Morphological diversity and phylogenetic analysis of wing circulatory organs in insects, part I: Non-Holometabola

HARALD W. KRENN and GÜNTHER PASS

Institute of Zoology, University of Vienna, Austria

Received: 29. 11. 1993; revised version: 1. 3. 1994; accepted: 10. 3. 1994

Key words: accessory pulsatile organ, wing circulatory organ, functional morphology, organ evolution, dorsal vessel, phylogeny

Summary

The accessory pulsatile organs for hemolymph transport in the wings were investigated in 45 species including representatives of most non-holometabolan insect orders by means of serial seminiferous sections and SEM. All accessory wing circulatory organs are located in the posterior region of winged thoracic segments. They consist of a part of the dorsal cuticle, the scutellum, and an associated pulsatile apparatus. The scutellum constitutes the pump casing and forms a small hemolymph space beneath the cuticle which communicates via cuticular tubes with the posterior wing veins. The pulsatile apparatus is composed of muscular structures which are attached to the cuticle by various connective tissue elements. The anatomical design of the pulsatile apparatus varies conspicuously among the investigated species at the order level. It can be comprised of either a portion of the dorsal vessel or of a separate pumping organ. The pleiomorphic condition is characterized by a laterally enlarged dorsal vessel equipped with a pair of incumbent ostia that open into the subsutellar hemocoele above (all Pauperaetabola). Apomorphic conditions are ampullary vessel formations (Pterygoptera), long diverticula of the dorsal vessel (Cordata, Paucoptera), and different slit openings between the subsutellar hemocoele and the thoracic cavity (Pterygota, Plesoptera). Muscular diverticula constitute the other kind of pulsatile apparatus which is also associated with the scutellum but independent of the dorsal vessel (Hemiptera). Brachypterous species exhibit all principal components of wing circulatory organs although they are less well-developed. In all sequitarily apterygote insects, however, these characters are completely absent. Despite the various arrangements of the pulsatile apparatus, it can be concluded from functional morphology that all wing circulatory organs function as sucking pumps. The one-way hemolymph pathway from the posterior of wing veins to the incotax is ensured by valves that prevent backflow. Key innovations in the evolution of the wing circulatory organs are: the formation of the scutellum and the scutellar arms, some specializations of the dorsal vessel in the region of the ostia, and the separation of the subsutellar hemocoele from the thoracic cavity. The pulsatile diverticula in Hemiptera represent an evolutionary novelty.

Introduction

In the open circulatory system of insects the pumping action of the large dorsal vessel ("heart") alone does not result in hemolymph transport to and from long body appendages. This is accomplished in antenaea, legs, wings, and cerci by accessory pulsatile organs. These organs are either specialized parts of the dorsal vessel or, in structure and function, independent hemolymph pumps (reviews: JOHNS 1977, MILLER 1985, PASS in press). For a long time these organs received little attention and were mentioned only in passing in entomological textbooks. Recently, it could be shown that these auxiliary pumps exhibit a surprisingly great variety of contractions and functional mechanisms making them especially interesting for phylogenetic analyses and

Abbreviations: a – ampulla; ac – axillary cord; ant – antera; d – diverticulum; dm – dorsal longitudinal muscle; dor – dorsal; dv – dorsal vessel; eo – excurrent ostium; fwc – forewing; fwh – hindwing; ls – lateral slit; i – incumbent ostium; pd – pulsatile diverticula; pm – pericardial hemocoele; postn – postnotum; s – scutellum; sc – mesoscutellum; sc2 – metascutellum; sa – scutellar arm; sc – scutum; sc2 – mesoscutum; sc3 – metascutum; se – suspending septum; sh – subsutellar hemocoele; sv – suspending strand; sv – septum valve; v – valve; w – wing; vv – wing vein.
Insect wings are supplied with hemolymph running through the tubular veins of the wing. The presence of special circulatory organs responsible for the exchange of hemolymph in these tubes has been reported for members of several insect orders (reviews and complete references: Jones 1977). Most of these descriptions, however, are merely brief notes found in general morphological monographs or in studies of the circulatory system with a view to a larger perspective. Details on the structural and functional morphology of these hemolymph pumps are given for only a few species (Broicher 1919, Friedenersten 1928, Meyer 1931, Whiton 1938, Thomsen 1938, Seiman 1965, Jensen 1978, Krienn and Pass 1993). Although different constructional types of wing circulatory organs have been found, adequate comparative investigations are lacking. Our scant knowledge is essentially due to difficulties in using classical histological methods to investigate these minute and delicate structures which are located directly beneath the strongly sclerotized cuticle. However, improved sectioning techniques and SEM permit a first detailed and comparative approach.

