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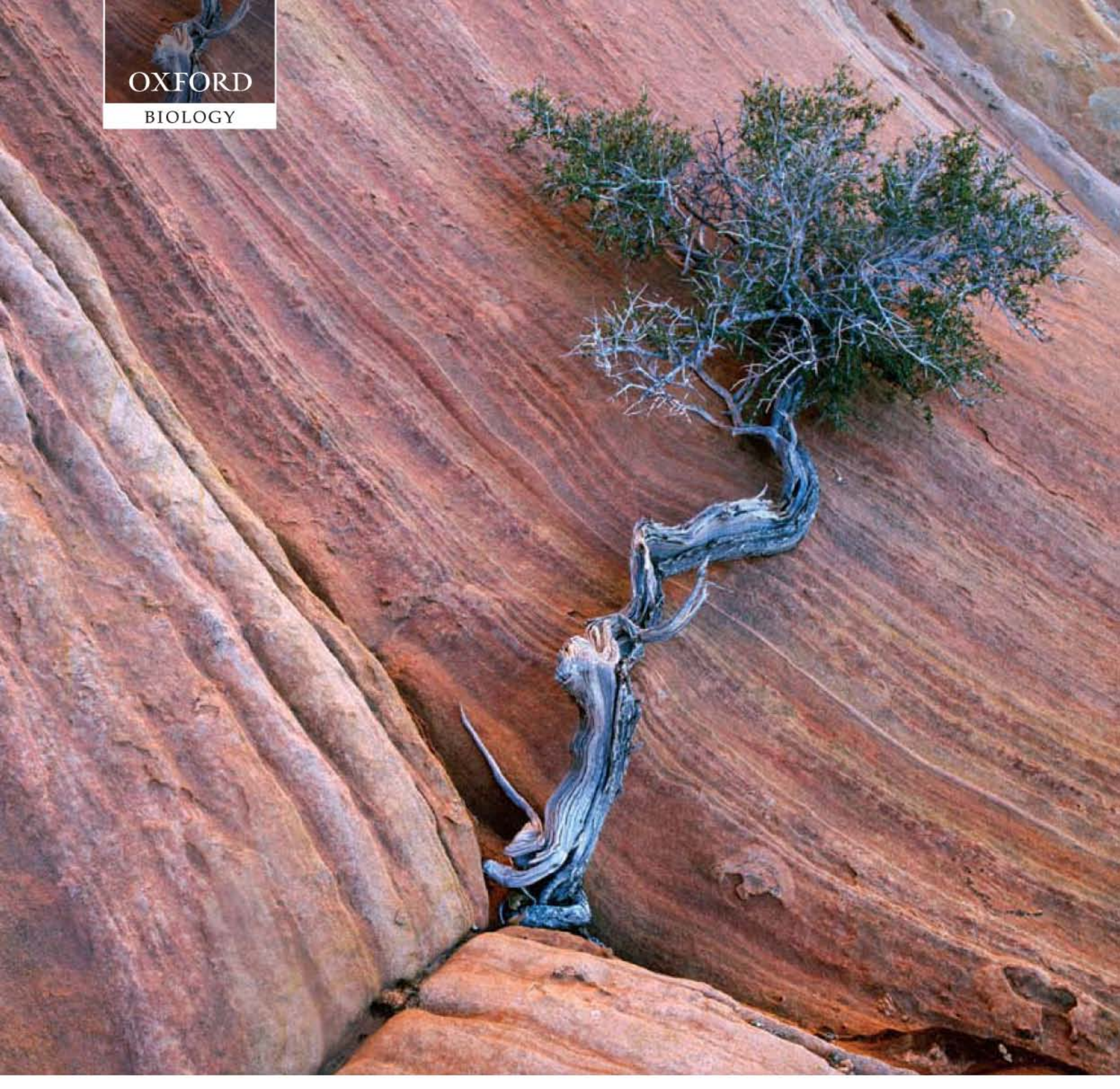

the TIMETREE of LIFE

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True flies (Diptera)

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Abstract

With over 150,000 described species in ~180 families, the insect Order Diptera (true flies) is one of the largest and most diverse groups of organisms. Flies exhibit an extremely wide range of morphological characters that have supported or confounded phylogenetic inferences within the group. Though molecular phylogenies exist, few have addressed macroevolutionary questions within the order and none has comprehensively addressed the order as a whole. Fossil and molecular data indicate that the earliest divergences among living dipterans occurred in the late Paleozoic, 270–251 million years ago (Ma). Most divergences among families occurred in the Triassic and Jurassic, 251–146 Ma.

