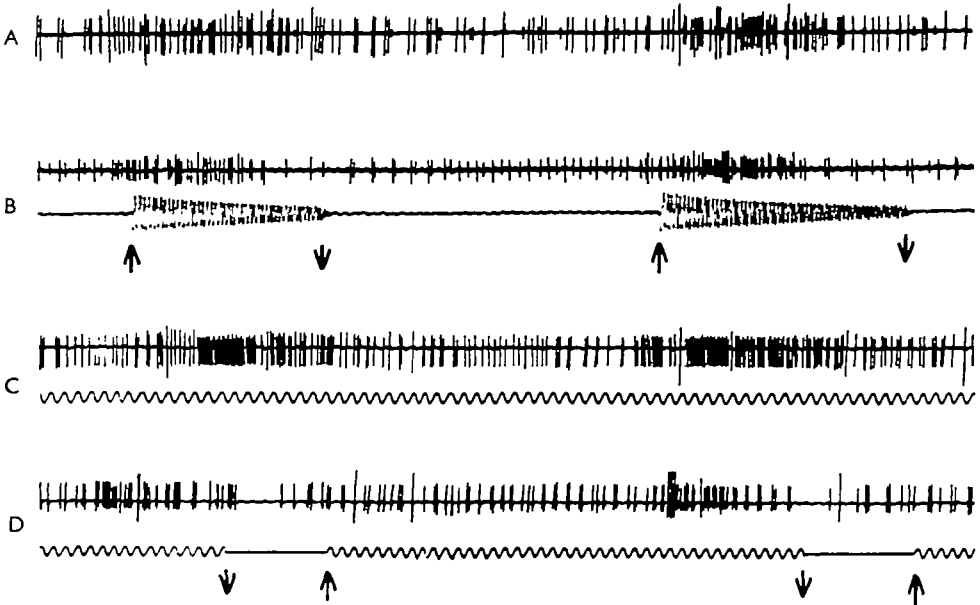


Fig. 5. Traced oscilloscope records from the transverse nerve to spiracle 2 of *Hadrothemis defecta* (A and B), *Urothemis edwardsi* (C) and *Pantala flavescens* (D). A, At rest, spiracle 2 is closed but rhythm persists in spiracle nerve; B, at rest, with 5% carbon dioxide introduced into spiracle 2. Pattern in motor nerve is unchanged, but the valve performs closing (upright arrow) and opening (inverted arrow) movements. C, at rest. The valve remains closed but there is a strongly marked rhythm. D, at rest. The valve opens (inverted arrow) during the short silent phase in the nerve and then re-closes (upright arrow). Time marker for all records, 50 cyc./sec.

In *P. flavescens* (12 insects) the pattern is similar to that in *H. defecta* with the addition of a brief silent phase, usually lasting 20–40 msec. (about 5% of the whole cycle), during which the spiracle rapidly flicks open as already described. It occurs shortly after the high-frequency burst (Fig. 5 D). The steady discharge is at 100–150 per sec., the high-frequency burst at 200–250 per sec. As in the other species the nerve is silent in flight, and afterwards the pattern is resumed, initially at lower frequencies and with a silent phase lasting up to 200 msec.

Frequencies of motor nerve impulses in the transverse nerve are summarized in Table 1.

Records from the motor nerve to spiracle 1. The closer muscle of spiracle 1 is innervated by the median nerve from the prothoracic ganglion. As already described spiracle 1 remains at least slightly open in resting dragonflies. Records from the

transverse nerve to spiracle 1 in *A. villosa* show a resting frequency of impulses at 30–35 per sec. (2 axons), while simultaneous records from the transverse nerve of spiracle 2 show a frequency of about 130 per sec. In *I. ferox* the corresponding frequencies are 15–25 per sec. and 50–60 per sec. The low frequency in the motor nerve of spiracle 1 does not bring about complete tetanus of the muscle and moreover allows the spiracle valve to react to very low concentrations of carbon dioxide (about 1%). Consequently adequate ventilation of the head and thoracic ganglia through the median orifice of this spiracle can continue in resting insects. After decapitation of *A. villosa* the frequency in the motor nerve of spiracle 1 is unchanged, while the frequency in the motor nerve of spiracle 2 falls to a value similar to that in the motor nerve of spiracle 1, and the valve usually opens. The presence of the head would appear to increase the firing frequency of the motor neurones of spiracle 2, but not those of spiracle 1, and this results in prolonged closure of the former spiracle in the resting insect. The effect of decapitation on spiracle 3 is similar to the effect on spiracle 2.

