

The evolution of myiasis in humans and other animals in the Old and New Worlds (part II): biological and life-history studies

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Myiasis, which is the dipteran parasitism of living vertebrates, occurs in several forms – ranging from benign to fatal, opportunistic to obligate – and seems to have evolved through two distinct routes: saprophagous and sanguinivorous. However, the convergent evolution of morphological and life-history traits seems to have had a major role in confusing the overall picture of how myiasis evolved and this 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. As discussed in part I of this review, if we are to elucidate how the different forms of parasitism arose, it is essential to separate the evolution of the various groups of myiasis-causing flies from the evolution of the myiasis habit *per se*. Accordingly, whereas we focused on recent landmark phylogenetics studies in part I, we use this framework to analyse relevant biochemical, immunological, behavioural, biogeographical and fossil evidence to elucidate the evolution of myiasis in part II.

Interpreting the evolution of myiasis through life-history characteristics

A robust phylogenetic framework enables the evolutionary relationships between myiasis-causing flies to be interpreted and patterns of the evolution of myiasis *per se* to be reconstructed, as discussed in part I of this review [1]. Here, we adopt Zumpt's [2] definition of myiasis as being the infestation of the tissues of live vertebrates (humans and/or animals) by dipterous larvae (see Box 1 in part I for full explanation [1]). Given such phylogenies, a wealth of morphological, biochemical and immunological data can also be used to provide independent evidence with which to interpret

the evolution of life-history traits such as myiasis. Additionally, such data can be supplemented by: (i) the addition of studies of host biology to help interpret patterns of parasite–host coevolution; and (ii) fossil data, which can help to pinpoint the dates of divergence events observed in phylogenies. Without fossil data, tree-derived divergence times often depend on rates of molecular evolution that are calculated from molecular clocks, the reliability and characteristics of which vary widely among genes, taxa and timescales.

A better understanding of the evolution of myiasis is not only of intrinsic scientific interest but might also have applied value, as alluded to in part I [1]. For example, knowledge of the immunological, physiological and biochemical strategies that enable the larvae of some myiasis-causing species to survive nonspecific (e.g. natural killer cells and complement proteins) and specific (e.g. antibodies and T cells) immunological responses of a host provides ideal targets for the development of immunological methods for the early detection of these species in livestock [3,4]. Similarly, an understanding of larval secretions has proven beneficial for understanding the basis of larval therapy [5].

The recent studies that have reconstructed evolutionary relationships among the key lineages of myiasis-causing flies – Calliphoridae (blowflies), Sarcophagidae (flesh flies) and Oestridae *sensu lato* (bot flies) – were reviewed in part I [1]. Having established a working knowledge of the phylogenies available for Diptera that parasitize vertebrates, we now focus on the diverse forms of data available from a range of independent biological and archaeological sources that are key to corroborating or, indeed, contradicting the relationships defined by these phylogenies.

Mechanisms of myiasis

Characteristically, members of the Oestridae family are all obligate, host-specific gastrointestinal or subdermal

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Available online 28 February 2006

larval parasites that undergo long periods of association with the host and usually have relatively low levels of pathogenicity. By contrast, not all species of Calliphoridae and Sarcophagidae are parasitic. However, the species of these families that are agents of myiasis are characterized by lower host-specificity, shorter periods of larval feeding, a relatively superficial or cutaneous location in the host and relatively high levels of pathogenicity. Nevertheless, fundamental common changes in behaviour and physiology are likely to have been associated with the evolution of all species towards parasitism. For example, the larvae of all of the parasitic species must be able to survive the immune challenge, the high body-temperatures of vertebrates [6] and the differences in diet presented by a living vertebrate host.

In the Oestridae family, such general adaptations to a parasitic lifestyle have been accompanied by changes in larval morphology and enzymic repertoire. For example, the larvae of Oestrinae and Gasterophilinae (which infest the nasopharyngeal region and the gastrointestinal tract, respectively) have characteristic, relatively large mouth-hooks and rows of well-developed spines on the dorsal and/or ventral side of each body segment. These spines are thought to be crucial for the migration of the larvae within the host and, at an intergeneric level, their arrangement is distinctive; within genera, however, they are unreliable for phylogenetic analyses. The proteases produced by these larvae are involved largely in enabling them to survive the host immune system [3]. Nevertheless, such morphological features are not universal in the Oestridae, and in the Hypodermatinae the larval stages have only vestigial mouth-hooks and poorly developed larval spinulation. In contrast to the relatively limited larval protease repertoire of Oestrinae and Gasterophilinae, however, the first-stage larvae of *Hypoderma* spp. secrete three major serine proteases (chymotrypsin and hypodermins) during their migration through host tissue [3]. Importantly, these proteases are involved not only in combating the host immune responses but also in larval migration [7,8]. Thus, changes in larval morphology in Oestridae complement the development of appropriate enzymic characteristics and seem to be associated with larval migration through the tissues of the host.

