

Tree of Lepidoptera showing the phylogenetic relationships among the most significant groups (45 taxonomic superfamilies). Colored boxes indicate high taxonomic ranks. Branches with thick lines indicate robust clades, and branches with thin lines indicate less-supported clades. The number in the green circle indicates the chapter in which lepidopterans are also included. The orange circle marks the most internal node and its age. Photographs illustrate principal clades; boxed numbers associate photographs with clades.

Lepidopterans

Butterflies and Moths

Roger Vila

SUMMARY Both professional researchers and aficionados, motivated by the appeal and popularity of Lepidoptera (butterflies and moths), have compiled a huge amount of knowledge on their biology, taxonomy, and distribution over the centuries, comparable only to the amount of information we have on vertebrates. However, it is only recently that a clear picture of the branch of the tree of life belonging to these insects has begun to emerge. The huge diversity of lepidopterans poses considerable difficulties for studying their evolution: with 170,000 species described, Lepidoptera represents the largest lineage of herbivorous organisms and one of the four most diverse orders on the planet. The limited fossil record has been another obstacle, both for understanding the evolution of morphological characters and for calibrating molecular clocks. The results of phylogenetic studies based on nucleotide sequences generally support hypotheses based on morphological characters. Indeed, both methods are advancing in parallel, and as more characters are added, the results are generally congruent. The taxonomy is gradually being adapted to phylogenetic results as they are consolidated and confirmed in numerous independent studies. Today, the most basal relationships are quite well resolved, as is the general structure of the tree. Along with fossil information, it tells the story of lepidopterans: their emergence in the Triassic or Early Jurassic from a common ancestor resembling the caddisfly (Trichoptera), the evolution from mandibulates to microlepidoptera with a proboscis, and the progressive acquisition of other specialized characters throughout one of the lineages, leading to the huge radiations of Ditrysia, which include the large moths and butterflies. Most of the relationships within the Ditrysia have yet to be satisfactorily resolved, due largely to this group's extremely rapid diversification. Projects under way include research on the phylogeny of the order based on morphological data and a large number of nuclear genes, the use of phylogenomics to resolve the most difficult cases, and investigation of multiple phylogenies of superfamilies and lower taxa. These promise exciting results that will clear up many obscure points in lepidopteran phylogeny and evolution.

WITH SOME 170,000 SPECIES DESCRIBED, LEPIDOPTERANS, along with coleopterans, hymenopterans, and dipterans, constitute one of the most diverse orders on the planet. Their beauty has captured the interest of scientists and aficionados alike, and the information compiled on their biology, taxonomy, and distribution is

the most extensive for any group of such great biodiversity. Their evolutionary success is based at least in part on their holometabolism, which allows for a high degree of specialization in their different life stages (see **Figure 36.1**). Another key factor is their specialized mouthpart (**proboscis** or **haustellum**), which al-

What is a lepidopteran?

A lepidopteran (from the Greek *lepis* [scale] and *pteron* [wing]) is a **holometabolous** (undergoing complete metamorphosis) insect whose larvae, called caterpillars, are herbivorous, with rare exceptions. It has six true legs and multiple prolegs with diminutive hooks on them. The adults develop two pairs of wings densely covered in scales that

contain pigments and microstructures with optical properties, giving their color patterns enormous plasticity. The order Lepidoptera, one of the most diverse orders on the planet, is the sister clade of the trichopterans or caddisflies (Trichoptera), and together these clades form the superorder Amphiesmenoptera.

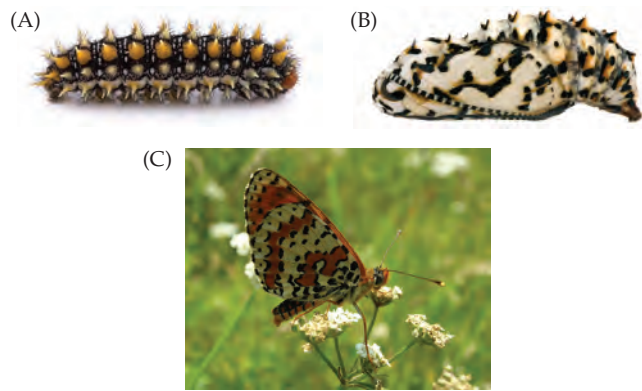


Figure 36.1 Postembryonic stages of a holometabolous insect, the lepidopteran *Melitaea didyma* (Papilionoidea: Nymphalidae). (A) Larva, (B) pupa, and (C) adult.

allows them to sip nectar from flowering plants (angiosperms). Lepidopterans are the largest lineage of phytophagous organisms in existence, as their larvae feed mostly on plants, especially angiosperms. These two groups have therefore evolved in tandem, and it is not surprising that the explosive radiation of angiosperms (see Chapter 12, Angiosperms) coincides with that of butterflies and moths, as well as that of other pollinating insects like bees (see Chapter 33, Hymenoptera). Another very important characteristic in the basic anatomy of lepidopterans is the dense covering of scale on their wings. This serves a function in both flight and thermoregulation, and also gives their color patterns enormous plasticity, a trait with profound implications for intra- and inter-specific relationships.

