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The Insect Circulatory System: Structure, Function, and Evolution

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Abstract

Although the insect circulatory system is involved in a multitude of vital physiological processes, it has gone grossly understudied. This review highlights this critical physiological system by detailing the structure and function of the circulatory organs, including the dorsal heart and the accessory pulsatile organs that supply hemolymph to the appendages. It also emphasizes how the circulatory system develops and ages and how, by means of reflex bleeding and functional integration with the immune system, it supports mechanisms for defense against predators and microbial invaders, respectively. Beyond that, this review details evolutionary trends and novelties associated with this system, as well as the ways in which this system also plays critical roles in thermoregulation and tracheal ventilation in high-performance fliers. Finally, this review highlights how novel discoveries could be harnessed for the control of vector-borne diseases and for translational medicine, and it details principal knowledge gaps that necessitate further investigation.

1. INTRODUCTION

The insect circulatory system is at the center of most physiological processes. It delivers nutrients and hormones to cells and removes waste (18, 68, 74). The circulatory system also coordinates defense mechanisms, modulates heat transfer, assists in gas exchange, facilitates ecdysis, maintains homeostasis, and more (**Figure 1**) (18, 54, 57, 68, 162). In its most basic sense, the circulatory system is composed of a fluid medium called hemolymph, a body cavity called the hemocoel, and a series of muscular pumps (99, 103, 118, 121, 173). The main driver of hemolymph circulation in the central body cavity is the dorsal vessel, which is usually divided into an aorta in the thorax and a heart in the abdomen. Peripheral circulation in the appendages, however, is driven by autonomous pumps known as accessory pulsatile organs, or auxiliary hearts. Although insects have an open circulatory system, hemolymph does not diffuse freely throughout the hemocoel, and instead flows along distinct channel-like routes that are created by the structural organization of the internal organs and by fibromuscular septa or diaphragms.

Despite the importance of this physiological system, our knowledge of how it works remains meager. This was recently noted in an insect physiology textbook, where the authors wrote, "in our view, the circulatory system of insects is the most understudied of insect physiological systems, and it seems likely that further research will bring many important advances in ecological and environmental physiology" (51, p. 46). This article seeks to bring attention to this grossly understudied physiological system by reviewing its structure, its function, and how it integrates with other systems and by highlighting areas that are ripe for investigation.
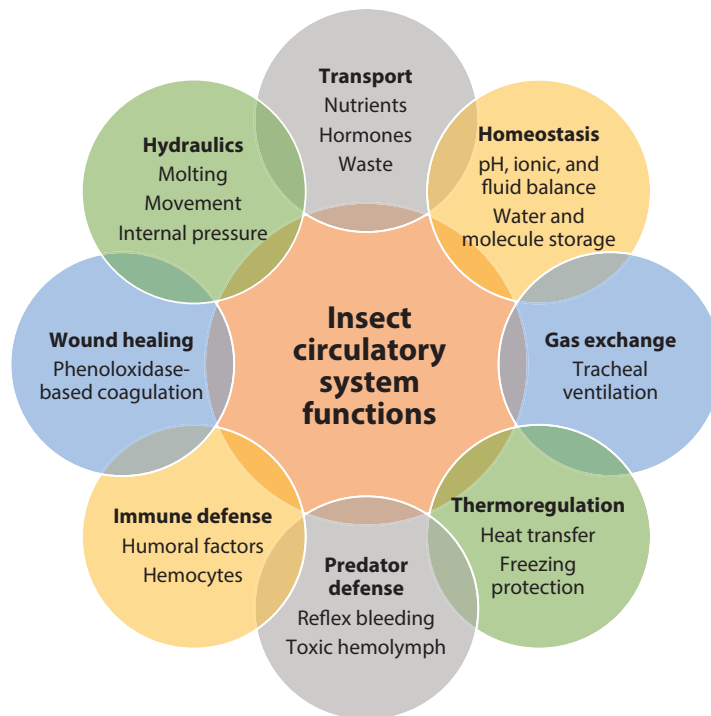

Figure 1

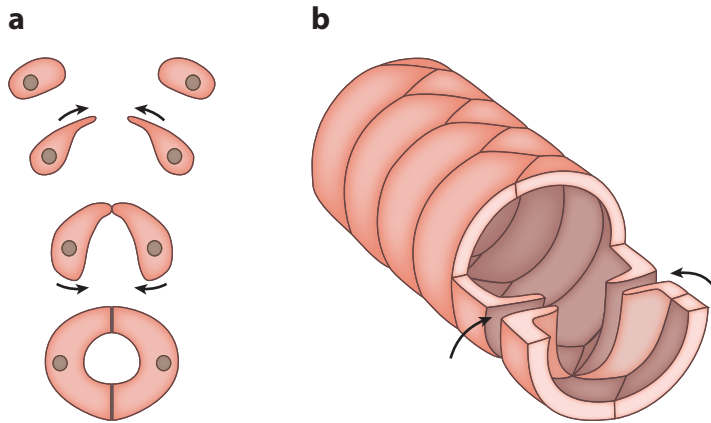The many functions of the circulatory system of insects.

Figure 2

Development and structure of the dorsal vessel. (*a*) Consecutive stages of morphogenesis, from top to bottom. Cardioblasts become polarized and extend leading processes toward the dorsal midline until they meet their contralateral counterparts. The trailing edges of the cardioblasts then extend posteriorly and medially and contact each other, thereby forming the lumen of the dorsal vessel. (*b*) The mature dorsal vessel consists of two rows of semicircular cardiomyocytes. Their staggering and contact points in the dorsal and ventral median line result in a spiral-like arrangement. The anterior front cut shows the organization of the ostial cells; the two inward directed flaps act together as a valve that allows hemolymph to enter into the vessel but hinders outward flow.

2. FUNCTIONAL MORPHOLOGY OF CIRCULATORY ORGANS

2.1. The Dorsal Vessel

The dorsal vessel is a cylindrical structure that extends the entire length of the insect and is organized by serial repetition of building blocks. Each module is comprised of cardiomyocytes, a pair of alary muscles, and connective tissue. During early embryogenesis, the cardioblasts move to the dorsal midline and form two regular, opposing rows (**Figure 2a**). The cardioblasts then extend processes that meet their contralateral counterparts in the midline, thus forming a cylindrical, spiral arrangement that encloses a luminal space (101). In each segment, a pair of cardioblasts on each side of the body differentiate into specialized cells that form inflow openings called ostia. Usually, each of these ostial cardioblasts develops a flap-like extension that protrudes into the lumen of the dorsal vessel (**Figure 2b**). The ostial cells are indistinguishable from ordinary cardiomyocytes at the ultrastructural level but express a suite of specific marker genes (91, 105, 127). In some insects, other cardioblasts form additional flap-like intracardiac valves or muscular pads that regulate flow within the lumen of the dorsal vessel (84, 121). The development of the dorsal vessel has been extensively studied in *Drosophila* (11); it is probably similar in all hexapods and may even represent the plesiomorphic state of the entire arthropod clade (42).