Such characteristics are not seen in the parasitic Calliphoridae and Sarcophagidae, in which there is no feeding stage that migrates through the tissues. Many species of Calliphoridae are saprophages that feed on animal carcasses, whereas others are obligate parasites. Indeed, for each of the calliphorid species that parasitizes vertebrates, there is at least one closely related species that does not display this behaviour [1,9]. For example, the blowfly *Lucilia sericata* is an important facultative ectoparasite in many parts of Europe [10] – particularly the UK, where it infests more than one million sheep annually [11]. The blowfly *Lucilia richardsi*, which is sympatric in many parts of its range, is closely related to *L. sericata* phylogenetically [9,12] and is also extremely similar to it morphologically [13]. There is, however, no record of the involvement of *L. richardsi* in ovine myiasis and, despite their close relationship, *L. richardsi* and *L. sericata* exhibit different larval behaviour. Similarly,

recent phylogenetic analysis [9] has highlighted the close relationships between important myiasis-causing flies and non-parasitic, or facultatively parasitic, carrion-breeding sister taxa, including both Old World and New World screwworm flies (see part I [1]). In the Calliphoridae and Sarcophagidae, the saprophagous obligate or facultatively parasitic sister species are all morphologically similar and are distinguished by only minor character differences.

It is unknown why groups of morphologically similar and closely related species of Calliphoridae and Sarcophagidae behave in such different ways. But, in the absence of obvious morphological differences between the larvae, it can be speculated that these distinct behaviours might be mediated by differences in temperature tolerance, response to immune challenge and/or the production of different arrays of enzymes, particularly those associated with feeding on the tissues of a living host. Differences between adults of parasitic (obligate and facultative) and strictly saprophagous species might be controlled by their responses to semiochemical cues that enable the parasitic species to locate and oviposit on a living host, although there is no evidence to confirm this hypothesis at present.

Morphology, convergent evolution and homoplasy

It is usually accepted that both molecular and morphological characters can be subject to convergent evolution and homoplasy. Certainly, the lack of congruence observed between phylogenies based on morphological characters [14] and molecular sequence data [9,15] can be explained only by the existence of homoplasy. Within each family, however, the limitations of employing morphological characters for phylogenetic and taxonomic purposes seem different, especially with regard to immatures.

The Calliphoridae and Sarcophagidae are characterized by having typical musciform larvae that are segmented and pointed at the anterior end with no head-capsule, whereas the posterior end is broad and usually truncate; the head and first thoracic segment contain the cephaloskeleton, which bears the mouth-hooks, whereas the last segment bears the posterior spiracles and the anus. Perhaps surprisingly, an obvious relationship between the structure and size of larval mouth-hooks and the life-history strategy (i.e. saprophagy, secondary parasitism or obligate parasitism) is not apparent, and available information about associated enzymic repertoires is inconclusive.

Within the Oestridae, however, a high level of differentiation in larvae belonging to the four subfamilies is apparent and could reflect the longer period in which they have been parasites since their divergence from a common ancestor [16]. In addition to substantial morphological differences between species of the various subfamilies, this hypothesis is also supported by a high level of specialization in their life cycles and by some immunological data, namely that some proteases produced by Hypodermatinae share epitopes with antigens of other members of the same subfamily but do not crossreact with members of the Oestrinae and Gasterophilinae families [3]. Therefore, morphology-based

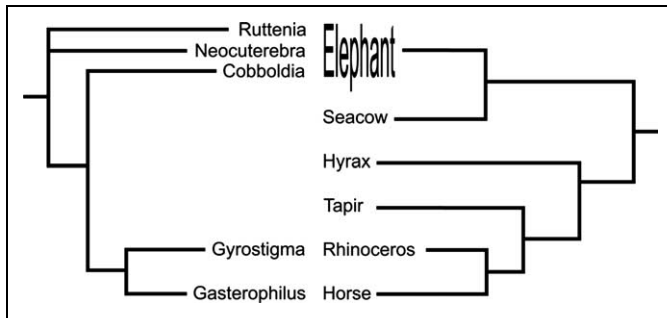


Figure 1. The evolution of host choice in the Gasterophilinae subfamily. Parasite and host phylogenies are compared. Modified, with permission, from Ref. [20].

phylogenies of oestrids seem to be less equivocal than comparable calliphorid and sarcophagid phylogenies [14,17,18], although there is still much less support for oestrid-subfamily-level phylogeny than for generic-level relationships.