The Order Diptera (true flies) comprises an ecologically and morphologically diverse assemblage of holometabolous insects. A number of morphological characters unite this lineage (*1–3*), the most recognizable one being the extremely reduced, knob-like, metathoracic wings, or halteres (Fig. 1). The majority of true flies also bear specialized sponging mouthparts that differ markedly from the chewing mouthparts found in most insects. Approximately 150,000 species of Diptera have been described in ~180 families, although total species diversity undoubtedly exceeds twice that number (*1, 2, 4*). Myriad species of Diptera are economically important vectors of human and animal pathogens (e.g., Culicidae) and many are destructive to crops and livestock (e.g., Tephritidae and Oestridae, respectively). Flies are also important ecologically as predators, decomposers, parasitoids, and pollinators (*5, 6*). Here we review the relationships and divergence times of major events in dipteran evolution, including the origin of the order and its constituent suborders, infraorders, and families.

Traditionally flies have been divided into two suborders: Nematocera ("thread-horn" flies) and Brachycera ("short-horn" flies). This division was based primarily on characters of the adult antennae and larval head capsule (*1, 2, 7*). Although a number of shared-derived characters support the monophyly of the Brachycera, the Nematocera is now widely regarded as a paraphyletic assemblage of infraorders, or suborders (*8*), from/within which the Brachycera originated (*7, 9, 10*). For a detailed overview of the current state of Diptera systematics, see Yeates and Wiegmann (*1, 2*).

Evolutionary relationships among the lower Diptera (= "Nematocera") have been particularly difficult to resolve. Morphology-based hypotheses disagree with respect to the composition and interrelationships of the nematoceran infraorders (*7, 9, 11–13*). Interpretations of character homology, polarity, and homoplasy, as well as incongruence between adult characters vs. those of the larvae and pupae, have contributed to disagreement concerning the higher-level relationships of these flies (*1, 2*). Comprehensive reviews of the relationships within the nematocerous Diptera are presented elsewhere (*7, 9*).

Determination of the closest relatives of the hyperdiverse Brachycera has been equally difficult. Hennig (*12, 13*) gave evidence, taken largely from adult characters,

Fig. 1 A predatory robber fly (Asilidae: *Ommatius gemma*) from Mississippi, USA. Credit: G. and J. Strickland.

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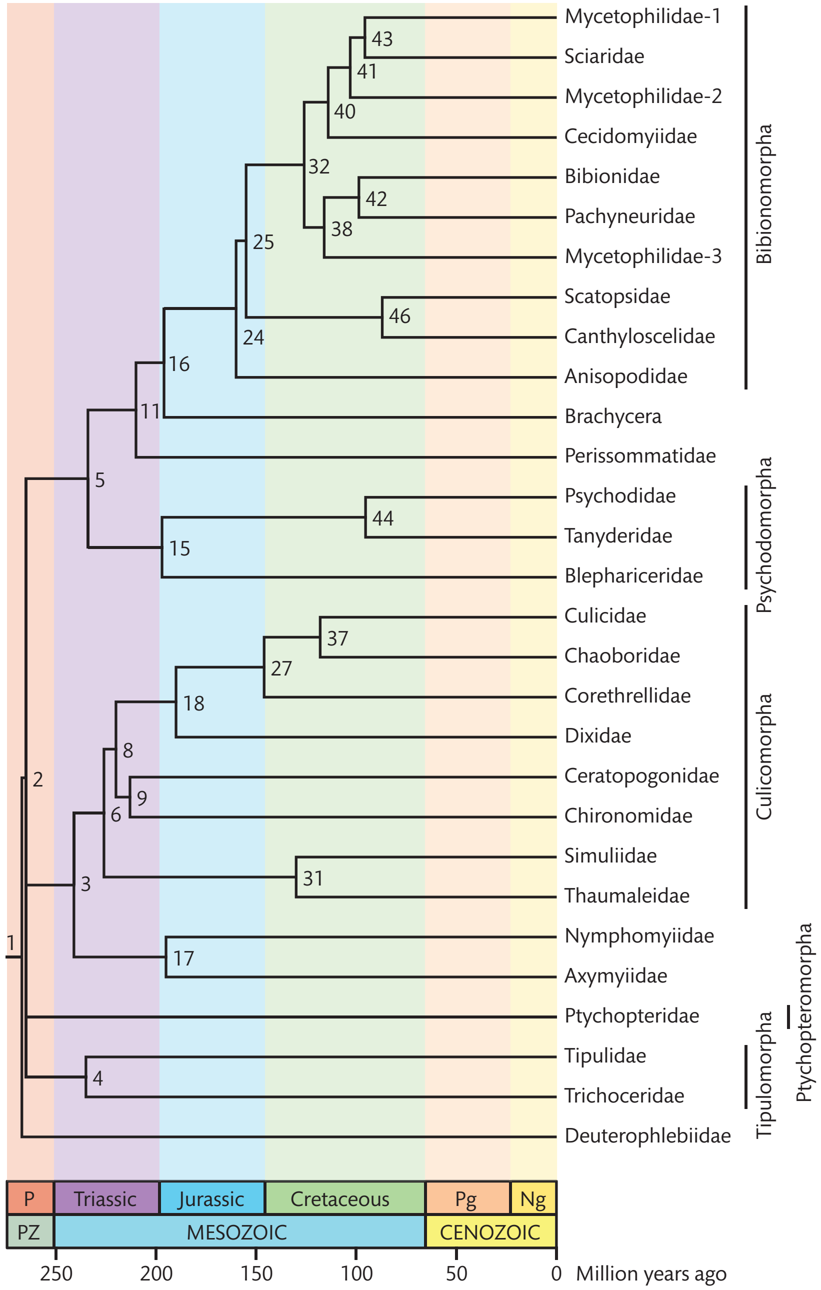