The body plan ground pattern of the hexapod dorsal vessel is a tube that is uniform in diameter along its length and contains a pair of ostia in each thoracic and abdominal segment. Such a conformation is largely present in most apterygotes, some ancestral pterygotes, and the larvae of many holometabolans (121). A general trend in more derived insects is a regional differentiation of the dorsal vessel based on functional bipartition. The anterior part, known as the aorta, has a narrow luminal diameter and lacks incurrent ostia as well as alary muscles; it is a simple conduit that extends through the thorax and into the head, where the hemolymph pours out into the hemocoel (139). The posterior part, known as the heart, is confined to the abdomen and has a wider luminal
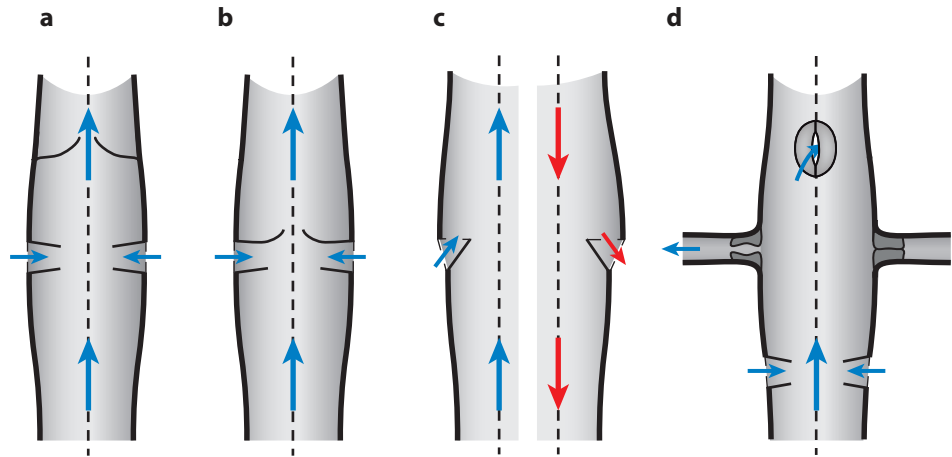
Figure 3

Various kinds of ostia present in the dorsal vessel. (*a*) Incurrent ostia with paired lips and an intracardiac valve. (*b*) Incurrent ostia with elongate lips that function as a pouch valve. (*c*) Two-way ostia with a single lip. (*Left*) Incurrent flow during the anterograde phase. (*Right*) Excurrent flow during the retrograde phase. (*d*) Incurrent ostia (*bottom*), excurrent ostia with sphincter-like valves at the base of lateral vessels (*middle*), and an unpaired excurrent ostium (*top*). The sphincter-like valve on the left is shown in the open position, whereas the valve on the right is shown in the closed position. Figure adapted with permission from Reference 121.

diameter, incurrent ostia, alary muscles, and a much thicker wall; it is the portion that drives flow across the dorsal vessel (41, 163). Specific modifications of the dorsal vessel, as well as a reduction in the number of ostia, are common in both primitive and derived insects (68, 103, 121). In some more ancestral pterygotes, the dorsal vessel also has excurrent ostia, such that hemolymph can exit at distinct points along the length of the vessel (31, 110). Some of these excurrent ostia open into lateral arteries, with the outflow being controlled by muscular valves located at their base (**Figure 3**) (104, 110).

Among the various insects, there are different modes of flow within the dorsal vessel (**Figure 4**). The plesiomorphic condition, as found in most apterygotes and mayflies, is a bidirectional flow whereby a valve located near the posterior of the dorsal vessel dictates the direction of hemolymph propulsion: Hemolymph anterior to the valve is propelled toward the head—called anterograde flow—and hemolymph posterior to the valve is propelled toward the posterior terminus of the abdomen and into the long caudal appendages—called retrograde flow (36, 37, 102). In most ancestral pterygotes, the dorsal vessel is posteriorly closed, and the hemolymph flows only in the anterograde direction. However, in many advanced pterygotes, such as Lepidoptera, Coleoptera, and Diptera, the peristaltic waves of the dorsal vessel periodically change direction, a phenomenon known as heartbeat reversal (41, 142, 161). The periods of anterograde and retrograde contractions are usually interrupted by a short stop and may have different duration and contraction rates (41, 163). The retrograde flow leaves the dorsal vessel either via excurrent openings at the posterior end of the heart or via two-way ostia, whereas the anterograde flow leaves the vessel through an excurrent opening in the head (**Figures 4c** and **5a**; **Supplemental Videos 1** and **2**). Although the adults of advanced pterygotes feature heartbeat reversals, their larvae may feature only anterograde flow (87). Heartbeat reversal is likely widespread among holometabolan insects, but the reconstruction of the evolutionary history of this phenomenon requires further investigation (38, 68).
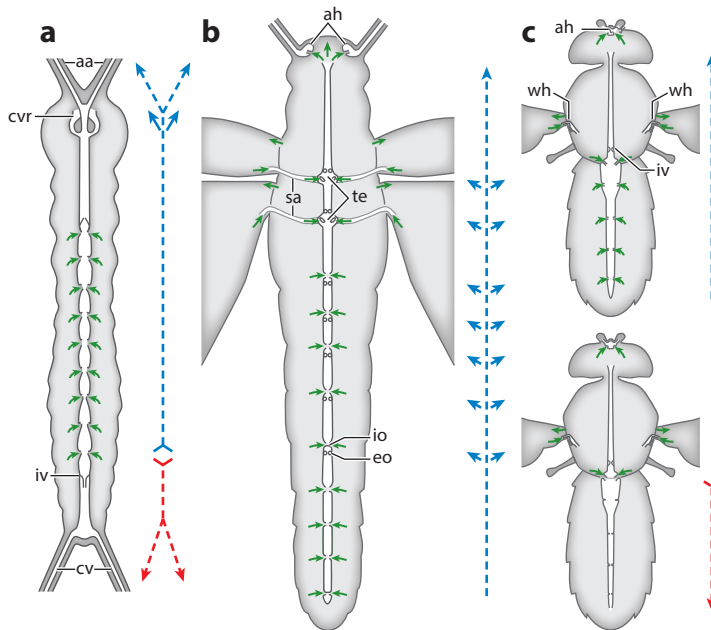
Figure 4

