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THE SUPPLY OF OXYGEN TO THE ACTIVE FLIGHT MUSCLES OF SOME LARGE BEETLES

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INTRODUCTION

Oxygen reaches the flight muscles of insects in flight as a result of diffusion and ventilation. Weis-Fogh (1964*b*) has shown that small insects of the size of *Drosophila* depend on diffusion alone, and according to Miller (1966) in species up to the size of *Eristalis tenax*, with a broad latero-linear tracheal supply, diffusion may still be adequate. In larger species the circulation of air in the primary tracheae is brought about by abdominal pumping and by thoracic volume changes which accompany each wing beat (thoracic pump). An additional method of assisting gaseous exchange near the spiracles was suggested by Stride (1958); he envisaged air as moving past the open lips of the thoracic spiracles in a flying locust and promoting exchange by a Bernoulli effect, but there is no experimental evidence to support this suggestion. In the large African cerambycid *Petrognatha gigas*, Amos & Miller (1965) showed that when the dead beetle was placed in the flight position in a moving airstream air passed along the large metathoracic trunks entering through spiracle 2 and leaving from spiracle 3. Further experiments are reported here which show that such a circulation takes place in the living beetle in flight and in which measurements are made of the bulk flow of air through the metathorax.

In insects such as locusts, dragonflies and moths the thoracic pump is believed to contribute most of the oxygen consumed by the flight muscles. By contrast, in many Hymenoptera the abdominal pump probably has a more important role (Weis-Fogh, 1964*a*). In beetles the thoracic pump is thought to be the major contributor and the metanotum of *Oryctes* is known to move through 0·5 mm. with each wing beat (Burton, 1962). Indeed in some species, such as *Melolontha* (Fraenkel, 1932) and *Geotrupes* (Fraenkel, 1932; Krogh & Zeuthen, 1941), it is reported that the abdominal pump ceases to function at least during the early stages of flight, although it may be very active during the preceding phase of warming up. Only a few species of beetles have been observed in flight, and most of these belong to the superfamily Scarabaeoidea. Measurements on the flight of beetles belonging to five families are reported here and in a number of cases efforts have been made to measure the stroke volume of the thoracic pump. One can conclude that large species in the Cerambycidae and some other families may depend on a thoracic pump and a through-conducting system for their oxygen supply during flight, whereas large species of Scarabaeidae and other families depend on the thoracic and the abdominal pumps exclusively.

MATERIAL

Much of the work was carried out on *Petrognatha gigas* (F.) (Cerambycidae) in Uganda. Altogether twelve specimens were found on the trunks of bark-cloth trees (*Ficus* sp.) near Kampala during August 1965. Larvae were found in the living wood of the same trees. Adults were kept in cages in the laboratory and provided with freshly sawn *Ficus* logs. Although they ate the bark they did not live for more than 2 weeks, whereas the one female imprisoned by Mr K. Brown (personal communication) on the trunk of a living tree survived for 76 days.

Other species of beetle were obtained in September 1965 in forests in Uganda and at ultraviolet light-traps. Altogether some members of six families were examined: Scarabaeidae (15 species), Cerambycidae (14 species), Buprestidae (3 species), Anthribidae (3 species), Elateridae (3 species) and Curculionidae (2 species). The species which were flown successfully are listed in Table 1. Other species which did not fly, or were examined only as preserved material (not necessarily from Uganda), include *Prionoplus reticularis*, *Acanthophorus maculatus*, *Prionomma* sp. and *Phryneta* sp. (all Cerambycidae), *Goliathus regius* and *Ceratorrhina* sp. (Scarabaeidae) and *Sipalinus* sp. (Curculionidae).

METHODS

Experiments on living bettles were carried out in the Department of Zoology at Makerere College. A few species (indicated in Table 1) were examined alive in England. Beetles were mounted on a rigid flight-stand and flown for periods of up to 1 hr. in a wind of from 3 to 5 m./sec. in front of a wind-tunnel. During flight, measurements of the wing-beat frequency were made using a microphone and tape recorder with subsequent display on an oscilloscope. For those species which flew in England a stroboscope was employed. Wing-stroke amplitude and stroke plane were measured on photographs of the flying beetles. The frequency of abdominal ventilation was counted and the amplitude estimated.

According to Sotavalta (1947) weak flight in tethered beetles, which are not producing positive lift, is accompanied by wing beats at normal frequency but reduced amplitude. In consequence, measurements were made only on beetles when the wing amplitude was near the steady maximum for the species. In addition a pivoted flight stand was employed in some cases which gave an indication of the occurrence of positive lift and thrust, although these parameters were not measured. Beetles were flown at 25°C. Even in large species no preceding phase of warming up seemed necessary at this temperature; strong flight often began as soon as the air flow was started and tarsal contact lost (cf. Krogh & Zeuthen, 1941; Leston, Pringle & White, 1965).

The thoracic volume changes which accompany wing movements were measured on the same specimens, after return to England, by a method similar to that of Weis-Fogh (1964*a*). The beetles were preserved either dry or in alcohol or formalin. Dry specimens were soaked in 10% alcohol before measurements were made. The different methods of preservation did not seem to affect the values obtained. The flight muscles were first removed and the metathoracic sternum was then cemented firmly to a solid base. The wings were moved through the path which they followed in flight, and the dorso-ventral excursion of the leading edge of the metanotum was measured

Table 1. *Flight parameters measured on beetles*

Family Species	Identification by	Weight (g.)	Size index	Flight duration (min.)	Wing-beat frequency (per sec.)	Stroke amplitude (degrees)	Angle of stroke plane to long axis of body (degrees)	Body angle to horizontal used in measurements (degrees)	*Abdominal* Amplitude	*Ventilation* Frequency (per min.)
Cerambycidae										
Petrognatha gigas	E. A. J. D.	7·6	1500	30	20–27	150–180	80	20	0	0
P. gigas	—	5·5	1190	25	27	160–180	80	20	Small	Occasional
P. gigas	—	3·9	950	25	27–30	180	80	20	0	0
Mallodon downesi	—	4·1	788	20	38–42	170	70–80	20	Small	30
Macrotoma serripes	—	4·3	770	15	30	140	80–90	20	0	0
Phryneta spinata	K. B.	1·1	223	15	42–45	180	80	20	Small	*c.* 25
Prosopocera belzebuth	E. A. J. D.	—	257	20	48	180	80	20	0	0
Sternotomos pulchra	K. B.	0·5	156	10	50–60	165–180	90	20	0	0
S. semirufescens	K. B.	0·27	148	10	60	170	90	20	0	0
Plocaederus viridipennis	K. B.	—	74	8	42–44	180	80	20	0	0
Chromacilla purpureocincta	K. B.	—	64	—	47–52	180	80	20	0	0
Anthribidae										
Deuterocrates sp.	K. B.	—	91	25	50	170	80	20	0	0
Mecocerus oculatus	K. B.	0·22	102	1	80 (at take-off)	—	—	—	—	—
Elateridae										
Tetralobus sp.	—	1·3	443	10	42–45	150	70–80	20	Small	Irregular
Tetralobus sp.	—	1·8	—	10	50	150	70–80	20	Small	*c.* 20/sec.
Scarabaeidae										
* *Oryctes rhinoceros* (in England)	—	5·2	—	—	41	—	—	14	Strong	—
* *Chalcosoma* sp. (in England)	—	7·03	1250	—	26–35	—	—	—	—	—
Heliocopris colossus	J. B. B.	14·75	1930	—	—	—	—	—	—	—
H. colossus (in England)	—	9·0	1190	30	40	100	60	0	Strong	60
Onthophagus multicornis	J. B. B.	0·15	65	6	112	150–160	45	0	0	0
Scarabaeus femoralis (in England)		5·5	860	30	60	100	50–60	0	Strong	60
Fornasinius (= *Goliathus*) *russus*	K. B.	9·18	1650	30	40–45	90	45	10	Strong	100 (max. 120
Diplognatha gagates	J. B. B.	0·58	260	30	90–92	110	50	0	Strong or 0	90 (max. 200) or 0
Diplognatha viridula	J. B. B.	0·6	264	15	85–95	100–120	50	0	Strong	80
Heterorrhina africana	—	0·47	—	19	85–100	100–140	45–60	0	0	0 or 4 pumps then pause
Chordodera quinquelineata	—	0·43	162	15	75	110	50	0	0	0
Oxythyrea plebeja	—	0·06	45	10	135–140	135	35	0	0	0
Buprestidae										
Megactenodes westermanni	K. B.	0·59	255	10	97–112	90–110	45–65	10	Strong	190
* *Sternocera castanea* (in England)	—	—	950	—	55	—	—	—	—	—
Agrilus sp.	—	—	7·5	5	155	180	70	0	0	0

* Values determined by Dr D. J. Aidley. E. A. J. D. = E. A. J. Duffy; K. B. = K. Brown; J. B. B. = J. Balfour Browne.

with a micrometer eyepiece in a microscope. The metanotum rises as the wings are depressed and falls as the wings are elevated: in most species it moves as though hinged near the posterior margin. Hence the volume change with each wing beat (i.e. the volume swept out by the metanotum) was calculated by multiplying the area of the metanotum by half the dorso-ventral movement of the leading edge. The volume pumped per unit time could be calculated using the wing-beat frequency.