Table 1. *Motor impulse frequency per second in the transverse nerves (2 axons in each) to spiracles 1 and 2 in four species of dragonfly*

	<i>Acanthagyna villosa</i>	<i>Ictinogomphus ferox</i>	<i>Hadrothemis defecta</i>	<i>Pantala flavescens</i>
Spiracle 2 steady discharge	120–140	60–75	80–90	100–150
Spiracle 2 high-frequency burst	—	200–250	220–250	200–250
Spiracle 2 steady discharge after cord section	120–140	—	90–100	140–150
Spiracle 1 steady discharge	30–35	15–25	—	—

Action of carbon dioxide on spiracle 2. Motor impulses in the transverse nerve to spiracle 2 of all four species have been recorded at the same time as small doses of various mixtures of carbon dioxide and air were introduced from a fine pipette into spiracle 2, by gently lifting the valve with forceps. 10% carbon dioxide causes full opening of the valve, the motor impulses meanwhile continuing without change and the other spiracle of the pair remaining closed. Weaker concentrations cause partial opening or, in *H. defecta* and *P. flavescens*, the temporary appearance of synchronized movements (Fig. 5 B). If gassing is continued other spiracles open and ventilation is stimulated with consequent modifications of the impulse patterns in the transverse nerves. Similar applications of mixtures of nitrogen and air, or of 100% nitrogen, have no immediate effect on the spiracles.

The response of the closer muscle to carbon dioxide, which takes place in spite of maintained motor impulses in the transverse nerve, suggests that the mechanism involved is similar to that in spiracle 2 of the locust (Hoyle, 1960). This conclusion is supported by tests which show that after damage or occlusion of the tracheal supply of the closer muscle the response is delayed, whereas after removal of other neighbouring structures there is no change. The tracheal supply to the closer muscle of spiracle 2 is illustrated in Fig. 1 E. Two tracheae, which run between a pair of air-sacs, pass on either side of the closer muscle sending numerous branches into the muscle fibres. The air-sacs are each joined by a short branch to a large trachea close to the spiracle atrium; both belong to the system of superficial air-sacs which lies between

the flight muscles and the cuticle and probably plays some part in heat regulation (Church, 1960*b*). Similar 'through tracheae' have been described in the muscle of spiracle 2 in the locust (Miller, 1960*b*), and they may allow the muscle to sample gases more effectively than would be possible with a supply of blind-ending tracheae.

In *H. defecta* and *P. flavescens* at rest a rhythmic variation in the frequency of motor impulses in the transverse nerve usually persists when the spiracle remains closed (except for the brief opening flick, corresponding to the silent phase in the nerve of the latter species). Under these conditions the lower frequency is adequate to maintain a tetanus in the closer muscle. In the presence of 5% carbon dioxide, however, the lower frequency becomes partially or completely ineffectual presumably as a result of the direct action of the gas on the neuromuscular junction, whereas the higher frequency still causes muscular contraction; the valve therefore alternately opens and closes. With higher concentrations of carbon dioxide both frequencies are ineffectual and the valve remains more or less fully open. It follows that alterations of the frequency of motor impulses can vary the sensitivity of the response of the closer muscle to carbon dioxide. Such a mechanism, providing graded opening of the valve in response to the tensions of carbon dioxide at the spiracle muscle, could apparently account for all the observed behaviour of the spiracle. There would be no need for alteration of the patterns of impulses in the transverse nerve except during flight. However, modifications of the nerve impulse pattern, which occur after flight, have already been described, and the observed behaviour must depend on the interaction of a peripheral mechanism (carbon dioxide) and a central mechanism affecting the spiracle through the motor nerve.

CENTRAL CONTROL OF THE SPIRACLES

The central inhibitory reflex. As already suggested, the transverse nerves to spiracles 1, 2 and 3 probably remain silent throughout flapping flight. Records from the motor nerve of spiracle 2 show that tapping the abdomen or a sudden movement in front of the head may produce an immediate inhibition of impulses not necessarily followed by flight movements. In Fig. 6A the first wing beat (arrow) starts soon after inhibition in the transverse nerve; in Fig. 6B, however, there is a delay of about 240 msec. before the first wing beat. Inhibition cannot therefore be caused by the wing movements. Moreover, it is still possible to obtain inhibition after section of all the lateral nerves in the pterothorax, although more often tighter spiracle closing now follows tapping the abdomen. During flight-preparatory movements, which involve wing whirring to raise the thoracic temperature (Krogh & Zeuthen, 1941), the spiracles flutter about the half-open position and impulses are not inhibited. It appears, then, that a central inhibitory reflex is responsible for the immediate opening of the spiracles at the start of flight. The reflex is excited by stimuli which normally give rise to flight (tapping the abdomen and quick movements in front of the eyes), but it can appear in the absence of flight movements.

