

Episodes in insect evolution

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Synopsis This article derives from a society-wide symposium organized by Timothy Bradley and Adriana Briscoe and presented at the 2009 annual meeting of the Society for Integrative and Comparative Biology in Boston, Massachusetts. David Grimaldi provided the opening presentation in which he outlined the major evolutionary events in the formation and subsequent diversification of the insect clade. This presentation was followed by speakers who detailed the evolutionary history of specific physiological and/or behavioral traits that have caused insects to be both ecologically successful and fascinating as subjects for biological study. These include a review of the evolutionary history of the insects, the origins of flight, osmoregulation, the evolution of tracheal systems, the evolution of color vision, circadian clocks, and the evolution of eusociality. These topics, as covered by the speakers, provide an overview of the pattern and timing of evolutionary diversification and specialization in the group of animals we know as insects.

Episodes in insect evolution

Biologists often refer to the “success” of a group of organisms, which typically means one of two things: evolutionary success—measured in terms of species diversity, geological duration, and/or geographic spread—and ecological success, as measured in terms of the impacts of a species or group of species upon an ecosystem. By either measure, insects are the most successful life form in the 450 million-year history of terrestrial living. They had appeared at least by the early Devonian, and by the Carboniferous some 80 million years later had evolved into a diverse array of winged forms. Shortly thereafter they evolved metamorphosis, and it was not until the Late Jurassic or Early Cretaceous, 150–140 million years ago, that the first complex societies evolved. These “episodes” correspond to what are probably the four major adaptive features of insects: terrestriality (the origin of hexapods),

flight (the origin of pterygote insects), complete metamorphosis (the origin of the Holometabola), and eusociality (Grimaldi and Engel 2005). While the first three can be credited with the astonishing diversity of millions of species of insects, the approximately 17,000 species of eusocial insects are most significant in terms of their collective impact on terrestrial environments (as well as their uniquely complex societies). Each of these adaptive features was a topic of discussion at the recent symposium on insect evolution at the SICB meeting in Boston.

When referring to “episodes” it must be cautioned against thinking of these as sudden events. In fact, all complex adaptive features progress through stages of gradual modification taking millions to tens of millions of years to refine. Wings and flight, for example, presumably evolved from lateral expansions of the insect body used for gliding, to fully articulated structures capable of powered flight.

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Refinement of the pteralia or axillary sclerites at the base of the wing and of wing muscles then led to various abilities, such as folding the wings over the back (neoptery), which then allowed for the invasion of tight spaces while retaining the ability to fly (even if just with the hind wings). Terrestrialization required not only a waxy cuticle, but a tracheal respiratory system, an excretory system composed of Malpighian tubules, as well as subsequent modifications of spiracular valves and cryptonephridia that reduced water loss.

Any consideration of terrestrialization in insects must address the origin of hexapods, which has been elusive. Traditionally, the living sister group to Hexapoda has been thought to be the Myriapoda (Snodgrass 1938; Boudreaux 1979; Hennig 1981; Kristensen 1991), based principally on the loss of the second pair of antennae commonly seen in Crustacea, and the presence of tracheal respiratory systems in both of the terrestrial groups. There are other morphological features, though, that appear to link myriapods and hexapods: loss of the mandibular palp (a structure that occurs in most Crustacea), presence of Malpighian tubules, a tentorium (the internal, cuticular strut inside the head capsule) bearing anterior arms, the presence of styli (pairs of small, nonsegmented, nonmusculated appendages on the coxae and/or abdominal sternites), and of eversible vesicles (eversible, membranous structures similarly distributed), as well as the presence of a specialized sensory structure at the base of the antenna, the postantennal (also called temporal, or Tömösváry) organ. Unfortunately, several of these characters are losses (mandibular palps, second pair of antennae), and absence of a structure is difficult to homologize. Based on the branching and fine structure of tracheae, and on the positions and structure of spiracles, tracheae clearly evolved multiple times in terrestrial arthropods, including the Hexapoda, Myriapoda, oniscoidean isopods, various arachnids, and Onychophora (Ripper 1931; Dohle 1988; Hilken 1997; Kraus 1998). The postantennal organ may actually be homologous to the frontal organ of Crustacea, and so may be a primitive feature of hexapods. There is insufficient comparative work on arthropods' Malpighian tubules to judge primary homology among the groups that possess them. Thus, morphological support for a myriapodian origin of hexapods is essentially limited to the structure of the tentorium and the presence of eversible vesicles and styli.

Evidence from nucleotide sequences presents a consistently different perspective: hexapods are

most closely related to Crustacea (Giribet et al. 2001; Hwang et al. 2001; Carapelli et al. 2005), and possibly they even are highly modified Crustacea (Nardi et al. 2003; Giribet et al. 2005; Regier et al. 2005; Mallatt and Giribet 2006). Interestingly, within Crustacea are groups that some of the molecular studies link to hexapods; these include species that inhabit freshwater and alkaline lakes (like Branchiopoda), and so would seem to represent an ecological intermediate between fully marine crustaceans and fully terrestrial insects. A crustacean origin for hexapods has some morphological support, too. This evidence includes, for example, gross brain structure (Nilsson and Osorio 1997; Whittington and Bacon, 1998), fine structure of the ventral nerve cord (Whittington et al. 1996), and structure of the ommatidia, or facets, of the eye (Paulus 2000).

Presumably, hexapods diverged from a common ancestor with Crustacea in the Silurian, more than 420 million years ago. By the Early Devonian there is definitive evidence in the Rhynie chert of Scotland (ca. 410 myo) for Collembola (Hirst and Maulik 1926; Whalley and Jarzembowski 1981) and true Insecta (Engel and Grimaldi 2004). The well preserved Devonian fossil *Devonohexapodus bocksbergensis*, from the famous Hunsrück Slate marine deposit of Germany, has been proposed as a stem-group hexapod (Haas et al. 2003). Intriguingly, this animal was reported to possess a single pair of antennae, a thorax with three long walking legs, and an abdomen with numerous pairs of small, segmented "leglets," nicely intermediate between hexapods and myriapods. This interpretation of *Devonohexapodus*, however, has been criticized (Willmann 2005), and as a result of the recent discovery of a large series of well-preserved specimens, it is now known that *Devonohexapodus* is actually a synonym of *Wingertschellicus* from the same deposit (Briggs and Bartel 2001; Kuehl and Rust 2009). The new material indicates that *Wingertschellicus* is an enigmatic, ancient Crustacean with two pairs of antennae, which is definitely not a hexapod. Thus, there is no known early stem-group hexapod, although fossil evidence has been very clear about the origins of another major adaptive feature of insects: an aquatic lifestyle.