No account is taken of lateral movements of the thoracic walls but these appear to be small. Increased lateral stiffness, which may be brought about by contraction of the pleuro-sternal muscles, may alter the amplitude of the movements, but again the effect is probably small (cf. Pringle, 1957). Removal of the flight muscles probably somewhat loosens the metanotum and so causes exaggeration of its movements when the wings are raised or lowered, but this is unavoidable since the fixed muscles allow little or no movement. Because of the extensive development of the tracheal system in the metathorax and since the spiracles are wide open in flight, we can assume that much of the volume changes will bring about gaseous exchange between the metathoracic tracheae and the outside air. This is known to take place in *Schistocerca* (Weis-Fogh, 1964*a*) but can only be inferred in the beetles considered here.

To facilitate comparisons between species, various parameters are considered per unit weight and per unit of the size index. The size index is found by multiplying the maximum metanotal width by the body length in millimetres (excluding the head).

THE MAJOR FEATURES OF THE TRACHEAL SYSTEM SUPPLYING THE FLIGHT MUSCLES

In all species examined it was possible to use Weis-Fogh's (1964*a*) classification of the air route from the spiracle to the fibrils of the flight muscles into primary, secondary and tertiary regions. In most insects only the primary region is ventilated, while diffusion accounts for the movement of gases in secondary and tertiary parts. In large insects, however, the secondary tracheae also may be ventilated. Here we are concerned only with those parts of the system in which ventilation brings about the bulk movement of gases.

Scarabaeidae and Buprestidae

In those species which have been examined (cf. Table 1) the supply routes have similar plans: several primary tubes arise from the atria of spiracles 2 and 3 and travel along the surface of the flight muscles, providing them with a latero-radial supply (cf. Weis-Fogh, 1964*a*). They may end blindly, but frequently they anastomose with other parts of the system. In species of other families, such as *Dytiscus* (Alt, 1912) and *Hydrophilus*, the primaries may be expanded into large sacs and provide the muscles with a latero-linear supply, but this type is not well developed in those scarabaeids and buprestids examined. Primary tubes are usually weak-walled and compressible and they may reach a diameter of over 1·25 mm. in *Goliathus regius* (size index 3250).

Numerous secondary tubes leave the primaries at right angles and pass between the muscle fibres. They are often expanded into sacs before penetrating the muscle; this was observed by Strauss-Dürckheim (1828) in *Melolontha*. In large species the secondaries may be so numerous that each row of fibres is separated from the next by a stack of tracheae which thus forms a discontinuous curtain of air (Miller, 1966). According to Darwin and Pringle (1959) 40–50% of the cross-section of the fibrillar flight muscles of the dynastine beetle *Orcytes* is filled with air tubes. Large scarabaeids have additional secondary branches which are wrapped round the outside of the muscle and from which smaller branches penetrate between the fibres; in *Heliocopris* they form an anastomosing web over the muscle surface.

Small tertiary tracheae leave the secondaries in large numbers to penetrate the fibres by indenting their surface membranes (Smith, 1961). Both primary and secondary systems are associated with numerous small airsacs which are usually blind-ending and lack taenidial thickenings. Together with the floppy primaries and secondaries, usually elliptical in cross-section, they form a large air space whose volume can be affected by pumping movements. Newport (1851) was struck by the large numbers of small sacs in *Lucanus*, and Strauss-Dürckheim (1828) by those in *Melolontha*, and this system has been described as the most highly evolved tracheal network found in insects (Imms, 1957).

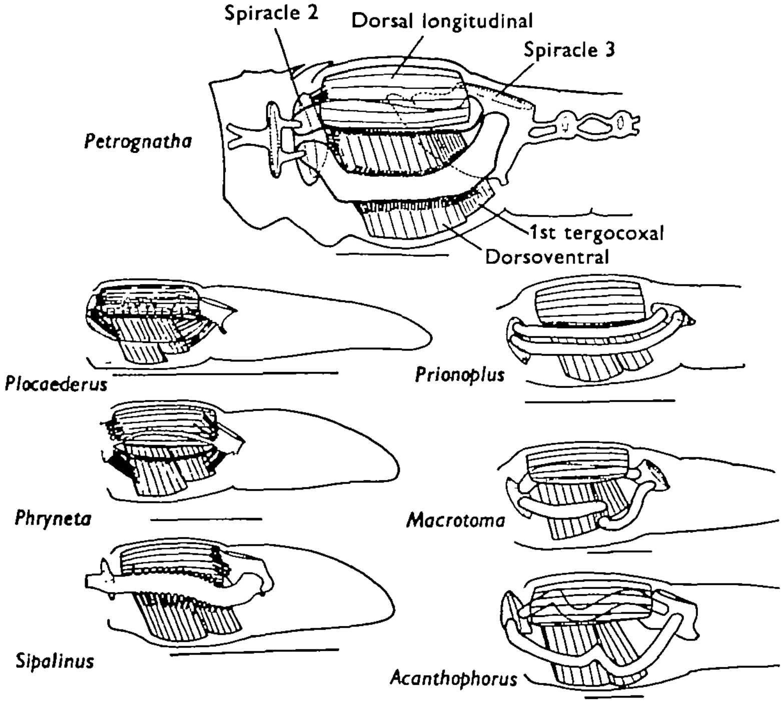
Cerambycidae, Anthribidae, Curculionidae and Elateridae

Together these will be called the cerambycid group, but it should be emphasized that this implies no evolutionary relationship. The system has been extensively explored only in the Cerambycidae, particularly in *Petrognatha*, but it appears to be similar in many other insects of this group. Blind-ending sacs, like those which are so abundant in scaraebaeids, are completely absent. Regions such as the elytra, mandibles and abdomen, typically containing numerous sacs in scarabaeids, are filled with many parallel-sided trachea. The failure to develop an extensive sac system in these families is correlated with a poorly developed abdominal pump and with the lack of abdominal ventilation in flight (see below).

In many large species one or two pairs of giant tracheal trunks pass through the metathorax joining the atria of spiracles 2 and 3 (spiracle 3 is the first abdominal) (Text-fig. 1). There may also be large tracheae in the prothorax running anteriorly from spiracle 1, but these are blind-ending. Abdominal tracheae, on the other hand, are of much smaller dimensions. The metathoracic giant tracheae, which form the primary supply to the flight muscles, are commonly thick-walled and gold or brown in colour. In *Petrognatha* they reach a diameter of 3 mm. and, where they give rise to numerous secondary branches, are further strengthened by the fusion of neighbouring turns of the taenidium into stout dark struts. Their course in various species is illustrated in Text-fig. 1.

Secondary tracheae radiate from the primaries and pass through the muscles between the fibres. In *Petrognatha* they are normally less than 300 μ in diameter, and like the primaries, they are lined with bristles 10–40 μ long which project into the lumen, as was noted by Marcu (1931) in other cerambycids. Their point of exit from the primary is guarded by longer bristles (Amos & Miller, 1965). Since each fibrillar flight muscle does not have its separate primary supply, secondary branches may either enter a muscle directly or they may travel some way before doing so. Thus the secondary routes may be 6 mm. or more in length. As in Scarabaeidae, stacks of secondary tubes pass between each row of fibres, the tubes diminishing in bore as they approach the muscle periphery. There is no dilatation of the tube beyond the muscle surface as occurs in Belostomatidae (Möller, 1921; Weis-Fogh, 1964*a*).

In *Aeshna* Weis-Fogh (1964*b*) has shown that with a hole-fraction of 0·1, an axial primary tube of radius 0·2 of that of the muscle, and with an oxygen consumption of 2·0 ml. O_2/g. muscle/min., the muscle could not exceed 2–4 mm. in diameter and the length of the secondary ***diffusion*** pathway must be less than 1–2 mm. Assuming similar values for the hole-fraction and the oxygen consumption in *Petrognatha*, secondary diffusion pathways of 5 mm. or more would probably be prohibitive, even with a much broader primary supply and indenting tracheoles. It follows therefore that much or all of each secondary tube is probably ventilated, although the tubes are not associated with sacs, nor do they protrude beyond the muscle. Their elliptical cross-section implies compressibility, and this has been verified (see below). The secondaries are probably affected by muscular contractions directly, but volume changes of the whole metathorax are likely to play a more important part. The primaries too may suffer volume changes when the pressure alters, but their stout walls and secondary thickening probably help to minimize such changes. Experiments described below show that the major part of the gas exchange in the primaries may be brought about by the through-conducting system.