Knowledge of the lepidopteran evolutionary tree is still only partial (see Tree of Lepidoptera), and until recently was based only on morphological characters. Studies using modern techniques of phylogenetic reconstruction have appeared more recently, as have studies based on DNA sequences, or on a combination of morphological and molecular data. This chapter describes the phylogeny and evolution of lepidopterans, with special reference to results of the most recent studies.

Characteristics of Lepidopteran Genomes

Our knowledge of lepidopteran cytogenetics, as well as that of trichoptera with whom they share the characteristics described below, is limited. Their **sex-determination system** is **ZW** (heterogametic females). Their chromosomes are holocentric, appearing almost circular during metaphase, and generally small and numerous. Lepidopterans have the widest range of chromosome numbers of all animals, ranging from 7 to approximately 223. The haploid modal number for lepidopterans is 30–31, which is unusually high for

insects. Although most species have this chromosome number, there are notable exceptions. For example, the butterfly family Lycaenidae has a modal number of 23–24. In some clades, the variability in chromosome number is huge. For example, the subgenus *Agrodiastus* (Papilionoidea, Lycaenidae) contains species with chromosome numbers ranging from 10 to 134. *Polyommatus atlanticus* (Figure 36.2), also a lycaenid, is the metazoan with the highest chromosome number: its haploid number is around 223. *Leptidea sinapis* (Papilionoidea, Pieridae) boasts another record: it exhibits a cline (continuous gradient) in number of chromosomes from $n = 53$ on the Iberian Peninsula to $n = 28$ in Central Asia. Surprisingly, the extremes have been observed to maintain a notable level of fertility when crossed. Another curiosity of lepidopteran cytogenetics is that they have **achiasmatic** oogenesis; in other words, there is no meiotic recombination of maternal genetic material. Knowledge of this group's chromosomal evolution, the type of rearrangements, and the reasons for their occurrence is practically nonexistent. It seems that karyotype changes occur as a result of fusion and fragmentation, given that the total amount of genetic material apparently remains constant (see also Chapter 12, Angiosperms). The DNA content (**C-value**) has been determined for 60 species of lepidopterans, with an average value of 0.66 ± 0.04 picogram (pg), ranging from a minimum of 0.29 to a maximum of 1.9 pg.

The most commonly used markers in phylogenetics include mitochondrial genes (*cox1*, *cox2*, *nad1* and *nad5*) and single-copy nuclear genes (*CAD*, *EF-1a*, *H3*, *wg*, etc.), as well as ribosomal regions (mainly the subunit 28S), and noncoding regions such as spacer regions (ITS-2) of the nuclear genome. Mitochondrial markers have a greater substitution rate than nuclear markers, and are therefore very helpful for studying the relationship between only slightly divergent taxa,

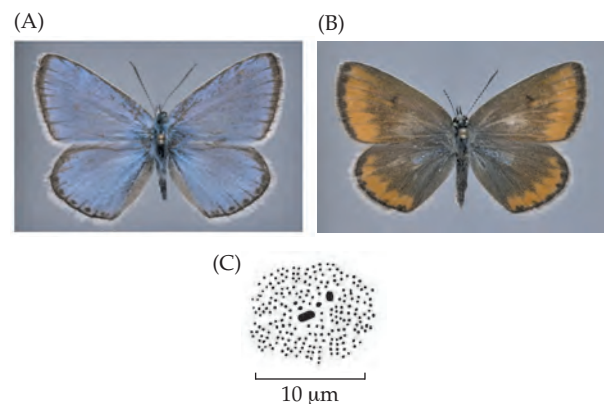


Figure 36.2 *Polyommatus atlanticus*, the species with the highest chromosome number of all metazoans studied. (A) Adult male. (B) Adult female. (C) Microscopic image of the nucleus of a male gamete in formation (metaphase 1) ($n =$ approximately 233).

Basic terms

Achiasmatic: Not forming a chiasma. The chiasma is the region where the filaments of non-sister chromatids cross during genetic recombination.

Apodeme: An infolding of the integument (exoskeleton) serving as an insertion point for musculature.

Aposematic coloration: Contrasted coloration, typically yellow and black, or red and black, that alerts potential predators and serves as an indication of toxicity or danger.

C-Value: The amount of DNA contained in a haploid nucleus of a eukaryotic organism.

Cerci (sing., *cercus*): A pair of appendages on the terminal segment of many arthropods' abdomens.

Epiphysis: Each of the ends of a long bone or joint.

Frenulum: A bristle located on the base of the hind wing, inserted into a hook or fold (*retinaculum*) at the base of the forewing. Forms part of the system joining the forewings and hind wings of most Heteroneura lepidopterans.

Galeae (sing., *galea*): External lobes on the maxilla which, in the case of lepidopterans, has been modified to form the haustellum.

Gonopore: The genital copulatory opening.

