

# 1 Diversity and Evolution

## INTRODUCTION

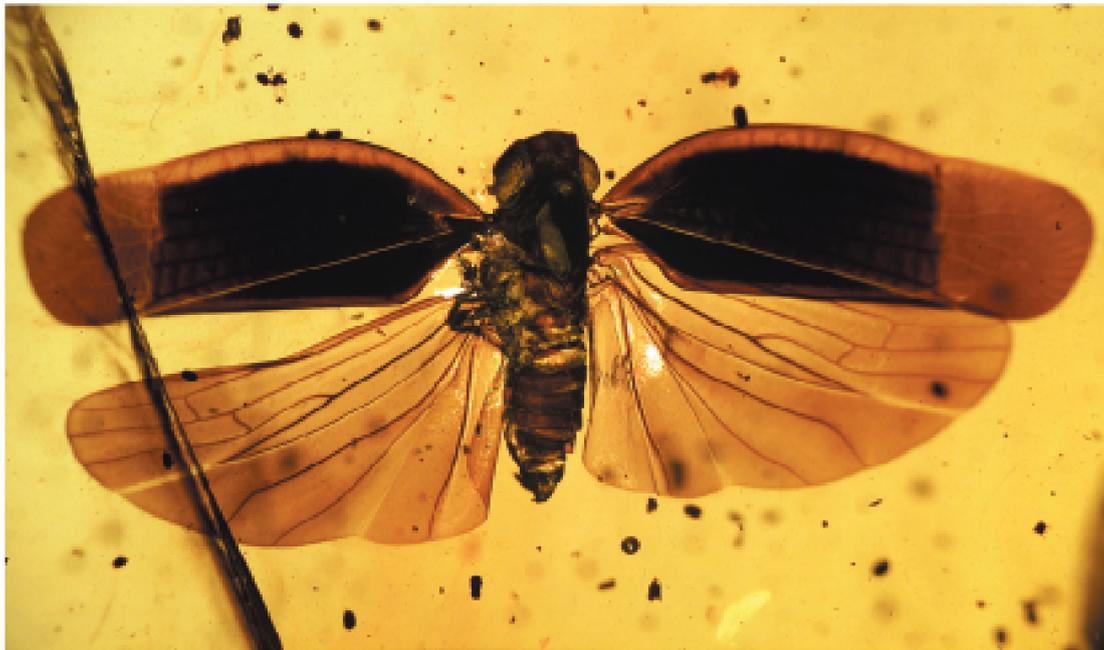
Evolution begets diversity, and insects are the most diverse organisms in the history of life, so insects should provide profound insight into evolution. By most measures of evolutionary success, insects are unmatched: the longevity of their lineage, their species numbers, the diversity of their adaptations, their biomass, and their ecological impact. The challenge is to reconstruct that existence and explain the unprecedented success of insects, knowing that just the veneer of a 400 MY sphere of insect existence has been peeled away.

**Age.** Insects have been in existence for at least 400 MY, and if they were winged for this amount of time (as evidence suggests), insects arguably arose in the Late Silurian about 420 MYA. That would make them among the earliest land animals. The only other terrestrial organisms of such antiquity are a few other arthropods, such as millipede-like arthropleurids and scorpion-like arachnids, and some plants. But age alone does not define success. Various living species belong to lineages that are hundreds of millions of years old, like horsetails (*Equisetum*), ginkgo, horseshoe “crabs” (*Limulus*), and the New Zealand tuatara (Rhynchocephalia), all of which, and many more species, are vestiges of past diversity. The living coelacanth (*Latimeria*), as another example, is the sole survivor of a 380 MYO lineage, and the very synonym for “relict.” Not so for the insects. While there are some very significant extinct insect lineages, such as the beaked Palaeodictyoptera, most modern insect orders appeared by 250 MYA, and many living insect families even extend to the Cretaceous about 120 MYA. Some living insect families, in fact, like staphylinid beetles and belostomatid water bugs, appeared in the Late Triassic approximately 230 MYA. By comparison, 120 MYA only the earliest and most primitive therian mammals had appeared, and not until 60 MY later did modern orders of mammals appear. Perhaps the most recited example of evolutionary persistence concerns 300 million years of

cockroaches, but this also brings up a very important aspect about fossils, which is their proper interpretation.

Fossil “roachoids” from 320 MYA to 150 MYA were actually early, primitive relatives of living roaches that retained a large, external ovipositor and other primitive features of insects (though they did have a shield-like pronotum and forewings similar to modern roaches). To interpret roachoids or any other fossil properly, indeed the origin and extinction of whole lineages, it is crucial to understand phylogenetic relationships. The incompleteness of fossils in space, time, and structure imposes challenges to understanding them, which is why most entomologists have avoided studying fossil insects, even beautifully preserved ones. Fortunately, there has never been more attention paid to the phylogenetic relationships of insects than at present (Kristensen, 1975, 1991, 1999a; Boudreaux, 1979; Hennig, 1981; Klass, 2003), including research based on DNA sequences (Whiting *et al.*, 1997; Wheeler *et al.*, 2001; Whiting, 2002), so an interpretive scaffolding exists and is being actively built. Entomologists are beguiled by the intricacy of living insects, their DNA, chemistry, behavior, and morphological detail, as the electron micrographs throughout this book partly reveal. But, ignoring fossils relegates us to a small fraction of all insects that have ever existed and seriously compromises our understanding of insect evolution.