Text-fig. 1. Median sagittal sections of the metathorax of six species of Cerambycidae and one of Curculionidae (*Sipalinus*) to show the primary tracheal supply to the flight muscles. Large paired primary tracheae are seen running between spiracles 2 and 3 in all Cerambycidae except in the small species *Plocaederus* and *Phryneta*. A single large primary is found in *Sipalinus*. The scale beneath each diagram represents 10 mm.

The atrium of spiracle 3 of *Petrognatha* is expanded anteriorly into a short blunt air-filled cavity which lies between the tergo-coxal and subalar flight muscles. It too gives rise to numerous secondary tracheae which invade the nearby muscles.

By pumping a latex solution through the trunks of a *Petrognatha* (size index 1500) and subsequently hardening it in acetic acid, a cast of the atria of spiracles 2 and 3 and of the trunks was made. By displacement of liquid, the volume of the cast was found to be 203 μl., of which 23 μl. was occupied by the atrium of spiracle 2, 100 μl. by the trunks and 80 μl. by the atrium of spiracle 3. Thus the whole system in a beetle occupies a volume of about 400 μl.

The spiracles of *Petrognatha* are very large: spiracle 2, when open, reveals a slit 9 mm. long and maximally 2 mm. wide. and it is guarded by naked lips. Spiracles 1 and 3 are each 6–8 mm. long and their lips bear a complex array of bristles. In scarabaeids and other families (e.g. Dystiscidae) spiracle 2 is naked while the lips of other spiracles are hirsute. Their dimensions are much smaller, however, than those of the cerambycid group. Even in *Goliathus regius* (size index 3250) spiracle 2 is not more than 5 mm. long, while spiracle 3 is only 2 mm. long. Very large spiracles therefore communicate with giant tracheal trunks.

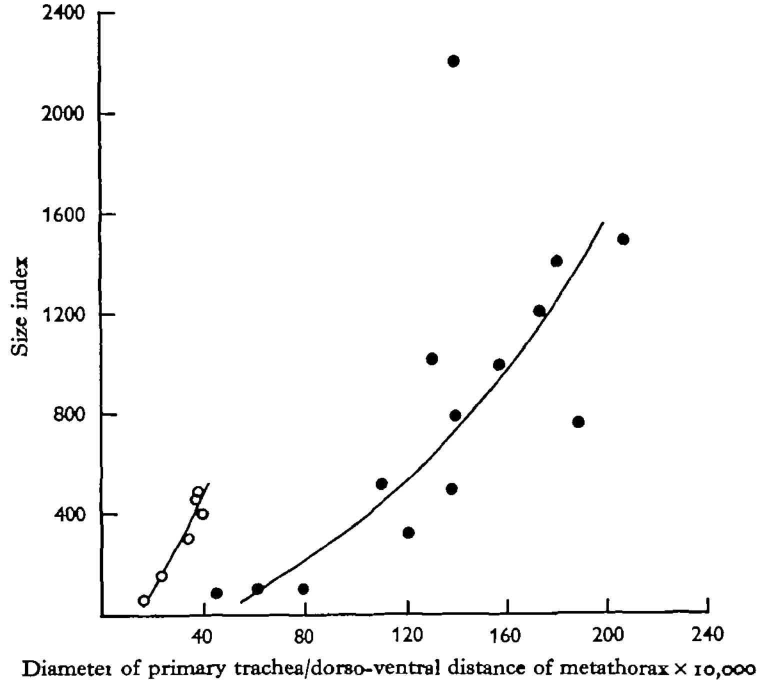
Weis-Fogh (1964*a*) has drawn attention to the isolation of the pterothoracic tracheal system of the locust. A similar situation is found in the cerambycid group. Anterior conduits into the prothoracic system and posterior ones into the abdominal tracheae are small and situated close to the atria of spiracles 2 and 3; there are no continuous ventral trunks comparable to those of the locust. Therefore, when these spiracles are open, pterothoracic air can not mix with air from other regions. Moreover no anastomoses have been found between the right and left sides of the metathoracic system. In flight and at rest, therefore, the insect is supplied through four more or less separate tracheal systems: one in the prothorax and head, one in the abdomen and two for the flight muscles. By contrast the scarabaeids and buprestids have a system in which there are well developed anastomoses between the tracheae in the metathorax and abdomen, and these are not situated close to spiracular atria.

In the curculionid *Sipalinus* (size index 162), the anthribids *Mecocerus oculatus* (102) and *Deuterocrates* (91) and in the elaterid *Tetralobus* (443), as well as in smaller elaterid species, the tracheal trunks are large and gold in colour, comparable to those of *Petrognatha*. In *Sipalinus* and *Mecocerus*, however, there is only one on each side (Fig. 1). Giant primary trunks have also been seen in other large cerambycids such as *Mallodon* (size index 788), *Macrotoma* (770), *Prionomma* (1030) and *Prionoplus* (525), whereas they have not been found in smaller cerambycids such as *Phryneta* (223), *Prosopocera* (257), *Sternotomis* (148, 156) *Plocaederus* (74) and *Chromacilla* (64). In these, their place is taken by silvery tracheae of much smaller size (Fig. 1) and many more tracheae run direct from the atria to the flight muscles.

In the very large prionine *Acanthophorus maculatus* (size index 2210) both dorsal and ventral primary trunks follow a W-shaped path. A similar tendency is found in the ventral trunks of *Macrotoma* and *Tetralobus* where the trunk turns ventrally to run between the dorso-ventral and tergo-coxal muscles and then bends dorsally again (Text-fig. 1). The possible consequences of this plan are discussed later.

In Text-fig. 2 the diameter of the primary trunk, relative to the dorso-ventral thickness of the metathorax, is plotted against the size index for a number of species. It shows that in large species the diameter of the trunks increases disproportionately. A separate curve is drawn through points derived from small cerambycids which lack a large through-trunk. Again, the possible consequences will be discussed later.

In summary, the principal features of the supply systems to the flight muscles of scarabaeids and cerambycids may be re-stated. In the scarabaeids there are separate primary tracheae to each muscle, a well-developed sac system and extensive connexions with the abdomen. In the cerambycids there are usually only two pairs of primary trunks; these are very large and run between the atria of spiracles 2 and 3. There is no sac system and connexions between the thoracic and abdominal systems are confined to the proximity of the spiracular atria. These morphological differences are shown below to be correlated with functional differences.



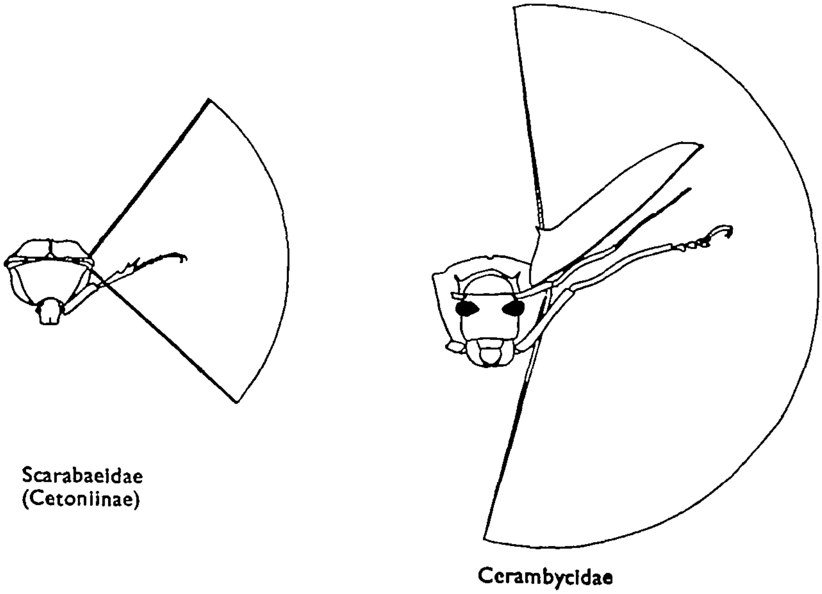
Text-fig. 2. Graph of the size index plotted against the maximum diameter of the primary trachea in the metathorax, divided by dorso-ventral distance of the metathorax (× 10,000). ○, Values from *Phryneta*, *Prosopocera*, *Sternotomis* and *Plocaederus* (species in which the primary tracheae are not large); ●, values from *Petrognatha*, *Prionomma*, *Prionoplus*, *Mallodon*, *Macrotoma*, *Acanthophorus*, *Deuterocrates*, *Sipalinus* and *Mecocerus* (species in which the primary trunks are well developed). Note that in large species an increasing proportion of the metathorax is occupied by the primary tracheae.

FLIGHT EXPERIMENTS

Attempts were made to examine the flight of all the beetles captured in Uganda. Measurements, which were commenced after not less than 5 min. flight, were made on the wing-beat frequency and amplitude, on the stroke plane and on abdominal ventilation as already described. The results are summarized in Table 1.