Haustellum: Mouthparts of the vast majority of lepidopterans, consisting of maxillae modified into a long tube that is kept rolled up in a spiral when not in use.

Holometabolous: Undergoing complete metamorphosis, through the postembryonic phases of larva, pupa, and adult (see Figure 36.1).

M veins: Central veins in the wings of insects running from the cell down to the termen, or external margin.

Macrolepidopteran: Belonging to one of the nine superfamilies that form the derived group Macrolepidoptera. Most are medium-sized to large species that have been recovered as a monophyletic clade in some studies, but not in others.

Metapopulation: A group of unstable local populations interconnected by migratory processes. This concept is of great significance for the study of habitat fragmentation and conservation.

Microlepidopteran: Belonging to one of the 36 non-Macrolepidoptera superfamilies. A nonmonophyletic group made up mostly of small-sized species (see Figure 36.4).

Myrmecophily: The ability to live in association with ants. In lepidopterans, myrmecophily is very common in the larvae and pupae of the families Lycaenidae and Riodinidae.

Ovipore: The female ovipositor opening.

Polyphenism: The expression of different phenotypes by a single genotype, depending on environmental conditions.

Preimaginal stages: Insect stages prior to the adult (imago) stage: egg, larva, and pupa (some of these are shown in Figure 36.1).

Proboscis: An elongated appendage on the head of an animal.

Retinaculum: A hook or fold on the base of the forewing into which a bristle (*frenulum*) extending from the base of the hindwing is inserted. It forms part of the system joining the forewings and hind wings of most Heteroneura lepidopterans.

Rs veins: Veins in the wings of insects running from the cell down to the apex or apical area.

Setae: Bristles on any part of an organism.

Sternite: Each of the ventral plates that form part of the cuticular lining of the segments in arthropods.

Tergite: Each of the dorsal plates forming part of the cuticular lining of the segments on arthropods.

ZW sex determination system: A system whereby the sex of offspring is determined by the ovum, not by the spermatozoid. The males are the homogametic sex (ZZ) and the females are the heterogametic sex (ZW). This system is also found in birds and certain actinopterygian fishes.

but saturation makes them much less useful for clarifying deeper relationships. The substitution rate in the nuclear markers is quite variable, and some also combine more or less conserved regions. Noncoding markers have insertions and deletions that make it difficult to align sequences between taxa at the level of tribe or higher for ITS-2 and at the level of family for 28S.

Phylogenetic Results Contrasted with Previous Classifications

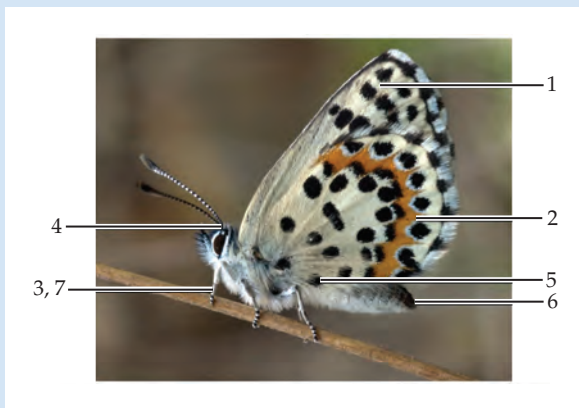
Molecular phylogenies are contributing a huge amount of new data on the origins of lepidopterans and their

evolutionary relationships. Information from the fossil record, valuable both for understanding the evolution of morphological characters and for calibrating molecular clocks, is very limited in the case of lepidopterans. The relative scarcity of lepidopteran fossils is due to the soft tissue of their larvae and the capacity of dead adult bodies to remain floating on water surfaces for long periods rather than sedimenting quickly. The oldest known lepidopteran fossil, *Archaeolepis mane* (190 million years old), belongs to the ancestral branch common to all extant lepidopterans. Based on this fossil, it would be reasonable to place the origin of the order in the Early Jurassic, some 200 million years ago.

BOX 36.1 Unique morphological characters of the Lepidoptera

Adult lepidopteran with some of the autapomorphies of the order indicated using the numbering from the list below. The characters listed are among the most evident of the more than 20 that have been described:

1. Wings with a dense scale covering on veins and membrane
2. **M Veins** of wings with three branches, instead of four or more (except in the only two known species of Agathiphagoidea)
3. Tibia of the forelegs with only one or no apical spine
4. Loss of central ocellus (simple eye)
5. First **tergite** very slightly sclerotized
6. Loss of **cerci** on the abdomen
7. Tibia of front legs with **epiphysis** articulated on inner surface.



However, the only molecular-clock dating performed for the order so far places its origin in the Triassic, some 240 million years ago.