The aim of our study is to analyze the accessory wing circulatory organs on a comparative basis considering structural and functional as well as phylogenetic and evolutionary aspects. The first part of this investigation includes representatives of all orders of non-hematoblastic Phygoptera except Zopastera and Phthiraptera. The Hemolobus will be treated in a separate and forthcoming paper. The present investigation focuses on the phylogenic development of the functional components of wing circulatory organs starting from the pleisiomorphic condition in the thorax of primarily apergynous insects. The results permit a reconstruction of the adaptations in the insect circulatory system due to the demands of the innovation of wings and the evolution of the flight apparatus.

Materials and Methods

List of investigated species:
Ephemeroptera: Calamites sp., Habrobatoideis contusa, Siphonarius, Schiapato sp., Ephemerella danica (Müller 1764).
Odonata: Ischnura elegans (Van der Linden 1820). Platycnemis peninsulae (Pallas 1771), Calopteryx virgo, Linné 1758.
Plecoptera: Dytogenus lunatus Ris 1866, Leuctra sp., Nemoura sp.
Diptera: Periplaneta americana (Linné 1758), Ectobius synthes (Poda 1763), Stenidea sp., Mantis religiosa Linné 1758.
Netoptera: Gryllotalpa campestriformis Walker 1914

Demaptera: Labidura riparia (Pallas 1773), Furcilia auricularia Linné 1758.
Orthoptera: Locusta migratoria Linné 1758, Chorthippus parallelus (Zettler 1821), Oedipoda caerulescens (Linné 1758), Arphia alpina (Kollar 1832), Chorthippus sp., Pholidoptera griseoaptera (Dröger 1773), Attagoptera rosea (Hagenbach 1822), Phylloicus gramineus (Fabricius 1791), Acrida dorsalis (Linné 1758), Philotoma alatula (Poda 1761), Leptophyes pulchrostoma (Boisduval 1792).
Phaenidae: Ectatomma caudatum (MacKay 1820), Leucopan sp.
Embiptera: Oligotoma nigra (Hagen 1866).
Thysanoptera: Aeolothrips sp., Gynaikothrips floridanus Marsh 1908.
Phasmatodea: Gryllophorus cruciger (Linné 1768).
Homoptera: Alydus castaneus (Linné 1758). Pyrrhocoris apterus (Linné 1758), Mantis religiosa (Linné 1758), Gerris sp., Ranatra linearis (Linné 1758), Nepa cinerea (Linné 1758), Psyllela sp., Cercops sauteri (Donner 1796), Capsus sp., Pteronara pholus (Linné 1758), Ophiogomphus pustulatus (Linné 1758), Ophiogomphus montana (Scopoli 1772).

Light microscopy: The animals were fixed in Duboscq-Brasil solution (Romero 1938) or 70% ethanol. The isolated meso- and metahemolymph were dehydrated with ethanol and, after an intermediate step in propylene oxide, embedded in ERL-4206 epoxy resin (Spurt medium) under vacuum impregnation. Semi-thin sections (1 μm) were made with a Reichert Om-U3 ultramicrotome using glass or diamond knives. The sections were stained with a mixture of 1% azure II and 1% methylene blue in an aqueous 1% borax solution (Romero 1938) for approximately 1 min at 80°C.

Scanning electron microscopy (SEM): After the fixation and dehydration procedure described above, hexamethyldisilazane was used as an intermediate medium before air-drying (Block 1987). The specimens were examined with a Joel JSM-35CF scanning electron microscope.

Results

The accessory wing circulatory organs are located just beneath the tegular cuticle in the posterior region of each pleurothoracic segment. There, the tegular cuticle forms a median dorso-ventral elevations, the scutelum (Fig. 1-A and all SEM figures). It extends laterally on both sides to the wing bases as projecting ridges, called scutellar arms. In cross section, they appear as tubes of rigid cuticle which are detached from the thoracic cavity (Figs. 1-19). A tube of flexible cuticle connects the distal ends of the scutellar arms on each side to the posterior wing veins (Figs. 1, 7, 15, 21, 23). This tube, called the axillary cord (Scho-

These structures form a tubular pathway between the suture of the wing veins and the small hemolymph space beneath the convex scutellum. This space is always separated from the

Zoology 98 (1994) 1
Fig. 1. Scheme of the cuticular components of wing circulatory organs in a thoracic segment (dorsal view). The domed scutellum is formed by the tegal cuticle. The subcuticular hemocoel is confluent with the posterior wing veins via the tubular scutellar arms and axillary cords on both sides. Figures below show sectional views of scutellum (1-1, 2-2), scutellar arm (3-3) and axillary cord (4-4).

1. Ephemeroptera

All-winged thoracic segments possess a well-developed scutellum with scutellar arms (Fig. 3). The dorsal vessel runs through the thorax just beneath the tergal cuticle. In each winged segment, the dorsal vessel forms an ampulla-like formation which extends into the subscutellar hemocoel (Fig. 2a). The wall of the ampulla is muscular and thicker than in other portions of the vessel. No ostia were found in the ampulla. The ampulla is attached to the scutellar cuticle directly along the frontal side (Fig. 4), and by a thin, non-muscular suspending septum on the other sides. Thus, the subscutellar hemocoel is separated from the general thoracic cavity. At the posterior side of the ampulla, the suspending septum forms a median valve through which the subscutellar hemocoel communicates with the thoracic cavity (Fig. 4, inset). In Ecdyonurus sp., a cluster of fat cells constricts the hemolymph pathway at the point where the scutellar arms enter the subscutellar hemocoel. Within the dorsal vessel a circular valve flap is located just anterior to each ampulla (Fig. 4).