The basal pterygote orders Ephemeroptera and Odonata have nymphs that live in fresh water, and for this reason it is commonly assumed that insects evolved from an aquatic ancestor. Evidence actually indicates that insects colonized freshwater some 200 million years after their origins. First, the most basal living hexapods [Collembola and other Entognatha, bristletails (Archaeognatha), and

silverfish (Thysanura)] are not aquatic. Second, the oldest remains of hexapods from the Devonian, Carboniferous, and Permian, are not in lake deposits, and there are no aquatic forms. Indeed, the earliest definitive evidence of aquatic insects (predaceous diving bugs [Nepomorpha: Heteroptera]), is from the Late Triassic, ca. 230 Mya. Lastly, location on the body and structure of gills reflect the convergent origins of these structures in the major groups of aquatic insects: Ephemeroptera, Odonata, Plecoptera, Trichoptera, as well as some Coleoptera and Diptera. The same system that adapted insects for terrestrial life, the tracheae, was co-opted in the form of evaginated tracheae or gills for an aquatic life.

The origins and diversification of insect flight

Dudley and Yanoviak addressed the evolution of flight in the insects. The origins of winged (pterygote) insects are both unresolved and deeply puzzling, given the absence of transitional fossil forms. Flying insects probably evolved in either the Upper Devonian or early Lower Carboniferous, and by the onset of the Upper Carboniferous (~325 Mya) were well diversified into about fifteen orders, many of which resemble taxa existing today (Grimaldi and Engel 2005). Fossils of these late Paleozoic winged insects and those of ancestrally wingless hexapods at ~390 Mya are separated by approximately 65 million years for which no apterygote, pterygote, or transitional fossil is recorded. The morphological origins of wings and their subsequent elaboration thus remain obscure; pterygote wings are not homologous with the legs (as is the case for volant vertebrates), and accordingly represent true evolutionary novelty.

Wings could have derived from fixed paranotal outgrowths of thoracic segments in terrestrial taxa (Rasnitsyn 1981; Bitsch 1994), with articulation and flapping motions being secondarily derived. Alternatively, wings might have arisen from ancestrally mobile gills or gill covers in aquatic forms (Wigglesworth 1973; Kukalova-Peck 1983), or possibly from mobile leg-derived structures (e.g., styli) on land. Fundamental to any such assessment of morphological origins is habitat association; are the pterygotes ancestrally aquatic or terrestrial? Because extant apterygote hexapods are exclusively land dwellers [with the few aquatic species of collembolans being highly derived (D'Haese 2002)], and given that all hexapods nest within a terrestrial crustacean lineage, the origins of flight are most parsimoniously

sought in terrestrial conditions (Pritchard et al. 1993; Samways 1996; Regier et al. 2005). Hypotheses positing aquatic origins of insect flight can accordingly be rejected (Dudley 2000a; Grimaldi and Engel 2005), and present-day aquatic larvae of the basal lineages Odonata and Ephemeroptera must then be viewed as secondarily derived. Similarly, fossil specimens suggesting that leg exites transformed into wings in putatively aquatic Paleozoic insect larvae (Kukaloveck 1978, 1983) have been critically assessed (Bethoux and Briggs 2008).

Independent of their morphological origins, wings today serve mostly aerodynamic purposes; but what would have been the use of a partial wing (i.e., a winglet) for an early pterygote? A variety of nonaerodynamic (and nonmutually exclusive) roles have been attributed to winglets, including use in courtship and in thermoregulation (see Dudley 2000a). However, even small fixed winglike structures might enhance glide trajectories, whereas mobile winglets could enhance maneuvers (Dudley et al. 2007). Increasing arborescence and geometrical complexity of terrestrial vegetation through the Devonian and into the Carboniferous (Dilcher et al. 2004) would have provided three-dimensional substrate suitable for recovery from inadvertent falls, maneuvers while gliding, and aerial escape from predators. Predatory pressure was likely intense on Devonian and Carboniferous insects given the wide contemporaneous diversity of insectivorous arachnids, amphibians, and reptiles (Shear and Kukaloveck 1990).

Studies of extant gliding arthropods also provide substantial evidence for the functional utility of both axial and appendicular structures in controlled and maneuvering aerial descent. Wingless ant workers either jump or fall from trees at high rates in the phenomenon known as "ant rain" (Haemig 1997). Many species can also direct their aerial descent to return to their home tree trunk (Yanoviak et al. 2005). Directed descent begins with an initial righting reflex and vertical drop with extended appendages, followed by a rapid, visually mediated turn that aligns the longitudinal body axis towards the target tree, and concluding in a steep backwards glide to the tree trunk (Yanoviak et al. 2005; Yanoviak and Dudley 2006). Such controlled gliding and maneuvering is now known to occur in at least seven arboreal ant genera, in larval instars of numerous hemimetabolous insects, and most significantly in the apterygote archaeognathans (Yanoviak et al. 2009). Controlled aerial behaviors thus precede phylogenetically the origin of wings. Only ants glide backwards, but all other gliding taxa identified

to date lead with the head, as must have characterized ancestral pterygotes. Steering mechanisms include use of the legs by ants, and abdominal ruddering in other taxa. The intentional control of body trajectories as wingless arthropods fall from heights likely characterizes many more groups than is currently recognized. Morphological and behavioral intermediates to large-amplitude, rapid flapping of true wings can then exhibit progressive functionality, particularly in steering, as flight capacity further evolves (Dudley et al. 2007).

Although gigantism was an important feature of the late Paleozoic insect fauna, in part enabled by a hyperoxic atmosphere (Graham et al. 1995; Dudley 2000b), a salient feature of the contemporary entomofauna is miniaturization. Relative to the Carboniferous giants and to an ancestral size of 2–4 cm, the mean body length of adult insects today is on the order of millimeters. Most of insect diversity derives from legions of small beetles and from miniaturized dipteran and hymenopteran parasitoids. Because wingbeat frequencies of volant animals in general, and of insects in particular, increase with decreasing body size, today's small insects exhibit rather high wingbeat frequencies, often in excess of 100 Hz (Dudley 2000a). Such extreme contraction frequencies for high-power muscles can be attained only by asynchronous flight muscle, a muscle type that is phylogenetically derived relative to synchronous precursors, and one that enables repeated stretch-induced contractions for a single activational neural impulse (Josephson et al. 2000). Asynchronous flight muscle has evolved many times in unrelated pterygote lineages (Dudley 2000a) and is found in ~75% of all described insect species, including three of the four largest orders (i.e., Coleoptera, Diptera, and Hymenoptera).

Such repeated evolutionary acquisition of asynchronous muscle may have facilitated the abundant taxonomic radiations of smaller insects that require concomitantly elevated wingbeat frequencies in order to retain the ability to fly. Comparison of sister insect lineages that differ in muscle type (i.e., synchronous versus asynchronous) statistically demonstrates the predicted decrease in mean body size and increase in species number when this physiological innovation is present (Dudley 2000a). Because higher wingbeat frequencies yield greatly increased aerodynamic forces (in approximate proportion to the square of flapping velocity), asynchronous muscle may also permit a reduced wing area relative to body mass if equivalent forces (e.g., weight offset) are to be generated. Suggestively,

both beetles and flies exhibit extreme nonaerodynamic modification of the forewings and hindwings, respectively, whereas these two wings are functionally fused in the Hymenoptera with typically much reduced hindwings. The one major insect order that does not possess asynchronous flight muscle is the Lepidoptera, an order characterized by relatively larger and nondifferentiated hindwings together with absolutely greater body size in comparison to asynchronous lineages. Thus, the presence of asynchronous muscle has had important consequences for patterns of morphological diversification among major insect orders. Much of insect diversity today derives from the biomechanical consequences of flight at small body size.