The results of all the phylogenetic studies based on DNA sequences published to date agree with previous classifications that placed Trichoptera as the sister order to Lepidoptera (Figure 36.3); together, they form the superorder called Amphiesmenoptera. This is one of the very few relationships between orders of holometabolous insects on which there is unanimous agreement. The monophyly of the Amphiesmenoptera is supported by more than 15 morphological and vital synapomorphies, notable among which are the following:

- ZW sex-determination system (i.e., the females are the heterogametic sex);
- presence of **setae**, more or less modified into scales, on the wing surface; and

- larvae with modified salivary glands that produce silk.
- The most striking ecological specialization of the trichoptera is that their larvae construct highly elaborate casings or shelters, for which they use silk and material from their environment. Lepidoptera also have these glands, and some groups use them to build shelters for their larvae or, more commonly, for their pupae (see Tree of Lepidoptera).

The basal structure of the Tree of Lepidoptera is now quite well defined, and the results of phylogenies based on molecular data generally concur with the results based on morphological characters. The most widely accepted classification today divides the order into four suborders (Zeugloptera, Aglossata, Heterobathmiina, and Glossata), although there are still some questions left unanswered, such as the position of Agathiphagoidea (Aglossata). Other divisions have been posited based on morphological characteristics (Heterocera and Rhopalocera; Microlepidoptera and Macrolepidoptera; Monotrysis and Ditrysis); all of these are still used at times, although more detailed morphological studies and results based on molecular data have shown that they contain paraphyletic groups. There are currently 45 accepted superfamilies and around 124 accepted families, whose monophyly has been proven, in many cases, by molecular studies. In addition, in the last few years, robust molecular phylogenies and revisions of the systematics of most major superfamilies have been published. However, the deep relationships between the lepidopteran Ditrysis, a monophyletic group representing 98% of the order's species, have proven hard to resolve, largely due to the fact that its prodigious diversity contains numerous adaptive radiations that occurred almost simultane-

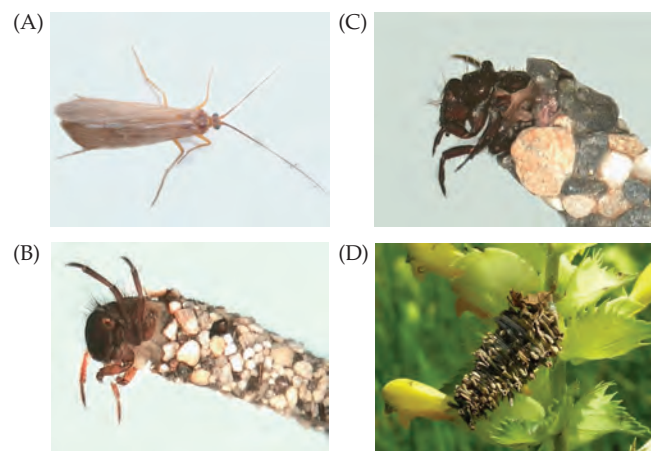


Figure 36.3 Trichoptera is the sister order of Lepidoptera. (A) Adult trichopteran and (B,C) Trichopteran larvae in their shelters. (D) Psychidae (Lepidoptera) larva in its shelter.

BOX 36.2 Lepidopterans by the numbers

- Number of species described: approximately 170,000
- Estimated number of species: 280,000–500,000
- Number of families: approximately 124
- Family with the largest number of species: Noctuidae (more than 35,000 species described), although the exact definition of this family is still being debated
- Origin: Triassic or Early Jurassic, 240–200 million years ago
- Oldest fossil: 190 million years old, *Archaeolepis mane* (Early Jurassic)
- Largest species: Wingspan of 280 mm in the adult *Thysania agrippina* (Noctuidae) (Central and South America). The largest wing surface, more than 400 cm², is that of *Attacus atlas* (Saturniidae) (Southeast Asia).
- Smallest species: Wingspan of <2 mm in adults of several species of the family Nepticulidae
- Approximate number of species described and estimated for the main groups:

Taxon	Species described	Species estimated (minimum)	Species estimated (maximum)
Basal groups	10,000	15,000	20,000
Gelechioidea	18,000	60,000	100,000
Tortricoidea	10,000	15,000	20,000
Pyraloidea	16,000	30,000	30,000
Papilionoidea	18,000	18,000	20,000
Geometroidea	21,000	30,000	45,000
Noctuoidea	70,000	100,000	150,000
Other	10,000	15,000	20,000
Total	173,000	283,000	405,000

ously. Although several relationships among Ditrysia superfamilies have been resolved in specific studies, each new study identifies contradictory relationships, making it almost impossible to reach any conclusion. Most of the discrepancies between traditional classifications and the new molecular phylogenies are found precisely in these highly diverse groups.

Evolution of Characters

Over the course of their evolution, lepidopterans (except for a few lineages whose habits and forms seem to have changed very little) have adapted their larvae and pupae to terrestrial environments. The adult forms have specialized in obtaining nectar and other fluids from flowers, with the development of

the proboscis. They have also improved their flight by modifying the venation, design, and attachment of their forewings and hind wings. The females have developed independent reproductive and excretory systems, and the copulatory opening has also been separated from the ovipositor opening for greater efficiency and control. It is also worth noting that on at least eight independent occasions, Ditrysia lepidopterans have developed tympanal organs that enable them to hear the echolocation signals of bats in order to avoid them.