Host–parasite coevolution?

The nature of the relationships between parasitic Diptera and vertebrate animals can take several forms but there are two main categories that are recognized. Oestridae are usually obligate host-specific endoparasites with relatively ancient associations with the host and relatively low pathogenicity. By contrast, the Calliphoridae and Sarcophagidae families are usually less host specific than Oestridae, with shorter periods of larval feeding and higher pathogenicity; importantly, they have a broad

range of life-history strategies and degrees of interaction with their hosts, from saprophagy, through facultative parasitism, to obligate parasitism. Some host specificity, however, is observed; for example, *Notochaeta bufonivora* and *Lucilia bufonivora* are parasites of toads, and *Cistudinomyia cistudinis* and *Notochaeta carolinensis* are obligate myiasis producers in reptiles.

Thus, the evolution of myiasis-causing Calliphoridae and Sarcophagidae does not seem to be intimately linked to the evolution of a particular host species (i.e. strict host–parasite coevolution is not a defining characteristic of these myiasis-causing flies). Fortunately, however, the range of life-history strategies within Calliphoridae (i.e. saprophagous, facultative and obligate), coupled with the robust phylogenies that are available [9,15], provides the essential prerequisites for meaningful comparative analyses of the evolution of parasitism within the Calliphoridae family (see Box 2 in part I [1]).

Conversely, although strict host–parasite coevolution is not usually observed in oestrid flies (which are all parasites that infect mammals), parallel evolution could have had at least some role in the speciation of these flies, with parasite lineages tracking host lineages through evolutionary time. It is unlikely that this association constitutes coevolution *sensu stricto* because, other than at an immunological level, the only obvious signs of adaptation of hosts to particular flies are some avoidance behaviours [19]. Certainly, with only ~150 extant species, oestrid flies cannot be considered successful in terms of their diversity. The strict association with mammals