Different types of circulatory organs and flow modes in insects. Green arrows show inflow into the dorsal vessel, and the dashed blue and red arrows beside each insect indicate intracardiac anterograde and retrograde flow, respectively. (*a*) *Campodea augens* (Diplura) represents an ancestral condition, with antennal and cercal arteries, a circumesophageal vessel ring in the head, and bidirectional flow in the dorsal vessel. Panel adapted with permission from Reference 36. (*b*) *Schistocerca shoshone* (Orthoptera) represents the condition in many basal pterygotes, with anterograde flow in the dorsal vessel and numerous paired incurrent and excurrent ostia. In the mesothorax and metathorax, the dorsal vessel has ampullary enlargements that suck hemolymph from the wing veins through scutellar arms formed of tergal cuticle. Panel created using descriptions in Reference 110. (*c*) *Drosophila melanogaster* (Diptera) represents the derived condition with heartbeat reversals, and thus, the dorsal vessel periodically alternates between contracting anterograde (*top*) and retrograde (*bottom*). Hemolymph is supplied to the antennae and wings by separate pulsatile organs. Panel adapted with permission from Reference 163. Abbreviations: aa, antennal arteries; ah, antennal heart; cv, cercal vessel; cvr, circumesophageal vessel ring with ventral opening; eo, excurrent ostium; io, incurrent ostium; iv, intracardiac valve; sa, scutellar arm; te, thoracic enlargement of dorsal vessel; wh, wing heart.

2.2. Diaphragms

Most insect species have two horizontal diaphragms that subdivide the body cavity into three compartments. The largest compartment, called the perivisceral sinus, is located between the dorsal diaphragm and the ventral diaphragm. Above the dorsal diaphragm is the pericardial sinus—which houses the dorsal vessel—and below the ventral diaphragm is the perineural sinus—which houses the ventral nerve cord.

The dorsal diaphragm is a layer of connective tissue and muscle—called alary muscle—that is located immediately ventral to the dorsal vessel. It usually extends the length of the abdomen, and its robustness varies among species and life stages. Typically, the connective tissue is fenestrated, which allows hemolymph to enter the pericardial sinus. The alary muscles maintain the dorsal vessel against the tergum by extending from a focal point at the tergum–pleuron suture and spreading medially until they attach to the dorsal vessel (103, 132). The muscle fibers attach below the heart and connect to fibers from the opposite side of the body or to fibers from adjacent
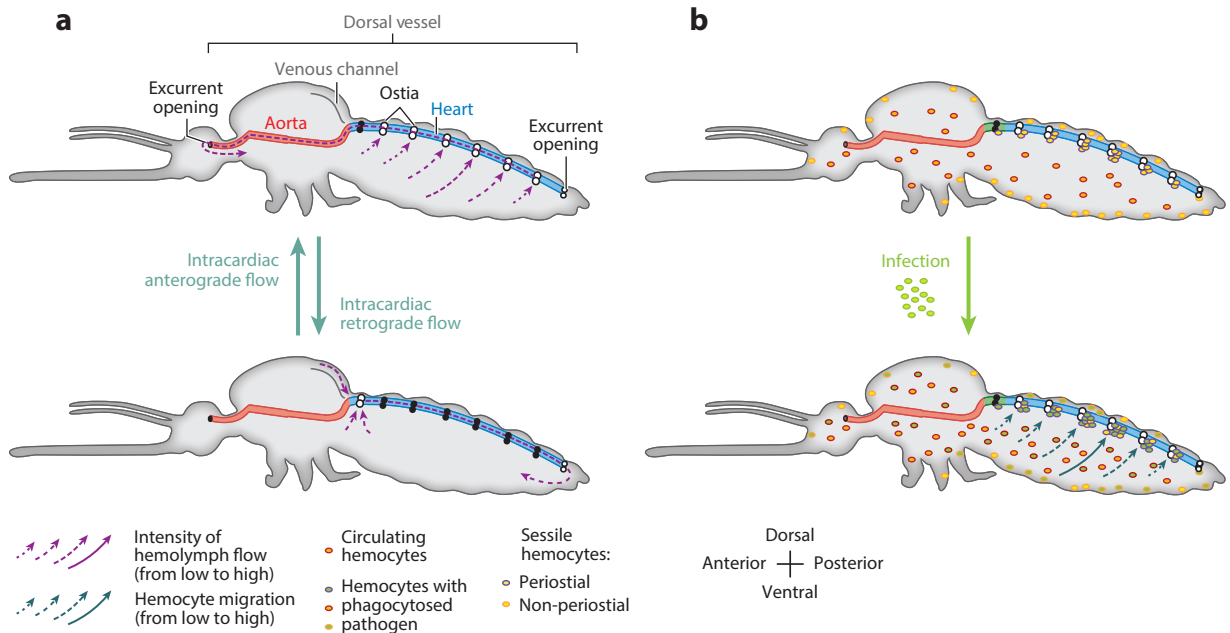
Figure 5

Functional integration of the circulatory and immune systems in the mosquito *Anopheles gambiae*. (*a*) The heart of an adult mosquito periodically alternates between anterograde (*top*) and retrograde (*bottom*) contraction. (*b*) Hemocytes exist in two spatial states: circulating and sessile. In uninfected mosquitoes (*top*), some sessile hemocytes attach to the extracardiac regions of the heart's ostia and are called periostial hemocytes. Upon infection (*bottom*), circulating hemocytes replicate by mitosis and increase in number. Some hemocytes migrate—and attach—to the periostial regions, where they phagocytose pathogens. The majority of periostial hemocytes reside around the ostia that receive the most flow. Figure adapted with permission from References 59 and 136.

abdominal segments (41). Attached to the alary muscles and flanking the heart are pericardial cells—also known as nephrocytes—that filter the hemolymph (72, 93, 170).

The ventral diaphragm of some species consists of connective tissue and bundles of transverse muscles that laterally attach to the sternal cuticle, whereas in other species, it is formed by longitudinal muscle fibers (130, 164). Coordinated contraction of the ventral diaphragm causes undulating movements that reinforce the retrograde flow of extracardiac hemolymph (1).