Listed below are the most significant apomorphic characters, from the oldest apparently monophyletic groups to the most subordinate:

- Glossata: Vestigial, nonfunctional mandibles; elongated **galeae** forming a proboscis (also called **haustellum**); larva with a spinneret, an organ used to create silk thread, located on the top of the prelabial-hypopharyngeal lobe
- Coelolepida: Hollow scales on the wing surface
- Heteroneura: Female genitalia with the **gonopore** separate from the **ovipore**, and no connection between the two; reduction in the number of branches of **Rs veins** in the hind wings; forewings and hind wings coupled by the **frenulum** and **retinaculum** system; loss of the first abdominal **sternite**
- Ditrysia: Internal connection between gonopore and ovipore; second abdominal sternite with large **apodemes**; muscles in the proboscis in short bands instead of long fibers
- Apoditrysia: Second abdominal sternite with specialized apodemes, with large, short bases
- Obtectomera: Pupa whose first to sixth abdominal segments are immobile; loss of dorsal rows of spines on pupal tergites
- Macrolepidoptera: Complete loss of the vein that ends at the anal angle (CuP vein); larval prolegs with diminutive hooks forming a crescent-shaped series on the inner part

Evolutionary Tendencies

A comparison of the Lepidoptera with other orders, especially their sister order, makes it possible to define evolutionary tendencies specific to butterflies and moths. One of the most obvious is the fact that lepidopterans have specialized in terrestrial larval development, while caddisflies have specialized their larval development in aquatic environments. Only a few lepidopterans have aquatic larvae, and only a few trichopterans have terrestrial larvae (although they are always associated with damp leaf litter). Another evolutionary feature of butterflies and moths is their extremely

Butterflies: In search of a robust phylogeny

In spite of their peculiarities, both in appearance and in habits, butterflies form a clade with relatively little diversity within the immense variety of lepidopterans. The number of butterfly species (18,000) is approximately 10% of the total, although it is the most extensively studied group. Its position in the phylogenetic tree indicates that it is a group of **macrolepidopterans** specializing in diurnal activity that probably first appeared in the early Cretaceous, some 110 million years ago.

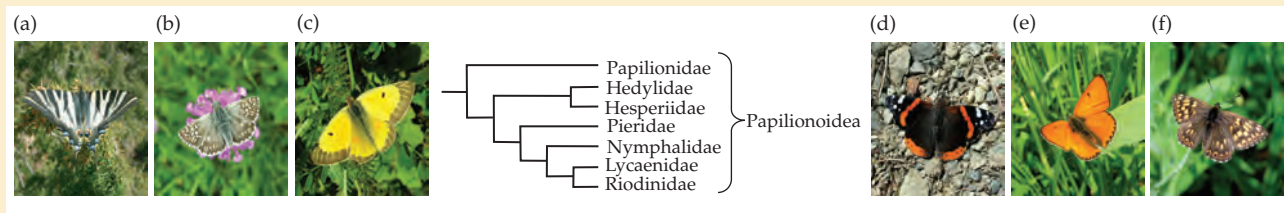
The beauty of butterflies has made them an object of study for nature lovers since ancient times. For this reason, data on their taxonomy, biology, and distribution is the most complete of all invertebrates. In spite of this wealth of information, the phylogenetic relationships between the main groups have been a source of considerable debate. In studies based mainly on morphological data on adult insects, various hypotheses have been posited with little empirical support. The first studies to include molecular data to address the problem were based on very short nucleotide fragments, and the conclusions were far from clear.

In light of this situation, an exemplary international collaboration project was established that brought together all the main molecular phylogenetics laboratories specializing in Rhopalocera, with one objective: to work together on the creation of a solid phylogenetic hypothesis based on a combination of morphological and molecular data. The resulting phylogeny identified Hesperioidea (made up of a single family, Hesperidae) as a

sister group to the rest (Papilionoidea). The five families within Papilionoidea were recovered as monophyletic, and the relationships between them appeared to be almost completely established.

However, the most recent phylogenies, which include a greater number of nuclear markers and revised morphological characters, have brought a new surprise: the family Papilionidae is the sister group to the rest; and Hedyliidae, in spite of its moth-like appearance (see Box *Macrosoma: The missing link?*), is a sister group of Hesperidae. The new relationships between families have resulted in the unification of all butterflies into a single superfamily: Papilionoidea (see **Figure**).

In the same way, by joining efforts and combining data, researchers are also trying to improve our understanding of the tree of all lepidopterans. In addition to the numerous groups studying the evolutionary relationships between subfamilies or lower groups, there are two especially noteworthy international collaboration projects. *LepTree*, a project funded by the US National Science Foundation's Assembling the Tree of Life Program (<http://www.phylo.org/atol/projects>), aims to resolve the phylogenetic tree by sequencing 24 genes (approximately 18 kb), from representatives of all of the families; and the European project *LepSys* (www.LepSys.eu) seeks to facilitate the integration of traditional morphological methods with the new molecular methods, as well as creating a catalogue and a classification of all lepidopterans.