Electrical stimulation of the metathoracic ganglion of *Aeshna juncea* with short bursts of impulses at 25 per sec. often produces immediate spiracle opening. With electrodes placed near the centre of the ventral surface of the ganglion the stimulus intensity can be reduced until no movement other than spiracle opening is detectable. Usually spiracles 1 alone open, but in successful preparations opening in all three pairs

has been obtained. In ageing preparations stimulation often produces tighter spiracle closing. Stimulation of the pro- and mesothoracic ganglia has produced only tighter spiracle closing.

The results of stimulation experiments suggest that at least some of the central connexions of the inhibitory reflex are located in the metathoracic ganglion, a conclusion which was tentatively reached for the locust (Miller, 1960c), but the hypothesis of a localized flight centre in that insect must clearly be abandoned as a result of the recent work of Wilson (1961).

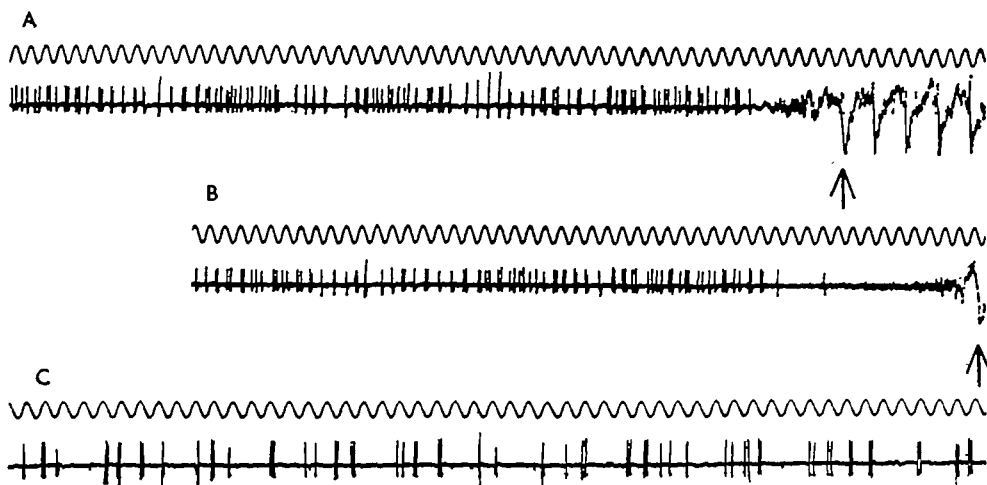


Fig. 6. Traced oscilloscope records from the transverse nerve to spiracle 2 of *Acanthagyna villosa*. A and B, the inhibition of impulses, brought about by tapping the abdomen, can be seen to occur well before the first flight movement (arrow). C, the reduced frequency of motor impulses which occurs 1 min. after introducing a gentle stream of nitrogen into spiracle 1. Time marker, 50 cyc./sec.

The effect of oxygen lack on spiracle control. Changes in the patterns of impulses in the transverse nerve to spiracle 2 after flight have already been described. In the following experiments the action of nitrogen and mixtures of nitrogen with air on the impulse patterns is examined. After directing a gentle stream of nitrogen into spiracle 1 of *A. villosa* for 1 min. the frequency of impulses in the motor nerve of spiracle 2 drops, the pattern becomes irregular and the spiracle valve starts to flutter (Fig. 6C). As gassing continues there is a further drop in frequency and the valve opens fully. The resting pattern is slowly resumed when gassing ceases. If the gas is directed into opened air-sacs in connexion with the thoracic ganglia the response appears more quickly. Gases containing more than 50% air have only a slight effect.

When the insect is enclosed in a small Perspex box and perfused continuously for 5 min. with nitrogen, fluttering movements of the spiracle valves may persist, ventilation continues to be strong and struggling occurs periodically. Stimulation of the hairs surrounding the spiracle produces immediate valve closing. Thus the drop in frequency of impulses in the transverse nerve does not seem to be a symptom of general debility but is probably a specific response to anoxia. The adult dragonfly is surprisingly 'resistant' to anoxia since under the same conditions a locust is completely motionless

in about one minute and recovers slowly on return to air. A dragonfly, on the other hand, kept for even longer periods in nitrogen recovers rapidly in air. It is possible that the ability of the aquatic larva to tolerate low oxygen tensions in the water (Wallengren, 1915) may persist in the adult dragonfly.

The other three dragonfly species show comparable reductions of frequency in the transverse nerve with nitrogen, both high and low frequencies being affected. In *P. flavescens* the silent phase is lengthened, as was noted after flight.

The experiments show that spiracle activity is affected peripherally by the direct action of carbon dioxide on the closer muscle and via the central nervous system by partial anoxia. Receptors which respond to anoxia have not been located, but they may lie close to or within the ganglia. Since partial anoxia reduces the frequency of motor impulses to the spiracle this will have the effect of increasing the sensitivity of the muscle to carbon dioxide.