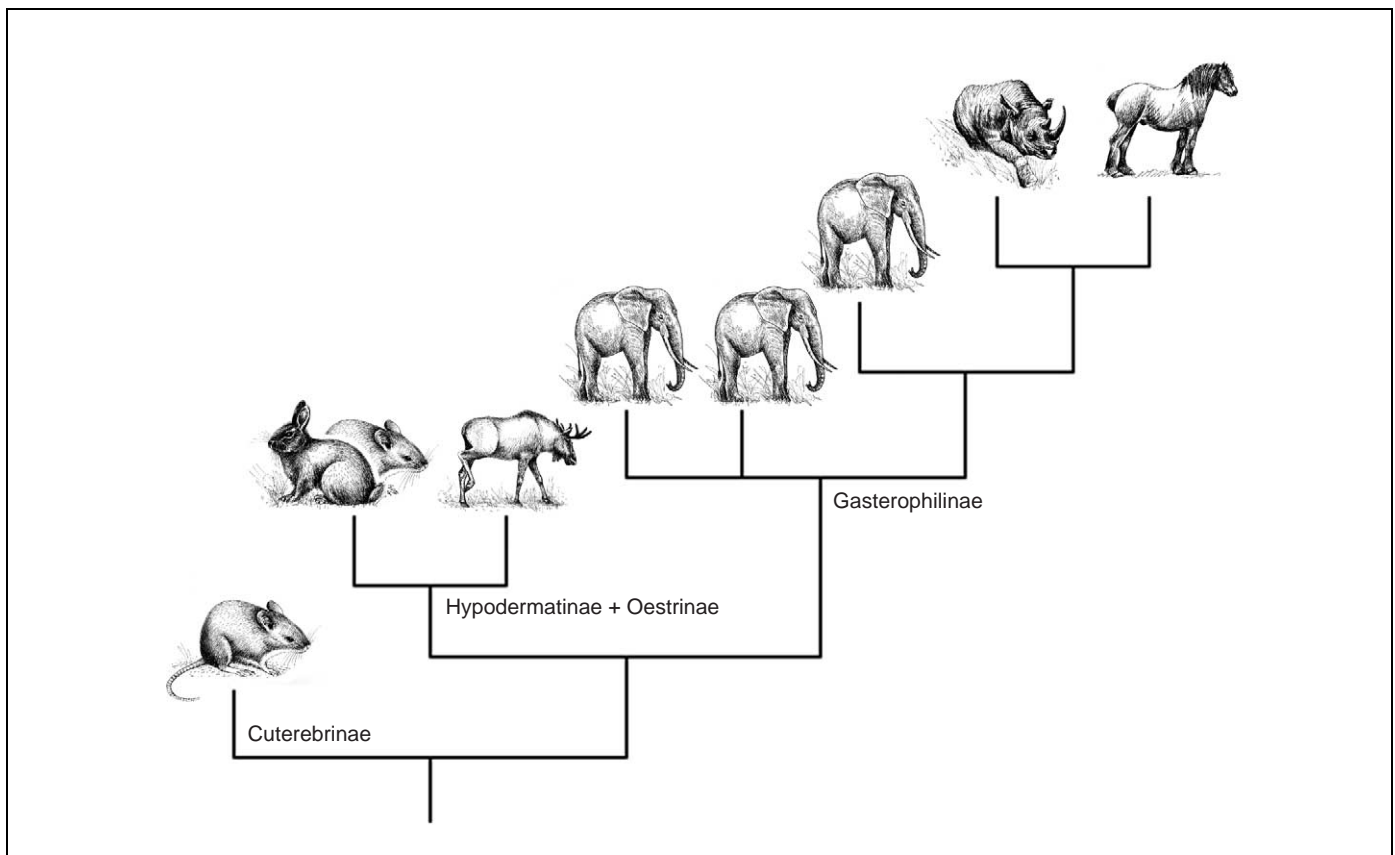


Figure 2. Schematic oestrid fly phylogeny. Possible ancestral hosts of extant fly lineages are mapped. Modified, with permission, from Ref. [20].

probably constrained Oestridae fly radiation by providing restricted niche opportunities.

Recent work by Pape [20] indicates that oestrid flies could have evolved originally from rodent (or rodent-lagomorph) parasitism. In exploring this issue, Pape used the program DIVA [21] to reconstruct the ancestral host by coding hosts rather than geographic areas for the terminal

taxa (DIVA is primarily a program for biogeographical analysis). Pape's results indicate that: (i) the large majority of extant oestrid flies are – and, therefore, probably were – restricted to one or a small number of host species (e.g. Gasterophilinae) (Figure 1); and (ii) parallel host–parasite evolution is not apparent. Pape concludes that the oestrid fly ancestral host was either a

