The evolution of myiasis in humans and other animals in the Old and New Worlds (part I): phylogenetic analyses

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Myiasis, the infestation of live vertebrates with dipterous larvae, seems to take two distinct forms that, it has been suggested, evolved from two distinct phylogenetic roots: saprophagous and sanguinivorous. However, the convergent evolution of morphological and life-history traits seems to have had a major role in simplifying this overall assessment of the evolutionary routes by which myiasis arose. Moreover, this somewhat simplistic division is further complicated by the existence of both ectoparasitic and endoparasitic species of myiasis-causing Diptera, the evolutionary affinities of which remain to be resolved. To understand how different forms of parasitism arose, the evolution of the various groups of myiasis-causing flies must be separated from the evolution of the myiasis habit *per se*. Until recently, evolutionary studies of myiasis-causing flies were little more than discussions of morphology-based taxonomy. Since the mid-1990s, however, several formal phylogenies – based on both morphological and, increasingly, molecular data – have been published, enabling reassessment of the hypotheses concerning myiasis evolution. In part I of this review, we focus on some recent landmark studies in this often-neglected branch of parasitology and draw together phylogenetic studies based on molecular and morphological data to provide a framework for the subsequent analysis of biochemical, immunological, behavioural, biogeographical and fossil evidence relating to the evolution of myiasis.

Reconstructing the evolution of myiasis

'It is not too difficult to reconstruct the evolution of myiasis' [1], wrote Zumpt in his landmark treatise about myiasis, before suggesting that 'two evolutionary trends of myiasis are obvious…they may be referred to respectively as the saprophagous and the sanguinivorous roots' [1]*. Thus, in the largely pre-cladistic and certainly pre-molecular era of the 1960s, the issue seemed relatively straightforward and readily resolved. With the benefit of hindsight, however, it is apparent that the convergent evolution of morphological and life-history traits almost

certainly had a part in simplifying Zumpt's overall assessment of the evolutionary routes by which myiasis arose. Fortunately, with the advent of modern molecular techniques, it is now possible to construct robust phylogenies that enable rigorous reassessment of the hypothetical evolution of dipteran vertebrate parasitism. So, how do the various scenarios proposed by Zumpt [1] and others since (e.g. Erzinçlioglu [2]) fit the phylogenetic pattern of parasitic life-histories observed across various dipteran families and subfamilies? And are the results supported sufficiently to be credible?

Given the various forms of insect–host relationship exhibited among the Diptera (e.g. myiasis, parasitoidism, micropredation and phytophagy), a working definition of myiasis is essential. Here, we adopt the definition of Zumpt [1] [i.e. that myiasis is the infestation of live vertebrates (humans and/or animals) with dipterous larvae] (Box 1). So, if we suspect that myiasis is a non-monophyletic trait, why place taxonomic limits on the topic? Is it pertinent to focus on the evolution of myiasis or should we be addressing broader issues relating to the evolution of parasitism in Diptera? What, for example, are the biological, chemical and behavioural commonalities relating to myiasis that are not found in the parasitism of invertebrates [e.g. woodlice (as exhibited by flies of the family Rhinophoridae), insects in general (as in the family Tachinidae), spiders (as in the family Acroceridae) and plants (as in the families Cecidomyiidae, Agromyzidae and Tephritidae)]? What are the defining characteristics or inherent properties of myiasis? Are they 'simply' the ability to overcome the immune defence response of a live vertebrate host?

The applied and immunological benefits of understanding the nature of myiasis are considerable. Among the ectoparasites that affect vertebrates, myiasis-causing larvae have evolved biological, physiological and biochemical strategies to cope with the nonspecific (e.g. natural killer cells and complement proteins) and/or the specific (e.g. antibodies and T cells) immunological responses of a host. The result of parasite modulation of a host immune system is the establishment of a dynamic equilibrium between the parasite and the host and, particularly in oestrids, the complexity of the mechanisms by which myiasis-causing larvae downregulate host immunological function indicates that larvae are

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* Zumpt's use of the term 'root' could be interpreted as meaning at the base of evolutionary trees, rather than pathways (i.e. routes).

Box 1. Myiasis

Myiases have been recognized since ancient times but, at the beginning of the third millennium, they remain an unresolved problem for animal production; they are responsible (through abortion, reduced milk production, weight and fertility loss, and reduced hide quality) for severe economic losses to the livestock industry in both developed and developing countries. Some myiasis-causing larvae, mainly in poor socioeconomic contexts, also parasitize humans, causing extant dermal, urogenital or internal infections [1,6,36].

Among the diseases of vertebrate animals that are caused directly by arthropods, myiases cause a broad range of infections depending on the location of larvae on or in the host body (e.g. dermal or subdermal, nasopharyngeal, intestinal and urogenital myiases) or on the relationship of the larvae with the host. The latter myiases can be defined as either obligate or facultative. Obligate-myiasis-producing species can complete their development only by parasitizing live hosts, whereas facultative species can develop on both living and dead organic matter. Primary facultative species initiate myiasis, whereas secondary species invade the host after primary or obligate species [1].