2.3. Circulation in Body Appendages

Insect appendages are usually hollow, dead-end tubes filled with hemolymph. Because of their narrow openings, the pumping activity of the dorsal vessel does not meaningfully influence the movement of hemolymph into and out of these elongate structures, and diffusion is largely ineffective over such large distances. Therefore, accessory pulsatile organs manage the exchange of hemolymph between the hemocoel and the appendages.

In the head, the antennae are supplied by specialized circulatory organs whose anatomy varies greatly across taxa (114, 117, 118, 121). The plesiomorphic condition, found only in apterygotan Diplura, is made up of antennal arteries that are connected to the dorsal vessel (36). In Pterygota, autonomous pumping organs in the head are separate from the dorsal vessel and supply hemolymph to the antennae (12, 116). These antennal hearts are composed of muscle and basal ampulla to which antennal vessels are connected. Remarkably, the antennal hearts also

function as neurohemal organs that are probably involved in regulating processes in the antenna's sensilla (3, 56, 85, 120, 122, 128). The insect head may house additional auxiliary hearts, such as the hemolymph pump that drives the hydraulic uncoiling of the proboscis of Lepidoptera (77).

Each leg of an insect contains a longitudinal diaphragm of connective tissue that subdivides the leg hemocoel into two sinuses. The sinuses join at the tip of the limb and experience countercurrent hemolymph flow. In Orthoptera and in heteropteran Hemiptera, pumping muscles facilitate hemolymph circulation through the legs (47, 63). In other insects, hemolymph flow appears to be driven by either the periodic pumping of the abdomen (65) or by the alternating deformation of elastic tracheal sacs that extend into the leg (162).

The wings contain sensory structures and other living tissues and must remain constantly hydrated to maintain the flexible, mechanical properties needed for flight (119). Hemolymph is propelled throughout the wings by the contractile action of circulatory pumps located in the thorax (79, 80, 123). Depending on the insect taxon, these organs are either modifications of the dorsal vessel or independent pulsatile diaphragms that attach to the scutellum. In the plesiomorphic condition, circulation through the wing veins is accomplished by the aspiration of hemolymph by auxiliary hearts located in the thorax (19, 78). In Lepidoptera and scarabaeid Coleoptera, in contrast, complex interactions among the dorsal vessel (including heartbeat reversals), accessory pulsatile organs in the thorax, and elastic wing tracheae oscillate hemolymph into and out of the wing veins (160).

Hemolymph is also propelled into the long caudal appendages of the abdomen by circulatory organs. For the paired cerci located in the posterior of the abdomen, the plesiomorphic state is for hemolymph to be delivered via vessels that are connected to the posterior end of the dorsal vessel (36). In more derived insects, these cercal arteries are decoupled from the dorsal vessel and instead either are connected to an autonomous pulsatile apparatus or contain diaphragms that guide the countercurrent flow of hemolymph (115, 121). Finally, the elongated ovipositor of the house cricket—and presumably other insects—has its own auxiliary heart (64).

3. HEART RHYTHMICITY AND ITS CONTROL

The dorsal vessel and accessory pulsatile organs are innervated, but physical or pharmacological disruption of nervous input does not abolish heart contractions (15, 26, 27, 31, 34, 45, 143). For example, the heart continues to contract after it is treated with neurotoxins, and when the heart is cut into several sections, all pieces continue to contract. This indicates a myogenic automatism of the dorsal vessel, which is consistent with the cardiomyocytes exhibiting action potentials with pacemaker characteristics (11). However, endogenous factors modulate heart rhythmicity and directionality (21, 59).

Perhaps the best-studied cardiomodulatory neuropeptide in arthropods is crustacean cardioactive peptide (CCAP). CCAP is cardioacceleratory in many insect orders (28, 31, 34, 89, 95, 158). CCAP increases the velocity of intracardiac hemolymph flow and has a larger effect during anterograde contraction periods, which suggests that it is part of the anterograde pacemaker (28, 34). In addition, FMRFamide, myosuppressins, neuropeptide F (NPF), short neuropeptide F (sNPF), and sulfakinins—collectively referred to as FMRFamide-like peptides (FLPs)—impact heart physiology in varying ways (29, 60, 88, 108, 134, 158). Finally, proctolin and allatotropin increase the heart rate, whereas allatostatins decrease it (31, 95, 152). However, the effect of neuropeptides on circulatory physiology is not restricted to the dorsal vessel; proctolin, some FLPs, and CCAP increase the contraction rate of the antennal hearts, resulting in an increase in hemolymph flow velocity within the antennal space (56, 148).

Several neurotransmitters modulate the heart contraction rate (59). Serotonin and octopamine are cardioacceleratory; glutamate is cardioacceleratory in some insects and has no effect or conflicting effects in others (22, 27, 61, 66, 108, 178). Octopamine may regulate heartbeat reversals, and glutamate has been implicated in the retrograde pacemaker of fruit flies but not mosquitoes (27, 61, 98). However, cardiomodulatory factors exhibit significant functional complexity; the activity of some FLPs, for example, depends on the presence of serotonin, and allatotropin and serotonin synergize to increase the heart contraction rate, but this synergism is abrogated by allatostatin (108, 153, 154). Remarkably, several cardiomodulatory substances colocalize in the same neuron; in the locust, for example, a neuron that innervates the heart contains octopamine, taurine, nitric oxide, and an RFamide-like peptide (15).

Abiotic factors, such as nutritional status and environmental temperature, impact circulatory physiology. As pertains to nutrition, studies on fruit flies have demonstrated that a balanced, low-calorie diet, or time-restricted feeding, improves cardiac performance, whereas diets that are high in sugar or fat induce cardiomyopathies (7, 8, 40, 46, 48, 70, 107). Cardiac dysfunction induced by a high-fat diet persists for two subsequent generations, indicating a transgenerational effect, yet exercise training reduces high-fat diet–induced cardiac aging (46, 171). In cockroach nymphs, food deprivation does not noticeably change heart physiology, but food deprivation in mosquito larvae decreases the heart rate (67, 68). In mosquito adults, however, deprivation of both sucrose and water, but not the deprivation of sucrose alone, reduces the heart contraction rate (33). As for temperature, increased ambient temperature increases the insect heart rate (30, 82, 129), and closely related species with different cold tolerances vary in their ability to maintain cardiac function as the temperature becomes colder (2).