Fig. 2 *Continues*

for a relationship between the Brachycera and his concept of the Bibionomorpha. Although not explicit in their published tree, Wood and Borkent (*7*) suggested a relationship between Brachycera and their Psychodomorpha (including Scatopsidae, Canthyloscelidae (as Synneuridae), Perissommatidae, and Anisopodidae, all of which were in Hennig's Bibionomorpha). This hypothesis was based predominately on characters of the larval mandible and was later supported (*14*). Oosterbroek and Courtney's (*9*) analysis of characters from all life stages found a single family—Anisopodidae—to be the closest relative of the Brachycera. This group was placed at the tip of a clade they termed the "higher Nematocera + Brachycera" (the "higher Nematocera" including all of Wood and Borkent's

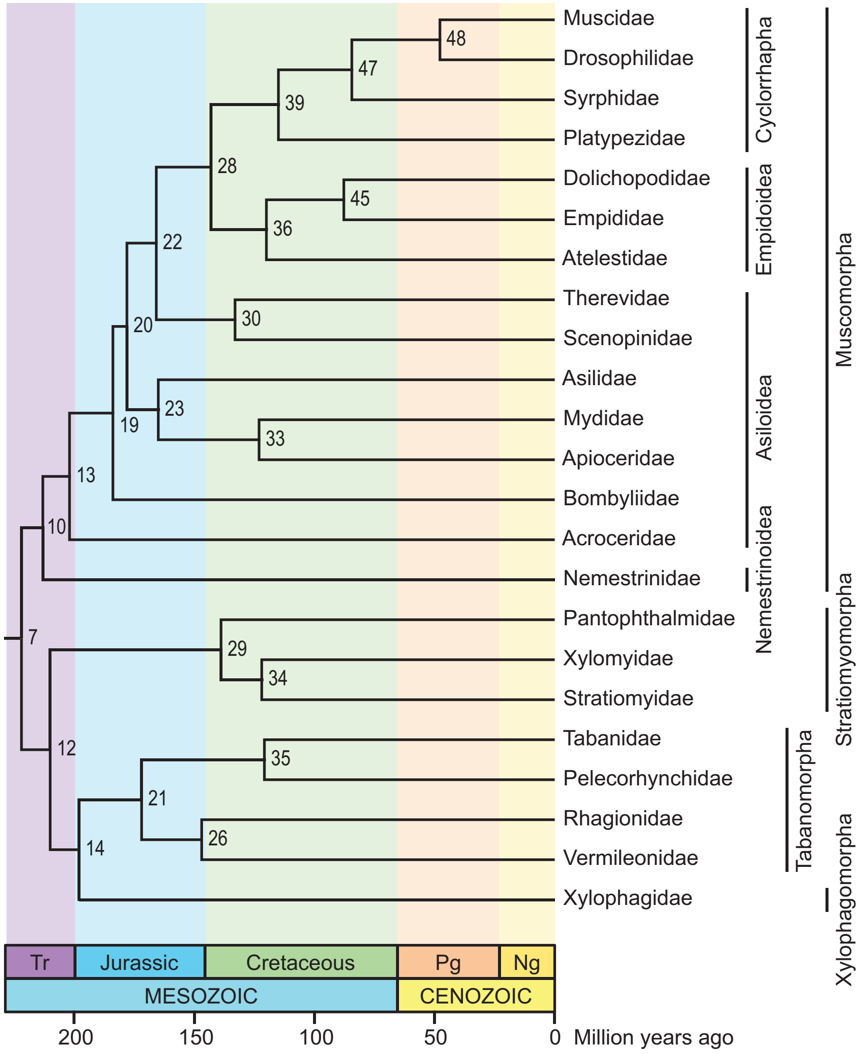

Fig. 2 A timetree of true flies (Diptera). Divergence times are shown in Table 1. The timetree for Brachycera is continued in a separate panel. Mycetophilidae-1 (Diadocidiidae, Mycetophilidae *sensu stricto*), Mycetophilidae-2 (Keroplatidae and Lygistorrhinidae), Mycetophilidae-3 (Ditomyiidae). *Abbreviations*: Ng (Neogene), P (Permian), Pg (Paleogene), PZ (Paleozoic), and Tr (Triassic).

Psychodomorpha and the Tipulidae). Subsequently, Michelsen (*11*) reunited the Brachycera with a Hennigian Bibionomorpha (as "Neodiptera") based on adult thoracic sclerites and musculature.

Divisions within the Brachycera have traditionally followed a trend of paraphyletic stem grades (e.g., Orthorrhapha and Aschiza) giving rise to monophyletic clades (e.g., Cyclorrhapha and Schizophora). The lower Brachycera (= Orthorrhapha) are generally small to very large flies, many of which are predators or parasitoids as larvae. The basal Infraorders Xylophagomorpha, Stratiomyomorpha, and Tabanomorpha are each represented by one, three, and five families, respectively (*1, 2, 15*). The Tabanomorpha is