Evolutionary patterns of osmoregulatory capacity in insects

Contreras and Bradley discussed the evolutionary history of salt and water balance in insects. As outlined above, phylogenetic analyses reveal that the insects, as a subset of the hexapods, arose as a terrestrial group. Within the insects, distantly related orders all possess similar adaptations that provide the capacity to survive in highly desiccating terrestrial conditions. Almost every genus of insects contains species in which the adult reproductive stages disperse in the terrestrial, or even aerial, habitat.

Two main osmoregulatory adaptations have been identified that permit insects to thrive in the terrestrial environment: a waterproofed cuticle and the capacity to produce a hyperosmotic excreta. The cuticle, which is secreted by the epidermis, contains multiple proteins and the carbohydrate chitin. These form the flexible, yet tough, exoskeleton of insects. The waterproofing aspects of the cuticle are thought to be due almost entirely to an epicuticular layer of waxes and oils secreted by the underlying dermal glands (Rourke and Gibbs 1999). The second adaptation to terrestrial life, the capacity to produce hyperosmotic excreta, derives in insects from the functions of the gut. The midgut of insects is the site of digestion and absorption of ingested food and fluids (Dow 1986). The Malpighian tubules, which empty into the gut near the midgut, are the site of primary urine production (Maddrell 1980). The fluids in the lumina of both the midgut and Malpighian tubules are iso-osmotic to the hemolymph. This fluid moves within the gut to the rectum. There, a single cell type is used to extract water from the gut contents in the rectal lumen, thereby transporting a hypoosmotic fluid to the

hemolymph (Bradley 1985). As a result of this activity, a hyperosmotic excreta is expelled via the anus.

These two adaptations, a cuticle highly impermeable to water and a rectum capable of producing hyperosmotic excreta, are found in virtually all insects lineages. It is clear that these are primitive characters that occurred early in insect evolution and were central to the success of insects on land.

Among terrestrial insects, a few species have evolved another very useful trait, namely the capacity to extract water from air. A number of species can extract water from air using the rectum (Noble-Nesbitt 1998). In these species, the ion-transporting capacities of the Malpighian tubules are employed, however a highly impermeable cryptonephridial barrier surrounding both the tubules and the rectum prevents water from the hemolymph from following. As ions accumulate in the cryptonephridial space, a hyperosmotic fluid is produced which draws not from the hemolymph but rather from the excreta in the rectal lumen. These same insects can pump air into and out of the rectal lumen via the anus. Under these circumstances, water can move down its activity gradient from the air in the rectum to the cryptonephridial space. From there, the water flows into the hemolymph. Other insects can remove water from subsaturated air by secreting a hyperosmotic, hygroscopic fluid onto surfaces near the mouth (O'Donnell 1977). After the fluid absorbs water, it is ingested. Phylogenetic analysis reveals that both of these strategies have evolved rarely and independently in terrestrial species. These capacities are not a primitive trait.

Many insect species have exploited aquatic habitats, particularly during their larval stages. The number of species and their abundance is testimony to the ecological success of insects in this habitat. Most of the aquatic insects are restricted to fresh water. The principle adaptation leading to success in fresh water involves the rectum (Bradley 2008). In freshwater insects, the rectum is the site of ion uptake from the excreta and primary urine, with little water following. The rectal cells of freshwater insects actively resorb ions, particularly sodium, chloride, and potassium. In many very oligotrophic habitats, however, the urine cannot be made as dilute as the surrounding waters. In these circumstances "extrarenal sites" of ion uptake are employed that absorb ions from the external medium, with little water following. These functions are carried out by specialized chloride cells as found, for example, on the gills of mayflies, or in the anal papillae of mosquito larvae (Komnick and Schmidt 1977;

Donini and O'Donnell 2005). Phylogenetic analysis demonstrates that adaptation to freshwater habitats occurred independently several times in the insects; however, terrestrial existence was the primitive condition (Fig. 1).

A few insect orders contain species that can survive in saline waters. Again, the principal adaptation involves cells in the hindgut. In saline-water mosquitoes in the genus *Ochlerotatus*, the rectum is divided into two segments (Bradley 2002). The anterior segment serves to take up ions from the primary urine and excreta. This function is vital when the larvae find themselves in fresh water, but also is useful in saline waters as a means of recycling valuable ions from the primary urine. The posterior rectal segment functions as a salt gland. It is capable of transporting sodium, magnesium, chloride and possibly bicarbonate, as necessary to remove ions obtained by ingesting the external medium. These ions are transported with little water following, resulting in a highly saline excreta. The insects that can survive in saline waters are all in orders also containing freshwater species. It would seem that adaptation to saline water is a highly derived characteristic, probably arising in species already adapted to fresh water. Saline tolerance occurs very infrequently in the insects and is clearly not a primitive state for the group.

In summary, it appears that cuticular and rectal adaptations that support terrestrial life occurred very early in the insects and are primitive characters for the entire clade. Some terrestrial insects also evolved a capacity to take up water from a subsaturated atmosphere. Adaptation to an aquatic existence occurred much later during insect evolution, and occurred frequently and independently in several insect orders. Adaptation to saline waters seems to have originated in freshwater ancestors. The principle adaptations for aquatic insects are modifications of rectal morphology and function; in freshwater forms, "chloride cells" take up ions from the external medium.

Tracheal systems and the evolution of insects

VandenBrooks, Harrison and Kaiser provided an overview of the role of the tracheal system in insect evolution. Most insects respire via an air-filled tracheal respiratory system. The tracheal system generally consists of cells joined to form walls of hollow tubes that run throughout the body, terminating in blind-ended tracheoles in close proximity to the cells. The use of air instead