Blowflies and screwworm flies (Calliphoridae), and flesh flies (Sarcophagidae) cause obligate [e.g. Calliphoridae: Old World screwworm fly (*Chrysomya bezziana*) and New World screwworm fly (*Cochliomyia hominivorax*); Sarcophagidae: *Wohlfahrtia magnifica* and *Wohlfahrtia vigil*] and/or facultative [e.g. Calliphoridae: sheep blowflies (*Lucilia cuprina* and *Lucilia sericata*); Sarcophagidae: *Sarcophaga carnaria*] myiases of relatively short duration, in which larvae mature within four to seven days, usually in wounds or body orifices. Bot flies (Oestridae or oestrids) are obligate parasites that are harboured within a host for several weeks to months. They feed in, for example, the host nasopharyngeal tract [e.g. sheep bot fly (*Oestrus*

ovis) and deer bot fly (*Cephenemyia* spp.)], gut system [e.g. horse and zebra bots (*Gasterophilus* spp.), rhinoceros bots (*Gyrostigma* spp.) and elephant bots (*Platycobboldia* spp.)] and/or internal organs and subcutaneous tissues [e.g. cattle warble flies (*Hypoderma bovis* and *Hypoderma lineatum*) and goat warble fly (*Przhevalskiana silenus*)]. It is not surprising, therefore, that differences among the larvae of Calliphoridae, Sarcophagidae and Oestridae – in terms of location within the host body, biology and pathogenesis, and the host immune response to larval infestation – have steered research along somewhat different lines [11]. This could have inadvertently hampered research into these parasites, in terms of both evolutionary and applied studies.

Humans are the one host notable by its absence from the above (incomplete) list. Today, even in the developing world, cases of human myiasis are relatively uncommon. In the West, such infections are something of a novelty, mostly occurring only in tourists returning from the tropics – predominately Central and South America, and Africa [37,38]. As cases of myiasis in humans are apparently becoming less common, are the species that were previously implicated in human myiasis becoming rarer? Our opinion is 'probably not', or at least not by reason of a reduced association with humans: no myiasis-causing flies are specific to humans – even the evocatively named *Chloropyga anthropophaga* (Tumbu fly) and *C. hominivorax* (New World screwworm fly) are parasites of a wide range of wild and domestic animals. Numerous factors have probably changed the broader ecology of such flies so that their opportunities to parasitize humans have been greatly reduced or eliminated. Depending on species and environment, some or all of the following factors have had a part in this: (i) changing socioeconomic circumstances of human populations; (ii) improved access to primary healthcare; (iii) improvements in housing; (iv) improved sanitation; and (v) a reduction in the proximity of human habitations to livestock.

specifically adapted to a parasitic existence in 'their own host' [3]. The complex interactions between calliphorid and oestrid larvae and their hosts have enabled the development of immunological methods for the early detection of the larvae as a reliable alternative to clinical and post mortem examination [3,4].

We suggest that the parasitism of vertebrates by Diptera is also of intrinsic evolutionary interest because the arthropod parasitism of vertebrates represents a switch (probably several switches) to parasitism that is independent of other classical parasite groups (e.g. protozoa, nematodes and platyhelminths).

Given the importance of the myiasis habit in defining the medical and veterinary relevance of flies that exhibit this life-history trait, one must first – in attempting to trace the evolution of myiasis – reconstruct (as others have done [5]) the evolutionary relationships among the key lineages of myiasis-causing flies: first and foremost, the relevant taxa of Calliphoridae (blowflies), Sarcophagidae (flesh flies) and Oestridae sensu lato (bots and warble flies). Here, if we are to avoid circular logic, it is essential to separate the evolution of each of these groups from the evolution of myiasis *per se*. So, what phylogenies are available for Diptera that parasitize vertebrates and how robust or well corroborated are these phylogenies?

Higher phylogenies

Constructions of the taxonomic and evolutionary relationships of myiasis-causing flies have undergone many revisions, a process that continues in light of an ever-expanding body of morphological, biological and molecular

data. Of the many early morphology-based studies, those of James [6] and Zumpt [1] are probably the most relevant to the subject of this review. Although never explicitly proposing a phylogeny, Zumpt recognized the following families when defining relationships among key groups of myiasis-causing flies: Calliphoridae (e.g. calliphorid spp., *Sarcophaga* spp. and *Wohlfahrtia* spp.), Gasterophilidae (e.g. *Gasterophilus* spp.) and Oestridae (e.g. *Oestrus* spp., *Hypoderma* spp. and *Cephenemyia* spp.). Subsequently, Hennig [7] and Pont [8] made revisions, before McAlpine [9] proposed the system that is used today in most cases, by grouping Oestrinae, Hypodermatinae, Gasterophilinae and Cuterebrinae as subfamilies within the family Oestridae, while continuing to recognize Calliphoridae and Sarcophagidae as separate families.

Recently, morphology-based studies [5,10] have provided support for the hypothesis that members of the Oestridae family occupy a phylogenetic position within the Calliphoridae[†]. However, molecular analyses [11,12], although lacking comprehensive taxon coverage, do not support such a topology, tending instead to confirm the sister-group status of the Calliphoridae and Sarcophagidae families, distinct from Oestridae. Thus, the exact pattern of the phylogenetic relationships among the key groups of myiasis-causing species – as defined on the basis of morphological, life-history and molecular characteristics – remains the subject of continuing revision.

Since 1998, several studies addressing long-running phylogenetic questions regarding Diptera have been undertaken using molecular data [13–15]. However,

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† To be taxonomically precise, within the non-rhiniine Calliphoridae.

most of them have focused on high-level phylogenetic questions and do not include sufficient taxa relevant to the focus of this review. Indeed, only the study by Nirmala *et al.* [15], which addresses the phylogeny of the Calyptratae, provides anywhere near sufficient taxon coverage to provide even a preliminary insight into questions concerning the evolution of myiasis.