Observations on the behaviour of spiracle 2 of *A. villosa* after a flight, together with records from the nerve of spiracle 2, show that the resting pattern of impulses is often restored before the spiracle is fully closed. When this occurs carbon dioxide is probably responsible for the delay in closing. Wigglesworth (1935) has demonstrated in the flea that although the spiracle opens in response to oxygen lack, opening is maintained by the outward diffusion of carbon dioxide coming from solution in the blood. Apparently the same factors are responsible for regulation of the dragonfly spiracle after flight, when the oxygen deficit seems to be made good before the carbon dioxide excess has diffused away. After very short flights consisting of only a few wing strokes, however, spiracle closing usually occurs before the resting pattern of impulses is completely restored; under these conditions excess carbon dioxide has probably disappeared before the oxygen is entirely replenished. The mechanism of spiracle control is likely to be the same in both insects, a conclusion reached by Schneiderman (1960) for the silk moth pupa and the flea. Schneiderman also describes fluttering movements of the pupal spiracle valves appearing with oxygen lack, as described above for the dragonfly. Moreover, both Case (1956) in flies and Schneiderman (1960) in the silk moth pupa have shown that the prevailing oxygen tension has a marked effect on the spiracle sensitivity to carbon dioxide. Their findings would be explicable if partial anoxia reduces the frequency of motor impulses to the spiracles in these insects.

The action of carbon dioxide on ventilation. In *A. villosa* 5–10% carbon dioxide causes hyperventilation, principally through an increase in amplitude; there is usually a coincident drop in frequency as the pumping strokes get deeper. These changes in ventilation do not have any effect on the streams of impulses in the transverse nerve to spiracle 2. The response to carbon dioxide in *P. flavescens* is similar, but there are also corresponding changes in the patterns of impulses in the transverse nerve to spiracle 2; the silent phase is prolonged and there is usually an increase in the frequency of the preceding burst. In other words, with hyperventilation the rhythm in the transverse nerve becomes more pronounced. A similar change has been recorded in *H. defecta*.

Experiments on the origin of the rhythmic discharge in the transverse nerves. After section of the nerve cord of *A. villosa* between the metathoracic ganglion and the first separate ganglion in the abdomen there is no change in the spiracle behaviour or in the transverse nerve impulses. In similarly treated *P. flavescens* and *H. defecta*, however, the rhythmic discharge in the transverse nerve is replaced by a steady stream of

Impulses at 140–150 per sec. in the former species (Fig. 7A) and at 90–100 per sec. in the latter (Fig. 7B). These frequencies are close to the steady discharge frequencies in the intact insects. Moreover, the spiracle behaviour after flight and at other times is now very like that in intact *A. villosa*. Observations have been continued for several hours but there is no return of synchronized movements in the thorax.

Records from the intact cord between the metathoracic ganglion and the first separate abdominal ganglion in *A. villosa* show no rhythmic discharge synchronized with abdominal ventilation, but similar records from *P. flavescens* always do show a