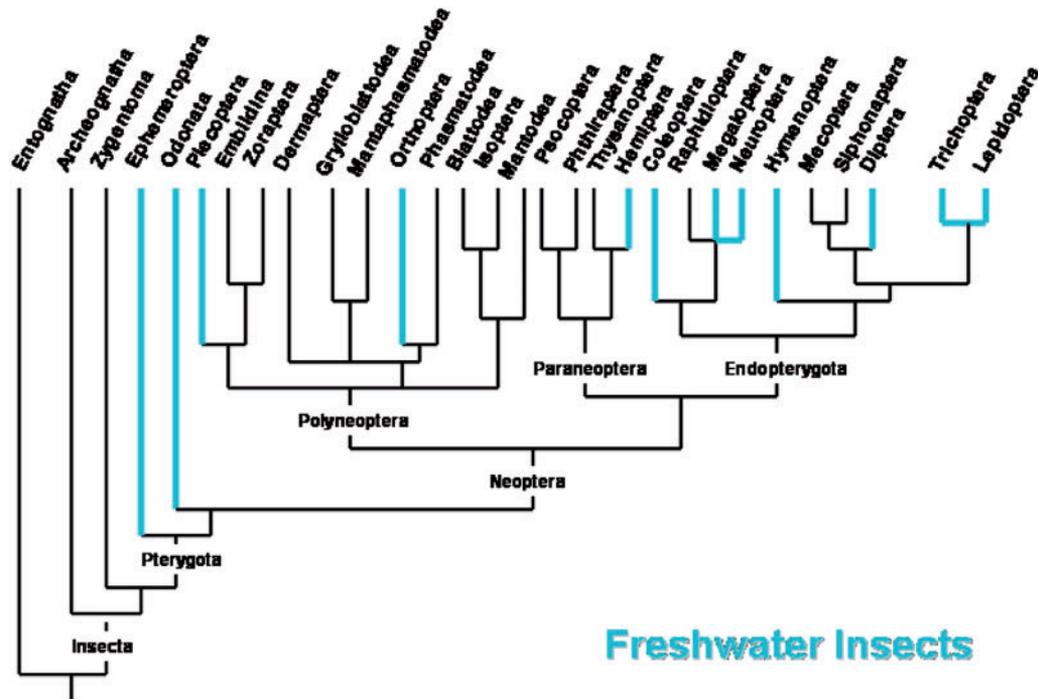


Fig. 1 A depiction of phylogenetic relationships in the insects (Grimaldi and Engel 2005). The lines in blue indicate lineages in which some species can inhabit fresh water. The relationships shown would suggest that the capacity to inhabit fresh water evolved repeatedly and independently in various lineages within the insect clade.

of a liquid (e.g., blood) as a medium for delivering oxygen comes with several major advantages: (1) It is light-weight and requires less energy for production, maintenance, and transport compared to blood-based systems. (2) It supports aerobic metabolism during active flight (Komai 2001), which demands the highest mass-specific rates of oxygen consumption in the animal kingdom (Casey et al. 1985, Nation 2002). The evolution of flight is a very important key to the great biodiversity and the ecological success of insects. (3) It provides an internal reservoir for oxygen, which allows prolonged periods of asphyxia (e.g., in hypoxic environments or under water). (4) If insects eventually become paralyzed by exposure to anoxia, they can recover quickly when re-exposed to oxygen, because oxygen can be delivered by passive diffusion, which is rarely true in vertebrates. However, the buoyancy associated with possession of a tracheal system may cause insects to be excluded from pelagic and deepwater environments (Maddrell 1998).

Despite the physiological importance of the tracheal system to insect function and success, the evolution (origin and diversification) of the tracheal system is very poorly understood. Tracheal systems appear in all three related clades of Ecdysozoa (animals that shed their exoskeleton in a molt) that

achieve large size and terrestriality—arthropods, onychophorans, and tardigrades (Fig. 2). Yet, all three tracheal systems would appear to have evolved independently since we know that primitive arthropods and tardigrades were aquatic. Within arthropods, all three major groups—the myriapods, chelicerates, and pancrustaceans (the taxonomic group containing hexapods and crustaceans)—exhibit tracheae. Again, there is evidence for multiple independent evolutions of the tracheal system—(1) current phylogenies have the closest ancestor to hexapods being an aquatic crustacean that lacks tracheae, a branchiopod (Glennner 2006), indicating an independent evolution of tracheae within hexapods, (2) most of the chelicerates are aquatic and lack tracheae pointing to an independent evolution of tracheae within the arachnids, and (3) if we accept a branchiopod relationship to hexapods, the myriapods, which are all terrestrial and possess tracheae, must also have an independently evolved tracheal system.

Within hexapods, the vast majority of insects possess tracheae and it has generally been assumed that these systems all derive from a common ancestor, but given the number of independent evolutionary events apparent at the basal parts of the tree and examples of secondary loss in aquatic and small

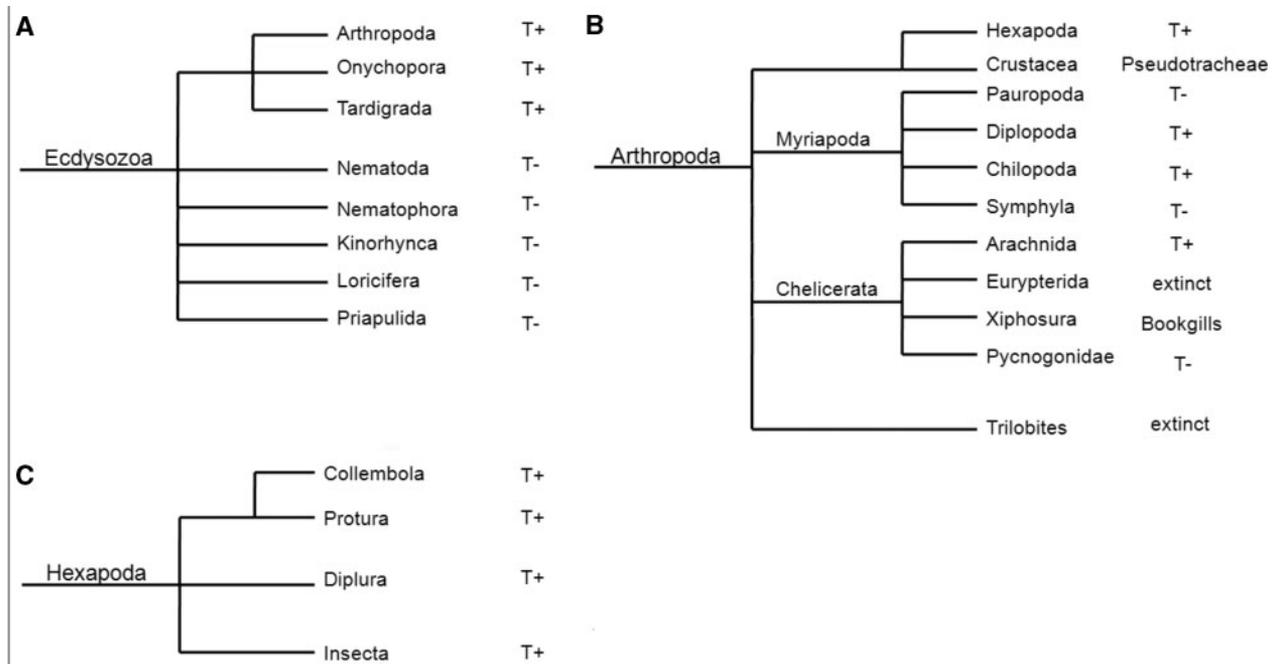


Fig. 2 T+ represents the presence of tracheae in at least one species, T– represents no tracheae present in any species (A) Tracheal distribution across Ecdysozoa. (B) Tracheal distribution across Arthropoda. (C) Tracheal distribution across Hexapoda.