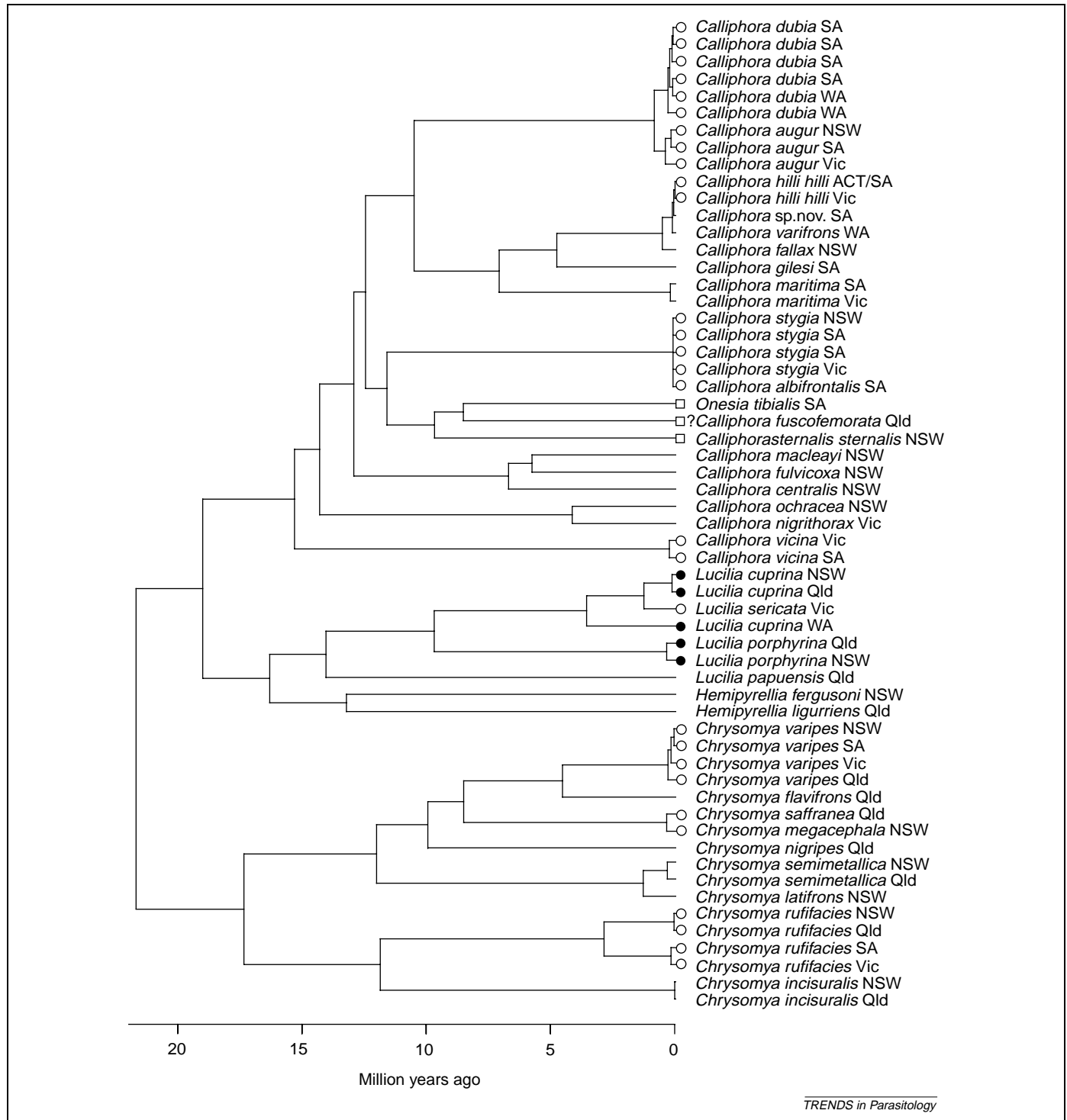


Figure 3. Linearized phylogenetic tree. Tree was calibrated with the preliminary mitochondrial DNA clock of Brower [43] based on mitochondrial cytochrome oxidase subunit (COI), COII, ND4 and ND4L sequences from Australian blowflies. Abbreviations denote the Australian state or territory in which each specimen was collected: ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; SA, South Australia; Vic, Victoria; WA, Western Australia. Black circles denote facultative, mostly or exclusively primary, myiasis species; white circles represent facultative, mostly or exclusively secondary, myiasis species; white squares represent obligate parasites of invertebrates (earthworms). The role of *Lucilia sericata* as a predominantly secondary species in Australia is at odds with its behaviour elsewhere – especially the UK, where it is a primary agent of myiasis. Figure redrawn using data from Ref. [15].

rodent or a form of rodent–lagomorph during the Cretaceous–Tertiary boundary and that the Oestridae probably began to diversify in the midst of an extensive mammal radiation. Host migrations, vicariance events and host switches, followed by subsequent evolution within different host lineages, all then had a part in shaping the small but biologically and morphologically diverse oestrid fly fauna that is present today [20] (Figure 2).

Patterns in the evolution of myiasis: fossils, biogeography and clocks

Calliphoridae

Evaluation of the distribution of taxa across phylogenies (see Box 2 in part I [1]) can provide further clues to the timing and pattern of blowfly evolution. In particular, the association of various blowfly genera and subfamilies with particular geographical regions (e.g. *Cochliomyia* in the New World, *Chrysomya* in the Old World and *Auchmeromyiinae* in the Afrotropical and Oriental regions) indicates that many parasitic blowfly groups could have come into existence after major geographical isolation and that they subsequently underwent localized divergence and speciation. In geological time, such a situation was present at the beginning of the Late Cretaceous period, following the breakup of the southern supercontinent of Gondwana, ~105 million years before the present (mybp) [22]. This was accompanied by the evolution and spread of flowering plants in the Cretaceous period (146–65 mybp) and the rapid diversification in the Tertiary period (65–1.8 mybp) of the mammals that now constitute many of the host species for Calliphoridae.

Unfortunately, fossil evidence that is relevant to this issue is sparse and its interpretation is far from unequivocal, with most reports not dealing with epochs earlier than the Lower Eocene [23,24]. There are, however, some exceptions. McAlpine [25] described the puparia of the extinct calliphorid *Cretaphormia fowleri* from Upper Cretaceous (105–65 mybp) deposits in Canada, although this material has subsequently been reassessed as probably not being calliphorid in origin [26] (Y.Z. Erzinçlioglu, PhD thesis, University of Durham, 1984). A second report concerns fly larvae, possibly Calliphoridae, found in the interior of bones of the extinct tapir *Lophiodon* from Eocene (54–38 mybp) deposits in the Geiseltal in Germany [27]. Gautier and Schumann [28] note that such associations between maggots and fossilized mammals might be more frequent than is commonly realized; larvae are likely to preserve poorly and be hidden from view inside cavities, thus escaping attention.