of particular interest for containing three families in which at least some female flies suck vertebrate blood (Tabanidae, Athericidae, and Rhagionidae) (*16*). Other notably diverse families within the lower Brachycera include the Bombyliidae (bee flies), Asilidae (robber flies), Empididae (dance flies), and Dolichopodidae (long-legged flies). Woodley (*17*), Sinclair *et al.* (*18*), and Yeates (*15*) present morphological evidence supporting relationships among the lower

Table 1. Divergence times (Ma) and their credibility/confidence intervals (CI) among true flies (Diptera).

Timetree		Estimates				Timetree		Estimates			
Node	Time	Ref. (*10*, *68*)		Refs. (*39*, *40*)		Node	Time	Ref. (*10*)		Refs. (*39*, *40*)	
		Time	CI	Time	CI			Time	CI	Time	CI
1	267	267	269–260	–	–	25	155	155	195–114	–	–
2	265	265	269–256	–	–	26	147	147	190–104	–	–
3	241	241	260–224	–	–	27	146	146	188–97	–	–
4	235	235	261–221	–	–	28	143	143	173–122	–	–
5	234	234	259–209	–	–	29	139	139	186–94	–	–
6	226	226	243–215	–	–	30	133	133	170–96	–	–
7	222	222	239–195	–	–	31	130	130	181–74	–	–
8	220	220	234–212	–	–	32	126	126	168–84	–	–
9	213	213	223–210	–	–	33	123	123	159–87	–	–
10	213	213	235–188	233(*40*)	239–217	34	122	122	171–78	129(*39*)	194–83
11	210	210	243–179	–	–	35	121	121	168–80	–	–
12	210	210	234–180	–	–	36	120	120	153–92	–	–
13	202	202	226–179	–	–	37	118	118	163–71	–	–
14	198	198	227–163	–	–	38	116	116	158–75	–	–
15	197	197	225–181	–	–	39	115	115	145–89	–	–
16	196	196	230–160	–	–	40	114	114	157–74	–	–
17	195	195	236–138	–	–	41	103	103	144–64	–	–
18	190	190	216–155	–	–	42	98.6	98.6	139–60	–	–
19	184	184	209–171	–	–	43	95.6	95.6	137–57	–	–
20	178	178	203–161	–	–	44	95.2	95.2	140–57	–	–
21	172	172	211–131	–	–	45	87.7	87.7	122–59	–	–
22	166	166	193–143	–	–	46	87.1	87.1	136–45	–	–
23	165	165	194–140	–	–	47	84.5	84.5	115–71	–	–
24	160	160	200–120	–	–	48	47.9	47.9	76–29	–	–

Note: Node times in the timetree are from ref. (*10*, *68*).