forms, even this may no longer be certain. However, while the tracheae or tracheal-like structures in a vast diversity of ecdysozoan groups (Fig. 2) may not derive from a common ancestor, we posit that all Ecdysozoa possess some fundamental developmental pathways that can be utilized in the formation of tracheae. All Ecdysozoa possess a cuticle and perhaps developmental signals inducing an invagination of this cuticle may underlie tracheal formation. This hypothesis is supported by the fact that all terrestrial groups (although not all species) of Ecdysozoa exhibit tracheae. At the moment, there is only one species in which the molecular mechanisms of tracheal development have been well-studied: the fruitfly *Drosophila melanogaster*. The fates of tracheal cells are specified by the interactions of the heterodimeric transcription factor encoded by *trachealess* and *tango*, which together cause tracheal precursor cells to undergo cytoskeletal rearrangements necessary for invagination (Ghabrial et al. 2003). It would be very interesting to examine the developmental pathways and genetic controls of the tracheal system in a much wider array of Ecdysozoa to discern if genes similar to *trachealess* and *tango* determine the fate of tracheal cells in the various independently evolved tracheal systems. Also, understanding the developmental role of such genes in aquatic Ecdysozoa that lack tracheae might help reveal the evolutionary pathway. Interestingly, *tango* codes for the protein that functions as the constitutive component of

hypoxia-inducible factor, an oxygen sensor in all animal groups (Gorr et al. 2006), suggesting that it might function in gills or other exoskeletal structures that might respond to oxygen levels in aquatic organisms.

On an evolutionary scale, the possession of a tracheal system may make insects more susceptible to changes in atmospheric composition through geologic time than is true for other groups. One of the most striking examples of this is the arthropod gigantism in the Carboniferous and Permian, coincident with hyperoxic atmospheres reaching 30% oxygen. During these times, there were Protodonata with 70 cm wingspans and Arthropleura that were 2 m in length. The mechanism behind this gigantism may be linked to tracheal respiratory systems. The tracheal system limitation hypothesis (Kaiser et al. 2007) suggests that as insects get larger, the blind-ended tracheal respiratory system leads to increasing challenges for gas exchange due to the effects of distance on diffusion rates. To compensate, insects increase their mass-specific investment in the tracheal system and utilize more convection. Eventually, this increased investment leads to spatial constraints that limit insect size. When animals are reared under higher levels of atmospheric oxygen, their tracheae are smaller, mass-specific investment in the tracheal system is reduced, and the insects can therefore attain larger sizes before reaching the limits set by spatial constraints. This provides one

possible mechanism whereby increasing atmospheric oxygen through time could lead to insect gigantism. In addition to effects on tracheal structures, rearing extant insects under different oxygen levels has strong effects on average body size and developmental rates in a variety of insect groups, suggesting that changes in atmospheric composition in the past would have greatly influenced insect ecology and evolution (Harrison et al. 2008). Both of these mechanisms indicate that the insects' unique physiology and possession of a tracheal system have been fundamental to their evolutionary history and the impact that environmental change has had on their evolutionary trajectory.

Evolution of color vision in Holometabolous insects

Briscoe presented a paper on the evolution of color vision in insects. The eyes of insects are remarkable. Much of the diversity in eyes can be traced to alterations in the number, spectral properties, and spatial distribution of the visual pigments. Visual pigments are light-sensitive molecules composed of an opsin protein covalently linked to a chromophore that in insects is either 11-*cis*-retinal or 11-*cis*-3-hydroxyretinal. Most insects have eyes that contain at least three visual pigments with a wavelength of peak absorbance, λ_{\max} , in the ultraviolet (UV) (300–400 nm), blue (B) (400–500 nm) and long wavelength (LW) (500–600 nm) part of the visible light spectrum, respectively, encoded by distinct UV, B, and LW opsin genes. Most of what we know about the molecular basis of vision in insects is based upon studies of holometabolous insects—insects that have four life stages consisting of an embryo, larva, pupa, and adult. In the compound eye of beetles, flies, bees, moths and butterflies, each individual ommatidium is composed of eight (i.e., flies and beetles) or nine (bees, moths and butterflies) photoreceptor cells (R1–9) that generally express only one opsin mRNA per cell, although in the eyes of some beetles, flies and butterflies, there are ommatidial subtypes in which two opsins are co-expressed in the same photoreceptor cell (Kitamoto et al. 1998; Sison-Mangus et al. 2006; Jackowska et al. 2007; Mazzoni et al. 2008). Based on a phylogenetic analysis of opsin sequences from red flour beetles (*Tribolium castaneum*), honey bees (*Apis mellifera*), silkworms (*Bombyx mori*), sphingid moths (*Manduca sexta*) and butterflies (*Danaus plexippus*), and comparative analysis of opsin gene-expression patterns (White et al. 2003; Sauman et al. 2005; Jackowska et al. 2007), the

patterning of the ancestral holometabolous insect eye most closely resembled the eye of the bee (Spaethe and Briscoe 2005; Wawakuwa et al. 2005) and the nymphalid butterfly (Briscoe et al. 2003). The R1 and R2 cells of the main retina of these insects express either UV–UV, UV–B, or B–B absorbing visual pigments while the R3–9 cells express an LW-absorbing visual pigment.

Following the evolution of this basic pattern for insect eyes, the visual systems of derived insects then underwent an adaptive expansion based on lineage-specific UV, B, and LW opsin gene duplications and on alterations in the spatial expression of opsins within the eye. This pattern of opsin expansion is especially notable in the butterflies, where in every butterfly family, lineage-specific opsin gene duplications, especially of the B and LW opsins, have been detected (Fig. 3) (Arikawa et al. 2005; Sison-Mangus et al. 2006; Briscoe 2008; Frentiu et al. 2007; Frentiu and Briscoe 2008). In the case of the fruitfly *D. melanogaster*, the majority of photoreceptor cells (R1–6) in the main retina express a 'blue-green'-sensitive (480 nm) visual pigment, *Rh1*, that is the result of an ancient gene duplication of the LW opsin but which was lost in all other studied lineages, including mosquitos. In the case of the red flour beetle, *T. castaneum*, the ancestral blue opsin was lost and an expansion of the pattern of expression of the LW opsin occurred; it included not only expression in the six outer photoreceptor cells but also in the R7 and R8 cells (equivalent to R1 and R2 in butterflies and moths), thus producing R7 cells that co-express the UV and LW opsins (Jackowska et al. 2007).

So far, opsin expression in more primitive insects remains elusive but is likely to produce additional surprises. Among them are whether or not this basic plan for photoreceptor patterning evolved early or late in insect evolution, and the extent to which sexually dimorphic eyes with respect to opsin expression pattern exists (e.g., dragonflies). Understanding the molecular sophistication and complexity of insect eyes is a challenge, which if met, has broad biological implications.