Based on the analysis of 16S and 18S rDNA sequences, Nirmala *et al.* [15] constructed a molecular phylogeny of Calyptratae ('higher' Diptera), a group that includes the Hippoboscoidea (e.g. keds, louseflies, batflies and tsetse flies), the Muscoidea and the Oestroidea (i.e. the overwhelming majority of flies reported to cause myiasis) [1]. Their study included sequences from one or two species for each gene from each calyptrate family and – from the point of view of exploring the origins of myiasis – key subfamilies, together with a range of acalyptrate and nematoceran outgroup taxa. Surprisingly, whereas taxa from the Hypodermatinae, Oestrinae and Gasterophilinae were grouped together as a clade, overall oestrid fly monophyly could not be established because the single Cuterebrinae species analysed was placed apart in a polytomy with numerous other non-oestrid fly species.

In terms of understanding the evolution of the myiasis habit, the relationship of the oestrid fly species responsible for predominantly endoparasitic myiases[‡] with those species responsible for ectoparasitic myiases [i.e. blowflies and screwworm flies (Calliphoridae), and flesh flies (Sarcophagidae)] is crucial. Here, the findings of Nirmala *et al.* offer insight into another long-debated relationship [5,16] – clarification of the sister-group status of Sarcophagidae and Calliphoridae. Comparison of the secondary structures of two variable regions within 18S rDNA indicates that Sarcophagidae are more closely related to Calliphoridae than to Tachinidae (as proposed by Pape [16]) and that Tachinidae seem to be more closely related to Oestridae than to Sarcophagidae. However, subsequent studies using many more taxa across a reduced taxonomic range (i.e. within a superfamily) indicate that some of the outcomes of the study by Nirmala *et al.* [15] were due to inadequate taxon coverage. The effects of inadequate taxon coverage on phylogenetic topology (e.g. long-branch attraction [17]) are well documented for several parasite groups (e.g. trypanosomes [18,19]) and, indeed, the potential effects of improved taxon coverage on family-level relationships are alluded to by Nirmala *et al.* [15] themselves.

Calliphoridae

Owing to their status as parasites of livestock and their role as primary indicator species in forensic entomology, the Calliphoridae are among the most phylogenetically well studied of myiasis-causing flies [5,12,20–27], although debate continues concerning the precise nature of their constituent taxa [5,12,28].

Of these studies, Rognes [5], and Stevens and Wall [22] were the first to focus specifically on the evolution of vertebrate parasitism in blowflies. Using a combination of morphological and molecular data (mitochondrial DNA and 12S rDNA sequences), Stevens and Wall [22] provided an insight into the apparently independent evolution of the ectoparasitic habit in multiple *Lucilia* species (i.e. in *Lucilia cuprina* + *Lucilia sericata* and in *Lucilia caesar* + *Lucilia illustris*), highlighting the evolution of blowfly myiasis in association with the domestication of sheep in relatively recent history, together with a unique form of parasitism of toads and frogs (by *Lucilia bufonivora*). Rognes [5] presented an in-depth morphology-based phylogenetic analysis of all major taxonomic groups within the family Calliphoridae, together with a comprehensive range of outgroup taxa. Rognes' analysis placed Sarcophagidae far apart from key calliphorid subfamilies (and as a sister group to Tachinidae) and grouped the family Oestridae in a clade containing the calliphorid subfamilies – Helicoboscinae and Melanomyinae (all snail parasites), Toxotarsinae (which includes saprophagous species), Chrysomyinae (which includes screwworm flies and bird blowflies), Calliphorinae (which includes bluebottle blowflies) and Luciliinae (which includes greenbottle blowflies) – which is a result that is somewhat at odds with existing classifications and subsequent molecular analyses [12,15]. Rognes' cladogram also identified Chrysomyinae + Toxotarsinae and Calliphorinae as sister groups; this apparent close relationship between Chrysomyinae and Calliphorinae is not suggested by recent molecular studies [12,27] (although it should be noted that these studies did not include any toxotarsine specimens).

More recently, two comprehensive molecular-based analyses of the family Calliphoridae have been undertaken using 28S rDNA, cytochrome oxidase subunits I and II (COI + II) and NADH dehydrogenase subunit 4 (ND4)–ND4L (NADH dehydrogenase subunit 4L) sequences [12,27]; mostly, the findings of these two studies seem to be in agreement (Box 2). Specifically, the analysis by Stevens [12] provided some support for the monophyly of Calliphoridae (based on the taxa analysed), with calliphorid subfamilies forming a well-supported grouping that is distinct from the family Oestridae. However, although the relationship of the calliphorid taxa with a single sarcophagid taxon, *Sarcophaga carnaria*, was resolved less well, neither parsimony nor maximum-likelihood analysis grouped *S. carnaria* within the Calliphoridae clade, thus indicating a sister-group relationship. Within Calliphoridae, the taxon complement of the subfamilies Chrysomyinae, Calliphorinae and Luciliinae was mostly in agreement with the taxonomic status of the taxa as defined by morphology-based analysis, although support for monophyly of the subfamily Chrysomyinae was not robust; in the molecular analysis, taxa from this subfamily (as classically defined) formed an unresolved polytomy of at least two lineages. These findings were supported by the analysis of COI + II gene sequences from a reduced taxon set. Inter-subfamily phylogenetic relationships derived from the molecular data did not concur fully with the pattern of relationships defined by morphological analysis [5]; notably, the analysis of both nuclear (28S rRNA) and mitochondrial (COI + II) sequences classified Calliphorinae and Luciliinae as sister groups.