In members of the cerambycid group the amplitude of wing beats was always found to be large (often approaching 180°) and in many species the wings regularly clapped together at the top or at the bottom of the stroke (but it is not known if this occurs in free flight). The frequency was low when compared with that of scarabaeids of similar size, and the stroke plane was usually about 80° to the long axis of the beetle (Text-fig. 3). Observations on a few free-flying cerambycids suggest that the normal body angle in flight may be more than 25° to the horizontal. Scarabaeids fly with a lower wing-stroke amplitude and higher frequency; the stroke plane is often about 45° to the body axis and the normal body angle in free flight is close to the horizontal. Many cetoniines and buprestids are extremely fast active fliers and the latter were found to be hard to catch when spotted in sunlit patches of Ugandan forests.

Most beetles fly with their elytra held out laterally and in this position it has been shown in *Oryctes* that they generate a small amount of lift (Burton & Sandeman, 1961).



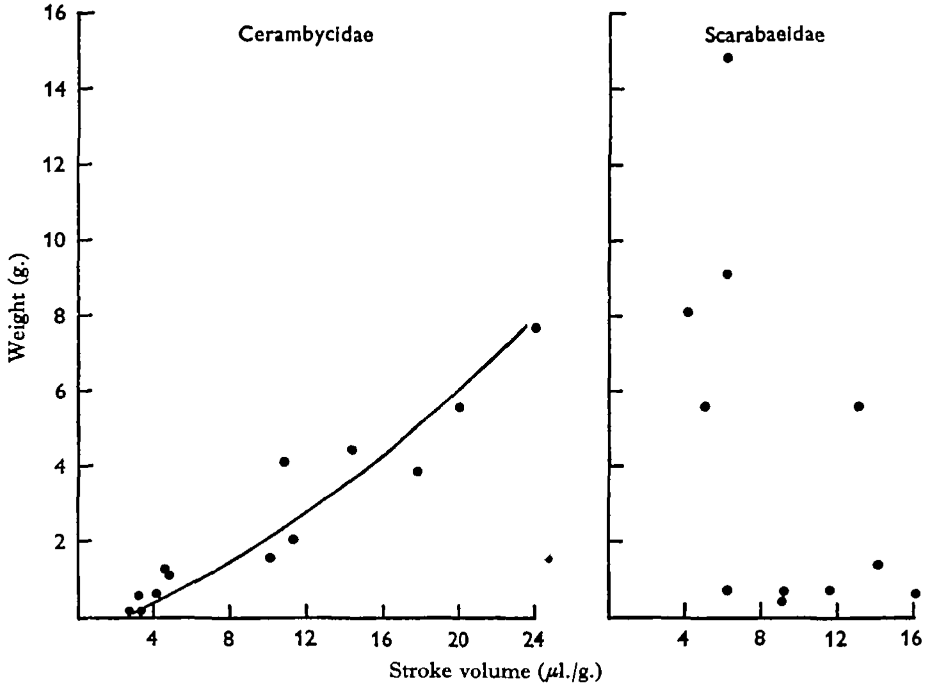
Text-fig. 3. Diagram to illustrate the normal wing amplitudes and positions of the elytra during tethered flight of scarabaeid (cetoniine) and cerambycid beetles.

In cerambycids, as in other families, vibrations are transmitted to the elytra via the meta- and mesonota and they are moved through 15–20° with each wing stroke. In cetoniines, however, the elytra are lifted only a small amount from the abdomen in flight (Magnan, 1934). Prasse (1960) observed that the elytra of the coprine beetles *Onthophagus fracticornis* and *Sisyphus schaefferi* remained more or less closed in flight, and similar observations were made here on *Onthophagus multicornis* and *Scarabaeus femoralis* as well as on all the cetoniines flown. In these species, therefore, one elytron was removed to allow observations to be made on abdominal ventilation.

Spiracles 2 and 3 were seen to be held fully open throughout flight in all species.

THE ABDOMINAL PUMP

Table 1 indicates that abdominal ventilation was weak or absent during flight in all species flown belonging to the cerambycid group. In a few (e.g. *Mallodon*) strong abdominal pumping with synchronized prothoracic pumping movements was observed before and after flight, whereas in *Petrognatha* pumping was very weak and of short duration after flight. In contrast, all scarabaeids and buprestids over 0·6 g. in weight pumped vigorously and continually in flight and for some time afterwards. There was no cessation of movements at the start of flight, such as has been described in *Melolontha* and *Geotrupes* (Fraenkel, 1932; Krogh & Zeuthen, 1951). In Ugandan cetoniines below 0·6 g. pumping was either absent or intermittent. For example in *Chordodera* (0·3 g.) it was never seen, while in *Diplognatha* (0·58 g.) it was seen to occur continually in some individuals but only after several minutes' flight in others.



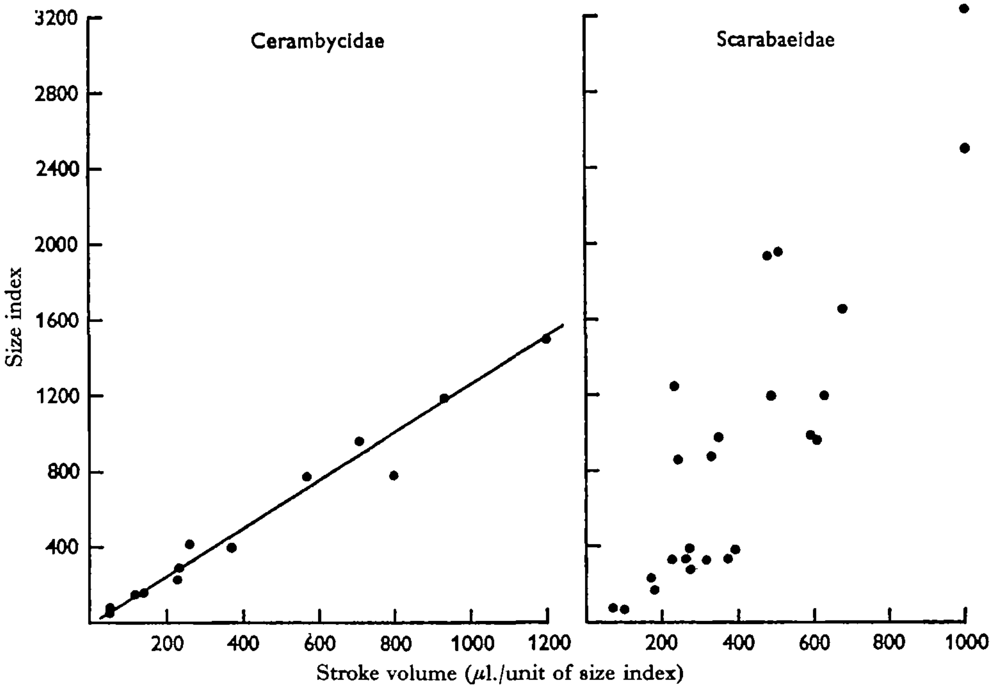
Text-fig. 4. Graphs of the live weights of beetles plotted against the stroke volumes of the thoracic pump per gram, when the wings are moved through the flight positions.

THE THORACIC PUMP

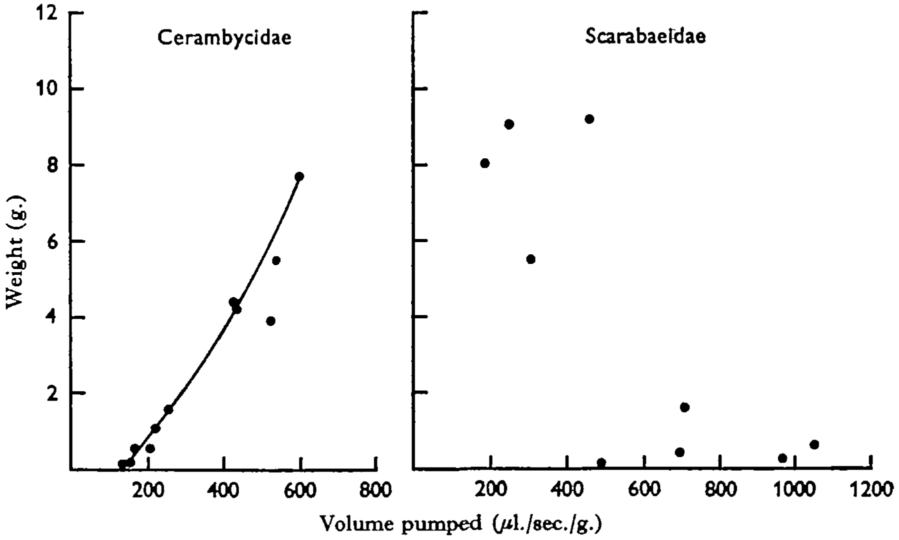
The stroke volume of the thoracic pump was measured after removal of the flight muscles by moving the wings through the known flight positions, as already described. For reasons already given this volume change is believed to bring about the exchange of a roughly equivalent volume of gas between the metathoracic tracheal system and the outside air.