The evolution of circadian clocks in insects

Merlin and Reppert presented an overview of the evolutionary history of circadian clocks in insects. Like most organisms, insects have evolved the ability to co-ordinate their activities with the day–night cycle caused by the Earth's rotation. This has given rise to a genetically programmed timekeeping mechanism, the circadian clock, whose intracellular

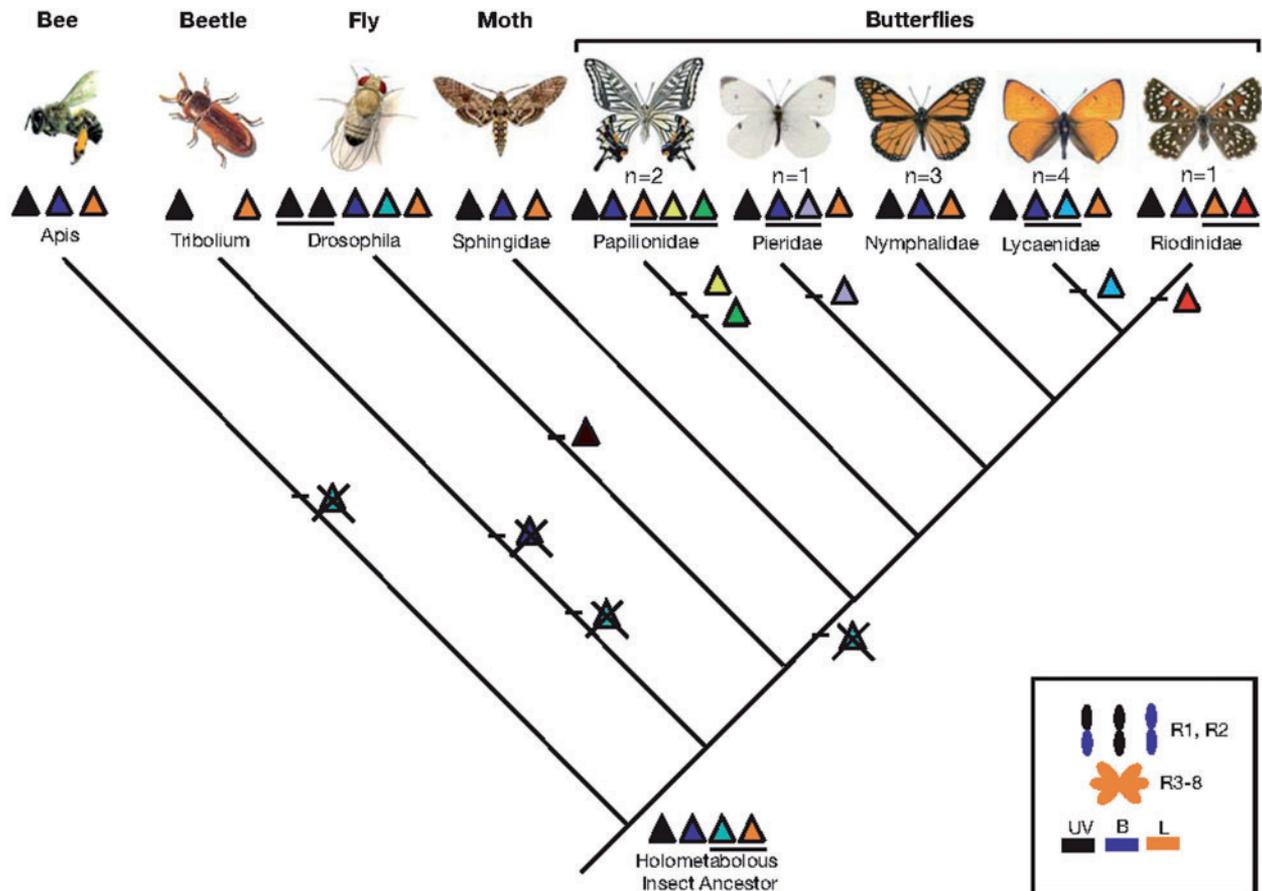


Fig. 3 Phylogeny of the holometabolous insects based on Savard et al. (2006) and Wahlberg et al. (2005) and on the pattern of visual pigment loss and gain in the adult compound eye. The ancestral holometabolous adult insect eye is likely to have had four types of visual pigments. Underlines represent visual pigments that are related by recent opsin gene duplication. Crosses represent visual pigment (opsin) losses. Inset: diagram of the ancestral holometabolous ommatidial subtypes in the main retina. Black, UV opsin; dark blue, B opsin; orange, LW opsin.

molecular machinery involves transcriptional feedback loops that drive persistent 24-h rhythms in mRNA and protein levels of key clock components.

Within holometabolous insects, a molecular clock mechanism has been most extensively studied in the fruitfly *D. melanogaster* (Hardin et al. 2005), the housefly *Musca domestica* (Codd et al. 2007), the monarch butterfly *D. plexippus* (Zhu et al. 2008) and the Chinese oak silkworm *Antheraea pernyi* (Chang et al. 2003), with more limited studies of the commercial silkworm *B. mori*, the honeybee *A. mellifera* (Rubin et al. 2006; Yuan et al. 2007) and the beetle *T. castaneum* (Yuan et al. 2007). In *D. melanogaster*, which has served as a model for the molecular dissection of the circadian system, the transcriptional activators CLOCK (CLK) and CYCLE (CYC) heterodimerize and bind to E-box enhancers in the promoters of the *period* and *timeless* genes, thus initiating their transcription. As the proteins are translated in the cytoplasm,

PERIOD (PER) and TIMELESS form heterodimers that, after the appropriate delay in time, are translocated back into the nucleus in which PER inhibits CLK/CYC-activated transcription. Resetting of the clock by light is mediated via a blue-light photoreceptor, CRYPTOCHROME (CRY), responsible for the light-dependent degradation of TIMELESS, which ultimately resets the molecular clock's feedback loop and keeps the clock coordinated to the 24-h day.

The recent discovery of a new clade of CRYs in insects, characterized most extensively in the monarch butterfly (Zhu et al. 2008), but also present in all non-drosophilid species examined so far (mosquitoes, butterfly/moths, beetles and bees) (Zhu et al. 2005; Yuan et al. 2007), revealed a feature important for understanding the evolution of insects' circadian clocks. Phylogenetic analyses revealed that this new CRY family (designated insect CRY2) aligned more closely to the vertebrate

CRYs, which function as the main transcriptional repressor at the core of the clock in a light-independent way (Reppert and Weaver 2002). These CRY2s constitute a vertebrate-like CRY family in insects, in addition to the *Drosophila*-like CRY family (insect CRY1). Based on phylogenetic analyses, these two families of insect CRYs appear to be the result of at least two rounds of gene duplication at the base of the metazoan radiation (Yuan et al 2007). Functional assays in cell culture have shown that insect CRY2s are light insensitive and are potent transcriptional repressors of the clockwork, as are their orthologs in vertebrates, whereas insect CRY1s are light sensitive with no transcriptional repressive activity.