Relationships among Papilionoidea families based on the combination of morphological and molecular data. Photographs of adult representatives of some of the families on the

tree. (a) Papilionidae, (b) Hesperidae, (c) Pieridae, (d) Nymphalidae, (e) Lycaenidae, and (f) Riodinidae.

high rate of diversification. In spite of being a relatively young order, lepidopterans have diversified considerably since their origins, especially in comparison with trichopterans. There are close to 170,000 known lepidopteran species, compared with 11,500 described species of trichopterans. This difference of more than one order of magnitude might be associated with the adaptation of butterflies and moths to diverse environments, to the high level of specialization of many species, and to the importance of sexual selection in this order. However, the Tree of Lepidopterans is extremely asymmetrical, and several basal lineages have very few ex-

tant species. Three of the four suborders (Zeugloptera, Aglossata, and Heterobathmiina) contain only around 250 known species. By placing the fossils and morphological characters on the evolutionary tree, it can be deduced that these lineages retain many ancestral characters and have remained almost unchanged for millions of years (evolutionary stasis). These species are superficially similar to the smallest trichopterans and probably resemble the common ancestor of all lepidopterans. The adults still have mandibles (functional in some groups, atrophied in others), an ancestral character that has been replaced by the proboscis in all other lepidop-

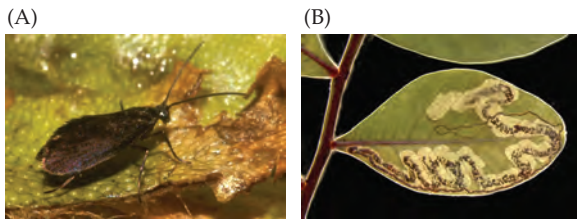


Figure 36.4 Microlepidopterans. (A) Adult micropterigid, of a genus and species not yet described, in northern Madagascar. (B) Mastic (*Pistacia lentiscus*) leaf mined by a larva of the species *Acalyptis minimella* (Nepticuloidea) in the Sierra de Grazalema, Spain.

terans. Micropterigidae (**Figure 36.4A**) are the only extant representatives of the suborder Zeugloptera, and seem to be the sister lineage to all other lepidopterans. Their larvae are detritivores; they feed on plant remains and fungi in damp soil. The only two known species of the suborder Aglossata, whose phylogenetic position is not yet resolved, have leaf-miner larvae that feed on seeds of relict conifers (Araucariaceae) in Oceania, and which have probably maintained characters from an evolutionary stage prior to the angiosperm radiation. On the other hand, the Heterobathmiina specialize in angiosperms, and their known species, numbering around 10, have leaf-mining larvae that feed on the austral relict genus *Nothofagus*. In short, lepidopterans seem to have followed the evolution of the plants, and for much of their evolutionary history they have been small in size (**microlepidopterans**) and generally with leaf-miner larvae (**Figure 36.4B**).

One lineage in particular has acquired a large number of apomorphies, key innovations that led to its ecological success: the Ditrysia lepidopterans, which probably appeared in the mid-Cretaceous at the beginning of the angiosperm radiation. The Ditrysia represent the vast majority of species of the order and include all of the major radiations, such as that of the Noctuoidea (some 70,000 known species) (**Box 36.2**).

Following is a summary of other evolutionary tendencies specific to lepidopterans:

- There is a very clear tendency toward an increase in average size over the course of their evolution.
- The chromosome number is relatively stable, although it is highly variable in specific genera.
- The cases of chromosome number variability appear to be due to fusion or fragmentation.
- Speciation is nearly always allopatric or parapatric.
- Obligate **myrmecophily** and ant parasitism entail a high degree of specialization that poses a greater risk of extinction, while also appearing to increase the rate of speciation.

- The greatest difficulties in phylogenetic studies, and most of the discrepancies between the results of different methods, are found in the most diverse groups, which have undergone significant adaptive radiations.

Biogeography and Biodiversity

Lepidoptera is the fourth most diverse order on the planet. Most of its biodiversity is concentrated in the tropics, no doubt because of the good conditions for butterflies and moths found in these regions. The biogeographical origin of the group is unknown. The oldest known fossil, which appears to be a common ancestor of all extant lepidopterans, was found in Great Britain. The two main lineages into which lepidopterans are divided (micropterigids and the rest of the lepidopterans) are distributed across all continents. It is important to remember that lepidopterans probably appeared at the time that the supercontinent Pangea was breaking apart. It is therefore not surprising that they were able to spread very quickly over the planet. Although no biogeographical study based on the whole lepidopteran tree has been conducted, there are numerous studies of families and lower levels. These studies have made it possible to determine the origin, for example, of Parnassiinae (Papilionoidea: Papilionidae), in Central Asia, and of Pseudopontiinae + Dismorphiinae (Papilionoidea: Pieridae) in western Gondwana. However, in some cases the interpretation of the results continues to be debated due to the dispersal capacity of butterflies and moths. Indeed, recent molecular studies with groups have confirmed the dispersal of butterflies and moths between continents, as well as their colonization of remote islands. This capacity for dispersal needs to be taken into account when calibrating the molecular clock based on vicariant paleogeographic events.