[‡] Some Hypodermatinae (e.g. *Przhevalskiana silenus* and *Oestromyia* spp.) cause cutaneous myiasis in a similar way to Sarcophagidae and Calliphoridae.

The findings of Wallman *et al.* [27], who focused on flies from Australia, showed a similar trend. The results showed each of the major calliphorid subfamilies – Calliphorinae, Luciliinae and Chrysomyinae – to be monophyletic within a monophyletic Calliphoridae clade relative to two sarcophagid outgroup taxa. The analysis also confirmed the sister-taxa status of Calliphorinae and Luciliinae. The lack of oestrid taxa within the analysis meant that oestrid–calliphorid relationships could not be investigated.

Oestridae

The family Oestridae includes 18 genera and ∼151 species of fly [1], with an enormous diversity in terms of lifestyle, spread and parasitic behaviour within a host. Until now, comparatively few phylogenetic studies of this group have been undertaken. Nevertheless, the analysis of morphological and life-history characteristics provides strong support for oestrid fly monophyly [16,29,30] – a position supported by recent molecular studies of myiasis-causing Oestridae and Calliphoridae [11,12]. As discussed, however, an earlier study by Nirmala *et al.* [15], which included only one or two species from each oestrid fly subfamily as part of a broad study of calyptrate phylogeny based on 16S and 18S rDNA, did not establish oestrid fly monophyly.

In their taxonomically inclusive study, Otranto and Stevens [11] presented a molecular cladogram (Figure 1) based on mitochondrial COI sequences in which each of the four constituent Oestridae subfamilies emerged as monophyletic groups within a monophyletic Oestridae clade, as previously postulated on the basis of morphological differences [16,29,30]. In particular, confirmation of the monophyly of the Calliphoridae and Oestridae families agreed with previously established relationships.

The only conflict of the COI-based phylogeny with the morphology-based classifications of Pape [30] and Pont [8] (which grouped Hypodermatinae and Oestrinae as subfamilies within the family Oestridae) is that Otranto and Stevens showed the Gasterophilinae to be the sister group of the Hypodermatinae rather than of a combined Hypodermatinae + Oestrinae clade; similarly, the rDNA-based study of Nirmala *et al.* [15] did not support a sister-group relationship between Hypodermatinae and Oestrinae. However, although the relationships described by the COI sequence data are robust in most cases (Figure 1), levels of bootstrap support at key nodes within the phylogeny suggest the need for further work to verify this topology, together (preferably) with supporting analysis of molecular data from other complementary (e.g. nuclear) genes. As demonstrated for *Lucilia* [31], primarily because of the non-recombinatory inheritance of mitochondrial genomes, the phylogenies described by mitochondrial genes are not always congruent with nuclear phylogenies.

Most recently, phylogenetic analyses (maximum-likelihood and parsimony) of the COI, 16S and 28S rRNA genes [32] have confirmed the monophyly of gasterophiline species that affect horses and their relatives (i.e. *Gasterophilus haemorrhoidalis*, *Gasterophilus inermis*, *Gasterophilus intestinalis*, *Gasterophilus nasalis* and *Gasterophilus pecorum*), together with *Gyrostigma pavesii*, which parasitizes rhinoceroses.

Sarcophagidae

The molecular phylogenetic study by Wells *et al.* [33] supports the recognition of Sarcophagidae as a monophyletic clade with respect to the single calliphorid taxon – *Chrysomya albiceps* – that was included in their analysis.

Box 2. Evolution of myiasis in Calliphoridae: molecular phylogenies and comparative analyses

Whereas oestrid flies are characterized by universal obligate parasitism [1], calliphorids demonstrate a range of life-history strategies, the distribution of which across blowfly phylogenies has been the subject of several recent studies.

Stevens [12] produced a 28S-rRNA-based phylogeny (Figure I) as the basis for a comparative analysis of myiasis evolution within Calliphoridae, from which it seemed that obligate parasitism arose on several (probably at least five) independent occasions in the taxa included in his study. The most parsimonious interpretation of the phylogeny also suggested that parasitism arose independently in Calliphoridae and Sarcophagidae, and in Oestridae. Focusing on blowflies (Calliphoridae), the distribution of obligate parasites of warm-blooded vertebrates indicated (based on the taxa analysed) that obligate parasitism of vertebrates arose independently on at least three separate occasions within this family. Alternatively, it could be hypothesized that obligate parasitism was the ancestral state and that it was subsequently lost from blowflies that are not obligate parasites (e.g. *Chrysomya albiceps* and *Cochliomyia macellaria*). The existence of ectoparasitic species, albeit facultative, in the subfamily Luciliinae indicated a fourth apparently independent evolution of the myiasis habit within Calliphoridae. The placement of the facultative primary myiasis species – *Lucilia cuprina* and *Lucilia sericata* – in a clade with other secondary and/or saprophagous blowfly species (i.e. *Lucilia richardsi* and *Lucilia silvarum*) also suggested the possible multiple independent evolution of the myiasis habit within this subfamily.

The analysis by Wallman *et al.* [27] of carrion-breeding calliphorids from Australia was based on multiple mitochondrial genes (Figure II).