Fossils provide unique data on the ages of lineages, on radiations, and on extinctions (Figure 1.1). Social bees, for example, occur today throughout the world’s tropics. However, based on diverse fossils in amber from the Baltic region – an area today devoid of native advanced social bees aside from the western honey bee, *Apis mellifera* – they were unexpectedly diverse in the Eocene 40–45 MYA (Engel, 2001a,b). Ants and termites existed for 50–100 MY before they became diverse and abundant (Grimaldi and Agosti, 2000; Dlussky and Rasnitsyn, 2003), indicating that sociality per se is insufficient for ecological dominance (rather, highly advanced societies in huge colonies make *certain* ants and termites ecologically dominant today). Tsetse flies (Glossinidae)

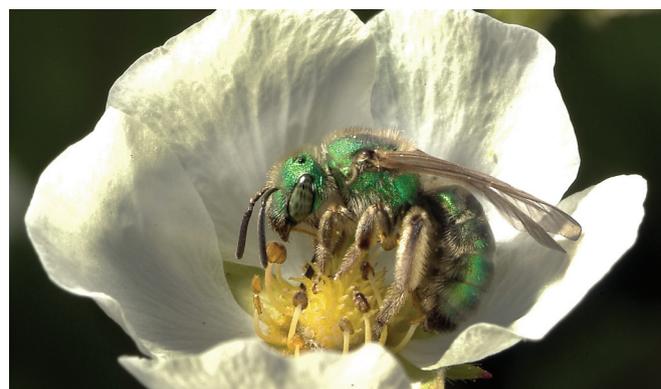


1.1. A fossil plant hopper of the living family Issidae, in Miocene amber from the Dominican Republic. Fossils are the only direct evidence of extinct life so they contribute unique insight into reconstructing evolutionary history. M3445; wingspan 8 mm; Photo: R. Larimer.

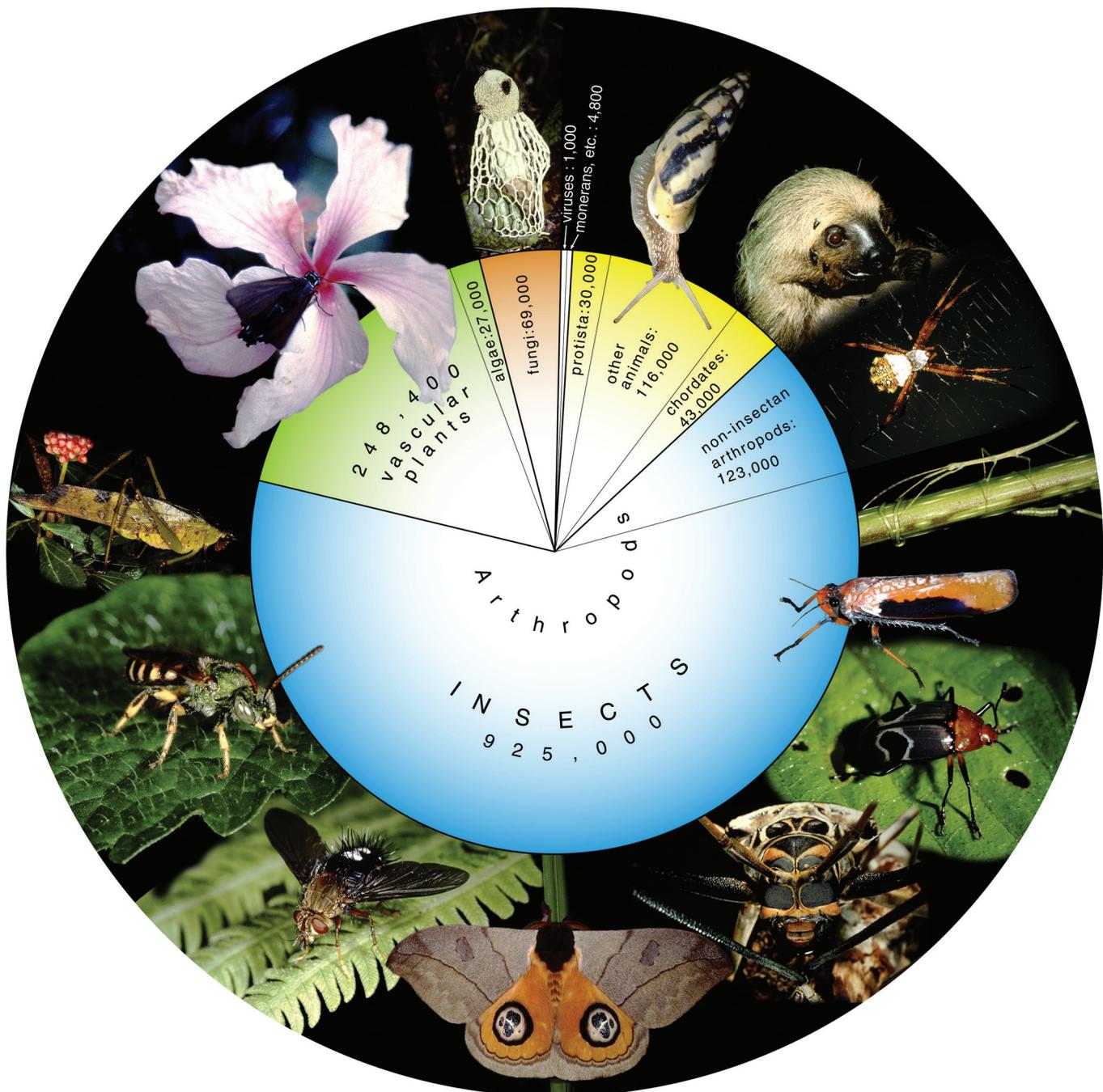
occurred in Europe and North America in the Oligocene and latest Eocene, 30–40 MYA, far outside their range in Africa today. Giant odonatopterans – griffenflies – cruised the Permian skies, their size possibly enabled by the high oxygen content of the atmospheres at the time (Dudley, 2000). When fossils provide insights like these, the greatest sin of omission arguably is avoidance of the fossil record, despite the challenges to studying fossils. Such avoidance is certainly not for a shortage of insect fossils.

The insect fossil record is surprisingly diverse and far more extensive than most entomologists and paleontologists realize. Hundreds of deposits on all continents harbor fossil insects (Rasnitsyn and Quicke, 2002; Chapter 2). Also, the manner in which insects have become fossilized exceed that of probably all other organisms except plants (Chapter 2). Insects are commonly preserved as compressions in rock (particularly their wings), but they are also preserved as exquisite three-dimensional replicas in carbon, phosphate, pyrite, and silica; as original cuticular remains from Pleistocene and Holocene tar pits, bogs, and mammalian mummies; as remains of their galleries and nests; and as inclusions in chert, onyx, gypsum, and of course amber. Insects are the most diverse and abundant fossils in ambers around the world (Grimaldi, 1996), though fossil resin records only the last third of insect evolutionary history. More recent exploration of fossilized plants has revealed a wealth of insect feeding damage (Scott, 1991; Scott *et al.*, 1991; Labandeira, 1998), including specialized relationships between insects and plants.