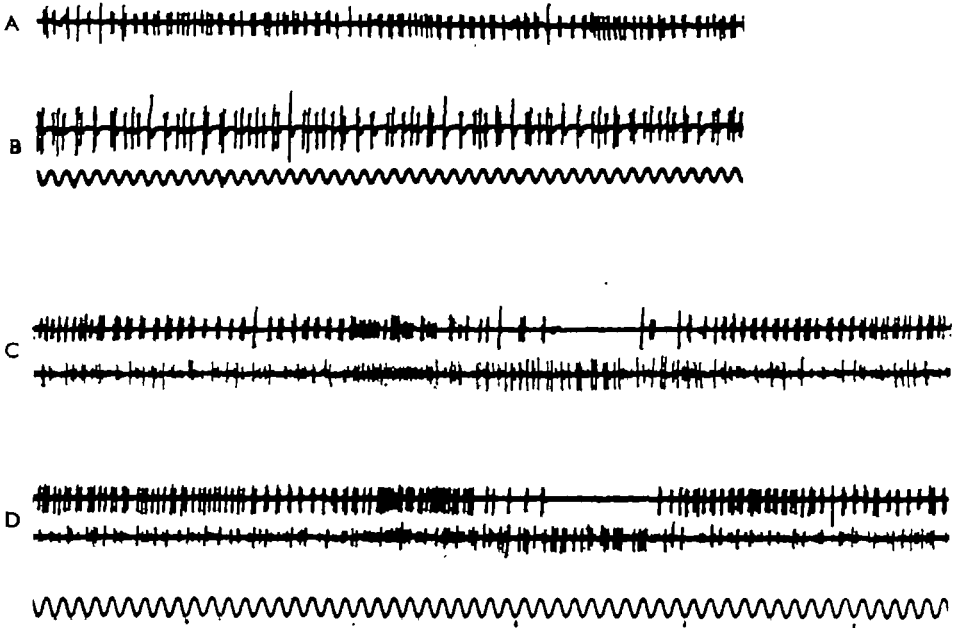


Fig. 7. Traced oscilloscope records from the transverse nerve to spiracle 2 of *Pantala flavescens* (A, C and D) and *Hadrothemis defecta* (B). A and B, uninterrupted stream of impulses after section of the nerve cord between the metathoracic ganglion and the first separate abdominal ganglion. C and D, simultaneous records from the transverse nerve to spiracle 2 (upper record in each) and from the nerve cord between the metathoracic ganglion and the first separate abdominal ganglion (lower record). Further explanation in the text. Time marker, 50 cyc./sec.

synchronized rhythm. When simultaneous records are taken from the cord of *P. flavescens* and from the transverse nerve to spiracle 2, a volley of small impulses in the cord is seen to correspond to the high-frequency burst in the transverse nerve, and a second volley of larger impulses coincides with the silent phase in the transverse nerve (Figs. 7C and D). Records taken first anterior and then posterior to a crushed section of cord show that these volleys are ascending the cord from the abdomen. No rhythmic discharge has been detected anterior to the damaged area. It seems probable that the ascending volleys in the cord are derived from abdominal ventilation centres and are responsible for synchronized movements in the thoracic spiracles.

To summarize, the motor neurones of the thoracic spiracles in all species examined fire regularly and continually, except in flight when firing is inhibited and with partial anoxia when the frequency is reduced. In addition, continual firing may be regularly

interrupted in some species by volleys of impulses which ascend the cord from the abdomen. These volleys are synchronized with abdominal ventilation and give rise to a temporary increase in frequency during expiration, and subsequently in *P. flavescens* to a brief silent phase. This silent phase might be achieved through the same central pathways which produce inhibition as a result of tapping the abdomen. When ventilation is stimulated the relayed patterns in the cord change correspondingly, and when the abdomen is removed uninterrupted firing of the motor neurones replaces the rhythmic discharge.

In no species examined does the head or thorax contain a centre whose rhythmic firing directly controls ventilation, but the head and thoracic ganglia do affect the frequency and amplitude of ventilation via abdominal centres.

POSSIBLE FUNCTIONAL IMPLICATIONS OF VENTILATION SYNCHRONIZATION

While several authors have described the tracheal system of the adult dragonfly (see Introduction), comparative studies of different dragonfly families are not known. The tracheal system has been re-examined during the present investigation and comparisons have been made between the four selected species. The details will be described elsewhere and only such aspects as seem to be related to the different types of spiracle behaviour will be mentioned here. Discussion of the movement of air in one direction along certain tracheae is speculative only and deduced from the study of synchronized spiracle movements. Experiments of the type performed by Fraenkel (1932*b*) on the locust are needed to support the hypotheses presented here.

In all four species the tracheal supply to the head and thoracic ganglia is more or less separate from the rest of the system. Large tracheae arising from the median orifice of spiracle 1 supply the head. Branches from them turn posteriorly to supply the thoracic ganglia and then communicate with spiracles 2 and 3 through narrow tracheae. As already described, spiracle 1 remains open at rest thereby allowing continuous ventilation of the head and thoracic ganglia to take place. When the valve is less than 10% open only the median orifice makes contact with the outside air, the lateral orifice being shut off externally by the valve and internally by the apodeme of the closer muscle and probably by part of the comb which is attached to the inside of the valve. The lateral orifice supplies tracheae to the anterior flight muscles (principally to the mesothoracic first subalar depressors) while the remainder are supplied from spiracles 2 and 3 (Fig. 8). Tracheae enter the flight muscles near their ventral insertions and in some also about half-way up; they travel axially through the muscles sending off numerous radial branches and then emerge dorsally often through a notch in the cap tendon. They immediately expand into air-sacs which intercommunicate and lie just below the nota. Tracheae from muscles supplied by spiracle 1 differ in not uniting with the remainder. The larger flight muscles possess two or three axial tracheae (see Clark, 1940, Fig. 5). The dorsal system of interconnected air-sacs is joined through small branches to the cephalic trunks close to spiracle 1; it communicates with the visceral trunks about the level of the metathoracic first subalar depressor; and posteriorly it is joined to the large dorsal tracheal trunks. The visceral trunks run down either side of the gut and open into spiracles 10. Their anterior

connexions with the flight-muscle air-sacs are large in *I. ferox* and *P. flavescens* but small in *A. villosa*. The dorsal trunks travel down the abdomen for much of the way as a string of elongated air-sacs and they are connected to each of spiracles 4 to 10 by broad tracheae. In most species examined spiracle 10 opens during expiration and spiracles 4-9 during inspiration (unless closed); under these circumstances air entering spiracles 4-9 is probably pumped anteriorly along the dorsal trunks into the thorax, and simultaneously air leaving the thorax along the visceral trunks will pass out through spiracles 10. In resting insects, when only spiracles 1 and 10 are opening, there may be a weakly directed airstream entering spiracle 1, passing dorsally through the pterothoracic air-sacs and then posteriorly along the visceral trunks.