In Caenis sp., the hindwings are completely reduced and neither a scutellum nor an ampullary vessel formation is present in the metathorax.

2. Odonata

The scutellum is located near the middle of each winged segment. It is relatively small and circular with markedly projecting scutellar arms originating at the posterior sides (Fig. 5). The dorsal vessel runs through the thorax at some distance from the tergal cuticle and exhibits a dorsal diverticule in the meso- and metathorax (Fig. 2b). The ending of each diverticule forms a small swelling which extends into the subscutellar hemocoel (Fig. 6). It is attached directly to the basal scutellar ridge and to the dorsal cuticle by numerous suspending strands. The muscular wall of each diverticule ending is considerably thicker than that of other parts of the dorsal vessel and bears a pair of slit-shaped ostia with two-tipped valves (Fig. 6).

3. Plecoptera

The scutellum is transversely arched, but not distinctly elevated longitudinally. The scutellar arms originate at the posterior end of the scutellum (Fig. 7). The dorsal vessel runs just beneath the tergal cuticle. It is attached to the tergal cuticle both anterior and posterior to the scutellum (Fig. 2c). Beneath the scutellum, the dorsal vessel is enlarged laterally. Its dorsal side is distinctly thickened compared to that of other regions in the thorax (Figs. 8-10). A non-porous suspending septum extends laterally from the dorsal side of the vessel to the basal ridge of the scutellum (Fig. 8). For a short distance, however, the septum is not attached to the cuticle and forms a lateral slit-opening on each side where the subscutellar hemocoel becomes confluent with the thoracic cavity (Fig. 9). Inside the subscutellar hemocoel a median membranous back-flow valve exists at the points of confluence with the scutellar arms (Figs. 8, 10). An unpaired excurrent opening exists in all species on the ventral side of the dorsal vessel anterior to the enlargement in the metathorax (Fig. 2c).
Fig. 2. Wing circulatory organs of selected species, showing reconstructions of mesothorax and metathorax (sagittal view). Arrows indicate flow of hemolymph: a: Dorsal vessel close to tergal cuticle with ampullary formations arching into each subscutellar hemocoel; hemolymph flow through median septum valve from subscutellar hemocoel into thoracic cavity. b: Dorsal vessel with diverticules bearing incipient ostia which open into the subscutellar hemocoels. c: Dorsal vessel close to tergal cuticle, paired incipient ostia and unpaired excurrent ostium only in mesothorax; hemolymph flow from subscutellar hemocoel into vessel and through lateral septum sits into thoracic cavity. d: Dorsal vessel enlargement close to tergal cuticle, paired incipient and excurrent ostia in mesothorax and metathorax. e: Centrally running dorsal vessel with a diverticule extending into each subscutellar hemocoel bearing incipient ostia. f: Pulsatile dilatations below the mesoscutellum and metascutellum are separate from dorsal vessel, incipient ostia only in metathorax.

Fig. 3. Habroleptoides confusa (Ephemeroptera). Dorsal view of posterior mesothorax and metathorax. The scutellum is the median tergal elevation, its posterior margin forms the scutellar arms.

Fig. 4. Ecdyonurus sp. (Ephemeroptera). Metascutellum and dorsal vessel in sagittal section. Ampullary formation of dorsal vessel arches forward into the subscutellar hemocoel; circular valve flap anterior to ampulla. Inset contains enlargement of bordered area, but from another section, in order to show the median septum valve between subscutellar hemocoel and thoracic cavity.

Fig. 5. Platycentrus penipes (Odonata). Dorsal view of metathorax. Scutellar arms arise from posterior edges of the small scutellum.

Fig. 6. Ischnura elegans (Odonata). Sagittal section through metathorax. Dorsal vessel diverticule with invaginated incipient ostia extends into the subscutellar hemocoel.

Fig. 7. Leuctra sp. (Plecoptera). Dorsal view of mesothorax and metathorax. Posterior end of the scutellum connected by scutellar arms and armerculated axillary cords with posterior wing veins.

10 Zoology 98 (1994)
Fig. 8. *Dichogenus fontium* (Plecoptera). Cross section through posterior region of mesoscutellum. Scutellar arm (longitudinally cut) is confluent with axillary cord. Subscutellar hemocoel separated from thoracic cavity by dorsal side of the vessel and lateral suspending septum. Membranous back-flow valve within subscutellar hemocoel.