Differentiation and Speciation

The study of speciation processes in lepidopterans has focused almost exclusively on butterflies. Sexual selection associated with the pronounced sexual dimorphism of wing coloration in butterflies (see for example **Figure 36.2**) provided Darwin with a key example for his theory. Although much less studied, there is also a chemical component to sexual selection and speciation in lepidopterans. Butterflies and moths produce pheromones characteristic of each species that enable the sexes of the same species to find and recognize one another. However, hybridization sometimes occurs, and a large number of natural hybrids can be found in collections. Several of the few proven cases of homoploid speciation resulting from the hybridization of two parental species in animals are butterflies (for

Lepidopterans as model organisms

Silkworm (*Bombyx mori*)

This moth from the superfamily Bombycoidea has been grown in China for silk for at least 5000 years. It is the only insect that has been totally domesticated to the point that it is incapable of living in the wild. Because it is easy to raise, and because of its economic importance, the silkworm has come to be used as a model organism. This has led to some major discoveries about pheromones, hormones, and the structure of the nervous system. Its pheromone, called bombykol, was the first to be characterized. For this study, Nobel Prize winner Adolph Butenandt had to collect extracts from 500,000 individuals. Since then, hundreds of lines and mutations have been described, and *Bombyx mori* was the first lepidopteran to have its nuclear genome sequenced. Several genetic engineering projects have enabled silkworms to produce proteins for medical or industrial uses, instead of for silk.

Heliconians

Most heliconians (Papilionoidea: Nymphalidae) are toxic or foul tasting. This characteristic has favored the development of a conspicuous **aposematic coloration** and widespread mimicry among heliconian species. The genetics and genomics of speciation and wing patterns in this group are being studied in depth.

Monarch butterfly (*Danaus plexippus*)

Monarch butterflies (Papilionoidea: Nymphalidae) make spectacular migrations between Mexico, where they gather in a few select forests to spend the winter, and the northern regions of North America. Both their extraordinary journey of thousands of kilometers and their beauty have made them one of the world's most popular butterflies. The monarch is a model for research on migration in invertebrates; marking and recapture campaigns and sightings of specimens in migration are conducted with the help of both amateurs and scholars. It is a lesser-known fact that this species is widely distributed over the planet; it includes colonies on the Atlantic and Pacific Islands, as well as in Australia and

New Zealand. It is also one of the few butterfly species whose genome has been sequenced.

Squinting Bush Brown (*Bicyclus anynana*)

This African woodland butterfly (Papilionoidea: Nymphalidae) exhibits a marked seasonal *polyphenism* (phenotypic plasticity) in its wing color pattern. It is used as a model for studying the development and genetics of wing patterns. The sequencing of its genome is currently underway.

Melitaeini

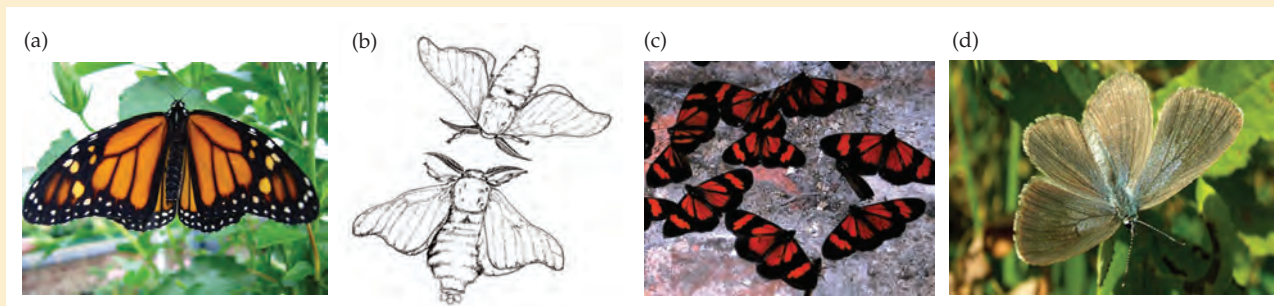
Several species of this group (Papilionoidea: Nymphalidae), especially *Euphydryas editha* in the United States and *Melitaea cinxia* in Europe, have been used for years in ecological and population dynamics research. Their usefulness for study lies mainly in their structure in **metapopulations**, along with the fact that they are easily marked and monitored in the field.

Large blues (genus *Maculinea*)

The larvae of the families Lycaenidae and Riodinidae (Papilionoidea), like many hemipterans, often sustain mutualistic relationships with ants (*myrmecophily*) (see Chapter 32, Hemipterans). However, the relationship sustained with ants by the genus *Maculinea* is parasitic. In several European countries, large blues are either extinct or are in extreme danger of extinction. They are used as a model organism for studying parasitism and are a featured organism in invertebrate conservation efforts. The number of studies conducted on large blues, as well as the number of initiatives to monitor, protect, and even reintroduce these butterflies in Europe, is comparable only with the campaigns carried out for certain endangered vertebrates.