Fortunately, the voluminous and scattered primary literature on fossil insects is now summarized in several compendia. The treatise by Carpenter (1992) is a catalogue of fossil insect genera described up to 1983, illustrated with reproduced drawings of the type species for many genera. Since 1983 about 500 families and 1,000 genera have been added to the insect fossil record. Carpenter's treatise is nicely complemented by the volume by Rasnitsyn and Quicke (2002), since the latter reviews major fossil insect deposits, insects in ancient ecosystems, and the fossil record and relationships within orders, particularly of extinct families. The volume by Rasnitsyn and Quicke, though, uses names of insect groups from Laicharting (1781), which no one else uses or even



1.2. A common halictine bee, visiting a flower in Vancouver, Canada. Flowering plants, and therefore much of terrestrial life, depend in large part on insect pollinators. Nearly half of all living insects directly interact with plants. Photo: R. Swanson.



1.3. The diversity of life shown as proportions of named species.

recognizes, and their systems of relationships (based almost entirely on fossil evidence) often conflict with phylogenies based on expansive evidence from living insects. Short reviews of the fossil record of insects include Wootton (1981, for Paleozoic insects only), Carpenter and Burnham (1985, now rather dated), Kukalová-Peck (1991), Ross and Jarzembowski (1993), Willmann (1997, 2003), Labandeira (1999, 2001), and Grimaldi (2001, 2003a). The volume by Hennig (1981) attempted to synthesize the geological record of insects with relationships of living insects, but the evidence he drew from was very limited compared to what is

now known. We have adopted Hennig's approach here, drawing fossils into the fold of the spectacular Recent diversity of insects, but in a much more comprehensive treatment and based on original study of many fossils.

Species and Adaptive Diversity. The daunting number of Recent species of insects is well known to naturalists (Figures 1.2 and 1.3). Though there are nearly one million described (named) species, the total number of insects is believed to be between 2.5 million and 10 million, perhaps around 5 million species. In an age of such technological

sophistication and achievement, it is remarkable that there is an error range for estimates of insect species in the millions. Despite this fundamental problem, without a doubt the diversity of any other group of organisms has never been more than a fraction of that of insects. The enduring question, of course, is: Why? The arthropod design of an exoskeleton with repetitive segments and appendages preadapted insects for terrestrial existence, and wings further refined this design by vastly improving mobility, dispersal, and escape. Judging just from Recent species, though, a more recent innovation in insect evolution spurred their success, which is holometabolous development. Just four orders today, Coleoptera, Diptera, Hymenoptera, and Lepidoptera account for approximately 80% of all insects, and these have a larva, or “complete” metamorphosis. It is uncertain, though, why a larval stage is so advantageous, as we discuss later. Two lineages within the holometabolous “big four” contain the two largest lineages of plant-feeding animals: the Lepidoptera (150,000 species) and phytophagous beetles (100,000 species). In each of these two lineages, almost all species feed on angiosperms, and many are restricted to particular species or genera of angiosperms. Indications are that these and other insect groups (indeed, nearly half of all insects) have co-radiated with the angiosperms beginning 130 MYA, but exactly how host plant specialization promotes speciation still needs to be resolved.

Another measure of diversity besides number of species is the variety of structures and behaviors that adapt insects to environmental challenges. The most obvious of these is wings. Insects are one of only four lineages of animals that had or have powered flight, the others being (in order of appearance) pterosaurs, birds, and bats. Insects evolved flight just once (based on the apparent common ancestry of all winged insects, or pterygotes), at least 100 MY before pterosaurs and perhaps 170 MY before them if *Rhyniognatha* (Figure 5.8) was actually winged. A time traveler going into the mid-Carboniferous to the mid-Triassic, 330–240 MYA, would have seen only insects in the air. Insects indeed. During the Permian, giants like *Meganeuropsis permiana* had a 27 inch (70 cm) wingspan and were the apex of aerial predators. Today, the flight of most insects outperforms that of birds and bats in energetic efficiency, wing beat frequency, and agility, though not speed. Birds and bats are the major vertebrate predators of Recent insects, but they clearly didn't wrest the air from insects; insects may have even spurred the evolution of flight in early insectivorous ancestors of these vertebrates. As birds and bats improved their abilities in flight, insects evolved an arsenal of defenses against them. No group of animals, for example, matches the camouflage and mimicry seen in insects (e.g., Figures 7.24 to 7.27, 13.62, 13.77, 13.87). Night-flying insects repeatedly evolved hearing organs sensitive to the ultrasonic calls of bats so they divebomb or fly in loops to escape an approaching bat. Myriad day-flying insects

have evolved warning, or aposematic, coloration either to advertise their venomous or toxic defenses or to mimic such species (e.g., Figures 13.88, 13.90). No group of animals possesses the chemical repertoire of insects from pheromones to toxic defensive secretions (Eisner, 2003). Only plants are as diverse in their chemical defenses, and in many cases phytophagous insects sequester host plant toxins for their own use.

Our time traveler to 330–240 MYA would also have noticed no chorusing frogs or song birds, not even dinosaurs. Other than the occasional squawk or grunt of a labyrinthodont or other early tetrapod, animal sounds would have been largely from singing insects. Fossilized wings of orthopterans are preserved complete with stridulatory structures, and in one case were used to reconstruct the song (Rust *et al.*, 1999). One can only imagine that Triassic Titanoptera (Figure 7.43) had a deep, resonant song, like a bullfrog. By the Jurassic the familiar nocturnal trill of crickets filled the air.

Sociality is perhaps the most striking and sophisticated innovation by insects (Wilson, 1971). Only one mammal (the naked mole rat of Africa) has advanced sociality, a behavior involving closely related individuals of different generations living together and specialized for particular tasks, particularly reproduction. Otherwise, sociality is entirely an arthropod innovation that occurs in groups as diverse as mantis shrimps and some spiders (Choe and Crespi, 1997) but that has evolved approximately 20 times in insects (Chapter 11; Table 11.7). The colonies of some attine (leaf cutter) ants, army and driver ants, and termitid termites contain millions of individuals housed in labyrinthine nests – the most elaborate constructions in nature. Such large colonies usually have extreme specialization: major and minor workers, soldiers, a queen replete with huge ovaries to produce thousands of eggs per year, and expendable males. No societies, including those of humans, have such efficiency.