Fig. 9. *Dichogenus fontium* (Plecoptera). Cross section through anterior region of mesoscutellum (right half). Suspens- ding septum is not fixed to cuticle and forms a lateral septum sit between subscutellar hemocoel and thoracic cavity.

Fig. 10. *Dichogenus fontium* (Plecoptera). Sagittal section through metascutellum and dorsal vessel. Vessel enlargement with thickened dorsal wall without incurrent cells. Membranous back-flow valve in the subscutellar hemocoel.
In *Leucia* sp., a pair of small, incurved ostia is present in the dorsoventral wall which connects the dorsal vessel directly with the subcuticular hemocoel, although lateral slit-openings of the septum were also found in this species (Fig. 2c). The incurved ostia were detected unambiguously only in the mesothorax.

4. Dictyoptera (Blattodea, Isoperta, Mantodea)

The formation of the scutellum differs among the investigated species. In most of the lepisma *Stenide* sp., the tergal cuticle of the meso- and metathorax is flat and no scutellum is detectable. However, the posterior margin of the scutum is elevated at both sides and forms voluminous scutellar arms which run to the posterior wing bases. In Blattodea, as well, the tergal cuticle is rather flat, yet in cross-section the posterior regions of the meso- and metathorax are slightly elevated in the middle (Figs. 11, 12). In family *Mantis religiosa*, however, a distinct shield-shaped scutelum is recognizable in the meso- and metathorax (Fig. 13).

Suspending septa of the dorsal vessel are present in all Dictyoptera studied. If the scutellum is well-developed, the septum is extended in the horizontal plane from the lateral sides of the dorsal vessel to the tergal cuticle. The septum in *Stenide* sp., which lacks a scutellum, runs in a nearly vertical direction toward the tergal cuticle. The dorsal vessel is enlarged (Fig. 13) and bears: an incipient and incipient ostia in the posterior region of each segment (Fig. 2d). The incipient ostia lie on the lateral sides of the vessel at an obtuse angle. They open into the hemolymph spaces above and below the suspending septum (Fig. 12). Thus, there is no strict separation between the subtergital hemolymph space and the subcuticular cavity above the dorsal vasculature. The incipient ostia are especially thick dorsally in the region of the ostia. The dorsal diaphragm is always found beneath the vessel (Figs. 12, 13).

A median membranaceous valve can be found within the posterior region of the subcuticular hemocoel in *Periplaneta americana*, *Ectobius* sylvestris, and *Mantis religiosa*. Similar to the valves of Plecoptera, they are situated in front of the confluence of the scutellar arms.

5. Grylloblattodea

In the wings of *Grylloblatta campodeiformis*, the form of the tergal cuticle and the arrangement of the dorsal vessel does not differ greatly among the thoracic and abdominal segments. The tergal cuticle in the meso- and metathorax lacks both a scutellum and scutellar arms. The dorsal vessel runs just beneath the cuticle. Near the middle of the segments it bears a pair of incumbent ostia in a lateral position. The wall of the dorsal vessel always has the same thickness in cross section. The dorsal diaphragm lies below the vessel.

6. Dermaptera

The mesoscutellum and metascutellum differ in size and shape in both investigated species. The tergal elevation is less prominent in the metathorax, but the scutellar arms are distinct tubes as in the mesothorax. The dorsal vessel runs near the cuticle and is enlarged beneath each scutellum. Its dorsal side is markedly thicker in this region. It is attached laterally to the scutellum by the suspending septum and dorsally by many thin strands (Fig. 14). Beneath the subcuticular hemocoel it bears a pair of incumbent ostia in the dorsal position (Fig. 14). A median back-flow valve exists where the scutellar arms join at the posterior end of the subcuticular hemocoel. Below the dorsal vessel lies the dorsal diaphragm in addition to the suspending septum.

7. Orthoptera

In all winged segments of *Orthoptera*, prominent scutellum and lateral scutellar arms are present (Fig. 15). The dorsal vessel lies just beneath the tergal cuticle. It is directly attached to the posterior and anterior end of the scutellum. The subcuticular hemocoel is separated by the enlarged dorsal vessel together with its suspending septum, which extends laterally from the dorsal vessel to the basal margin of the scutellum. The dorsal vessel has a triangular shape in cross section beneath the scutellum. The thick dorsal wall is fixed to the cuticle by many thin vertical suspending strands (Fig. 16). In this enlarged region, the dorsal vessel bears a pair of invaginated incumbent ostia (Figs. 17, 18). Anterior to them is a pair of lateral excurrent openings.

In the investigated Ensifera, the incumbent ostia are situated below the posterior end of the scutellum close to the lateral excurrent openings. The incumbent ostia are obliquely as slit-like invaginations from the dorsal side of the dorsal vessel to the lateral side. They open dorsally into the subcuticular hemocoel and laterally — beneath the horizontal suspending septa — into a kind of atriun which is formed by thin tissue structures originating from the dorsal diaphragm (Fig. 17).