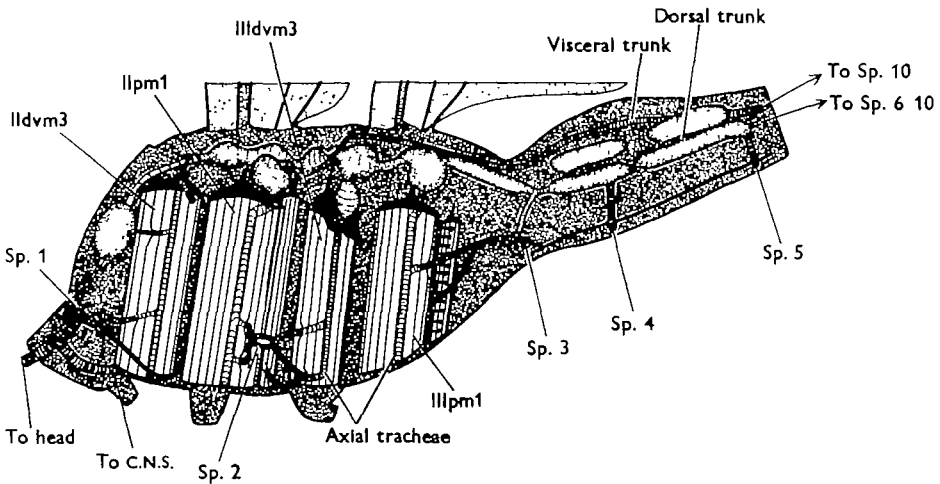


Fig. 8. Highly simplified diagram of the principal tracheal supply to the more lateral flight muscles in an anisopteran dragonfly. Sp, spiracle; II and III dvm3, meso- and metathoracic first subalar depressors; II and III lpm1, meso- and metathoracic pleuroradial muscles.

Ventilation of the flight muscles is most probably brought about by the flight movements as in other insects (Krogh & Weis-Fogh, 1951) through the open spiracles 1, 2 and 3. Weis-Fogh (1962) has shown that volume changes in the pterothorax during flight are more than adequate to provide for a metabolic rate of 110 kcal./kg./hr., which he has measured in flying *Aeshna*. In most species examined here, synchronized movements of spiracles 1, 2 and 3 are best seen during recovery from flight, and it seems likely that they are concerned with the continued ventilation of the flight muscles immediately flight ceases. We may make some attempt to predict the directions taken by airstreams through the tracheal system during this period.

In *A. villosa*, immediately after flight, spiracles 1, 2 and 3 remain open whereas spiracles 10 (and probably others on the abdomen) are closed; hence abdominal ventilation will pump air in and out of spiracles 1, 2 and 3 (two-way ventilation; Fig. 9A). The route from pterothorax to abdomen is probably less important than in other species and the anterior connexions of the visceral trunks are slender. In *I. ferox* (when synchronized movements appear) and in *H. defecta* spiracles 1, 2 and 3 open during inspiration but are closed for most of the expiratory stroke and a partially directed air-stream is likely to occur. Air entering these spiracles probably passes up

through the flight muscles and then posteriorly along the visceral trunks to escape from spiracle 10 (one-way ventilation; Fig. 9B). Routes between the thorax and abdomen are important and the anterior connexions of the visceral trunks are large. In *P. flavescens* the spiracle movements are more complex. During the first stage the situation is probably similar to that in *I. ferox* and *H. defecta*. During the third stage when the spiracles open briefly towards the end of expiration (and at rest when this activity continues) there may be an anteriorly directed airstream which enters through spiracles 4-9, travels along the dorsal trunks and then passes down through the flight muscles to leave from spiracles 1, 2 and 3 (Fig. 9C). The second stage of recovery