Further insights into the timing of blowfly evolution can be derived from the work of Wallman *et al.* [15], who applied a molecular-clock approach to studying the divergence of Australian carrion-breeding calliphorids. In the resultant tree (Figure 3), the age of the root node – the node separating the *Chrysomyinae* from the *Luciliinae* and *Calliphorinae* – was estimated to be 21.7 mybp. Based on this estimate, the divergence of the *Calliphorinae* and *Luciliinae* occurred approximately two million years later, whereas divergences within genera occurred mostly between approximately one and 15 mybp.

The origins of the clades within which myiasis occurs seem to be mainly in the period between five and 15 mybp.

Other evidence of a relatively recent evolution of a parasitic lifestyle within Calliphoridae comes from studies of *Lucilia* blowflies. The artificial selection of animals for domestication and greater meat, milk and wool productivity has frequently been associated with a reduction in resistance to ectoparasites and the unintentional exaggeration of features that confer greater susceptibility to ectoparasite infestation (Box 1). For example, the outer coat of primitive sheep such as the mouflon (*Ovis musimon*) is stiff and hairy, and covers a woolly undercoat; this outer coat is absent from domesticated sheep (*Ovis aries*), the fleece of which consists entirely of the woolly undercoat. Selection for a longer, thicker fleece has increased the susceptibility of sheep to various diseases and ectoparasites, particularly blowflies. Other than in domesticated sheep, however, blowfly myiasis of healthy hosts is uncommon and it seems likely that parasitism of sheep by *Lucilia* blowflies has arisen in response to the opportunities created by sheep domestication, probably in the relatively recent past.

Sarcophagidae

Fossil evidence of flesh flies is scarce, as is the case for all calyptrate families. An unidentified *Sarcophaga* has been recorded from Baltic amber [29] but this requires verification. No other specific indications of the timing of the origins of this family have been forthcoming.

Oestridae

Dating the origin and subsequent evolution of oestrid flies seems to be equally difficult because of a similar lack of conclusive evidence. As such, several Eocene fossils that were initially thought to have affinity with Oestridae [30] have been discounted [17,20], although there remains the possibility of oestrids described from Baltic amber (35–40 mybp) and a Tertiary deposit of unknown origin [31].

Despite the paucity of fossil evidence, species distribution and knowledge of vicariance events and biogeography could help to date major divergence events. Certainly, the presence in Australia of the nasal bot fly *Tracheomyia macropi* has important implications for the minimum age of bot fly ancestry [20]. With a host-borne immigration from South America into Australia, via Antarctica, being most likely [32], separation of the *Tracheomyia* lineage from its sister group within the Oestrinae should have a minimum age of at least 38 mybp, after which South America became separated from Antarctica [33]. Considering the basal dichotomy of the Oestridae between the New World and the Old World as a vicariance event [20], the minimum age of the oestrid flies would be pushed even further back to the separation of South America and Africa (~100–90 mybp [34]), although an absence of clear-cut schizophoran fossils from before the Cretaceous–Tertiary boundary is at odds with an age of Oestridae that is more than 65 mybp.

Clock calibration

A note of caution is needed with regard to clock calibration. Estimates of the divergence times of the

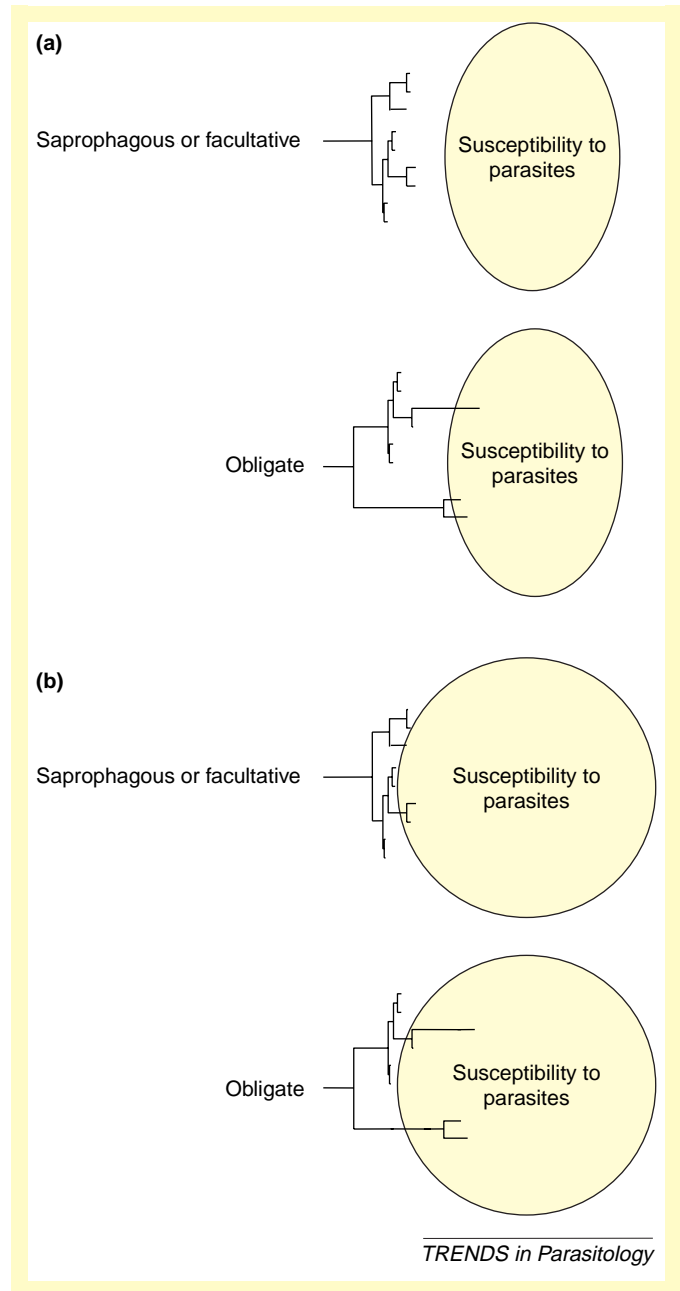
Box 1. Domestication creates new opportunities for parasitism