Therefore, within insects, it appears that a single clockwork mechanism evolved that has undergone specialized changes in various lineages, through the processes of gene duplication and loss to produce at least three types of clock mechanisms (Fig. 4) (Yuan et al. 2007): (1) one form is the ancestral clock (apparent in the monarch butterfly, silkworms and mosquitoes) in which both insect CRY1 and CRY2 exist and function differentially within the clockwork; insect CRY1s function in light-entrainment of the clock, while insect CRY2s function as the main transcriptional repressor of the clockwork; (2) *Drosophila* has a derived clock in which the vertebrate-like CRY2 has been lost and *Drosophila*-like CRY1 functions primarily as a circadian photoreceptor in clock neurons in the brains of flies (Emery et al. 1998); and (3) beetles and bees also have derived clocks in which the light-sensitive CRY1 has been lost and only the insect CRY2 exists and functions within the clockwork.

In conclusion, *Drosophila*, as a genetically tractable model, has been invaluable for understanding the molecular basis of the clockwork mechanism in animals. However, studies in other insects, like the monarch butterfly, have provided an “ancestral model” for comparison of clockwork mechanisms among insects, and between insects and mammals. Importantly, the recent development of comparative analysis of the clockwork mechanisms within holometabolous insects would not have been possible without the availability of their genomes. With the recent explosion in genome-sequencing methods, extension of molecular studies to the circadian clocks of hemimetabolous insects (e.g., cockroaches, locust, and crickets), in which much physiological and anatomical analyses have been performed, should be forthcoming.

The evolution of eusocial insects

Brady and Danforth reviewed recent progress toward understanding the evolutionary history of eusocial insect societies. The repeated origins of eusocial organisms can be considered one of the major evolutionary transitions in the history of life (Maynard Smith and Szathmary 1995). Most eusocial animals are insects, and several lineages including termites, ants, wasps, and bees have evolved diverse forms of social organization and often display considerable ecological dominance (Wilson 1971, 1990). Eusociality traditionally has been defined to include three key criteria: generation overlap, cooperative brood care, and reproductive division of labor (Michener 1969; Wilson 1971). These societies can also be viewed as displaying high reproductive skew where relatively few individuals monopolize reproductive output (Crespi and Yanega 1995; Gadagkar 1995; Sherman et al. 1995), leading some to consider as eusocial gall-inhabiting aphids (Ito 1989; Benton and Foster 1992) and thrips (Crespi 1992), and one species of beetle (Kent and Simpson 1992). The major lineages of eusocial insects, however, are termites (summarized by Grimaldi and Engel 2005) and several groups within the order Hymenoptera (ants, bees, and wasps) that provide the focus of the present survey.

Ants (Formicidae) comprise the largest eusocial insect lineage in terms of described species (<12,000) and often are ecologically dominant members of their communities (Holl Dobler and Wilson 1990; Ward 2006). All ants are eusocial, and the origin of eusociality is clearly quite old in this group. The most recent molecular divergence-dating analysis estimates an age of 115–135 Ma for crown group ants (Brady et al. 2006a), and thus eusociality must have originated sometime before that. Phylogenetic work shows that some taxa previously considered to reflect early ant eusociality, such as the bulldog and dinosaur ants of Australia (Ward and Brady 2003), instead are nested well within the phylogeny of ants. Several subterranean lineages with cryptobiotic characteristics resolve at or near the base of the ant tree in several studies (Brady et al. 2006a; Moreau et al. 2006; Rabeling et al. 2008). It remains possible, however, that the closest stem-group fossils, the Sphecomyrminae, which were large-eyed and probably lived in exposed environments, better represent the groundplan for early ants (Brady et al. 2006a). Ants throughout their history have used eusociality as a platform on which many behavioral specializations have evolved

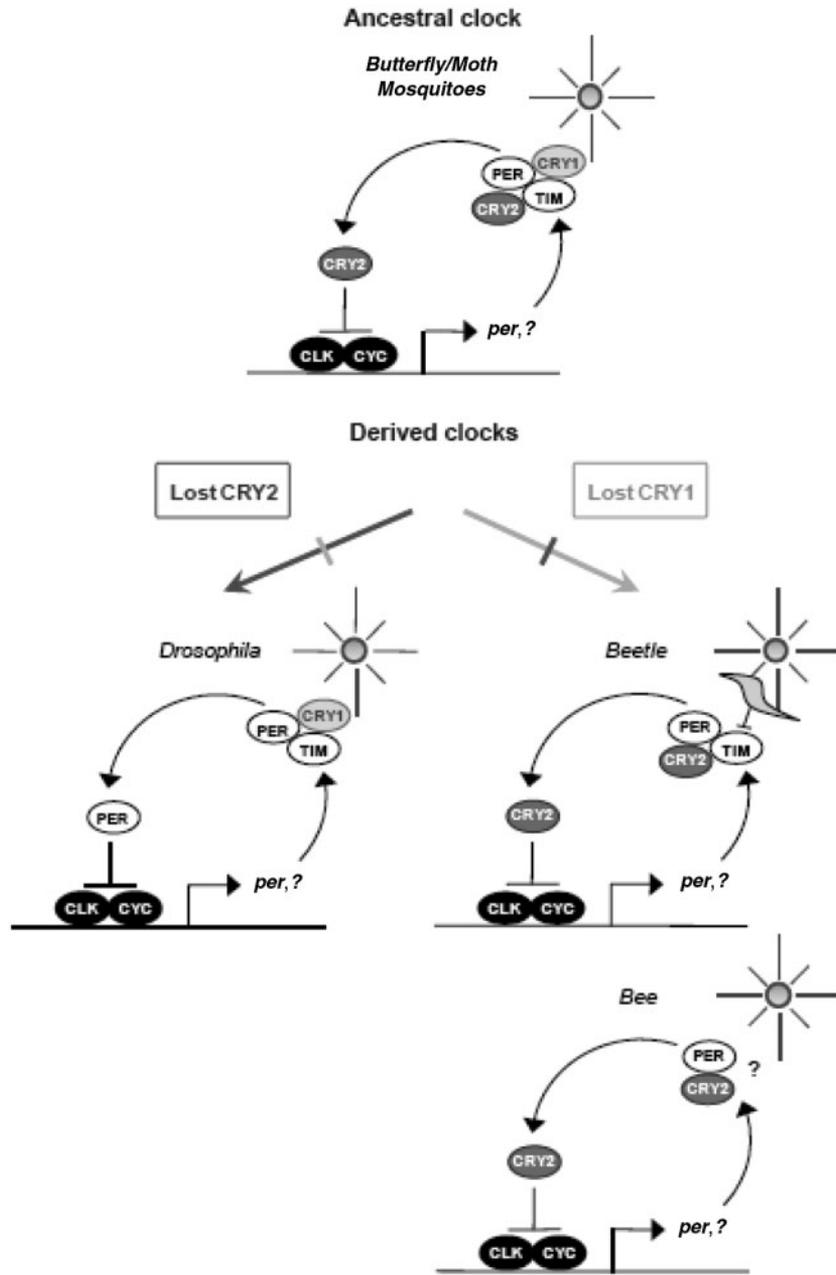


Fig. 4 Evolution of insect clockwork mechanisms. The ancestral clock (butterfly/moths and mosquitoes) possesses the two types of cryptochromes (insect CRY1 and insect CRY2). Gene losses gave rise to two types of derived clocks, one possessing only CRY1 (*Drosophila*) and one possessing only CRY2 (beetle and bee). CLK, clock; CYC, cycle; PER, period; TIM, timeless. The sun represents the light input to the clock. In the derived clock found in the beetle, the flag represents a putative photoreceptor involved in TIM degradation for clock entrainment, and in the bee clock, the exclamation point represents a novel light input pathway to the clock. Adapted from Yuan et al. (2007).