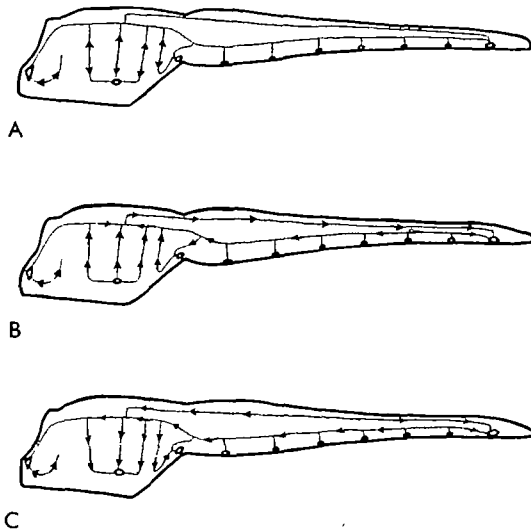


Fig. 9. Diagrams illustrating hypothetical directions followed by ventilating airstreams immediately after flight in various dragonflies. A, *Acanthagyna villosa*, two-way ventilation; B, *Hadrothemis defecta*, one-way ventilation; C, *Pantala flavescens*, one-way ventilation as occurs towards the end of recovery and probably at rest.

seems to be intermediate between the first and third. It follows that at rest there is often likely to be continued ventilation of the flight muscles due to the maintained activity of the thoracic spiracles—a point to which we shall return later.

In *Anax imperator* Leach (Aeshnidae) from Uganda yet another pattern of synchronized movements has been observed (2 specimens examined). During recovery from flight spiracles 1, 2 and 3 open briefly during expiration but are otherwise closed. Records from the transverse nerve to spiracle 2 show that a short silent phase in the nerve corresponds to valve opening, but there is no preceding burst at high frequency as occurs in *P. flavescens*. This type of synchronization probably provides an anteriorly directed airstream, entering through the abdominal spiracles (spiracle 10 opens sometimes during inspiration as well as expiration) and passing downwards through the flight muscles to leave from spiracles 1, 2 and 3. Thus during recovery from flight ventilation is similar to that in resting *P. flavescens* (Fig. 9C). Although the dorsal trunks of *A. imperator* are large the branch connecting them to the main trachea close to spiracle 3 is small. Most of the air pumped anteriorly along the dorsal trunks is therefore unable to leave directly from spiracle 3, but must

first circulate through the flight muscles. The reduction of this branch, which is not seen in the other species studied, seems to be correlated with the expiratory function of the thoracic spiracles.

DISCUSSION

The confusing picture, presented above, of ventilation and directed airstreams through the tracheae of the different species examined may be clarified by arranging the examples into four groups. The possible ecological implications of the different types of ventilation will then be discussed.

Group I. A two-way or tidal ventilation system in which the thoracic spiracles make no synchronized movements. Expiration and inspiration take place through the same spiracles so that a considerable 'dead space' results, and the method will be less efficient than that in other groups. This type is exemplified by *A. villosa* and four other Aeshnidae have been found which are similar (*Acanthagyna* sp., *Heliaeshna* spp). All are crepuscular species whose activity is limited to short flight periods at dawn and at dusk, or to occasional activity at other times in densely shaded forests (Corbet, 1962). Many other species have crepuscular flight periods but are also active in exposed situations during the day, and in all these which have been examined synchronized movements do occur. The *Acanthagyna* and *Heliaeshna* species examined therefore stand apart in being active only in twilight periods and in lacking spiracle synchronization in the thorax. (In four species of Aeshnidae examined in England spiracle synchronization has not been observed in spiracles 1, 2 or 3, but it does occur in all the Libellulidae studied there.)

Group II. A one-way ventilation system in which air enters spiracles 1, 2 and 3, is pumped dorsally through the flight muscles and then posteriorly along the abdomen. This type is exemplified by *I. ferox* (in which it does not always occur) and by *H. defecta*. Both species are active throughout the day, but much time is spent perching and flights are usually of short duration.

Group III. A one-way ventilation system in which air is pumped anteriorly along the abdomen and then ventrally through the flight muscles. This system is exemplified by *A. imperator* and it has been observed and recorded in two other aeshnid species, *Aeshna ellioti* Kirby and *Anaciaeshna triangulifera* McLachlan. All three species have been observed flying actively throughout the day in exposed situations.

Group IV. A more complex one-way ventilation system in which air is initially pumped posteriorly, but at a later stage of recovery it may be pumped anteriorly. It combines the methods in groups II and III. *P. flavescens* provides our example and it appears to be common in many other Libellulidae. Only in *P. flavescens*, however, have synchronized movements been seen to continue at rest. This species is a seasonal migrant (see Corbet, 1962), but even when not migrating it spends most of the day on the wing, unlike most Libellulidae in Uganda which make short flights from perches. In bright sun it spends much time gliding and there are only short intervals of flapping flight; Hankin (1921) has measured glides in this species of up to 15 sec. duration. Heat generated by the flight muscles (Sotavalta, 1954) plus the heat gained due to radiant energy from the sun (see Wigglesworth, 1953; Digby, 1955) in flying dragonflies is likely to be considerable, especially in the tropics. Corbet (1962) has discussed the temperature relations of tropical dragonflies and he suggests that overheating of

the pterothorax in flight is avoided since flapping flight is frequently interrupted by gliding or perching in different species. Continued ventilation of the flight muscles immediately the flight movements cease will cool the pterothorax due to the increased evaporation from the tracheal system. The heat loss through evaporation in many insects is only a small proportion of the total loss (Church, 1960*a*), but in the dragonfly, where a layer of superficial air-sacs insulates about 80% of the pterothoracic capsule and considerably reduces the heat loss due to convection (Church, 1960*b*), losses resulting from evaporation from the tracheal system may be more significant.