Text-fig. 4 shows the stroke volume per gram plotted against the weight of the beetle and in Text-fig. 5 the stroke volume per unit of size index (× 10,000) is plotted against the size index. The stroke volume per gram or per unit of size index can be seen to increase regularly with increase in size or weight in cerambycids whereas for scarabaeids the points are more widely scattered. Comparison with Table 1 shows that more insects have been measured than were flown; however, in those not flown the wing-beat frequency and amplitude were estimated from the same or closely related species which were flown.

In Text-figs. 6 and 7 the volumes pumped per second per gram and per second per unit of size index (× 10,000) are shown. Again, with increasing size cerambycids increase the volume pumped, relative to size or weight, whereas this does not appear



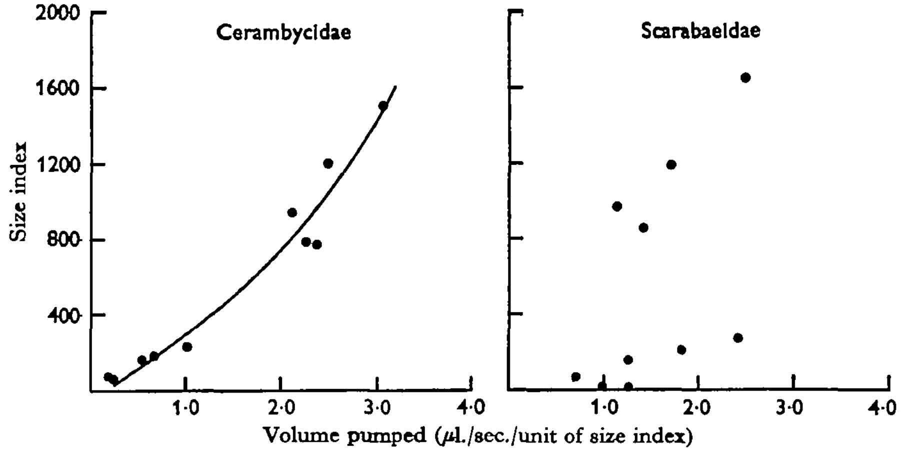
Text-fig. 5. Graphs of the size indices of beetles plotted against the stroke volumes per unit of size index of the thoracic pump, when the wings are moved through the normal flight positions.



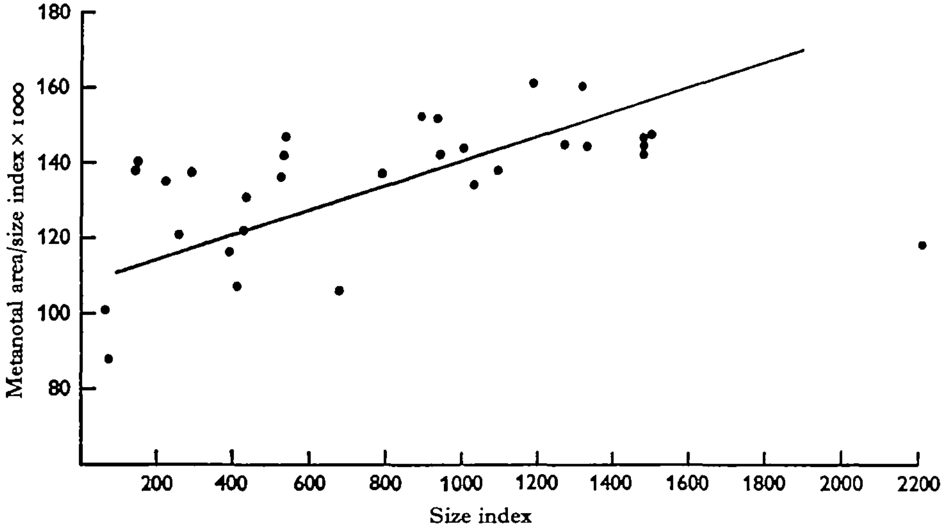
Text-fig. 6. Graphs of the live weights of beetles plotted against the volume pumped per second per gram by the thoracic pump, when the wings are moved through the normal flight positions.

to occur in scarabaeids. The high values for small scarabaeids may reflect their high wing-beat frequencies, but measurements on small species are probably rather inaccurate.

Two tentative conclusions can be reached. In the first place large cerambycids appear to pump more per gram than scarabaeids of similar size, probably due to the greater area of their metanota relative to overall size, and to the greater amplitude of their wing beat. Secondly, large cerambycids pump more per unit of size or weight than small ones. Despite the higher wing-beat frequencies of small species, this is true per second as well as per wing stroke. The increase may be partly due to a relative



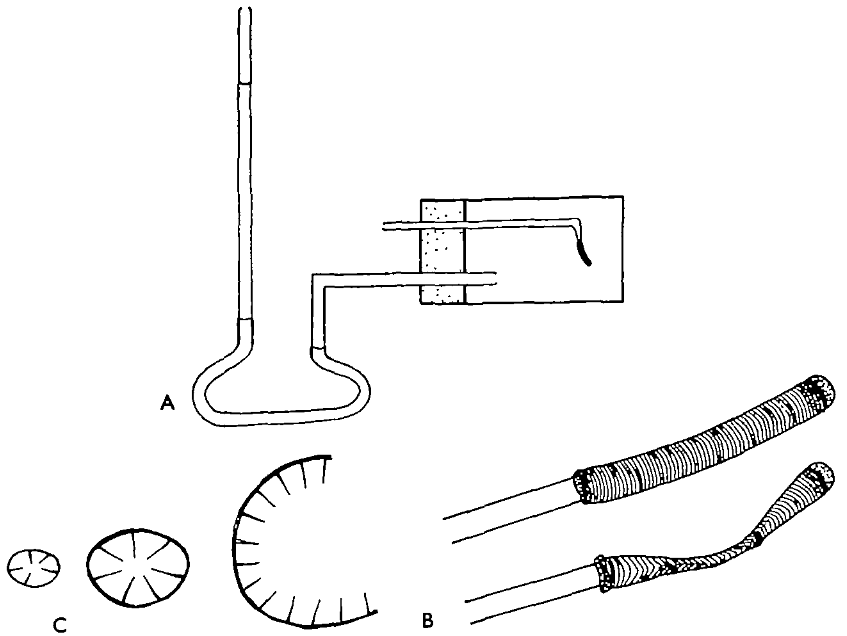
Text-fig. 7. Graphs of the size indices of beetles plotted against the volume pumped per second per unit of size index by the thoracic pump, when the wings are moved through the normal flight positions.



Text-fig. 8. Regression of the metanotal area of cerambycids per unit of size index on the size index (regression coefficient = 0·032 ± 0·0075).

increase in the area of the metanotum (Text-fig. 8) in larger species and partly to an increased amplitude of movement.

In a *Petrognatha* of 6 g. the stroke volume is about 120 μl., or 20 μl./g. This provides 540 μl./g./sec. In *Schistocerca* weighing 2 g. with a wing amplitude of 68° and a wing-beat frequency of 20/sec., the pump has a stroke volume of about 20 μl., equivalent to 10 μl./g. or 200 μl./g./sec. (Weis-Fogh, 1964*a*). Weis-Fogh has estimated that 5–7 % of the air in the pterothoracic tracheal system is exchanged with each beat. In *Petrognatha* the total volume of the metathorax is about 4·6 ml. If half of this volume is air-filled, then 5 % of the air may be exchanged with each wing beat. If the system contains less air, more can be exchanged per beat.



Text-fig. 9. Method for determining the compressibility of secondary tracheae removed from the metathorax of *Petrognatha*. A, Apparatus comprising a manometer and a water-filled vessel enclosing the trachea. B, The appearance of lengths of trachea before and after the application of increased pressure. C, The appearance of small tracheae in cross-section, showing the bristles which project into the lumen.