It included virtually all known species of *Calliphora* from carrion in Australia. Facultative myiasis is known to be caused by six species of this genus (including the introduced European bluebottle *Calliphora vicina*), with their evolutionary placement suggesting the independent development of this life-history trait on four occasions (Figure II). Each of the six species has been recorded as an instigator of myiasis (mostly in sheep) but secondary involvement might also be common in association with *L. cuprina* as the primary agent [39–42]. *L. cuprina* is the most aggressive agent of myiasis in Australia and was also included in the study by Wallman *et al.*, together with all known saprophagous species of Luciliinae and Chrysomyinae from that continent. The phylogenetic data obtained for Luciliinae point to a single origin of facultative primary myiasis, with the primary habit having been lost almost completely from Australian *L. sericata*. Among the genus *Chrysomya*, facultative secondary myiasis could have evolved on three separate occasions.

The exact status of many blowfly taxa as agents of myiasis is difficult to define and includes many facultative secondary myiasis agents and saprophagous species, the status of which might change according to local factors, particularly climate. For example, in northern regions of Europe such as Denmark and western Norway, *Lucilia caesar* and *Lucilia illustris* are both common in sheep myiasis (strike) [43,44]. The propensity of blowflies to cause both primary and secondary facultative myiasis and/or to develop in carrion indicates that the ancestral state of larval development in Calliphoridae could have been carrion feeding and opportunistic myiasis: a conclusion that is in general agreement with the saprophagous origins proposed by Zumpt [1] and Erzinçlioglu [2].
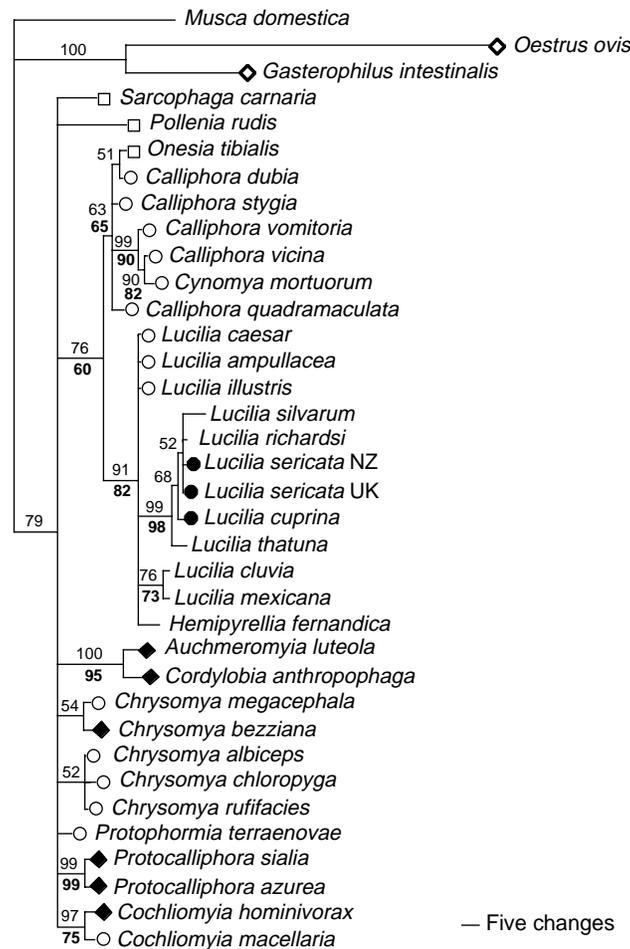
Figure I. Phylogeny constructed by parsimony analysis of 28S rRNA sequences. The percentage bootstrap support values were derived from 1000 pseudoreplicates by both parsimony (upper values) and maximum-likelihood (lower values in bold) analyses. Thirty-two sequences, representing 46 blowfly taxa from 32 species, from five subfamilies of Calliphoridae are shown – from top to bottom: Polleniinae, Calliphorinae, Luciliinae, Auchmeromyiinae and Chrysomyinae. The four outgroup taxa are *Musca domestica*, *Oestrus ovis*, *Gasterophilus intestinalis* and *Sarcophaga carnaria*. The branches of the tree receiving poor ((50%) bootstrap support by both methods of analysis have been collapsed. Black diamonds represent obligate parasites (Calliphoridae) of vertebrates; white squares represent obligate parasites (Calliphoridae and Sarcophagidae) of invertebrates (earthworms); white diamonds represent obligate endoparasites (Oestridae) of vertebrates; black circles represent facultative, exclusively or mostly primary, myiasis species; white circles represent facultative, exclusively or mostly secondary, myiasis species. It should be noted that *Lucilia sericata* is a major primary agent of myiasis in the UK, but in Australia it functions mostly as a secondary species (see Figure II). Image modified, with permission, from Ref. [12].

Within Sarcophagidae, on the basis of the taxa analysed, the recognition of two monophyletic subfamilies, Sarcophaginae and Paramachronychiinae (the only Sarcophagidae subfamilies that contain species involved in myiasis), was supported. Pape had earlier suggested a sister-group relationship between these two on morphological grounds [16,34]. To date, however, there has been only a small number of phylogenetic studies of Sarcophagidae, and additional research remains a priority if the evolution of this group, particularly its relationship to Calliphoridae, is to be fully understood.