In Diastatidae, the incumbent ostia are more dorsal in position than in Ensifera. The incumbent ostia
connect only the subscutellar hemocoel with the dorsal vessel (Fig. 1A).

In most species delicate flap-like structures are found within the subscutellar hemocoel where the scutellar arms enter (Fig. 1B). They are arranged in a way to permit only inflow of hemolymph from the scutellar arms (Fig. 20).

Among brachypterous species (Chortippus paralellos, Cryopihrochaeta dispar, Meteoptera wessell), no essential differences in structure are found when compared to fully-winged species. An enlarged region of the dorsal vessel with a thickened dorsal side of the vessel is present and bears the incipient ostia (Fig. 1C). In a completely apterous species (Micrella aegina), as well as in species with only one pair of wings (Leptophyes punctatissima, Prolleptera griseoclectera, Terex sp.), the scutellum, an enlargement of the dorsal vessel, and the thickened dorsal side of the vessel are lacking in the wingless segments.

8. Phasmatoidea

In the short-winged females of Extatosoma tiareum the tergal cuticle in the meso- and meta thorax is only slightly elevated in the posterior region. However, stouter orms exist at the posterior margins of
Fig. 15. Metacoptera roeseli (Ensiferata). Dorsal view of mesothorax and metathorax with scutellum and prominent scutellar arm in both segments.

Fig. 16. Metacoptera roeseli (Ensiferata). Cross section of the mesoscutellum. Enlarged dorsal vessel and lateral suspending septum separate the subscutellar hemocoel from thoracic cavity. Wall of dorsal vessel distinctly thickened and fused to the scutellum cuticle by suspending strands.

Fig. 17. Lepidoptera pulcherrissima (Ensiferata). Cross section of dorsal region of brachypterous mesothorax. Dorsal vessel bears lateral incipient ostia which open into the atrium formed by membranous tissue (marked by asterisk); thickened dorsal wall restricted to the area between ostia.

Fig. 18. Cockyera citrinula (Caelifera). Cross section through dorsal vessel enlargement below mesoscutellum. Incipient ostia dorso-ventrally positioned, thickened dorsal wall with attached suspending strands.

Fig. 19. Pheropoda falcata (Ensiferata). Cross section through the posterior margin of mesothorax showing connection to right scutellar arm.

Fig. 20. Pheropoda falcata (Ensiferata). Enlargement of bordered area in fig. 19. A back-flow valve is located at the site where the scutellar arms enter the subscutellar hemocoel. Arrow head indicates passing hemocytes.
the tergal cuticle. The dorsal vessel in this region is distinctly enlarged and attached to the dorsal cuticle by a horizontal suspending septum. A pair of incurved ostia open into the small hemocoel above. If the apterous {Baculum sp.}, the tergal cuticle is continuously arched and no scutellum or scutellar arms are discernible. The dorsal vessel has no ostia in the thorax.

9. Embioptera

In winged males of {Oligotoma nigra}, the scutellum is rather flat and clearly discernible only in the mesothorax. In the meso- and metathorax, each scutellum has a distinct tube from the posterior end of the scutellum to the wing base which lies unusually far forward. The scutellar arms contain valve flaps which together form a median back-flow valve at the opening into the subscutellar hemocoel. The thick muscular dorsal side of the vessel extends horizontally on both sides to the scutellum cuticle. The dorsal vessel is depressed in cross section and bears a pair of small incurved ostia in the dorsal wall. A single ventral excurrent opening is present in the middle of the segments.

10. Pscoptera

In {Graphopscopus cruciatus}, the scutellum is round-shaped in both winged segments. The scutellar arms originate from the posterior-lateral sides (Fig. 21). In the meso- and metathorax the dorsal vessel runs below the dorsal longitudinal flight muscles. In the posterior half of each winged segments, it shows a long diverticulus which extends between the flight muscles dorsally into the subscutellar hemocoel (Fig. 22). Each diverticulus terminates in a bladder-shaped enlargement bearing a pair of incurved ostia (Fig. 22). This vessel formation is attached to the scutellum by a horizontal suspending septum and thin vertical stands. Membranous flaps are discernible on both sides where the scutellar arms enter the subscutellar hemocoel.

11. Thysanoptera

The scutellum and scutellar arms are absent in both of the two investigated fully-winged species. The dorsal vessel runs straight through the thorax and shows no specific modifications in the meso- and metathorax.

12. Hemiptera

Among the investigated Hemiptera, differences were found in the shape of the scutellum and the course of the dorsal vessel through the thorax. In all species, however, the pulsatile apparatus of each wing circulatory organ is composed of a pulsatile diaphragm associated with the scutellum. In contrast with insects described above, no connection exists between the dorsal vessel and the wing circulatory organs (Fig. 26). The pulsatile diaphragm consists of transversely extended muscle fibres which are attached laterally to the scutellum (Fig. 24). In addition, this muscular plate is suspended to the scutellum by numerous thin strands.