4. MODULATION OF THE HEMOLYMPH PRESSURE IN THE CENTRAL BODY

Hemolymph pressure in the extracardiac hemocoel of insects is low relative to animals with a closed vascular system and can be modulated by the combined forces of abdominal muscles and the pumping of the dorsal vessel (141, 162). From the perspective of hemolymph circulation, swift flow is only possible if different pressure ratios are produced in different parts of the body. To facilitate this, the body of numerous adult insects is separated into an anterior compartment that is comprised of the head plus thorax and a posterior compartment that is comprised of the abdomen (41, 160, 162). This separation is often obvious at the narrow waist between the thorax and abdomen (as in Hymenoptera or Diptera) but is sometimes less discernible because it is formed by the anatomical arrangement of fat body, tracheal sacs, and other internal organs (as in Coleoptera). Due to this spatial constraint, hemolymph can only be exchanged between the anterior and posterior compartments through a narrow extracardiac sinus and the lumen of the dorsal vessel. Thus, coordinated contractions of the abdomen and dorsal vessel, including heartbeat reversals, temporarily increase pressure in one body compartment relative to the other, and this directs the movement of hemolymph (150). In some insects, abdominal contractions are vigorous, whereas in others, they are barely discernible (1, 141, 150). Barely visible compressions occur, for example, in the mosquito abdomen, where the contractions of the ventral diaphragm and intersegmental muscles are coordinated with heartbeat reversals to potentiate extracardiac retrograde flow during periods of anterograde heart contractions (1).

5. METAMORPHOSIS, AGING, AND CARDIOPATHIES

During metamorphosis, the dorsal vessel and associated diaphragms are remodeled, and drastic changes in circulatory physiology often ensue (23, 68, 133, 135). For example, the heart of larvae

contracts exclusively in the anterograde direction, yet during or immediately following metamorphosis, the heart of many insects begins to periodically alternate between contracting in the anterograde and retrograde directions (68, 87). In addition, new organs, such as the wing hearts, may develop during the pupal stage. Remarkably, in *Drosophila*, these organs originate from embryonic precursors of the pericardial cells instead of the cardioblast lineage (123, 151), and the mature muscle resembles adult somatic muscles (92).

Multiple key events during metamorphosis require an active circulatory system. During imaginal ecdysis, the action of the dorsal vessel and the wing hearts results in hydraulic effects that are critical for eclosion and the spreading of the wings (32, 106, 159). Immediately prior to eclosion, the heart beats anterograde at an accelerated rate, which, together with peristaltic contractions of the abdomen, increases hemolymph pressure in the anterior of the body. This facilitates the rupturing of the pupal cuticle, leading to adult emergence and the inflation of the initially folded wings. After wing inflation, the epidermal layer of the wing lamellae undergoes apolysis, the resulting cell fragments are removed by the sucking action of the wing hearts (151), and the dorsal and ventral cuticles are glued together with a matrix produced by special hemocytes in a manner that leaves spaces that become the wing veins (71). After eclosion is completed, heartbeat reversal sets in, thereby balancing hemolymph pressure throughout the body.

As insects age, their heart performance changes, and this can include changes in the proportion of anterograde and retrograde contractions (25, 100, 111, 112). Age-associated changes in cardiac physiology have been extensively studied in *Drosophila*; much of this research has sought to inform on cardiac health in general, given that the core genetic networks and signaling pathways involved in cardiogenesis and heart ageing are conserved among animals (9, 111, 174, 177). The heart rate of adult fruit flies slows with advanced age, and as the insect approaches the end of its lifetime, non-rhythmical contractions—including asystoles and fibrillations—become common (9, 111, 149).

6. INTERPLAY BETWEEN THE CIRCULATORY SYSTEM AND TRACHEAL VENTILATION

Respiratory gases are moved into and out of the insect body by diffusion and convection through a series of chitinized tubes that collectively comprise the tracheal system (50, 145, 162). The convective exchange of gases is primarily driven by abdominal contractions but can be assisted by the action of the dorsal vessel. In the blow fly, for example, heartbeat reversals, along with the coordinated action of an accessory pulsatile organ in the head and small abdominal movements, cause periodic shifts of hemolymph between the anterior body and the abdomen; this, in turn, leads to alternating pressure changes and the compression of tracheae and air sacs (165, 166). Because the frequency of heartbeat reversals is about four times higher during flight than during rest, this so-called cardiogenic inspiration and expiration of the tracheal system is thought to contribute significantly to gas exchange in these high-performance fliers (166, 167). Apart from these events, in vivo synchrotron X-ray imaging has revealed rhythmic collapse and reinflation of tracheal tubes that support ventilation (145). It is assumed that these cyclic compressions are based on very small extracardiac pressure changes—probably corresponding to what have previously been referred to as coelopulses (141)—that are caused by minute abdominal contractions (144). The tracheal spiracles close for extended periods of time during these cyclic compressions, which may facilitate the internal mixing of respiratory gases contained within the long tracheal tubes (169).

7. THE CIRCULATORY SYSTEM AND THERMOREGULATION

Insects are poikilotherms, and as such, changes in environmental temperature result in considerable changes in body temperature. Nevertheless, some insects utilize behavioral or physiological

strategies to alter their body temperature, and some of these strategies rely on the circulatory system. In some cases, insects utilize hemolymph flow to transfer heat from the thorax to the abdomen and vice versa, whereas in others, they utilize hemolymph flow to maintain different temperatures in different regions of the body (53, 54). Some moths and bumblebees, for example, elevate their thoracic temperatures by the shivering of flight muscles prior to or at the onset of flight, yet maintain a lower abdominal temperature. This temperature compartmentalization is accomplished by a countercurrent exchanger whereby heat produced in the thorax is contained within extracardiac hemolymph; as this warm hemolymph flows retrograde and reaches the posterior thorax, it transfers its heat to cooler hemolymph that is traveling anterograde via the dorsal vessel, thus maintaining a warm thorax and a cooler abdomen. However, when the temperature in the thorax rises above a physiologically acceptable level, the heart rate and stroke volume increase, which, together with abdominal contractions, circumvents the countercurrent exchanger and transfers heat between the thorax and abdomen (52). A similar countercurrent phenomenon was recently discovered in kissing bugs, whereby hemolymph flowing anterograde through the dorsal vessel cools ingested vertebrate blood that is moving toward the abdomen (83). In addition to transferring heat, the hemolymph of some insects also provides protection from extremely cold temperatures. Cold tolerance by Antarctic springtails, for example, is conferred by exceptionally high hemolymph osmolality, thermal hysteresis proteins, and glycerol (140).