Brachycera. However, as in the lower Diptera, anatomical characters uniting major lineages are often lacking, equivocal, or convergent.

The brachyceran clade Cyclorrhapha contains over half of all true flies. Extreme reduction of the larval head capsule and pupation of the third instar in the final larval skin (puparium) are the major innovations of this group (*1*, *2*). More homogeneous in morphology than the lower Diptera, Cyclorrhapha contains the stereotypical "higher" flies and familiar members of this group include the vinegar fly (commonly called the fruit fly by geneticists, *Drosophila melanogaster*) and the house fly (*Musca domestica*).

The lower Cyclorrhapha (= Aschiza) is a paraphyletic collection of families united only by plesiomorphic characters. Included within this lineage are ~8 families of flies, some of which are highly diverse (e.g., Syrphidae and Phoridae) (*19*, *20*). Relationships between families within the lower Cyclorrhapha remain ambiguous (*21*, *22*). The division Schizophora contains most of the family-level diversity in the Diptera, with at least 75 described families (*1*, *2*). All flies in this group possess a membranous head sac (ptilinum) that, when inflated, allows the adult to escape the puparium. After emergence the sac is withdrawn leaving a remnant, U-shaped, ptilinal fissure. Schizophora is further divided into two main groups: Acalyptratae and Calyptratae. While the Calyptratae is a well-supported monophyletic group (*1, 19*), the Acalyptratae may or may not be monophyletic (*1, 2, 19, 23, 24*) and most dipterists now suspect the latter.

Although containing only about 20% of fly species, Acalyptratae contains nearly half of the order's family-level diversity (~62 families). Remarkably, six common acalyptrate families (Tephritidae, Lauxaniidae, Agromyzidae, Chloropidae, Drosophilidae, and Ephydridae) make up >50% of the species diversity in the entire assemblage (*2*). Resolving the relationships within and among acalyptrate superfamilies has traditionally been difficult, due, in part, to the lack of convincing shared-derived characters for most major groupings.

Calyptrate flies are divided into ~13 families that are important medically (e.g., Glossinidae, Muscidae and Oestridae), forensically (e.g., Calliphoridae and Sarcophagidae), or as biological control agents (e.g., Tachinidae). Calyptratae also contains several groups of specialized, vertebrate ectoparasites (Hippoboscidae, Streblidae, and Nycteribiidae).

Despite the publication of 14 completed dipteran genomes (*Anopheles gambiae*, *Aedes aegypti*, and 12 *Drosophila* species), relatively few studies have used molecular markers to reconstruct higher-level relationships of flies. However, many new molecular studies are emerging (*73*) and these will certainly increase knowledge of relationships within the order.

Early nucleotide-based higher-level studies compared nuclear *28S* ribosomal DNA sequences among Culicomorpha (*25*), some Cyclorrhapha (*26*), and among the nematocerous Diptera (*27*). Major markers employed to date for phylogenetic inference include mitochondrial genes (*28–30*), elongation factor 1α (EF-1α) (*31, 32*), *28S ribosomal DNA* (*10, 16, 27*), and the nuclear protein-coding genes CPSase (CAD) (*33–36*) and white (*37*).

Friedrich and Tautz's (*27*) phylogeny of the earliest dipteran lineages strongly supported several infraorders; however, the relationships among the infraorders were not well resolved (<80% bootstrap support) and the taxon sampling was quite limited. Recently, Bertone *et al.* (*10*) analyzed nuclear genes to infer the relationships among all of the ca. 26 nematocerous fly families. Using full-length *28S rDNA* and three protein-coding genes (CAD, TPI, and PGD), they found support for several infraorders, both traditional and novel. The most surprising finding was a close relationship between the small, unusual Family Deuterophlebiidae and all remaining Diptera. In general accordance with Hennig and Michelsen, Bertone *et al.* also found support for the Neodiptera: Perissommatidae + Brachycera + Bibionomorpha (Fig. 2).