To some extent adaptive diversity is both the cause and the effect of species diversity, but it also seems to be an intrinsic aspect of insect design, with refinements building on the basic design. Having six legs allows for the front pair to become raptorial or fossorial without losing the ability to walk. Wings facilitate mobility, but when the fore pair is hardened as in Heteroptera and Coleoptera, they protect the flight pair and abdomen when the insect is wedged in tight spaces and burrowing into substrates. An impervious exoskeleton guards against injury and desiccation on land but also protects insects from their own toxic secretions (Blum, 1981).

**Ecological Dominance.** In terms of biomass and their interactions with other terrestrial organisms, insects are the most important group of terrestrial animals. Remove all vertebrates from earth, by contrast, and ecosystems would function flawlessly (particularly if humans were among them). Insects, moreover, have invaded virtually every niche except

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the benthic zone, including ocean shores and in one instance (the water strider *Halobates*) the open ocean. On land, though, insects reign.

Angiosperms are the defining terrestrial life form, but even these have co-radiated with the insects. Approximately 85% of the 250,000 species of angiosperms are pollinated by insects, and the inspiring diversity of flowers, in fact, is due in large part to insects lured to them (Figure 1.2). Thousands of generalized insect species visit and feed from flowers today, so similar liaisons in the Early Cretaceous must have spurred the diversification of angiosperms, and fossils indicate that specialized insect pollinators evolved quickly after angiosperms appeared. When bees evolved about 120 MYA, and later radiated eventually to form the current fauna of 20,000 species, the world truly blossomed. Bees are extremely efficient foragers and pollinators, and without doubt these insects alone are the most important agents of pollination.

The impact of insects, as plant-feeding organisms (phytophages), eclipses that of all other animals, the most impressive testament being crop pests. No other group of organisms affects agriculture and forestry as much as insects. A few of the more devastating ones include the boll weevil (*Anthonomus grandis*), Colorado potato beetle (*Leptinotarsa decemlineata*), and Mediterranean fruit fly (*Ceratitis capitata*), which alone inflict annual damage amounting to hundreds of millions of dollars, and for which tons of insecticides are broadcast. Migratory locusts (*Schistocerca*) form swarms of biblical proportions – billions of individuals covering several thousand square kilometers – and because they have indiscriminate diets, their swarms denude entire landscapes. Bark beetles (Scolytidae) and gypsy moths (*Lymantria*) can destroy or denude entire forests. In all, the cumulative effect of approximately 400,000 species of plant-feeding insects must be staggering. It has been estimated, in fact, that every species of plant has at least one species of insect that feeds on it, and probably all plants have many more than this (some host dozens of insect species). Even on the savannas of eastern Africa, renowned for the vast herds of ungulates, insects like orthopterans, beetles, caterpillars, and termites consume more cellulose than all mammalian herbivores combined. The array of plant chemical defenses is arguably attributed to the herbivory of insects, two groups that have been waging an arms race for 350 MY or more.

Insect vectors of pandemic diseases have probably affected humans more than any other eukaryotic animals. Tens of millions of people have died throughout historical times as a result of just six major insect-borne diseases: epidemic typhus (a spirochete carried by *Pediculus* lice), Chagas's disease (a trypanosome carried by triatomine bugs), sleeping sickness (another trypanosome, carried by *Glossina* tsetse), and the three big ones, malaria (*Plasmodium* carried by *Anopheles* mosquitoes), yellow fever (a virus carried by *Aedes* mosquitoes), and plague (a bacterium carried by

*Xenopsyllus* and *Pulex* fleas). Two mutations in humans, sickle cell anemia and the delta-32 gene, are actually genetic adaptations to millennia of selection by malaria and plague, respectively. While these microbes are the immediate agent of selection, their mosquito and flea vectors are the only metazoans known to have affected the evolution of humans. Given the scale with which humans have been affected, blood-feeding insects have obviously had an immense effect on natural populations of various land vertebrates.

While earthworms are absolutely essential for soil-building (humification), certain insect detritivores, particularly termites (Isoptera), play a role that earthworms can't. Termites comprise an estimated 10% of all animal biomass in the tropics; one virtually cannot kick into a rotting log in a tropical forest without having termites spill out. In tropical regions they consume an estimated 50–100% of the dead wood in forests, as well as dead grasses, humus, fungi, and herbivore dung, and so are absolutely essential in mineralization of plant biomass. The huge termite mounds on the savannas of Africa, South America, and Australia are chimneys for the waste gases from the huge underground nests. A large nest has the respiratory capacity of a cow, and it has even been estimated that termites contribute 2–5% of the annual global atmospheric methane. The amount of soil that is moved by these insects is prodigious: one geological formation in eastern Africa, formed between 10,000 and 100,000 years ago by the living mound-building species *Macrotermes falciger*, consists of 44 million cubic meters of soil (Crossley, 1986). Some ants vie with the excavation abilities of these termites, particularly leaf-cutter (attine) ants. Unrelated *Pogonomyrmex* ants, which form modest-sized colonies of approximately 5,000 individuals, excavate sand that is more than 100 times the weight of the colony in just 4 days (Tschinkel, 2001). Since the biomass of ants in the world's tropical river basins is estimated to be up to four times that of vertebrates, their impact on humification and mineralization, as well as the predation of other arthropods is likewise prodigious. But perhaps no other fact speaks to the ecological significance of ants as this: More than 2,000 species in 50 families of arthropods mimic ants, hundreds of plant species in 40 families have evolved specialized structures for housing ant colonies, and thousands of hemipteran species engage in intimate protective alliances with ants in exchange for honeydew. Ants have had a pervasive effect on the evolution of other insects and are clearly keystone consumers in the tropics.

Because insects have been so destructive to agriculture and human health, less informed people gladly imagine a world devoid of insects. But *if ants, bees, and termites alone were removed from the earth, terrestrial life would probably collapse*. Most angiosperms would die, the ensuing plant wreckage would molder and ferment for lack of termites, soil depleted of nutrients would barely be able to sustain the remaining plants; erosion would choke waterways with silt.

Vast tropical forests of the Amazon, Orinoco, Congo, and other river basins would die off, and the earth's atmosphere and oceans would become toxic.

Without a doubt, the ecological significance of insects, their diversity, and the longevity of the insect lineage makes this the most successful group of organisms in earth's history, and a subject completely worthy of our understanding.