Concluding remarks

Phylogenetic studies based on data from multiple genetic markers are now widely regarded as the 'gold standard' of methods for determining evolutionary relationships. In this article, we have demonstrated that molecular phylogenies can provide a robust framework for understanding the evolution of myiasis and myiasis-causing flies. For example, Stevens [12] interpreted the distribution of blowfly taxa in clades, as defined by phylogenetic analysis, as indicating that the majority of calliphorid subfamilies were already in existence and globally distributed at the time of the breakup of Gondwana: ~100 million years ago; however, the molecular-clock analysis by Wallman *et al.* [27] suggests that the extant subfamilies Calliphorinae, Chrysomyinae and Luciliinae could have shared a common ancestor as recently as ~25 million years ago. Further work is obviously required to resolve such large differences in divergence estimates (75 million years in this example) and it is expected that phylogenetic information from additional genes and classes of gene marker will be of value in resolving some of these issues. The absence of a relevant fly genome continues to hamper rapid development in this area – at present, the 'closest' available genome is that of *Drosophila* [35], which, while providing a general framework for dipteran research, lacks information about, for example,
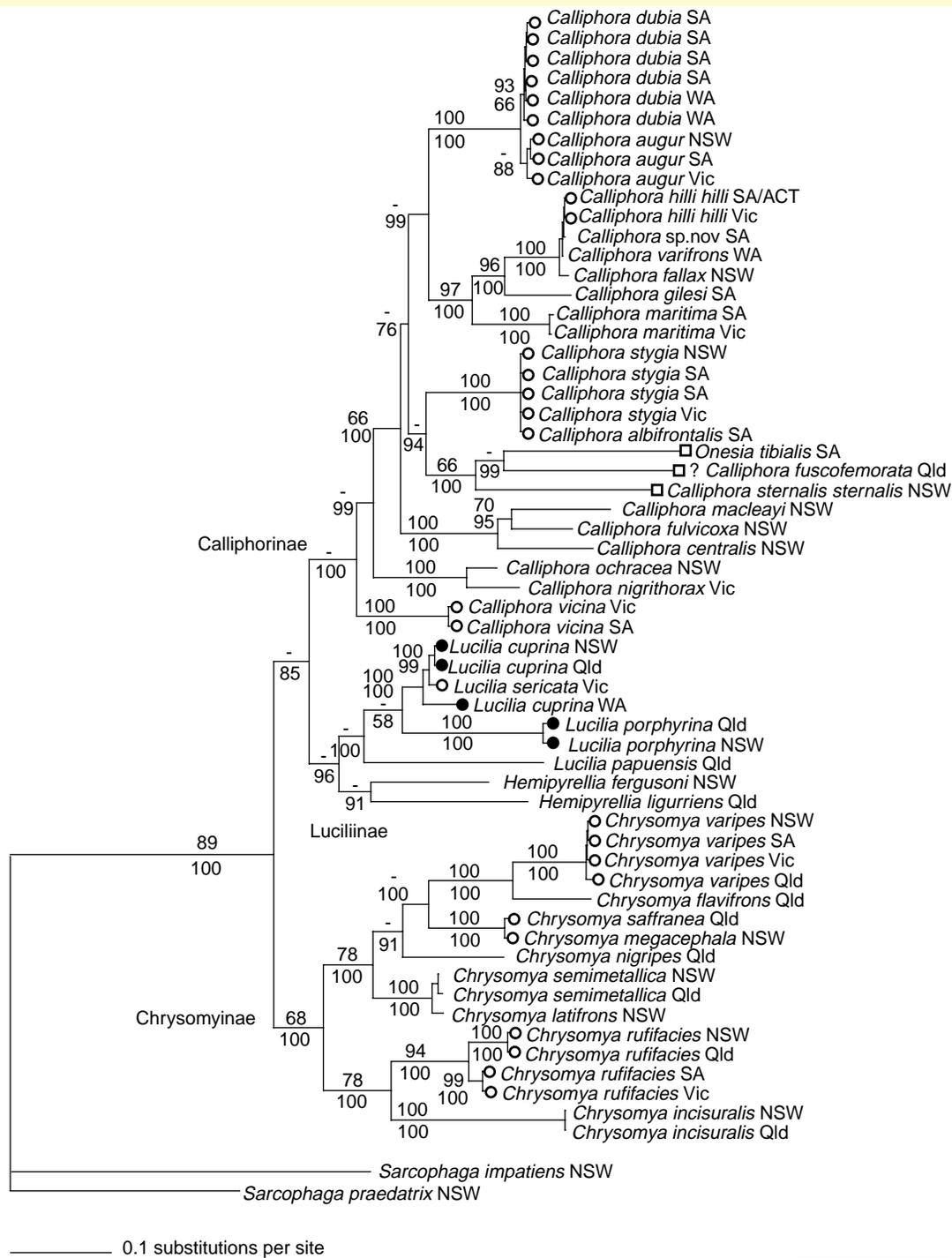
Figure II. Maximum-likelihood phylogram showing relationships among mitochondrial COI, COII, ND4 and ND4L sequences from blowflies (ingroups) and flesh flies (outgroups) from Australia. Abbreviations refer to the Australian state or territory in which each specimen was collected: ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia. Numbers above branches refer to bootstrap proportions among 1000 maximum-parsimony pseudoreplicates (>50%) and numbers below branches refer to posterior probabilities (from Bayesian analysis). Black circles represent facultative, exclusively or mostly primary, myiasis species; white circles represent facultative, exclusively or mostly secondary, myiasis species; white squares represent obligate primary parasites of invertebrates (earthworms). Image modified, with permission, from Ref. [27].

the proteins that must be expressed in myiasis-causing flies in relation to the parasitic phase of their lifecycles[§]. Moreover, as understanding of the limitations of genetic

data continues to increase (e.g. the effects of nucleotide saturation and horizontal gene transfer), it is apparent that gene-based phylogenies might not always be able to provide the unequivocal species phylogenies that were envisaged at the start of the molecular era. Accordingly, for the foreseeable future, evidence from other sources (e.g. biogeography and clade–taxon complement,