Nucleotide data have also been applied to phylogenetic questions among and within families of Brachycera. Wiegmann *et al.* (*16*) inferred relationships within the Tabanomorpha (including taxa from the Xylophagomorpha and Stratiomyomorpha as outgroups) using *28S rDNA* sequences. The resulting phylogeny reflected similar relationships to those based on morphology (*15, 17*). Motivated by the discovery of a new brachyceran family, Evocoidae [as Ocoidae], Yeates *et al.* (*38*) sampled *28S rDNA* across a limited set of asiloid taxa. Evocoidae was placed within the therevoid clade (Apsilocephalidae, Scenopinidae, and Therevidae), a position supported by their observations of its morphology. Nucleotide sequences have also been used to clarify higher-level relationships within the Stratiomyidae (*28S* and EF-1α) (*39*), Acroceridae (*16S*, *28S*, CAD and COI) (*40*), Therevidae (*28S* and EF-1α) (*31*), Apioceridae and Mydidae (*28S*) (*41*), Asilidae (*16S*, *18S*, *28S* and COII) (*42*), and Empidoidea (*28S*, EF-1α and CAD) (*32–34*).

Molecular studies of Cyclorrhapha have largely been limited to analyses of relationships within a family or major family group, and no single study has yet sampled across the clade's 80+ families. Notable higher-level studies published to date include analyses of relationships among aschizan families (*21, 22*), Syrphoidea (*43*), Tephritoidea (*44*), Calyptratae (*45*), and Hippoboscoidea (*36, 46*). A recent study of full mitochondrial genome sequences, from a diverse sampling of brachyceran flies, recovered expected higher-level relationships, including paraphyly for acalyptrates (*30*). Molecular studies below the family level are numerous and include intensive work on dipteran model systems such as *Drosophila* and Drosophilidae (*47–49*), Syrphidae (*50*), Diopsidae (*51, 52*), Tephritidae (*44, 53*), Agromyzidae (*35*), Coelopidae (*54*), Muscidae (*55*), and Calliphoridae (*56, 57*).

Much of the seminal work on molecular clocks and divergence times has been carried out in studies of *Drosophila* species groups and a handful of other Diptera using immunological distances (*58*), DNA–DNA hybridization (*59*), DNA/protein sequences (*60–62*), and various marker systems (*63, 64*). Nonetheless, few studies have yet applied these methods above the species level. Studies of evolutionary rate dynamics have revealed multiple examples of rate heterogeneity in many dipteran lineages and in various mitochondrial and nuclear genes (*60, 62*). Comparisons of evolutionary rates using multiple concatenated genes from completed dipteran genomes show a lineage specific increase in evolutionary rates in Diptera when compared to the beetle, *Tribolium casteneum* (*66*), a result concordant with a similar rate increase shown for fly ribosomal genes (*67*).

Wiegmann *et al.* (*68*) were the first to apply relaxed clock Bayesian methods to estimate dipteran divergence times (Fig. 2). This study used a combined molecular (*28S rDNA*) and morphology-based (*15*) tree to infer dates from *28S rDNA* sequences analyzed using the relaxed clock lognormal distribution of nucleotide rate evolution in the program Multidivtime (*69*). A similar Bayesian analysis of *28S rDNA*-based divergence times for early dipteran relationships is presented in Bertone *et al.* (*10*; Fig. 2). Relaxed clock methods have also been applied to estimate divergences within several dipteran families, including Acroceridae (*40*), Stratiomyidae (*39*), Drosophilidae (*65*), and Calliphoridae (*57*).

Divergence times based on *28S rDNA* (Fig. 2; Table 1), fossil, and phylogenetic data are largely congruent and provide useful approximations of the range of dates ascribable to major splits in the Diptera tree of life. The Diptera most likely arose in the late Permian or early Triassic, 270–245 Ma (*3*). The earliest fossil evidence of a putative dipteran (*Grauvogelia*) is recorded from the early Triassic (240 Ma) (*3, 70*). Most major dipteran lineages, from the nematocerous infraorders to the first Eremoneura (Empidoidea and Cyclorrhapha), originated between 240 and 150 Ma, which was a time of major innovation and radiation for many insect groups (*3*). It was also during this period in fly evolution that the origin and extinction of nearly 40 nematoceran families, or more than half of the group's historical diversity, occurred (*71*). By the Cretaceous (146–66 Ma) many of the order's extant, higher-level groups had arisen, though diversification of fly families and much of the Cyclorrhapha continued into the late Cretaceous and early Cenozoic (100–30 Ma).