### SPECIES: THEIR NATURE AND NUMBER

To understand evolution and its history, it is essential to understand what is a species. The concept of species is so entrenched in biology that it should be very easy to define or describe, but it has meant different things to different biologists. Species (singular and plural) have generated a great deal of discussion (perhaps too much), but it is important to review it briefly here because the hallmark of insects is that there are more species of them than any other group of organisms. Without question, species comprise a real unit – the fundamental unit of nature (Wilson, 1992) – and not a category defined at somewhat of an arbitrary level, like genera and families. Fortunately, we can draw on several intensively studied insects to illustrate the empirical nature of species.

Species have been recognized well before Linnaeus, who erected this as a formal category for classification (“species” means “kind” in Latin). In the first half of the twentieth century, the New Synthesis in evolutionary biology was preoccupied with variation and its significance in evolutionary change. One of its architects, Ernst Mayr, reacted strongly to the traditional systematic concept of species. To Mayr (1942, 1963), the concept of species up to that point was *typological*, wherein systematists grouped individual organisms into a species if they all conformed to a particular standard or ideal. Mayr, as a bird systematist, was familiar with the constant variation within species that sometimes confounded interpretations of species' boundaries. Most systematists dismissed the variation as trivial, but to Mayr and other evolutionists the variation was highly significant. Mayr's definition of species, the *biological species concept*, was “a group of actually or potentially interbreeding populations, which are reproductively isolated from other such groups.” In other words, if two individuals mate and produce offspring, they're the same species, because they share the same gene pool. There were difficulties with this concept. First, “potentially” was an unfortunate adverb to use. Many closely related species can be forced to breed in the laboratory, zoo, or barnyard, but they produce infertile offspring or hybrids, like mules, but hybrids of some species are fertile. It was argued, in response, that individuals within a species would only breed *naturally*, but, again, such hybrids also occur, like the “red wolf” of the southern United States, which is a wolf-

coyote hybrid. Also, what about parthenogenetic organisms, including bacteria, all bdelloid rotifers, many insects, and even some vertebrates, all of which are easily classifiable as species on the basis of morphology and DNA? Or fossils? Individuals separated by thousands of generations may belong to the same species, but they are hardly reproductively compatible. Lastly, the daily work of systematists is deciphering species from preserved specimens, so breeding experiments are just too impractical, and yet great progress has been made in deciphering species. In fact, Mayr (1942, 1963) used these traditional systematic studies with their “typological” concepts quite successfully in formulating the biological species concept.

Another major criticism leveled against the biological species concept is that it defines species on the basis of the process by which they arise: Species are formed when an isolated population or group of individuals becomes reproductively isolated from other populations. Defining species as reproductively isolated (or interbreeding) groups of individuals is thus circular. In response, some systematists defined species using different criteria, leading to evolutionary (Simpson, 1944; Wiley, 1978), phylogenetic (Wiley, 1978; De Queiroz and Donoghue, 1988; Cracraft, 1989; Wheeler and Meier, 2000), and other concepts of species (reviewed in Futuyma, 1998). The first two of these are actually not very different, and they also accommodate the process by which systematists work. A reasonable consensus of the evolutionary and phylogenetic definitions of a species is that it is *a discrete group of individual organisms that can be diagnosed, or defined on the basis of certain specialized features, and that had a common ancestor and unique evolutionary history*. The species could be defined on the basis of any feature of its genotype or phenotype, including morphology and behavior. Strict adherence to this definition, however, is not without its problems. First, how can a “unique evolutionary history” actually be observed? It can only be inferred, based on the strength of the evidence defining the species, like the morphological characters or the DNA sequences. If the sole criterion for circumscribing species is that they be discrete groups of individuals, then some variants could be called different species, like the color morphs of many butterflies or castes of an ant colony. A few phylogeneticists might not have any problem calling color morphs of a butterfly as different species, but we actually know that the morphs differ by just one or a few genes that affect coloration, and in all other respects they are identical.

In reality, systematists have been using a phylogenetic and evolutionary species concept all along. They assess variation and then lump individuals on the basis of consistent similarities. It is very reassuring that the results of this practice have largely agreed with results based on the biological species concept. This is well revealed by the study of two genera of insects, *Drosophila* fruitflies and *Apis* honey bees. Years of

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scrutiny of each of these two genera – their morphology, genetics, behavior, ecology, and hybrids – have provided probably more empirical evidence on the nature of species than have any other kind of organisms.