§ Ongoing research efforts in Australia, New Zealand, the UK and elsewhere suggest that a calliphorid (blowfly) genome could be available within five years; sequencing of the mitochondrial genome of the sheep blowfly *Lucilia sericata* has recently been completed at Exeter (see http://www.people.ex.ac.uk/jrsteve0/).
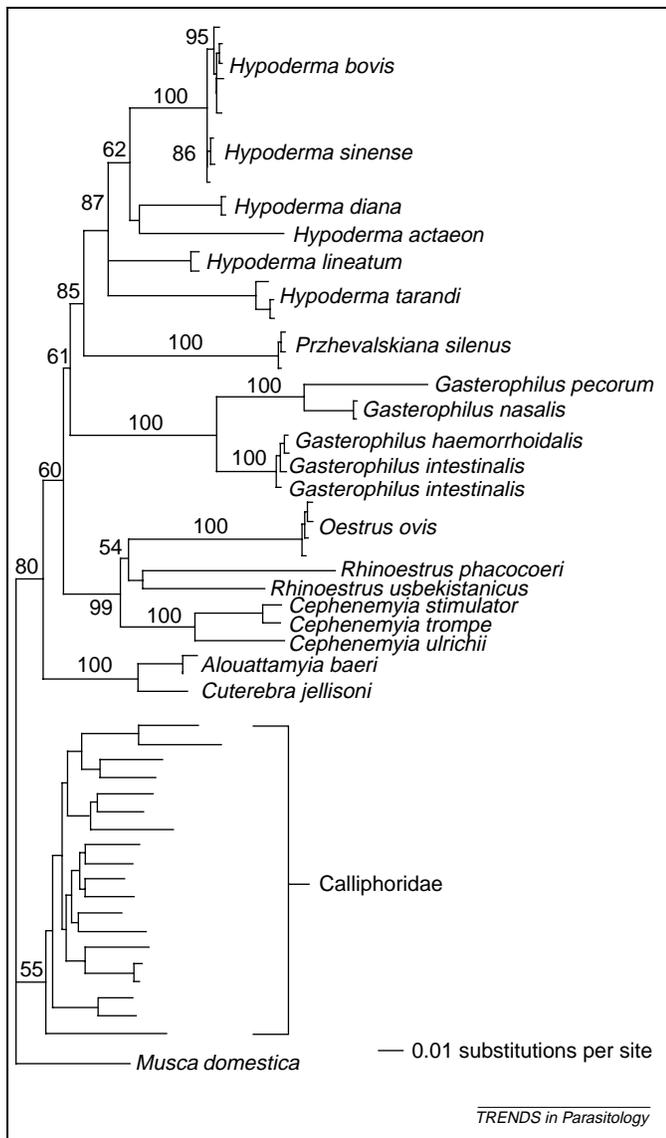
Figure 1. Phylogram showing phylogenetic relationships of Oestridae species. Phylogram was constructed by neighbour-joining analysis of COI nucleotide sequence data, using the Kimura two-parameter model. Percentage bootstrap values for key nodes are shown, based on 1000 pseudoreplicates. Figure created using data from Ref. [11].

molecular clocks and fossils) will remain essential for determining the timing of divergence events in the evolution of myiasis-causing flies. These factors, together with the phylogenetic evidence reviewed in this article, are addressed in part II of this review to provide a holistic view of the evolution of myiasis and its agents.

References
1 Zumpt, F. (1965) *Myiasis in Man and Animals in the Old World*, Butterworth
2 Erzinçlioglu, Y.Z. (1989) The origin of parasitism in blowflies. *Brit. J. Entomol. Nat. Hist.* 2, 125–127
3 Otranto, D. (2001) The immunology of myiasis: parasite survival and host defence strategies. *Trends Parasitol.* 17, 176–182