8. INTERPLAY BETWEEN THE CIRCULATORY SYSTEM AND DEFENSE MECHANISMS

The circulatory system plays an integral role in defense. This role plays out on both organismal and cellular scales: Reflex bleeding protects insects from predators, and the hemocytes—via the functional integration of the immune and circulatory systems—defend insects from microbes that have invaded the hemocoel.

Reflex bleeding is an antipredator defensive behavior that occurs in select members of the orders Plecoptera, Hemiptera, Hymenoptera, and Coleoptera (146). During reflex bleeding, insects increase their internal hydrostatic pressure via a series of muscle contractions, which induces the expulsion of hemolymph via breaches in thin cuticular membranes or through dedicated openings (75, 124, 131, 146). Many insects that undergo reflex bleeding display aposematic coloration (113), and the substances in the reflex bleed—by toxicity, taste, odor, or mechanical means—deter or neutralize predators such as birds or other insects (10, 97, 124, 131). Given the evolutionary distances among insects that engage in this process, reflex bleeding appears to be a case of convergent evolution in which a metabolically costly activity confers protection from predators. However, reflex bleeding is not the only manner in which insects use their hemolymph to ward off predators. Many insects shuttle toxic compounds via their hemolymph and warn potential predators via aposematic coloration, with perhaps the best-known example being the sequestration of cardenolides by monarch butterflies (69, 126).

Within the hemocoel, insects fight infection by mounting vigorous immune responses that are primarily mediated by immune cells called hemocytes (58). Hemocytes dynamically change from circulating with the hemolymph (circulating hemocytes) to attaching to tissues (sessile hemocytes) (5, 14, 96, 136, 138). Some sessile hemocytes attach to the heart, and in certain adult insects, they aggregate in the extracardiac space that surrounds the ostia—called the periostial regions (**Figure 5b**) (24, 39, 62, 72, 136). The activity of periostial hemocytes exemplifies the functional integration of the immune and circulatory systems, whereby hemocytes adhere to the locations of the body that experience the most hemolymph flow, thus increasing the probability of encountering and neutralizing pathogens (57). Periostial hemocytes phagocytose microbes

within seconds of infection, and an infection induces the migration of additional hemocytes to the periostial regions (72, 136). The periostial regions are the only locations in the adult body where hemocytes aggregate, and the majority of hemocytes aggregate around the ostia that receive the most flow (73, 136, 138). The molecular factors that drive this process—such as *Eater*, thioester-containing proteins, and pericardin—are only beginning to be elucidated (17, 76, 137, 175). There is also a link between myotropic factors and hemocytes: Hemocytes are major producers of the cardiomodulatory free radical nitric oxide, and allatotropin enhances phagocytic activity and melanization potential in the hemocoel (15, 24, 55).
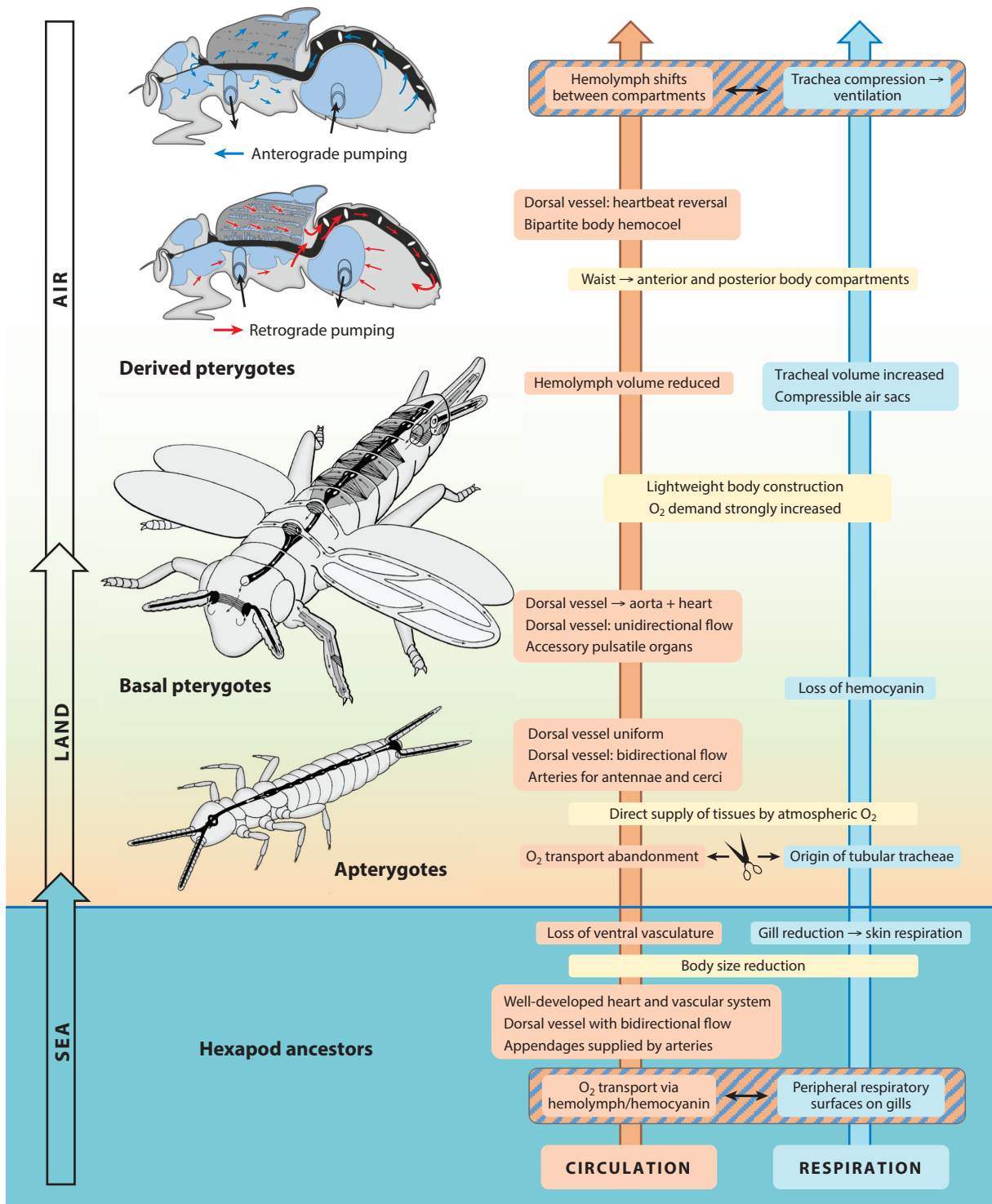
However, circulatory dynamics often vary among insect taxa and life stages. The heart of dipteran and lepidopteran adults reverses contraction direction and accepts hemolymph via the abdominal ostia, but the larval heart contracts exclusively anterograde and accepts hemolymph only through a posterior incurrent opening that only possesses excurrent function in adults (68, 87). In a process that is functionally analogous to what occurs in adults, the hemocytes of these larvae instead aggregate around the tracheal tufts that surround the posterior of the heart (86, 94).