Molecular divergence time estimates make it possible to approximate the timing of major morphological and ecological shifts within the Diptera. Wing vein characters have been important in dipteran systematics and are indispensable for determining early fly fossils, given that isolated wings are often the only structures preserved. Hypotheses of ancestral wing venation are usually based on extant taxa and either of two groups is considered to have the ancestral state due to retention of certain wing veins: the Tipulomorpha or the Diarchineura. The former have long been considered primitive (*7, 12, 13*) for possessing two complete anal veins (all other Diptera have at most one), while some paleoentomologists (*72*) have defined the latter (flies with a complete, four-branched radial sector, including the extant Families Psychodidae and Tanyderidae) as the most ancestral of all flies. Molecular analyses and dating (*10*), however, show that even groups with fewer wing veins (i.e., Culicomorpha and Ptychopteridae) or even extremely reduced wing venations (i.e., Deuterophlebiidae) were present early in dipteran evolution, contemporary with the Tipulomorpha and Diarchineura. This reflects the early and rapid evolution of major dipteran clades, groups which may have evolved along an array of wing venation trajectories.

A suite of changes have also occurred in the immature stages of the Diptera, and one of the most striking evolutionary trends is the reduction of the larval head capsule. Most extant nematoceran larvae have a well-sclerotized, identifiable head (eucephalic), the ancestral condition shared with most holometabolous insects. The origin of the Brachycera in the late Triassic or early Jurassic (220–195 Ma) was marked by desclerotization and movement of the posterior portions of the head capsule into the anterior of the thorax. By the time the Cyclorrhapha appeared in the Cretaceous (~143 Ma) the larval head was almost entirely desclerotized within the thorax, and consisted of only mouth hooks and their internally supporting rods. The antiquity of this type of larva (maggot) is shown in rare examples found in Cretaceous amber (New Jersey ~90 Ma; *3*), which possessed mouth hooks similar to those of recent flies. The other novelty of immature Cyclorrhapha, the puparium, probably developed during the same time period, and fossil puparia have been recorded from the late Cretaceous, 70 Ma (*3*).

Ancestral fly larvae were no doubt closely tied to moist or aquatic habitats. Within the lower Diptera, larvae of the Culicomorpha, Nymphomyiidae, Deuterophlebiidae, most Psychodomorpha, and many Tipulomorpha are aquatic, while Ptychopteridae and Axymyiidae are semiaquatic. Larvae developing in these habitats feed largely on particulate matter (detritus), or graze/filter diatoms, algae, or other aquatic plants (*5*). Diptera with terrestrial larvae (i.e., Perissommatidae, Bibionomorpha, and Brachycera) appear to have originated sometime in the Triassic (~234 Ma) and diversified predominately in the late Jurassic or early Cretaceous (180–115 Ma). Terrestrial fly larvae occupy a number of niches and trophic levels, including reversals to aquatic lifestyles. The first terrestrial larvae were probably saprophagous or mycophagous, as seen in most extant Bibionomorpha, Perissommatidae, and some lower Brachycera (i.e., Stratiomyidae). Major clades of predatory larvae originated within the lower Brachycera in the late Triassic or early Jurassic, and the first parasitic flies, including Nemestrinidae, Acroceridae, and Bombyliidae, may have originated as early as the late Triassic (~213 Ma). Lower Cyclorrhapha feed predominately on decaying plant material, a food source that may have been provided by some of the first angiosperms in the Cretaceous (130 Ma).

Adult flies are generally restricted to a few trophic types, including nectivory, pollinivory, predation, and hematophagy, though many are nonfeeding or feed facultatively on other energy-rich resources (e.g., plant sap flows or homopteran insect secretions). Based on phylogenetic evidence, the ancestral adult feeding type was probably nonfeeding, as exhibited in Deuterophlebiidae, Ptychopteridae, and most Tipulomorpha, or perhaps hematophagy, as is the case for most Culicomorpha. Though hematophagy has arisen in all major groups of flies, it is most common in the lower Diptera (Culicomorpha and Psychodidae) and probably evolved sometime in the Triassic (251–200 Ma). Early bloodfeeders most likely fed on reptile or amphibian hosts, groups which are still fed upon by flies. Most dipteran radiations occurred before the rise of flowering plants in the mid-Cretaceous (*5*), and the array of extant flies that are specialized for taking nectar and pollen probably evolved later in fly history.

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