Evidence accumulated from a broad range of mammal and bird groups indicates that artificial selection (domestication) of animals for greater meat, milk and wool productivity and the associated unintentional exaggeration of certain features have increased host susceptibility to exploitation (infection) by a range of previously non-parasitic organisms. In the case of blowflies, the domestication of sheep seems to have provided opportunities for previously nonspecifically adapted, probably saprophagous, organisms (ancestral blowflies) to be classified as parasites – not through evolution of the parasite but by changes in the nature of the potential host organism (the sheep).

As proposed by Stevens and Otranto [44], based on patterns observed in previous phylogenetic studies [9,45], the association between obligate parasites and their hosts and between facultative parasites and their hosts might also have a phylogenetic component. Stevens and Otranto proposed a possible link between parasite status (i.e. facultative or obligate) and the relative degree of molecular evolution (as measured by phylogenetic branch lengths); specifically, they suggested that obligate parasites, which often exhibit a range of specialist phenotypic adaptations to parasitism (e.g. marked larval spinulation and thickened larval tegument), also seem to be associated with phylogenetic lineages that exhibit relatively high degrees of molecular evolution (longer branch lengths). At the moment, however, the datasets required to test such a hypothesis fully are being gathered and, moreover, it should be remembered that long branches on their own might be caused simply by inadequate sampling of evolutionarily adjacent taxa.

Nevertheless, the two phenomena seem to be complementary (Figure 1) and might eventually provide further insight into the evolutionary basis of even relatively recent host–parasite associations. Certainly, it seems that the transition of species such as the sheep blowfly *Lucilia sericata* from saprophage and opportunistic exploiter of diseased, moribund or wounded living animals into a parasite of major veterinary importance could be more a result of changes in the conformation of the domesticated host than a marked evolutionary change in the adult or larval forms of the potentially parasitic fly.

Figure 1. Potential effects of ungulate domestication on host susceptibility profile in relation to dipteran parasitism. Schematic representation of how changes to a non-susceptible host (a), such as those caused by artificial selection (domestication) of animals for greater meat, milk and wool productivity, have provided new opportunities for exploitation by a range of previously non-parasitic organisms without requiring significant evolutionary change in the potential parasite (b). Phylogenies of obligate parasites in relation to the host are also shown in each panel for comparison, with changes in host susceptibility having little or no effect on the extent of host–parasite interaction.



Calliphoridae, Sarcophagidae and Oestridae families based on geographic distributions are valid only if the underlying assumption of the causative vicariance event is correct and that such distributions are not the product of more-recent biotic interchange (or, for that matter, were caused by even older vicariance events). Similarly, sequence divergence data are valid only if there is evidence that molecular rates of evolution have been inferred correctly. Portions of a phylogeny might exhibit true clock-like behaviour but rate variation among lineages is widespread, particularly among parasites – the evolution of a parasite lineage might be additionally affected by the evolution of their host (or hosts) [35]. Rate differences might also be apparent between genes and, although molecular clocks might be useful tools for evolutionary dating, a rigorous approach

should be taken in their calculation and calibration [36]. As Gaunt and Miles [37] highlight, given the lack of reliable fossils for calibrating evolutionary rates within phylogenies, calibrations often assume the isolation of populations following tectonic movements such as the breakup of Gondwana (as has been speculated for the oestrids). However, such geographic events might not be the only source of speciation. Pending refinement of the putative fossil evidence for families of myiasis fly, the dating of molecular phylogenies for these groups will continue to rely on assumptions from biogeography and from evolutionary rates among other arthropod lineages.