12.1. Heteroptera: The mesoscutellum is significantly elongated caudally and completely overlaps the small metathorax (Fig. 25). The metascutellum is only slightly elevated but occupies the middle of the tergal cuticle of this small segment (Fig. 25). The scutellar arms of meso- and metascutellum extend as distinctly projecting ridges along the lateral sides of the mesoscutellum to the wing bases (Fig. 23). Where they enter into the subscutellar hemocoel, aided membraneless valves flaps are found in {Alydus calcarius} and {Mimzi striata}.

The dorsal vessel extends straightly through the thorax below the dorsal longitudinal flight muscles. In the mesothorax, the pulsatile diaphragm is situated approximately in the middle of the subscutellar hemocoel where it assumes a nearly vertical position (Fig. 25). Openings are found on the dorsal and ventral margin of the pulsatile diaphragm. The dorsal margin merges into a thin membrane and appears as a widen opening (Figs. 24, 25). In the metathorax, the diaphragm is attached to the cuticle on the ventral side (Fig. 25).

In {Nepa cinerea}, the entire thorax is depressed and the dorsal vessel runs just beneath the tergal cuticle. Yet, as in other Heteroptera, no connection exists between the dorsal vessel and the scutellum or the pulsatile diaphragm. In wingless specimens of {Germis sp.}, no pulsatile diaphragm could be found beneath the fat tergal cuticle.

12.2. Homoptera: In {Cercopis sanguinolenta}, the mesoscutellum is triangular in dorsal view and overlaps the metascutellum. The dorsal vessel runs at some distance to the tergal cuticle. A pair of small lateral ostia is found only in the mesothorax (Fig. 25). In the hemocoel, which is formed by the elongated mesoscutellum, the pulsatile diaphragm is in a vertical position. The subscutellar hemocoel opens at its dorso-arterial side into a small hemolymph space which is formed by vertical membranous septa above the dorsal longitudinal flight muscles. The scutellar arms contain membranous valve flaps near the scutellum. In {Cicadetta montana}, the dorsal vessel runs close to the tergal cuticle in both winged segments. It passes around the very large posterior meso-
Fig. 21. Graphopsocus cruciatus (Pauroporida). Dorsal view of mesothorax and metathorax. The scutellar arms rise laterally from the round scutellum and lead into the axillary cords.

Fig. 22. Graphopsocus cruciatus (Pauroporida). Cross section of mesoscutellum. Vessel diverticle with ampulla-shaped enlargement bearing incurrent cilia attached to the scutellum base by the suspending septum.

Fig. 23. Miles affinis (Heteroptera). Dorsal view of the extremely elongated mesoscutellum which completely overlaps the metasternum. Scutellar arms of both winged segments run along the lateral side of the body in anterior direction to the posterior margin of the wing base.

Zoology 99 (1994) 1
In the above mentioned sense would refer to different portions of the dorsal vessel in the various insects.

1. Functional morphology

In general, the accessory circulatory organs of insect wings can be seen as functional units consisting of (a) a pump casing with supply tubes, and (b) an associated pulsatile apparatus.

a) Pump casing and supply tubes

In all investigated species, the scutellum constitutes the pump casing. The scutellar arms form the supply tubes through which the small subscutellar hemocoel is connected to the lumina of the posterior wing veins. The scutellar arms are lateral extensions of the scutellum cuticle folded up to form a closed tube separate from the general thoracic cavity (Brochier 1916, 1919, Wheldon 1936, Amold 1964, Bayer 1968, Krenn and Pass 1993).

So far, little has been published about the functional significance of the scutellum. Some authors assumed that it functions to support the pronotum or the folded wings (Matsuda 1970, La Grece 1987).

However, since the scutellum occurs in all winged insects and is always associated with the wing circulatory organs, regardless of different resting positions of the wings or pronotum size, it is suggested here that the primary function of the scutellum is to serve as a pump casing of these accessory pulsatile organs (Krenn 1993a).

b) Pulsatile apparatus

The pulsatile structures of the wing circulatory organs are composed either of specialized formations of the dorsal vessel or of pulsating muscular diaphragms which are separate from the dorsal vessel.

Dorsal vessel formations: In most investigated species a specialized region of the dorsal vessel...
constitutes the pulsatile apparatus of the wing circulatory organs. In those cases, where the dorsal ves-
sel runs just beneath the tergal cuticle, the dorsal side of the ampulla-like vessel formation are associated with the scutellum.

In Odonata and Psocoptera, where the dorsal ves-
sel runs a more central course through the thorax, long diverticules are present in the meso- and meta-
thorax.