Although after short flights in the middle of the day many dragonflies perch exposed to the sun they are often orientated so as to be nearly parallel to the incident radiation (Corbet, 1962). In these ventilation through the flight muscles soon ceases as respiratory equilibrium is restored and the thoracic spiracles close. In *P. flavescens*, however, which glides fully exposed to the sun between active flapping flights, continued ventilation of the flight muscles after the respiratory demands have been met may be necessary to prevent over-heating. In this way the persistence of synchronized movements by the thoracic spiracles in resting (or gliding) insects may be explained as a cooling mechanism. It is interesting to note that the thoracic cuticle of *P. flavescens* and many other species is transparent but a layer of pigment occurs between the flight muscles and the air-sacs. Hence air in these air-sacs is exposed to direct radiation and probably heats up quickly. Measurements of the pterothoracic temperature of flying and non-flying dragonflies in sunlight are clearly required. As Corbet (1962) points out, the need for the perching dragonfly to maintain a temperature in the pterothorax which allows instantaneous flight in pursuit of prey may conflict with the need to prevent over-heating once the insect is in flight and exposed to direct radiation.

The most striking difference lies between the members of group I and those of groups II, III and IV, both as regards spiracle behaviour and the habits of the species. (An exception has been found in the crepuscular libellulid, *Paraxyxomma flavicans* Martin (Corbet, 1959), in which synchronized movements of the thoracic spiracles do occur). The metabolic rate or the pterothoracic temperature of dragonflies which fly only in cool periods may never reach values high enough to make the more efficient one-way ventilation system necessary. These species may be limited to crepuscular activity by the lack of central connexions required for spiracle synchronization in the thorax. Such a limitation would seem to be disadvantageous to insects primarily dependent on sight, since although insect food is more abundant at dawn and dusk, other activities such as mate selection and oviposition may have to be confined to this period which is short in the tropics. A more likely possibility is that the crepuscular habit confers definite advantages (discussed by Corbet, 1962) on a species such as *A. villosa* which breeds in temporary pools (Corbet, 1961) and survives long periods as an adult between wet seasons (Gambles, 1960). Thus spiracle synchronization may have been lost by crepuscular species in which it is not needed.

To summarize, it is suggested that continued ventilation of the flight muscles immediately after flight by means of a one-way airstream is necessary for the fulfilment of respiratory demands and perhaps also for cooling in dragonflies which are continuously or intermittently active throughout the day in the tropics. On the other hand, a less efficient two-way ventilation stream provides adequately for the respiratory needs of species which are active only in cool periods (or places).

SUMMARY

1. Spiracle activity is described in four species of adult dragonfly (Anisoptera) at rest, in flight and during recovery from flight. The closer muscle of spiracle 2 reacts directly to locally applied carbon dioxide. In one species this results in maintained opening of the valve, and in the other three in movements synchronized with abdominal ventilation. Neither reaction involves a change in the patterns of nerve impulses in the motor nerve to the closer muscle.

2. Conditions of partial anoxia have no direct action on the spiracle but they reduce the frequency of impulses in the motor nerve, thereby increasing the sensitivity of the closer muscle to carbon dioxide.

3. A central inhibitory reflex accounts for spiracle opening at the start of flight. Various stimuli are described which excite this reflex.

4. Rhythmically-firing ventilation centres are confined to the abdomen, but the brain and thoracic ganglia exert a considerable influence over ventilation. In species whose thoracic spiracles are at times synchronized with ventilation, volleys of impulses ascend the cord and regularly superimpose a burst at high frequency (and in one species a subsequent brief inhibition) on the otherwise constant firing of the spiracle motor neurones. In the presence of carbon dioxide these bring about synchronized movements.

5. Synchronized spiracle movements probably give rise to a one-way airstream in tracheae which pass axially up through the flight muscles and then posteriorly along the abdomen. In some species the stream may pass in the reverse direction.

6. Among thirty-five species of dragonfly examined in Uganda synchronized movements of the thoracic spiracles are found in all species active during the day; in five species which are active only during crepuscular periods they are absent. The possibility is discussed that these movements are important for cooling as well as for respiration.

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