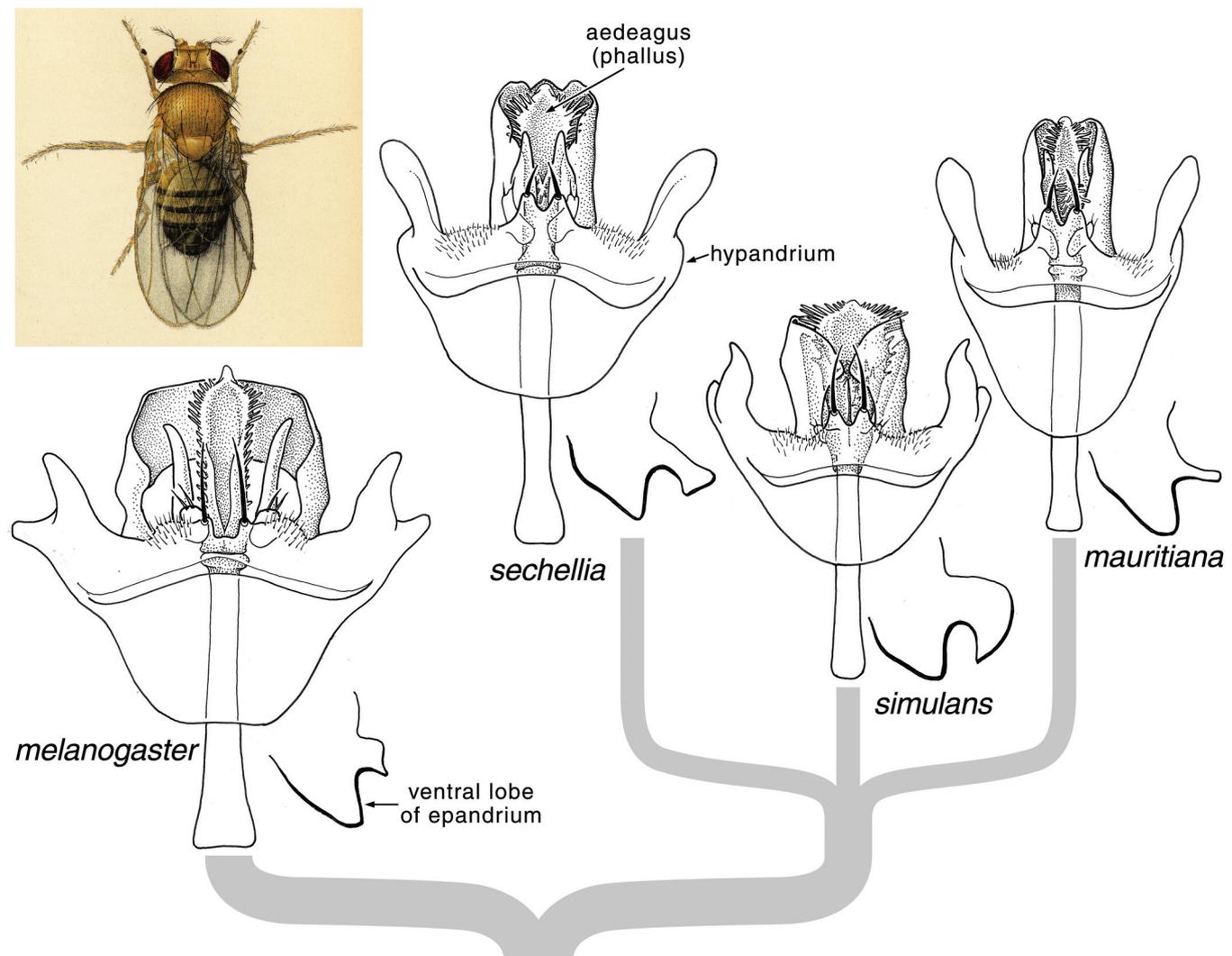
**DROSOPHILA**

That stupid little saprophyte.

–William Morton Wheeler, on *Drosophila melanogaster*

*Drosophila* fruitflies may not have the behavioral repertoire of ants that so fascinated the famous entomologist W. M. Wheeler, but *Drosophila* has revolutionized biology more than any other organism. Contrary to popular belief, *Drosophila* does not naturally live in little vials. There are approximately 1,000 species in the genus, which breed in a great variety of plants and other substrates. Some species are highly polyphagous and have followed humans around the

globe, the so-called tramp or garbage species. The laboratory fruitfly, *Drosophila melanogaster*, is one such tramp species. It was originally used by T. H. Morgan and his “fly group” at Columbia University for probing the elements of heredity and the behavior of chromosomes (see Sturtevant, 1965; Kohler, 1994). Because its genetics became so well known, *D. melanogaster* has been and is still used in all sorts of laboratory research, from cell biology, to physiology, behavior, and ecology (Lachaise *et al.*, 1988; Ashburner, 1989), making it, arguably, the best known eukaryotic organism. To better understand *D. melanogaster*, there has been intensive comparison of this species to its three closest relatives: *D. simulans*, which is a polyphagous African species introduced around the world; *D. mauritiana*, endemic to the islands of Mauritius and Rodriguez in the Indian Ocean; and *D. sechellia*, endemic to the Seychelles Islands, also in the Indian Ocean. The ancestral distribution of *D. melanogaster* is



1.4. Relationships among closely related species in the *Drosophila melanogaster* complex, differences being best reflected in the male genitalia (shown here). Relationships based on Hey and Kliman (1993) and Kliman *et al.* (2000).

believed to be central Africa. Collectively, these species comprise the *melanogaster* complex of species.

Individuals of the *melanogaster* complex are consistently separated and grouped on the basis of male and female genitalia (Figure 1.4), mating behavior (Cowling and Burnet, 1981; Cobb *et al.*, 1986), chromosomes (Ashburner and Lemeunier, 1976; Lemeunier and Ashburner, 1976), DNA sequences (Hey and Kliman, 1993; Kliman and Hey, 1993; Kliman *et al.*, 2000; Schawaroch, 2002), and other features, including larval diet. For example, even though *D. simulans* and *D. melanogaster* breed in a great variety of decaying fruits, *D. sechellia* is very specialized and breeds naturally only in fruits of *Morinda citrifolia* (Rubiaceae), which contain toxins that the other species can't tolerate. *Drosophila simulans*, *D. sechellia*, and *D. mauritiana* are most closely related, based on DNA sequences (Kliman *et al.*, 2000), their *homosequential* polytene chromosomes (there are no distinguishing inversions), and fertile F<sub>1</sub> hybrid females (F<sub>1</sub> males are sterile). In a comprehensive study of 14 genes and nearly 40 strains of these species (Hey and Kliman, 1993; Kliman and Hey, 1993; Kliman *et al.*, 2000), all or most strains of these species are grouped according to traditional separation using morphology and chromosomes. Interestingly, though, a few strains of *D. simulans* grouped with *D. sechellia* or *D. mauritiana*, but groupings varied depending on the gene.

Apparently, *D. sechellia* and *D. mauritiana* evolved nearly contemporaneously as peripheral, isolated populations of *D. simulans*. This has fundamental implications for systematics because in this case a living species is considered ancestral and not a simple two-branched divergence from an extinct common ancestor. In a mainstream phylogenetic view, at least some strains of *D. simulans* would not belong to that species, because they make *D. simulans* a *paraphyletic* taxon (basically everything left over after *D. mauritiana* and *D. sechellia* were extracted). Yet, *D. simulans* has distinctive (diagnosable) and consistent differences with other species in the complex. Also, a typical assumption in phylogenetic analyses is that divergence is bifurcating, or two-branched, even though traditional models of speciation allow for the simultaneous origin of species. Traditionally, it has been thought that isolated populations on the periphery of the range of an ancestral species can diverge into species, the old "Reisenkreiss" model of speciation, which may actually be the case for *D. simulans*, *D. mauritiana*, and *D. sechellia*. Most importantly, though, when all the evidence is considered in total, from DNA sequences to behavior, individual flies in the *melanogaster* complex are consistently categorized into discrete groups of individuals, which can be done even on the basis of morphology alone.