Tobacco hornworm (*Manduca sexta*)

This moth (Bombycoidea: Sphingidae) is used as a model species in studies of invertebrate neurobiology. Its large size facilitates the study and manipulation of its nervous and hormonal systems.

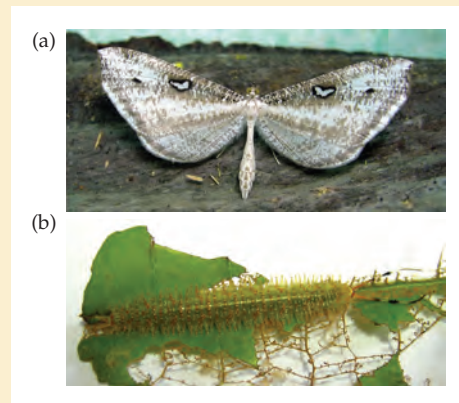


Lepidopterans used as model organisms. (a) Monarch butterfly (*Danaus plexippus*), (b) silkworm (*Bombyx mori*),

(c) heliconians (*Actinote* sp.), and (d) large blue (*Maculinea alcon*).

Macrosoma: The missing link?

The genus *Macrosoma* Hübner, with close to 40 species described (all restricted to Neotropical forests), is the only known representative of the family Hedylidae. Its external appearance (pictured) is typical of moths of the geometrid group (Geometroidea: Geometridae). It is therefore no surprise that the group was described in 1857 as belonging to Geometridae, a taxonomic position they occupied until 1986, when Scoble published his revision of the group. Scoble's conclusion was astonishing: in spite of their appearance and their mainly nocturnal habits, the Hedylidae are not moths, but butterflies. This extraordinary hypothesis identified *Macrosoma* as the only extant genus of a superfamily of butterflies, and a sister group to the rest. A detailed analysis reveals a large number of possibly synapomorphic characters for butterflies including *Macrosoma*. Indeed, their appearance in immature stages is surprisingly similar to that of several groups of butterflies. The first researcher to study the development of *Macrosoma* wrote, "I thought that the larvae might belong to a species of nymphalid [Papilionoidea: Nymphalidae], but when the first larva hatched I was sure that it was a pierid [Papilionoidea: Pieridae]. The first adult to emerge was a complete surprise." Recent studies based on molecular data and on a combination of molecular and morphological



The Hedylidae (genus *Macrosoma*) are butterflies (Papilionoidea) in spite of their moth-like appearance. Shown are *Macrosoma tipulata* (a) adult and (b) larva.

data have corroborated that the Hedylidae are butterflies and identify them as a sister group to HesperIIDae. The last revision of the group posits a single superfamily that encompasses all butterflies (Papilionoidea), and thus *Macrosoma* has ceased to be viewed as a missing link and is now considered a true butterfly.

example, the heliconian *Heliconius heurippa*, the swallowtail *Papilio appalachiensis*, and lycaenids of the genus *Plebejus*). The heliconians, a group that serves as a model for research into speciation, have also provided evidence of the possibility of a species dividing into two, due to specialization in mimicking two different toxic models. A recent study with lycaenids of the subgenus *Agrodiaetus* used their phylogeny to demonstrate the reinforcement of prezygotic barriers. Specifically, it demonstrated that natural selection favors differentiation in colors of males between nascent species that are in secondary geographic contact, and their selection by females of the same species, so as to prevent creating infertile hybrids. Chromosome fragmentation and fusion are common in this subgenus's process of speciation. With around 120 species produced over some three million years, it has one of the highest known rates of speciation, probably due to its great chromosomal instability. Studies conducted to date suggest that allopatric speciation is the most common type of speciation among lepidopterans, although parapatric speciation has also been documented in a few cases.

In spite of the close relationship between lepidopterans and food plants, and between ants and some butterfly larvae, hardly any cases of coevolution have been documented. In other words, the usual evolutionary pattern involves lepidopterans being able to diver-

sify by specializing on ants or plants, but the converse relationship rarely occurs. The famous case of the coevolution of yucca moths (Adeloidea: Prodoxidae) with their host plant is a notable exception.

Principal Questions Remaining

- Will it be possible to resolve the topology of the Tree of Lepidopterans and establish solid relationships among the 45 superfamilies?
- Given their rapid diversification, relationships within the Ditrysia clade seem practically impossible to resolve with current methods. Will phylogenomics provide new data on this group?
- Is it possible to create a complete matrix of morphological characters that would encompass the whole order and combine it with molecular data?
- Can the origin of lepidopterans be dated more precisely by calibrating the molecular clock from the fossil record?
- What is the biogeographical origin of the order?
- Can the taxonomy continue to be updated in response to new discoveries about the group's evolution?

- Will the phylogenetic tree of the order help to explain the origin and evolution of ecological specializations such as changes in diet, myrmecophily, diurnality, the aquatic life of **preimaginal stages**, and so on? Will it also help to explain their effects on generating biodiversity and its correlation with morphological adaptations?

Basic Bibliography

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