In general, these vessel formations are equipped with a pair of diverticules that connect the sub-
scutellar hemocoele with the lumen of the dorsal vessel. We confirm earlier reports that the Epimeraoptera
do not have diverticules in the thoracic portion of the dorsal vessel (Meyer 1931). Instead, a median septum valve is present which connects the sub-
scutellar hemocoele with the thoracic cavity. In Ple-
coptera, the situation varies among the investigated species. Schwermer (1914) described vessel swell-
ings without ostia in Penta marginata whereas no in-
current ostia were found in Pleurostictus californicus for each pleuroterothoracic segment (Nutting 1951). The latter could be verified in the present study only for Leuctra sp. Overlooked so far, however, are lateral septum slits which could be demonstrated for all in-
vestigated plecopteran species. They connect the subscutellar hemocoele directly with the thoracic cavity lateral to the dorsal vessel.

A feature common to all vessel formations is the thick dorsal muscular side. Ultrastructural investiga-
tions of some Odonata (Jennew 1976; Bhulai- 
karikar 1985) and Locusta migratoria (Krenn 1993b), re-
vealed that the muscle fibers in this region do not differ essentially from the rest of the dorsal vessel. These formations are attached by lateral suspending septa to the basal ridge of the domed scutellum whereby the subscutellar hemocoele is separated from the hemolymph. In Locusta migratoria, it was shown that the suspending septum consists of connective tissue only and contains no muscle fi-
bers (Krenn 1993b). Despite similarities with the
dorsal diaphragm, it is obvious that these suspending septa represents a separate structure since both can be found in the same section in many species. An additional attachment of these dorsal vessel enlargements to the scutellum cuticle is provided by the numerous vertically extended suspending strands. Such strands are common attachment struc-
tures of the dorsal vessel and are not restricted to the thorax (Crossley 1972, Lockie and Hue 1972, Jennew 1976). Ultrastructural investigations revel-
ed that these suspending strands consist of a finely filamentous matrix (Krenn and Pass 1993, Krenn 1993b) and hence they resemble elastic connective tissue (Ashburner 1986).

Pulsatile diaphragms: In Hemiptera, the pula-
satile apparatus is structurally different from all other investigated non-Holometabola. A small muscular
suspending strands and the suspending septum. The local effects of the elevation in the thoracic pressure forces haemolymph from the subcuticular hemocoel either through incipient ostia into the vessel lumen or through slit-openings directly into the thoracic cavity. Remarkably, measurements of the hemolymph pressure at different places of the body in Locusta migratoria, revealed the lowest pressure beneath the scutellum (Bayer 1968). The afferent flow from wings to thorax is ensured by back-flow valves, which could be demonstrated in a number of species. They comprise a number of structures within the subcuticular hemocoel or pained valve flaps in the lumen of the scutellar arms. In Ephemeropera and Acallophonatidae fat cell structures probably fulfill this function.

Although differences exist in the anatomy of the circulatory pumps, all accessory wing circulatory organs suck hemolymph from the posterior wing veins into the thorax. This function is closely linked to the access of ascarid and aphanopod nematodes. The cecus-heart in steniferflies which also sucks hemolymph out from these long abdominal aepandiges (Pass 1967). The antenna-hearts pump the hemolymph in the opposite direction from the cranial cavity into the antennae (Pawlowa 1895, Pass 1985, 1991). The leg-hearts of the Hemiptera lie within the legs, and the current flow in two hemolymph spaces separated by a thin diaphragm (De Riese 1868, Mantshou 1991).

d) Lack of wing circulatory organs

Nearly all insects with well-developed wings or at least wing rudiments have accessory wing circulatory organs in the thorax. Exceptions to this are the insects of the order Thysanoptera, whose lack of wing circulatory organs in these insects may be due to the small wing size and highly modified wing venation. In aphids, only a weak hemolymph circulation without regular pathways in the wing veins has been observed (Arnold 1964).

The secondary loss of wings is correlated with the absence of typical components of the wing circulatory organs in all investigated wingless species (Gryllloblattidae, Mirametina, Bacterium sp., Genetic sp.). A hemolymph space is not present above the dorsal vessel, and neither a scutellum nor scutellar arms are found in these insects. Furthermore, the enlargement of the dorsal vessel is absent and the vessel wall has the same thickness around the entire cross section as already noted by Nutino (1851). Also, in the secondarily apterous Phthiraptera, the dorsal vessel is described as a straight tube bearing no special characters in the thorax (Seguy 1951). In principle, this holds true also for the ontogeny. In early instars of Odonata and Locusta migratoria, wings which are still very small and veinless, the typical characters of a wing circulatory organs are absent (Wieden 1938, Kissin 1958).

2. Phylogenetic analysis

The constructional diversity of accessory wing circulatory organs permits the reconstruction of some of their phylogenetic pathways and requires consideration of their modification in correspondence with the evolution of the insect thorax.

a) Character judgement

The plesiomorphic condition of the circulatory organs in the thorax can be reconstructed by outgroup comparison with primarily aphygnotic insects. In Archaeognatha and Zygentoma, the dorsal vessel is uniform from the head to the end of the abdomen. It runs dorsally, attached to the tergal cuticle by thin suspending strands. Dorsal vessel enlargements bear a pair of dorso-lateral incipient ostia in the meso- and metathoracic and all abdominal segments (Biegen 1915, Rehmsmann 1961). No scutellar arms are present or scutellar arms were described for the thoracic segments of these insects (Matuda 1970).