Hybrids in the *melanogaster* complex have also been intensively studied, and the genetics of hybrid sterility are known to be controlled by at least five genes on the X

chromosome (Coyne and Charlesworth, 1986; Wu *et al.*, 1993), and probably many more loci overall (Wu and Palopoli, 1994). Interestingly, it has been estimated on the basis of molecular clock estimates (Kliman and Hey, 1993; Kliman *et al.*, 2000) that *D. sechellia* and *D. mauritiana* diverged from *D. simulans* merely 420,000 and 260,000 years ago, respectively.

A few other examples in *Drosophila* show more of a continuum of groupings or divergence among individuals, perhaps the best studied being in the *Drosophila willistoni* species group. The *willistoni* group consists of 25 Neotropical species, six of which are "sibling" (cryptic) species, and among these six there are 12 "semispecies" and "subspecies," most of them in *Drosophila paulistorum* (reviewed by Ehrman and Powell, 1982).<sup>1</sup> The semispecies of *paulistorum* are morphologically indistinguishable so far as is known (one is never sure that very subtle features are being overlooked), and were first identified on the basis of chromosomal inversions. They also have distinct male courtship songs (Kessler, 1962; Ritchie and Gleason, 1995), and the hybrids of most crosses produce sterile males (Ehrman and Powell, 1982). DNA sequences of some *paulistorum* semispecies were examined (Gleason *et al.*, 1998), and these also group discretely. Thus, under evolutionary and phylogenetic definitions of species, *Drosophila paulistorum* itself could be considered a complex of cryptic species, but more data are needed to address this.

Interestingly, mating behavior (usually male courtship behavior) appears to diverge in *Drosophila* more quickly and prior to noticeable differences in morphology (e.g., Chang and Miller, 1978; Gleason and Ritchie, 1998; Grimaldi *et al.*, 1992), and this appears to be the case as well in many insects (Henry, 1994). It is known that just a few amino acid changes in a protein can dramatically affect, for example, an important component of *Drosophila* courtship song, the pulse interval (coded by the *period* gene; Wheeler *et al.*, 1991). Most morphological characters, by contrast, such as merely the shape of a lobe on the male terminalia of *Drosophila* (Coyne *et al.*, 1991), are highly polygenic. Divergence in mating behavior probably leads to further divergence (Liou and Price, 1994), which is eventually expressed morphologically.

<sup>1</sup> Sibling species and semispecies are categories devised largely by drosophilists and can be ambiguous terms. Sibling species are morphologically very similar or even identical, but the word "sibling" implies a close relationship, much like "sister group" in phylogenetics (which we discuss later). In fact, there are six sibling species in the *willistoni* group, some of which are closest relatives. Thus, we prefer the term "cryptic species" to simply mean morphologically indistinguishable or very subtly different species. The terms "semispecies" and "incipient species" imply these are not quite species, but are perhaps in the process of becoming species. But, because we can't predict the future, simply calling them populations and forms also adequately conveys their nature.

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### APIS

I hate myself, I hate clover, and I hate bees!

—Charles R. Darwin, in letter to J. Lubbock (3 September 1862)

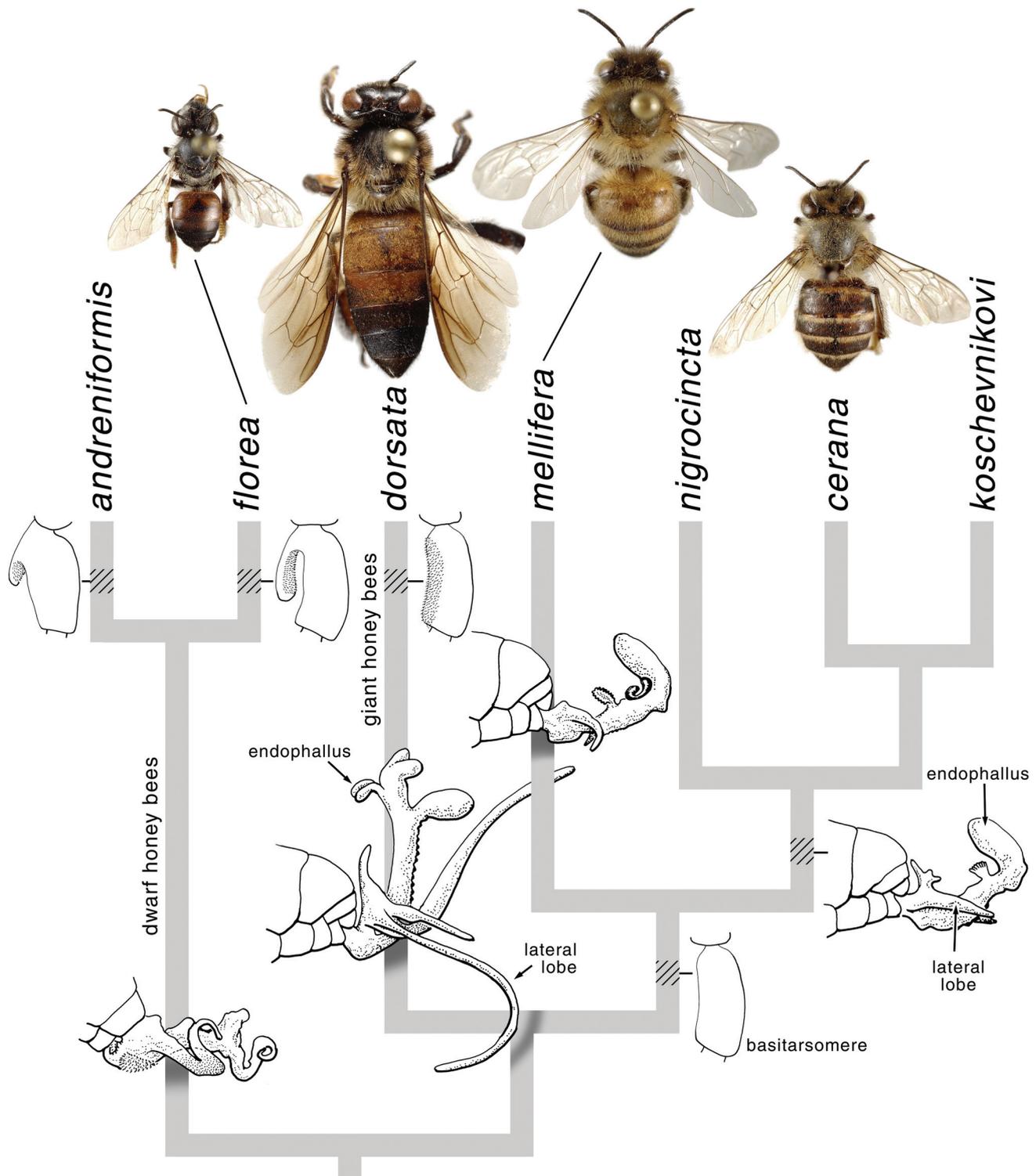
The western honey bee, *Apis mellifera*, has perhaps received more intensive study than any animal except *Drosophila melanogaster*, white mice, and humans. Like horses, dogs, and other domesticated animals, a cultural bond was forged between humans and honey bees from the earliest civilizations, and *A. mellifera* has even been woven into mythology and religions (Ransome, 1937; Crane, 1983, 1999). Honey bees are eusocial, living in perennial colonies within nests constructed principally of wax from the sternal glands of worker bees. The genus is native to the Old World (with the exception of the Australian Region and Pacific islands) but has been globally distributed by humans. There is, in fact, scarcely a vegetated place on earth where *Apis* is not found. While the pollination of honey bees is not always as efficient as that of wild bees (Buchmann and Nabhan, 1996), apiculture is a multibillion dollar industry, and the demand for honey alone makes it highly unlikely that *Apis* will be commercially displaced by native pollinators anytime soon.