The primary trunks may be compressed to a limited extent by thoracic volume changes but the main parts affected are probably the secondary tracheae, which are elliptical in cross-section. The compressible nature of such tracheae, as opposed to those with circular cross-section, was pointed out by Krogh (1941). That this is true for the secondary tracheae of *Petrognatha* has been established by direct measurements as follows. A small segment about 2 mm. long and 300 μ in diameter was removed from the metathorax and waxed to the end of a small glass pipette (Text-fig. 9). The other end of the trachea was sealed with a lump of wax. It was then placed in a glass vessel filled with water with the open end of the pipette passing through a hole in the cork. The pressure in the vessel was increased while the trachea was examined under a microscope. The pressure at which the first sign of tracheal collapse occurred and the pressure needed to produce complete collapse were recorded. Six tracheae, all removed from the proximal parts of secondary tubes, were measured and collapse was observed in each to start under a pressure of between 5 and 10 mm. Hg. Collapse was complete under 18 mm. Hg. Since rather short lengths were used, values in the living insect may be lower. In *Schistocera* a pressure change of 4·1 mm. Hg accompanies each wing beat (Weis-Fogh, in preparation), while with strong abdominal ventilation pressures of up to 10 mm. Hg have been recorded (Watts, 1951). The pressures occurring in *Petrognatha* have not been recorded but they are likely to be greater than those in *Schistocerca* and it is probable that regular deflation and inflation of the secondary tracheae occurs with each wing stroke. Attempts to measure the compressibility of primary trunks failed since in all tests water entered before collapse was observed.

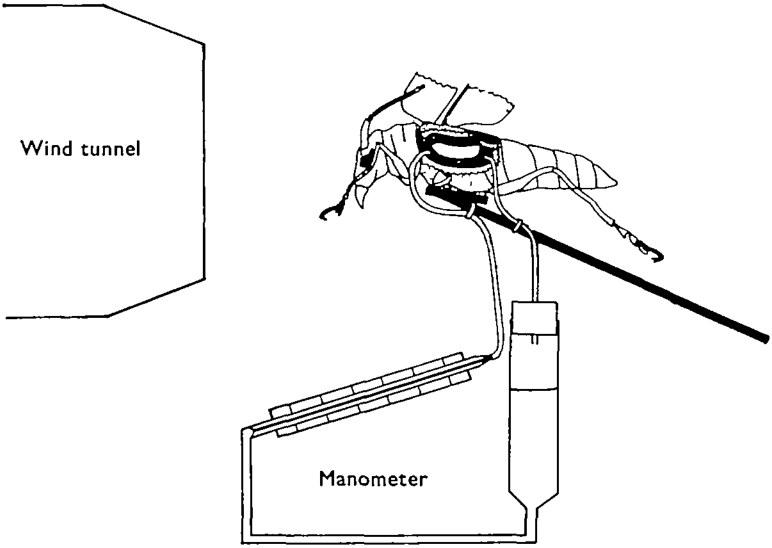
All tracheae in *Petrognatha* down to those of about 50 μ in diameter are lined with small bristles which protrude into the lumen. They may prevent the complete obliteration of the airway when a trachea collapses under pressure.

MEASUREMENTS OF THE AIRFLOW THROUGH THE PRIMARY TRUNKS OF *PETROGNATHA*

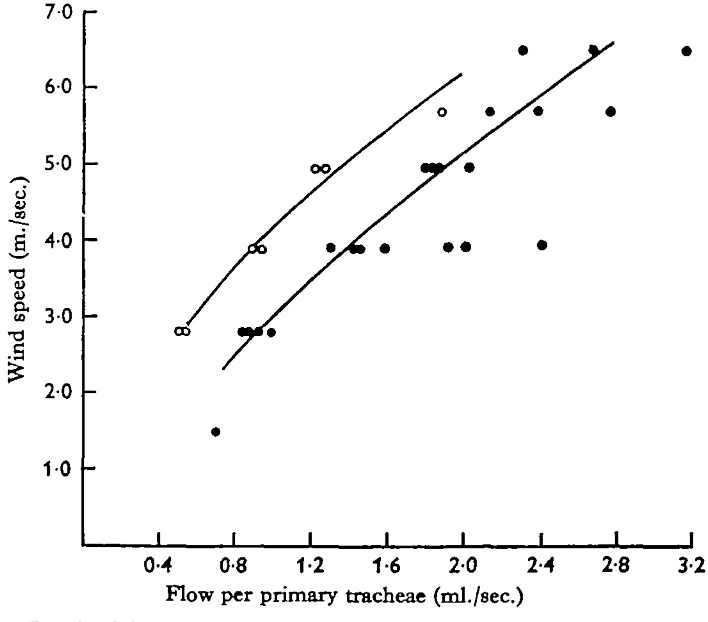
Amos & Miller (1965) demonstrated an airflow through the primary trunks of a dead *Petrognatha* suspended in an airstream. Comparable observations have now been made on flying *Petrognatha* by releasing dusts into the stream in front of the beetle and watching their emergence from spiracle 3 under a microscope. Subsequent dissections revealed the presence of dust particles on the walls of the atria and the trunks.

In order to measure the volume of air passing through a trunk, a dead *Petrognatha* was dissected so as to reveal the dorsal and ventral trunks on one side. Two holes were burnt into either the dorsal or the ventral trunk, one at each end, and a small polythene tube (internal diameter 0·5 mm.) was sealed into each hole with wax. The beetle was then reassembled round the tubes, which were joined to either end of a manometer. One arm of the manometer was formed by an inclined capillary tube and the other by a wide tube. In this way nearly all the movement of the manometer fluid (alcohol coloured with eosin), which was observed with a microscope, took place in the capillary, the movement being magnified by the slope (Text-fig. 10). The beetle was glued to a flight-stand and placed in front of the wind tunnel with wings and elytra in various flight positions. Spiracles 2 and 3 were held open with small wire clips. The pressure drop between the ends of the manometer was read when the beetle was subjected to different wind speeds. The volume passing through the tube could then be calculated from Poiseuille's equation: $V = \pi p r^4/8\, l\eta$, where V = flow (ml./sec.), l = length of trunk between the polythene tubes (0·9 cm.), r = average radius of the trunk, calculated from drawings of cross-sections on graph paper (0·117 cm. dorsal trunk, 0·019 cm. ventral trunk), p = pressure difference between the introduced tubes in dynes/cm.^{2} and η = viscosity of air = 0·0018 poises per dyne per cm.^{2} The pressure differences measured varied between 0·038 and 0·46 mm. water (= 3·8–45 dynes/cm.^{2}).

During readings each arm of the manometer was disconnected in turn so that the pressure at either end could be compared with atmospheric. This provided an additional check on the method. Calculations of the Reynolds number for the system with a flow velocity of 30 cm./sec, give a value of 20. Flow would therefore be expected to be laminar. The situation in the atria is probably more complex, but this does not affect the measurements made. Since the flow is being measured through a non-

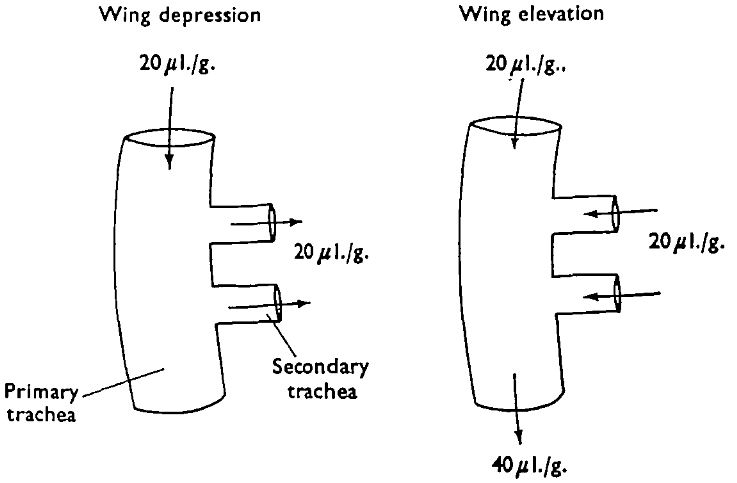


Text-fig. 10. Method for determining the air flow through the large primary tracheae oi *Petrognatha* in various wind speeds. The two ends of the manometer are shown joined to either end of a ventral primary.



Text-fig. 11. Graph of the wind speed plotted against the airflow, as measured with the manometer, through the dorsal (○) and ventral (●) primary trachea of *Petrognatha*.

uniform tube and Poiseuille's formula applies to hollow cylinders, the manometer was calibrated directly by passing air through the trunk at a known rate, This was done by sealing a small tube into spiracle 2 and passing air from a calibrated flowmeter through the trunk. The other trunk was blocked with wax so that all the air passed along the trunk from which the manometer readings were made. The readings from the calibrated flowmeter and those calculated from Poiseuille's formula were in good agreement.



Text-fig. 12. Diagram to summarize the movement of air in all the primary and secondary tracheae supplying the flight muscles during the upstroke and the downstroke in *Petrognatha*.

Some results from four *Petrognatha* (all about 6 g. in weight and with a size index of *c.* 1200) are shown in Text-fig. 11. The flows through ventral and dorsal tubes were determined independently, the other tube being left open. It can be seen that more air passes through the ventral tube than through the dorsal. Alterations of the body angle from 0–25° to the horizontal had little effect on the readings; at greater body angles the values rapidly declined. The wing position had no effect on the flow except near the bottom of the stroke where a small reduction occurred. Some conclusions which follow are set out in Table 2.