4 Boulard, C. *et al*. (1996) Cross-reactive, stage-specific antigens in the Oestridae family. *Vet. Res.* 27, 535–544
5 Rognes, K. (1997) The Calliphoridae (blowflies) (Diptera: Oestroidea) are not a monophyletic group. *Cladistics* 13, 27–66
6 James, M.T. (1947) *The Flies that Cause Myiasis in Man*, USDA Misc. Pub. No. 631
7 Hennig, W. (1973) Diptera (Zweiflüger). *Handb. Zool. Berlin* 4, 1–200
8 Pont, A.C. (1980) Family Oestridae. In *Catalogue of the Diptera of the Afrotropical Region* (Crosskey, R.W., ed.), pp. 886–888, British Museum (Natural History)
9 McAlpine, J.F. (1989) Phylogeny and classification of the Muscomorpha. In *Manual of Nearctic Diptera* (McAlpine, J.F. *et al.*, eds), pp. 1397–1518, Research Branch, Agriculture Canada
10 Pape, T. and Arnaud, P.H. (2001) *Bezzimyia* – a genus of native New World Rhinophoridae (Insecta, Diptera). *Zool. Scr.* 30, 257–297
11 Otranto, D. and Stevens, J.R. (2002) Molecular approaches to the study of myiasis-causing larvae. *Int. J. Parasitol.* 32, 1345–1360
12 Stevens, J.R. (2003) The evolution of myiasis in blowflies (Calliphoridae). *Int. J. Parasitol.* 33, 1105–1113
13 Carreno, R.A. and Barta, J.R. (1998) Small subunit ribosomal RNA genes of tabanids and hippoboscids (Diptera: Brachycera): evolutionary relationships and comparison with other Diptera. *J. Med. Entomol.* 35, 1002–1006
14 Bernasconi, M.V. *et al*. (2000) Phylogenetic relationships among Muscoidea (Diptera: Calyptratae) based on mitochondrial DNA sequences. *Insect Mol. Biol.* 9, 67–74
15 Nirmala, X. *et al*. (2001) Molecular phylogeny of Calyptratae (Diptera: Brachycera): the evolution of 18S and 16S ribosomal rDNAs in higher dipterans and their use in phylogenetic inference. *Insect Mol. Biol.* 10, 475–485
16 Pape, T. (1992) Phylogeny of the Tachinidae family-group (Diptera: Calyptratae). *Tijdschr. Entomol.* 135, 43–86
17 Felsenstein, J. (1978) Cases in which parsimony or compatibility will be positively misleading. *Syst. Zool.* 27, 401–410
18 Maslov, D.A. *et al*. (1996) Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa. *Mol. Biochem. Parasitol.* 75, 197–205
19 Stevens, J.R. *et al*. (2001) The molecular evolution of Trypanosomatidae. *Adv. Parasitol.* 48, 1–56
20 Sperling, F.A.H. *et al*. (1994) A DNA-based approach to the identification of insect species used for postmortem interval estimation. *J. Forensic Sci.* 39, 418–427
21 Stevens, J. and Wall, R. (1996) Species, sub-species and hybrid populations of the blowflies *Lucilia cuprina* and *Lucilia sericata* (Diptera: Calliphoridae). *Proc. Biol. Sci.* 263, 1335–1341
22 Stevens, J. and Wall, R. (1997) The evolution of ectoparasitism in the genus *Lucilia* (Diptera: Calliphoridae). *Int. J. Parasitol.* 27, 51–59
23 Stevens, J. and Wall, R. (2001) Genetic relationships between blowflies (Calliphoridae) of forensic importance. *Forensic Sci. Int.* 120, 116–123
24 Wells, J.D. and Sperling, F.A. (1999) Molecular phylogeny of *Chrysomya albiceps* and *C. rufifacies*. *J. Med. Entomol.* 36, 222–226
25 Wells, J.D. and Sperling, F.A. (2001) DNA-based identification of forensically important Chrysomyinae (Diptera: Calliphoridae). *Forensic Sci. Int.* 120, 110–115
26 Wallman, J.F. and Donnellan, S.C. (2001) The utility of mitochondrial DNA sequences for the identification of forensically important blowflies (Diptera: Calliphoridae) in southeastern Australia. *Forensic Sci. Int.* 120, 60–67
27 Wallman, J.F. *et al*. (2005) Molecular systematics of Australian carrion-breeding blowflies (Diptera: Calliphoridae) based on mitochondrial DNA. *Invert. Syst.* 19, 1–15
28 Gleeson, D.M. *et al*. (2000) The phylogenetic position of the New Zealand batfly. *Mystacinobia zelandica* (Mystacinobiidae: Oestroidea) inferred from mitochondrial 16S ribosomal DNA sequence data. *J. Roy. Soc. New Zeal.* 30, 155–168
29 Wood, D.M. (1987) Oestridae. In *Manual of Nearctic Diptera* (Vol. 2) (McAlpine, J.F., ed.), pp. 1147–1158, Research Branch, Agriculture Canada

30 Pape, T. (2001) Phylogeny of Oestridae (Insecta: Diptera). *Syst. Entomol.* 26, 133–171

31 Stevens, J.R. *et al*. (2002) Paraphyly in Hawaiian hybrid blowfly populations and the evolutionary history of anthropophilic species. *Insect Mol. Biol.* 11, 141–148

32 Otranto, D. *et al*. (2005) Utility of mitochondrial and ribosomal genes for the differentiation and phylogenesis of species of gastrointestinal botflies. *J. Econ. Entomol.* 98, 2235–2245

33 Wells, J.D. *et al*. (2001) DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). *J. Forensic Sci.* 46, 1098–1102

34 Pape, T. (1987) *The Sarcophagidae (Diptera) of Fennoscandia and Denmark*, Brill Academic Publishers

35 Adams, M.D. *et al*. (2000) The genome sequence of *Drosophila melanogaster. Science* 287, 2185–2195

36 Hall, M.J.R. and Wall, R. (1995) Myiasis of humans and domestic animals. *Adv. Parasitol.* 35, 257–334

37 Noutsis, C. and Millikan, L.E. (1994) Myiasis. *Dermatol. Clin.* 12, 729–736

38 Jelinek, T. *et al*. (1995) Cutaneous myiasis: review of 13 cases in travelers returning from tropical countries. *Int. J. Dermatol.* 34, 624–626

39 Ryan, A.F. (1954) The sheep blowfly problem in Tasmania. *Aust. Vet. J.* 30, 109–113

40 Norris, K.R. (1959) The ecology of sheep blowflies in Australia. *Monographiae Biologicae* 8, 514–544

41 Watts, J.E. *et al*. (1976) The species of flies reared from struck sheep in south-eastern Australia. *Aust. Vet. J.* 52, 488–489

42 Murray, M.D. (1978) The species of flies reared from struck sheep in Western Australia. *Aust. Vet. J.* 54, 262

43 Cragg, J.B. (1950) Studies on *Lucilia* species under Danish conditions. *Ann. Appl. Biol.* 37, 66–79

44 Brinkmann, A. (1976) Blowfly myiasis of sheep in Norway. *Norw. J. Zool.* 24, 325–330