(Hölldobler and Wilson 1990, 2009). A particularly astonishing example occurs in the fungus-growing ants, the most important group of agriculturalists other than humans (Schultz et al. 2005). Recent molecular phylogenetic and divergence dating analyses provide a historical timescale for this symbiotic system over the past 50 Ma and reveals that leaf-cutting ants, the dominant herbivores of

the Neotropics, evolved remarkably recently, only 8–12 Ma (Schultz and Brady 2008).

Within wasps of the family Vespidae, eusociality occurs in the Polistinae (paper wasps), Vespinae (yellowjackets), and Stenogastrinae (hover wasps) (Ross and Matthews 1991; Hunt 2007). Morphological and behavioral characters argue that these three groups form an exclusive clade, indicating

a single origin of eusociality (Carpenter 1983, 1991). A recent molecular phylogeny, however, suggests that Stenogastrinae is only distantly related to the other two eusocial groups, implying two eusocial origins (Hines et al. 2007). Several trace fossils of wasp nests are preserved throughout the Cenozoic (Genise and Cladera 2004), the oldest of which is a presumed nest of Polistinae or Vespinae at least 63 Ma (Wenzel 1990), establishing a minimum age for a eusocial origin in this group.

Eusociality also occurs in at least one species of *Microstigmus* wasp in the family Sphecidae (Matthews 1968; Ross and Matthews 1989) and may extend to related species and genera, although these taxa remain poorly studied (Matthews 1991).

The remaining instances of insect eusociality occur within the lineage Anthophila (bees). The vast majority of the ~18,000 described species of bees are solitary or cleptoparasitic, with only approximately 6% displaying eusociality (Danforth 2007; Michener 2007). Molecular phylogenetic analyses (Danforth et al. 2006) clearly indicate that a solitary lifestyle is the ancestral state for bees, and that eusociality evolved multiple times within bees. Eusociality occurs in three groups of bees, corbiculates, allodapines, and halictids, each of which are discussed below.

The corbiculate bees are divided into four tribes: Apini (honey bees, including *A. mellifera*), Meliponini (stingless bees), Bombini (bumblebees), and Euglossini (orchid bees). All tribes except Euglossini are eusocial, with Apini and Meliponini displaying especially elaborate eusocial colonies. In a situation similar to that in vespid wasps, morphology and molecules conflict over the phylogenetic resolution of the corbiculates. Morphological studies (Michener 1944; Roig-Alsina and Michener 1993; Chavarría and Carpenter 1994; Engel 2001; Schultz et al. 2001; Cardinal and Packer 2007) largely support a phylogenetic arrangement of {Euglossini, [Bombini, (Apini, Meliponini)]}, implying a single origin of the advanced eusociality seen in Apini and Meliponini. However, ever-increasing molecular data (Cameron 1993; Cameron and Mardulyn 2001; Kawakita et al. 2008) often favor the alternative view that Bombini and Meliponini form a sister group to the exclusion of Apini, challenging the notion of a single origin of advanced eusociality. The oldest known crown-group bee fossil at ~65 Ma (*Cretotrigona prisca*) falls within Meliponini (Michener and Grimaldi 1988; Engel 2000), placing a lower bound on the origin of eusociality in this group.

Eusociality also occurs in the allodapine bees, one of four tribes within the sub-family Xylocopinae. Allodapines show a broad range of eusocial behavior (Schwarz et al. 2007) and recent work has demonstrated that no allodapine group is strictly solitary (Tierney et al. 2008). Molecular divergence-dating studies suggest a single origin of eusociality in this group dating back 40–80 Ma (Schwarz et al. 2003, 2006).

Halictidae (sweat bees) exhibit particularly flexible forms of eusociality over both ecological and evolutionary timescales (Schwarz et al. 2007). Eusociality occurs in three halictid taxa: Augochlorini, *Halictus*, and *Lasioglossum*. These groups have experienced several evolutionary reversals back to a solitary state, as many as 4–6 times both in *Halictus* and *Lasioglossum* (Danforth 2002; Danforth et al. 2003). Molecular divergence dating infers recent origins for halictid eusociality, ~20–25 Ma in each of the three groups (Brady et al. 2006b). This time period coincides with a global warming trend during the late Oligocene warming and mid-Miocene climatic optimum (Zachos et al. 2001). A potential correlation between eusocial evolution and climatic warming is strengthened by observations that climatic factors influence the manifestation of eusociality in some modern species that have solitary forms in colder areas and eusocial forms in warmer areas (Sakagami and Munakata 1972; Eickwort et al. 1996; Miyanaga et al. 1999; Richards 2001; Soucy 2002; Soucy and Danforth 2002; Cronin and Hirata 2003); this switch may be due to an increased growing season necessary for producing multiple seasonal broods. These results combine to suggest that additional study of the taxonomy, phylogeny, and social evolution of halictid bees may provide an especially valuable window into the origins and early maintenance of insect eusociality.

Conclusions

The symposium speakers demonstrated that considerable progress has been made in recent years in our understanding of evolutionary processes and patterns in the insects. Molecular phylogenetic approaches, when combined with morphological data and evidence from the fossil record, have provided a much more robust and fine-grained understanding of phylogenetic relationships. With regard to the origin of the insects, while many details remain unclear, a consensus is developing that the hexapods arose from an aquatic crustacean ancestor. The primitive condition in the insects, however, was a terrestrial lifestyle. Evidence suggests that adaptations

to an aquatic lifestyle and the evolution of flight occurred subsequent to adaptation to a terrestrial existence.

A more fine-grained knowledge of evolutionary relationships within the insects has proven very useful for elucidating the evolutionary patterns of behavioral and physiological processes such as color vision, circadian rhythm generators, and eusociality. Mechanistic and selection studies have proven useful in elucidating evolutionary patterns related to ion transport and respiratory physiology in the insects. One of the most intriguing aspects of the insects is their capacity for sustained, powered flight. While a strong case can be made that flight evolved in the insects from the capacity for guided, gliding flight, the precise morphological and physiological processes by which wings and flight musculature arose in the insects remains an intriguing problem for further study.

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