Unlike *Drosophila*, with about 1,000 species, honey bees in the genus *Apis* have just seven currently recognized species (Engel, 1999e) (Figure 1.5), although some distinctive Asian populations are frequently elevated to specific status (e.g., Sakagami *et al.*, 1980; McEvoy and Underwood, 1988; Otis, 1991, 1996). This lack of species diversity, however, has not hindered systematists from classifying the extensive variation in honey bees. While drosophilists cite their sibling species and semispecies, apidologists refer to *subspecies* or *races*. Indeed, perhaps more scientific names (species, subspecies, and races) have been proposed for *Apis mellifera* than for any other organism, 90 to be precise (Engel, 1999e). Despite the effort concentrated on species of *Apis*, the recognition of natural groupings in the genus has been confusing. Numerous attempts to classify the variation in *Apis* have resulted in the recognition of from four to 24 species at any one time (e.g., Gerstäcker, 1862, 1863; Smith, 1865; Ashmead, 1904; Buttel-Reepen, 1906; Enderlein, 1906; Skorikov, 1929; Maa, 1953; Ruttner, 1988; Engel, 1999e).

Species of *Apis*, particularly *A. mellifera* and *A. cerana*, are widely distributed (even without the aid of humans), and they have a striking range of variation across their various habitats (Ruttner, 1988). The most noticeable variation is in coloration, but it also includes subtle morphological differences like the size and shape of cells in the wings. These variants were alternatively treated as species or subspecies in the past because they corresponded to geographical regions and climatic zones. As the New Synthesis began to influence apidologists, morphometric analyses (mostly of wing venation) were used to segregate individuals into “morphoclusters.”

Backed by the appearance of statistical rigor, these morphoclusters were then united into newly defined subspecies and species (Ruttner, 1988), and these studies became the norm for segregating honey bees into what were believed to be natural groups. Contradictions between the morphoclusters and numerous biological traits and molecular data were increasingly found (Hepburn and Radloff, 1998; Hepburn *et al.*, 2001), and large regions of hybridization further blurred the traditional distinctions of these forms. Subtle morphometrics of wing venation have proven to be of little systematic value.

Like most groups of insects, species of the genus *Apis* can be distinguished on the basis of differences in male genitalic structure to varying degrees (Ruttner, 1988; Koeniger *et al.*, 1991) and other morphological details of adults and even larvae (Ruttner, 1988; Engel, 1999e) (Figure 1.5). These differences are largely congruent with ecological, behavioral, chemical, and molecular features, and they serve to define most of the honey bee species, regardless of the preferred species concept. Adoption of the biological species concept, however, sent generations of apidologists into apiaries and fields seeking mating differences in honey bee populations that might be congruent with the traditional morphoclusters (i.e., subspecies). Differences potentially restrictive to gene flow were considered enough evidence to warrant species status for isolated subspecies. For example, the timing and location of mating flights is important in *Apis* biology because this is when virgin queens meet drones, with synchronization being critical for the two sexes to meet. Temporal segregation of drone flight times and spatial differentiation of drone congregation areas has therefore been used as evidence of reproductive isolation, and the separation of species in the absence of morphological features (e.g., Underwood, 1990; Hadisoelilo and Otis, 1996; Koeniger *et al.*, 1996). These behavioral differences are indeed significant because they likely represent incipient isolation, the first step in speciation. Such traits, however, are difficult to use for defining species. Even though forms can be segregated from each other at their point of contact, drone flight time varies considerably over its entire distribution within a species. On this basis, traits for species recognition are only applicable to one or a few locales and do not diagnose the species as a whole. It is difficult, if not impossible, to distinguish the species in its entirety from its peripherally distinct morphs. This is a common problem because the Biological Species Concept (BSC) is testable in regions of contact only. The BSC is not amenable to complete testing because some allopatric populations, such as the distinct island populations of giant honey bees (*Apis dorsata*), do not come into geographical contact. Most accounts ignore the historical relationships of the species and their populations and fail to think in terms of defining individual species on a global scale. In other words, how is it that we define *A. cerana* or *A. dorsata* across the



1.5. Relationships among species of Recent honey bees, genus *Apis*, showing important variations in tarsomeres and male genitalia. Relationships from Engel and Schultz (1997).

entirety of their ranges, distinct from regional morphotypes or ethotypes, and that may be reproductively isolated at fine geographical scales?

Perhaps the most dramatic development of variation is seen in the Cape honey bee, *Apis mellifera capensis*. This

subspecies is facultatively parthenogenetic and a social parasite on colonies of other honey bee subspecies. While *A. mellifera capensis* is still reproductively compatible with other subspecies of *A. mellifera*, gene flow is asymmetrical and the Cape bee dominates during introgressions (Johannsmeier,