In nearly all Pherygota, however, the scutellum and scutellar arms are found in winged segments. These circulatory structures are therefore regarded as a synapomorphy of Pherygota and must have evolved simultaneously with the wings (Kissin 1958a). In secondarily aphygnotic insects, the lack of a distinct scutellar elevation as well as scutellar arms is regarded a derived feature shared among various non-related taxa. Likewise, the absence of a distinct scutellum in Isoptera and Thysanoptera must be considered as a derived state with regard to the phylogenetic position of these taxa.

In the plesiomorphic state of the wing circulatory organs, the dorsal vessel is attached to the tergal cuticle by the suspending strands, and the incipient ostia are situated in segmental vessel enlargements which connect to the subcuticular hemocoel. Derived character states include the lack of ostia, a centrally running dorsal vessel, ampulla-like formations or diverticules, and, of course, the accessory pulsatilie diaphragms.

b) Phylogenetic pathways and constraints

The principle construction of the wing circulatory organs varies in the investigated insects only at the
level of insect orders. Therefore, the following analysis focuses on comparing the various characters of these accessory pulsatile organs using a widely accepted cladogram based in KRIENEN (1981, 1991) (Fig. 27). From a phylogenetic viewpoint, it is noteworthy that neither the Ephemeroptera nor the Odonata show the pleiomorphic condition in their wing circulatory organs. We found no synapomorphic character uniting either of these two taxa with the Neoptera. Thus, we are unable to contribute to the controversy dealing with the relatedness of these groups (cf. HENN 1969, KRIENEN 1981, BOUDREUX 1987). The wing circulatory organs of the Paurometabola, however, have a high number of pleiomorphic traits. Apparently, the key evolutionary innovations include development of the scutellum and scutellar arms, as well as the close association of the dorsal vessel to these cuticular structures. A further essential step was the complete separation of the scutellar hemocoele from the thoracic cavity by the suspending septum which is found in all insects aside from certain Dictyoptera.

In connection with this, a shift of the recurrent ostia from a lateral to a more dorsal position occurred resulting in a restriction of the inflow of hemolymph from the scutellar hemocoele and posterior wing veins. The lack of recurrent ostia in the thoracic portion of the dorsal vessel in Ephemeroptera, Plecoptera, Phasmatodea, Heteroptera, and Thysanoptera is considered a secondary loss. The recurrent ostia of the dorsal vessel in Paurometabola are probably not of functional importance for the wing circulatory organs. Clear autapomorphic traits are the median septum valve in Ephemeroptera and the lateral septum slits in Plecoptera. Without any doubt, apomorphic characters are the pulsatile diaphragms of Hemiptera. Whether they have the same phylogenetic origin as those described in Holometabola (FREDENBERG 1928, SELMAN 1966, KRIENEN and PASS 1993) is beyond the scope of this study. The ultrastructure of the pulsatile diaphragms in some Holometabola suggests that they originated from the dorsal vessel; this is further supported by species with transitional character states of the wing circulatory organs (KRIENEN and PASS 1992, unpublished data).

It is concluded that the common winged ancestors of the Pterygota already possessed wing circulatory organs made of the scutellum and the
slightly modified dorsal vessel. The fundamental modifications of the thorax during the evolution of the flight motor (review: MATSUIA 1970) appear to be of greater importance in explaining the diversity of wing circulatory organs than the differences in wing size and venation patterns. For example, as a consequence of a dorso-ventral extension of the winged segments, the dorsal vessel may take a more direct course at some distance from the tergal article, resulting in spatial constraints for long diverticula or the complete detachment of the dorsal vessel from the cuticle. In particular, the development of the pulsatile diverticula in Hemiptera might be due to the evolution of especially large phragmata which serve as attachment sites for the power-producing indirect wing depressors (GALENI 1974, KAMMER 1985). Detailed morphometric investigations are in preparation which will evaluate the evolutionary changes of the flight apparatus and how these changes correspond to the anatomy of the wing circulatory organs.

Acknowledgements

We are very grateful to A. HANTSCHE and G. ROTH for their excellent technical assistance, H. GLITTSCH, A. TOLEDER and C. EWIN for help with the illustrations, B. GERBER for critically reading the manuscript, and J. PLANT for correcting the English. Thanks also to a number of colleagues for providing determined specimens. This study was supported by the Austrian Science Foundation (Project 8022-Sio).

References

CROSSEY LC (1972): The ultrastructure and function of pericardial cells and other nephrocytes in an insect, Callopilla erythropoeca. Tissue & Cell 4: 529–560
KELLER S (1963): Entomologisches Wörterbuch. 3rd edition, Akademia Verlag, Berlin

Zoology 98 (1994) 1 21