Table 2. *Petrognatha of* 6 *g. in a wind speed of* 5 *m./sec.*

(1) Primary trunks	
Each dorsal trunk conducts	1250 μl./sec.
Each ventral trunk conducts	1900 μl./sec.
Total for beetle (4 tubes)	= 6300 μl./sec.
	= 1050 μl./sec./g.
At wing-stroke frequency of 27 beats/sec.	= 39 μl./stroke/g.
(2) Capacity of primary trunks and associated atria	= 400 μl.
	= 67 μl./g.
(Therefore air in primary trunks is renewed approximately every two wing strokes.)	
(3) Thoracic pump contribution	= 540 μl./sec./g.
	= 20 μl./stroke/g.

Assuming that the thoracic pump affects only the secondary tracheae it can withdraw 20 μl. from the primaries every downstroke ('inspiration') and add the same volume each upstroke ('expiration'). Overall it does not therefore affect the volume flowing along the primaries. The stroke volume of the pump (20 μl./g.) is about half the volume of air (39 μl./g.) entering the primaries each complete wing-beat cycle. During the downstroke, therefore, approximately as much air is removed from the primaries as enters at spiracle 2 in the same time, and it is replaced during the upstroke. The similarity of these values in a wind of 5 m./sec. is probably a coincidence and more important is the probability that at each downstroke more or less fresh air is sucked from the primaries into most of the secondaries. The atrium of spiracle 3 contains 40% of the air in the whole primary system; in flight this air will have already been 'used' by secondaries more anterior to the atrium and may be 'used' again by those which are connected to this atrium. The system is represented schematically in Text-fig. 12.

DISCUSSION

Air is blown through the two pairs of primary trunks of *Petrognatha* when the beetle flies. The speed of *Petrognatha* in free flight is not known but is probably between 2·5 and 5 m./sec.; the volume of air passing through the trunks at these speeds is known (Text-fig. 11). Smaller species with lower flight speeds and narrower tubes may be unable to drive air through the primaries in this way; however, measurements have been made only on *Petrognatha* so far.

In *Petrognatha* the primary system includes the expanded atria of spiracles 2 and 3, which form compact cavities from which numerous secondary tracheae penetrate the muscles. The air they contain is probably stirred by eddy currents produced by the through draught, thereby hastening its exchange with the outside air.

The absence of air sacs in those species of Cerambycidae, Anthribidae, Curculionidae and Elateridae which have been examined is striking. In other families and orders sacs permit the abdominal pump to work on a freely compressible region of the tracheal system; as we have seen, the abdominal pump is poorly developed in the cerambycid group. In many insects large sacs allow changes in the size of the internal organs to take place without a corresponding deformation of the external features. Cerambycids and other beetles, however, do tolerate such external deformations, and the expanded abdomen of gravid females is a familiar sight.

In the scarabaeid supply system the thoracic pump, aided by the abdomen, is responsible for moving air in the primary and to a large extent in the secondary tracheae. In cerambycids, however, the principal action of the thoracic pump is believed to be on the secondaries. The reduction in the number of primaries in most cerambycids to two on each side may testify to the efficiency of the through-draught system, but it may also reflect the fact that such a mechanism can work only in short broad tubes where the frictional resistance is low, since it depends on a small pressure difference between the ends of the tube.

In large species of both groups a greater *proportion* of the whole pathway between spiracles and tissues must be ventilated, since the length of the terminal region in which diffusion alone operates is independent of the size of the insect (except in so far as there may be a slight increase in bore). The thoracic pump must be made increasingly efficient, therefore, or the proportion of the system in cerambycids affected by the through draught must be increased. We have found indications of both mechanisms in large cerambycids. For example in *Petrognatha* the thoracic pump has a greater stroke volume per gram than in smaller cerambycids. However, since it is coupled to the wing movements, the increase in the volume which can be pumped per second per gram probably reaches a maximum. To effect exchange in a greater proportion of the secondary system, therefore, the volume of gas must be reduced. In this light the lack of air sacs in the cerambycid system may be interpreted, such sacs having their main function with respect to an abdominal pump of large amplitude but low frequency.

The second possibility is exemplified in *Prionomma* and *Tetralobus*, where the ventral trunk is lengthened, and most notably in *Acanthophorus* (the largest cerambycid examined) where both trunks follow contorted W-shaped routes. Secondaries, arising from such lengthened primaries, are therefore shorter and the proportion of the air which can be exchanged with each stroke may be increased. Text-fig. 2 suggests, however, that in large species an ever-increasing proportion of extra-muscular space becomes occupied by the primaries, and this may ultimately be limiting.

The capacity of the thoracic pump may therefore impose a limitation on the further increase in size of flying beetles. While the proportion of the route requiring ventilation increases, the contribution per gram made by the thoracic pump probably reaches a maximum. In the absence of a *combination* of the mechanisms found in cerambycids and scarabaeids, families containing some of the largest insects in volume alive today (e.g. *Titanus* and *Goliathus*), further increase in size could be achieved perhaps only with the loss of flight.

It is intriguing to contemplate the respiratory problems which such an insect as the giant Carboniferous dragonfly, *Meganeura*, with a pterothorax 10 cm. long and 5 cm. wide (Tillyard, 1917), must have solved if it flew actively. Weis-Fogh (1964*b*) has shown that the respiratory system in contemporary dragonflies is taxed to near its limit; if, therefore, *Meganeura* consumed oxygen at the same rate per gram, which seems likely if it was able to generate positive lift, it must have possessed tracheal modifications which are not found in present-day dragonflies. These may have included tracheoles which indented the muscle fibres and thoroughly ventilated secondary tracheae. In turn this might suggest that *Meganeura* is not in the direct ancestral line of contemporary species. However, its problems would have been alleviated if the Carboniferous atmosphere contained substantially more oxygen than our own (cf. Berkner & Marshall, 1964).

It is well known that the flight muscles of large insects get hot in flight (Krogh & Zeuthen, 1941). Amos & Miller (1965) suggested that the through-conducting trunks of *Petrognatha* might cool the pterothorax in flight, that is, that *Petrognatha* has an air-cooled engine. Temperature measurements were made in the metathorax of one *Petrognatha* in flight by introducing a small thermocouple between the dorso-longitudinal muscles. This showed that after 30 min. flight in a female (weight 7·6 g.) the internal temperature was 34·5° C. (9·5° C. above ambient). Since no measurements of the lift were made at the same time, the reading has limited significance, but it suggests that the primary trunks do not contribute an appreciable cooling effect. In the flight of *Orcytes rhinoceros*, Machin, Pringle & Tamasige (1962) argue that the expected pterothoracic temperature is about 40° C. (10° C. above ambient) since at this temperature the flight muscles work with the maximum efficiency. In *Petrognatha*, however, which probably flies mainly at night at environmental temperatures often not much above 20° C. in Uganda, the metathorax probably does not exceed 30° C. If the flight muscles are adapted to work with maximum efficiency at the pterothoracic temperature normally prevailing in flight, then the optimum temperature for the muscles of *Petrognatha* may be 10° C. below that for *Orcytes*. Such insects as moths, bees and dragonflies are well provided with heat-insulating devices, superficial air sacs or fur, which reduce the rate of heat loss from the pterothorax (Church, 1960). It has been argued that these play an important role during temporary cessations of flight when the thorax is maintained at a temperature at which renewed flight is immediately possible (Miller, 1964). Such mechanisms appear to be absent in those beetles which have been examined and this may reflect the different nature of their flight activity, at least in nocturnal species, which are believed to make long continuous flights rather than numerous short flights. In large species, with such behaviour patterns, therefore, one initial period of activity to raise the thorax to a temperature at which flight is possible is all that is required.

SUMMARY

1. Measurements of the wing-beat frequency, wing-stroke amplitude and stroke plane and of abdominal ventilation have been made during the tethered flight of twenty-six species of beetles belonging to five families, mainly in Uganda.

2. Abdominal ventilation is weak or absent in all species of Cerambycidae, Elateridae and Anthribidae examined in flight. The tracheal system in these families is characterized by the complete absence of air sacs, and in larger species by the presence of four giant trunks running through the metathorax between spiracles 2 and 3 and forming the primary supply to the flight muscles.

3. Abdominal ventilation is strong during the flight of all species over 0·6 g. in weight of the Scarabaeidae and Buprestidae which were examined. Their tracheal systems contain an abundance of air sacs while giant trunks are absent.

4. Measurements of the thoracic volume changes which accompany each wing beat show that the amount of air which can be pumped in this way increases in larger Cerambycidae per second per gram as compared with small species. Large Cerambycidae pump more per gram than Scarabaeidae of comparable size.

5. During the flight of the cerambycid *Petrognatha* the thoracic pump exchanges 540 μl. air/sec./g. Its action is mainly on the compressible secondary tracheae. In a wind speed of 5 m./sec. 1050 μl. air/sec./g. are driven through the four giant trunks, entering through spiracle 2 and leaving from spiracle 3. The trunks are stout-walled and probably unaffected by the thoracic pump.