In addition to hemocytes exerting their immune activity in areas of high hemolymph flow, the circulatory and immune systems are intricately linked by the frequent association between the hematopoietic organs of insects—and many other arthropods—and the dorsal vessel (6, 39, 43). Furthermore, hemolymph circulation is critical for coagulation because hemocyte recruitment and phenoloxidase-based melanization, among other things, close wounds following injury (4, 58, 172).

9. MAJOR EVOLUTIONARY TRENDS AND INNOVATIONS

The hexapod clade presumably evolved from an arthropod lineage that lived in a marine environment and had gills as respiratory organs (**Figure 6**) (49). Because the gills of marine arthropods are located in the periphery of their body, oxygen taken from the water is transported to organs and tissues by means of a powerful circulatory system that is composed of a well-developed heart, a complex arterial system, and hemolymph that contains the oxygen transport protein hemocyanin (16, 173). In the hexapod stem lineage, however, the tracheal system evolved as an evolutionary innovation in conjunction with terrestrialization (44, 49). In contrast to gills, the tracheal system is decentralized and directly supplies atmospheric oxygen to internal organs and tissues. Although some ancestral hexapod groups have retained hemocyanin in their hemolymph (16, 35), the dominant method for gas exchange is air-based transport through the tracheal system. For the hexapod circulatory system, abandoning oxygen delivery reduced the selection pressure for fast and even circulation. Thus, the hearts of hexapods have, over time, become weaker, and the vasculature has been drastically reduced. The reduction in the complexity and strength of the circulatory system was likely accelerated by the small size of primitive hexapods. As a result, hexapods only retained the dorsal part of the vasculature, which is now called the dorsal vessel. The loss of arteries necessitated the evolution of accessory pulsatile organs (118, 121). Such auxiliary pumps evolved for almost every long body appendage, and thus, several autonomous hearts beat in every insect body.

Acquiring the ability to fly also drastically shaped the evolution of the insect circulatory system. Its role in gas exchange increased due to the oxygen demands associated with the contraction of the flight muscles, and it also developed an essential role in stabilizing internal temperature. The efficiency of gas exchange was increased by evolving constrictions or valves that separate the body hemocoel into an anterior and a posterior compartment. Heart contractions, in conjunction with heartbeat reversals, create different pressure ratios in the two compartments, enabling the alternating compression and relaxation of the compliant parts of the tracheal system and thereby ventilating the respiratory air by convection. Dividing the body hemocoel into two largely separate compartments also facilitates thermoregulation because, for example, heat produced in the thorax

Anterograde pumping

Retrograde pumping

Derived pterygotes

Basal pterygotes

Apterygotes

Hexapod ancestors

AIR

LAND

SEA

Hemolymph shifts between compartments ←→ Trachea compression → ventilation

Dorsal vessel: heartbeat reversal
Bipartite body hemocoel

Waist → anterior and posterior body compartments

Hemolymph volume reduced

Tracheal volume increased
Compressible air sacs

Lightweight body construction
O_2 demand strongly increased

Dorsal vessel → aorta + heart
Dorsal vessel: unidirectional flow
Accessory pulsatile organs

Loss of hemocyanin

Dorsal vessel uniform
Dorsal vessel: bidirectional flow
Arteries for antennae and cerci

Direct supply of tissues by atmospheric O_2

O_2 transport abandonment ←✂→ Origin of tubular tracheae

Loss of ventral vasculature

Gill reduction → skin respiration

Body size reduction

Well-developed heart and vascular system
Dorsal vessel with bidirectional flow
Appendages supplied by arteries

O_2 transport via hemolymph/hemocyanin ←→ Peripheral respiratory surfaces on gills

CIRCULATION

RESPIRATION

(*Caption appears on following page*)

Figure 6 (*Figure appears on preceding page*)

Evolutionary history of the circulatory and respiratory systems in Hexapoda. The most drastic changes took place along the major ecological transitions: from sea to land and from land to air. Shown, from bottom to top, is the ancestral condition in hexapods, illustrated by the apterygote *Campodea augens* (adapted from Reference 36); a generalized basal pterygote (modified after Reference 118); and the derived pterygote condition in high-performance fliers, exemplified by *Calliphora vicina* (modified after Reference 162). In *C. vicina*, the hemocoel of the anterior compartment (head + thorax) is separated from the abdomen by both a waist and a large tracheal sac that functions as a valve (the tracheal system is in blue). (*Top*) During anterograde periods, hemolymph is drawn from the abdomen and pushed into the anterior body compartment, resulting in an expansion of the abdominal parts of the tracheal system and compression of those in the head and thorax; in turn, respiratory air is drawn through the abdominal spiracles and forced out through the thoracic spiracles. (*Bottom*) During retrograde periods, the events in the thorax and abdomen are reversed. Vessels are shown in solid black, and diaphragms and pumping muscles are shown in gray. The colored blue and red arrows indicate anterograde and retrograde hemolymph flow, respectively, and the black arrows indicate flow of the respiratory air through tracheal spiracles.

during flight can be transferred to the abdomen by the flow of hemolymph and radiated from there. This spatial arrangement of the body hemocoel can be regarded as a key innovation of high-performance fliers and evolved convergently in several groups of pterygotes, either by the formation of a structural waist between the thorax and abdomen or by the specific arrangement of the internal organs (125, 162).

Acquiring the ability to fly also selected for a lighter body; this was largely accomplished by reducing hemolymph volume, e.g., from up to 50% of body weight in caterpillars to approximately 15% in adult butterflies (109). This reduction occurred in parallel with an enormous increase in the volume of the tracheal system. As a result, the hemocoel of some insects was gradually replaced by, figuratively speaking, an aerocoel. In high-performance fliers, such as the blow fly, the tracheal system contains numerous compressible tracheal sacs and accounts for as much as 50% of the entire body volume (168).