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REFERENCES

ALT, W. (1912). Über das Respirationsystem von *Dytiscus marginalis* L. Ein Beitrag zur Morphologie des Insektenkörpers. *Z. wiss. Zool.* 99, 357–413.

AMOS, W. B. & MILLER, P. L. (1965). The supply of oxygen to the active flight muscles of *Petrognatha gigas* (F.) (Cerambycidae). *Entomologist* 98, 88–94.

BERKNER, L. V. & MARSHALL, L. C. (1964). The history of oxygenic concentration in the earth's atmosphere. *Disc. Faraday Soc.* 37, 122–41.

BURTON, A. J. (1962). (Quoted in T. Weis-Fogh, (1964*a*). *J. Exp. Biol.* 41, 207–27).

BURTON, A. J. & SANDEMAN, D. C. (1961). The lift provided by the elytra of the rhinoceros beetle, *Oryctes boas* Fabı. *S. Afr. J. Sci.* 57, 107–9.

CHURCH, N. S. (1960). Heat loss and body temperature of flying insects. II. Heat conduction within the body and its loss by radiation and convection. *J Exp. Biol.* 73, 186–212.

DARWIN, F. W. & PRINGLE, J. W. S. (1959). The physiology of insect fibrillar muscle. I. Anatomy and innervation of the basalar muscle of lamellicorn beetles. *Proc. Roy. Soc.* B 151, 194–203.

FRAENKEL, G. (1932). Untersuchungen über die Koordination von Reflexen und automatisch-nervösen Rhythmen bei Insekten. I. Die Flugreflexe der Insekten und ihre Koordination. II. Die nervöse Regulierung der Atmung wahrend des Fluges. *Z. vergl. Physiol*, 16, 371–93, 394–417.

IMMS, A. D. (1957). *A General Textbook of Entomology*, 9th ed. Revised by O. W. Richards and R. G. Davies. London: Methuen.

KROGH, A. (1941). *Comparative Physiology of Respiratory Mechanisms*. University of Pennysylvania.

KROGH, A. & ZEUTHEN, E. (1941). Mechanism of flight preparation in some insects. *J. Exp. Biol.* 18, 1–10.

LESTON, D., PRINGLE, J. W. S. & WHITE, D. C. S. (1965). Muscular activity during preparation for flight in a beetle. *J. Exp. Biol.* 42, 409–14.

MACHIN, K. E., PRINGLE, J. W. S. & TAMASIGE, M. (1962). The physiology of insect fibrillar muscle. IV. The effect of temperature on a beetle flight muscle. *Proc. Roy. Soc.* B 155, 493–99.

MAGNAN, A. (1934). *Le vol des insectes*. Paris: Hermann et Cie.

MARCU, O. (1931). Beitrag zur Kenntnis der Tracheen der Insekten. *Zool. Anz.* 93, 61–3.

MILLER, P. L. (1964). Notes on *Ictinogomphus ferox* Rambur (Odonata; Gomphidae). *Entomologist* 97, 52–66.

MILLER, P. L. (1966). The regulation of breathing in insects. In *Advances in Insect Physiology*, vol. III, pp. 279–354. Eds. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth.

MÖLLER, H. (1921). Über *Lethocerus uhleri* Mont. *Zool. Jb.* 42, 34–90.

NEWPORT, G. (1851). On the formation and use of the airsacs and dilated tracheae in insects. *Trans. Linn. Soc.* 20, 419–23.

PRASSE, J. (1960). Über den Start und Flug des *Sisyphus schaefferi* L. (Coleoptera:Scarabaeidae). *Beitr. zur Ent.* 10, 168–83.

PRINGLE, J. W. S. (1957). *Insect Flight*. Cambridge University Press.

SMITH, D. S. (1961). The structure of insect fibrillar flight muscle. *J. biophys. biochem. Cytol.* 10, 123–58.

SOTAVALTA, O. (1947). The flight tone (wing-stroke frequency) of insects. *Acta ent. fenn.* 4, 1–117.

STRAUSS-DÜRCKHEIM, H. E. G. (1828). *Considérations générales sur l'anatomie comparée des animaux articulées*. Paris: F. G. Levrault.

STRIDE, G. O. (1958). The application of a Bernoulli Equation to problems of insect respiration. *Proc. 10th Int. Congr. Ent.* 2, 335–6.

TILLYARD, R. J. (1917). *Biology of Dragonflies*. Cambridge.

WATTS, D. T. (1951). Intratracheal pressure in insect respiration. *Ann. ent. Soc. Amer.* 44, 527–38.

WEIS-FOGH, T. (1964*a*). Functional design of the tracheal system of flying insects as compared with the avian lung. *J. Exp. Biol.* 41, 207–27.

WEIS-FOGH, T. (1964*b*). Diffusion in insect wing muscle, the most active tissue known. *J. Exp. Biol.* 41, 229–56.

EXPLANATION OF PLATE

Fig. 1. Frontal view of a cerambycid (*Prosopocera belzebuth*) in tethered flight, showing the wing-beat amplitude of about 180°.

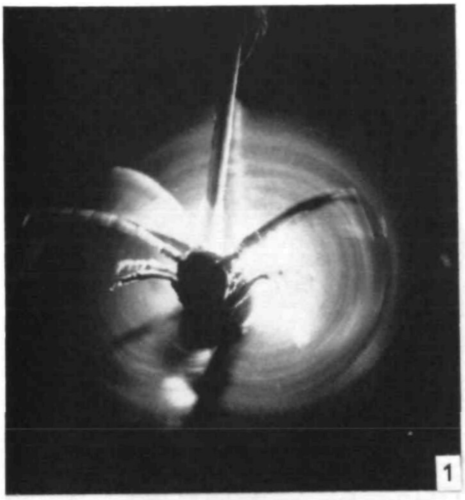
Fig. 2. Frontal view of a buprestid (*Megactenodes westermanni*) in tethered flight, showing the wing-beat amplitude of about 110°.

Fig. 3. Part of the dorsal longitudinal flight muscle of *Petrognatha gigas*, showing some of the secondary tracheae which pass between the fibres.

Fig. 4. Views of the inside of a giant primary trachea of *Petrognatha gigas*, showing the struts which reinforce the walls and the origins of secondary tracheae guarded by circlets of bristles.

Fig. 5. Median sagittal section of the metathorax of *Petrognatha gigas*, after removal of the dorsal longitudinal flight muscle, showing the dorsal (*d*) and ventral (*v*) giant tracheae on the left side.

Fig. 6. Transverse section of the right side of the metathorax of *Petrognatha gigas*, showing the dorsal and ventral giant tracheae and, more laterally, part of the expanded atrium of spiracle 3.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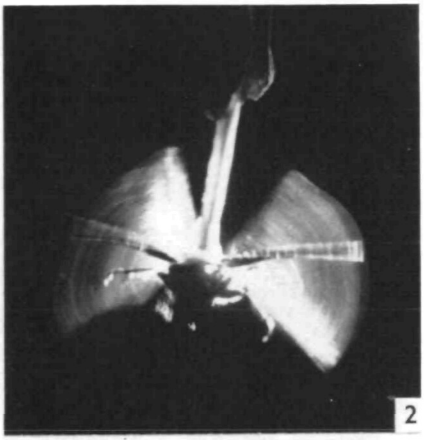
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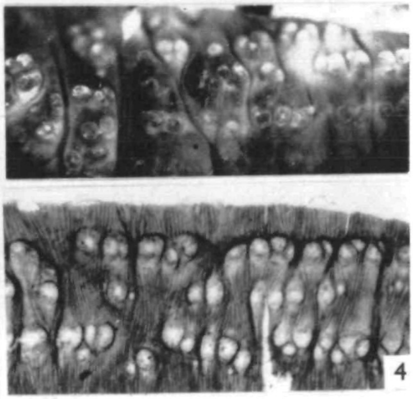
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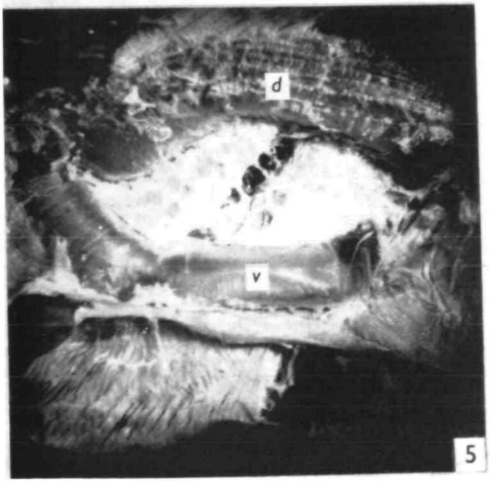

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