10. CONCLUSIONS AND PERSPECTIVES

Despite its critical importance for everyday processes, our understanding of the circulatory system lags far behind our knowledge of most other physiological systems in insects. For example, although it has long been known that the circulatory system functions in thermoregulation, little research has been conducted on this topic over the past 25 years (54). Likewise, the peptidergic regulation of heart rhythmicity has been studied in detail, yet how heart rhythmicity is modulated in vivo is unknown (21, 59).

Insects have been evolving for over 400 million years, but it is commonly believed that there is almost complete conservation in the circulatory system of members of this animal group. However, extensive comparative studies have revealed that there is significant diversity in the functional morphology of the circulatory organs, especially among the various accessory pulsatile organs. This structural and functional divergence is in part due to spatial constraints caused by the disparate evolution of internal and external structures, which necessitated fundamental changes in the anatomy of the circulatory organs and the flow of hemolymph (116, 121). Most accessory pulsatile organs are not homologous with any organ in the hexapod ancestor. Thus, they are prime examples of evolutionary novelties, especially because they have a simple organization and are formed from only a few construction elements (123, 151). Further studies in this area, using the concepts of modern evolutionary developmental biology (or evo-devo), should yield in-depth insights relevant to the largely neglected problem of the emergence of evolutionary novelties (156, 157).

Although much is known about the structure of the various circulatory organs, very little is known about hemolymph flow or about how the various circulatory organs are coordinated to maximize circulatory performance. The most comprehensive approach to studying both flow and coordination has been undertaken in the mosquito *Anopheles gambiae*; correlative bright field and

fluorescence imaging of microspheres has revealed the trajectory and velocity of hemolymph, as well as the pumping frequency and coordination of all relevant circulatory organs: the dorsal vessel (41, 87), the antennal hearts (12), the wing hearts (19), and the contractions of the ventral abdomen (1). These studies have benefited from the small size and relative translucency of the cuticle of this species. Studies in larger or more sclerotized insects will necessitate different approaches; perhaps synchrotron X-ray imaging, as has been used to visualize flow in a grasshopper (90), or Doppler optical coherence tomography, as has been used in fruit flies (20), will overcome what is arguably the largest obstacle: the need to avoid deeply invasive manipulations that disrupt the integrity of the hemocoel.

Circulatory physiology also impacts the transmission of blood-borne pathogens by hematophagous insects. Some of these pathogens—malaria being an example—must migrate across the hemocoel until they invade the salivary glands or reach the mouthparts (58, 72). This migratory process subjects the pathogen to hemolymph currents in an environment that is inherently hostile because of the vigorous immune responses aimed at eliminating the infection. Given the obligate nature of pathogen migration, and the functional integration between the immune and circulatory systems, gaining a better understanding of circulatory processes could aid in the development of novel strategies to control the spread of vector-borne diseases (57, 58).

Research over the past two decades has deciphered the genetic basis of cardiac development in *Drosophila*, and it turns out that it shares significant homology with heart development in humans (11). As a result, research on *Drosophila* has begun to utilize low-cost and efficient screening approaches to identify genes that drive or influence nutritional processes, metabolism, and cardiovascular health in a manner that provides insights into both insect physiology and human diseases such as diabetes and obesity (13, 81, 155, 174). Moreover, because the circulatory system of insects is not directly linked to oxygen transport, severe cardiac malformations or even heart failure can be explored in detail because such conditions do not always lead to immediate death. Lastly, *Drosophila*, with its short life cycle and high fertility, is also ideal for transgenerational studies to investigate epigenetic effects on cardiac health, such as the effects of hypoxia under high-altitude conditions (176) or diet (46, 48). Thus, research on the insect circulatory system may yield important findings with implications for translational medicine.

Finally, this review focuses on the function, structure, and mechanics of the insect circulatory system, and on how this system integrates with other systems. Due to space constraints, several topics are not discussed. For example, this review does not delve into the composition of hemolymph—e.g., pH and osmolarity—but this topic is reviewed elsewhere (18, 74). Likewise, the immune system, including the different types of hemocytes, is not discussed other than to highlight the functional integration between the immune and circulatory systems. The immune system of insects has received intense scrutiny over the past few decades, with recent reviews highlighting this topic (58, 147).

In conclusion, the insect circulatory system is vital, but it has been widely neglected. Further research is warranted, as it will illuminate basic aspects of insect physiology, vector-borne disease transmission, and even human disease.

SUMMARY POINTS

1. The circulatory system is composed of a fluid medium called hemolymph, a body cavity called the hemocoel, and a series of muscular pumps.

2. The main driver of hemolymph circulation in the central body cavity is the dorsal vessel, which in ancestral species is largely uniform but in derived insects is functionally bipartitioned into an aorta in the thorax and a heart in the abdomen.

3. Depending on the species, the dorsal vessel can propel hemolymph bidirectionally (simultaneous anterograde and retrograde propulsion) or unidirectionally (only anterograde propulsion) and can experience heartbeat reversals (periodic shifts between anterograde and retrograde propulsion).

4. Accessory pulsatile organs—also known as auxiliary hearts—supply hemolymph to the antennae, wings, and other appendages in a manner that is independent from circulation in the central body cavity.

5. Insect hearts feature a myogenic rhythmicity that is modulated by neural or hormonal input and by extracardiac pressure changes.

6. Heart rhythmicity is affected by temperature, age, the nutritional status of the insect, and the onset of key developmental events.

7. The circulatory system is functionally integrated with defense mechanisms: Reflex bleeding protects from predators, and the periostial hemocytes that aggregate on the heart drive immune responses against microbial invaders.

8. Due to the physiological challenges associated with high-performance flight, some insects evolved new roles for the circulatory system, such as involvement in thermoregulation and tracheal ventilation.

FUTURE ISSUES

1. What factors control the rate of hemolymph flow and the coordination of circulatory organs, particularly in large insects?

2. From an evolutionary perspective, how widespread are heartbeat reversals among insect orders, and which functional requirements induce their onset?

3. Several factors regulate heart rhythmicity, but which biological functions require rhythmicity modulation in vivo, and why?

4. How does the circulatory system, together with its integration with the immune system, affect the migration of vector-borne pathogens across the hemocoel?

5. What is the relative contribution of the circulatory system to gas exchange and thermoregulation?

6. The accessory pulsatile organs of pterygote insects are evolutionary novelties, so further research into their evolution will yield seminal discoveries related to how novel organs emerge.

7. Comprehensive examinations of fluid mechanics at a microscale, such as in the narrow antennal vessels or wing veins, will provide a deeper and clearer understanding of insect circulation and hemodynamics.

8. Can discoveries pertaining to the insect circulatory system be translated into treatments for human cardiovascular diseases?

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