Evolution of myiasis in humans

For thousands, possibly tens of thousands, of years, humans have lived in close proximity to their domesticated livestock and companion animals, with all the

parasites, flies and vermin that accompany them (see Box 1 in part I [1]). Although the thought of sharing our homes with thousands of flies might seem unpalatable, the findings from excavations of various ancient human settlements (e.g. the Viking Greenland settlements [38]) indicate that, until just a few hundred years ago, humans would have lived in continuous close proximity to many different species of flies, together with an assortment of lice, fleas and other parasites. Myiasis in humans could be a byproduct of a changed host–parasite interface that enabled predominantly animal-infecting species to become parasites of humans. In socioeconomically disadvantaged regions that have less-developed public health standards, myiasis in humans (mainly children and the elderly) still occurs; as recently as 1993, Zhang and Li [39] reported human hypodermosis rates of up to 7% in some regions of China. Even in wealthy societies with a technologically advanced health care system, elderly and homeless people without a social network could be prone to severe myiasis [40]. It should be mentioned, however, that the capacity of certain blowflies to cause myiasis can also be beneficial to humans; sterile-bred blowfly maggots are now widely used for larval therapy as an efficient alternative to traditional antibiotics to heal complicated wounds and persistent bedsores [41].

Concluding remarks

Evidence relating to the evolution of myiasis and the major groups of fly taxa that exhibit this life-history strategy can be compiled from several sources, including biogeography and clade–taxon complement, molecular clocks and fossils – all of which can be of value in helping to fix divergence events in evolutionary time. Certainly, the overall picture of myiasis evolution relies on information from each of these sources and can be summarized as follows. The Oestridae seem to have appeared 100–90 mybp at most and seem to have evolved and spread across the continents in close association with a rapidly radiating mammal fauna. At least one lineage (*Tracheomyia*) dispersed into Australia via Antarctica 38 mybp at least. The situation for the Calliphoridae and Sarcophagidae families is less straightforward. Stevens [9] interpreted the distribution of blowfly taxa in clades defined by his 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 mybp); subsequent divergence occurred in response to biogeographical vicariance and faunal movements. However, the molecular-clock analysis by Wallman *et al.* [15] indicates that the extant subfamilies Calliphorinae, Chrysomyinae and Luciliinae might have shared a common ancestor as recently as ~25 mybp. Although further work is obviously required to resolve a difference in divergence estimates of 75 million years, this disparity highlights the limitations of such evolutionary inferences when they cannot be based on direct fossil evidence. Indeed, the limitations of mitochondrial-only studies of blowflies are well recognized [42], reiterating the need for multigene, total-evidence-based analyses.

Finally, the analyses carried out so far indicate that Zumpt's hypothesis that the myiasis habit arose by one

of two evolutionary trends, 'the saprophagous and the sanguivorous roots [sic]' [2], is partly supported, although it might be oversimplistic. Certainly, it seems that myiasis has arisen by at least two distinct evolutionary pathways in Calliphoridae: one sanguivorous, as exhibited in several extant taxa such as the Congo floor maggot (*Auchmeromyia luteola*) and various species of bird blowflies (*Protocalliphora* spp.), another saprophagous, as exhibited by a broad range of other myiasis-causing calliphorid flies. However, both forms of myiasis are polyphyletic within this group, each having evolved independently on more than one occasion – the sanguivorous form on at least two occasions [9] and the saprophagous form numerous times, possibly also in other families of higher Diptera [1,9]. Where, if at all, the oestrid flies and flesh flies fit into these evolutionary scenarios remains to be resolved and the range of life histories exhibited by extant taxa in these groups indicates several possible alternative ancestral strategies.

Acknowledgements

J.R.S. thanks the Natural Environment Research Council for providing financial support for this work (grant NER/M/S/2003/00081). We thank Knut Rognes (Stavanger) for valuable comments about an earlier draft of this manuscript, and Mark Sandeman (La Trobe) for helpful discussions about some aspects of this work.

References

- 1 Stevens, J.R. and Wallman, J.F. The evolution of myiasis in humans and other animals in the Old and New Worlds (part I): phylogenetic analyses. *Trends Parasitol.* 22, 129–136
- 2 Zumpt, F. (1965) *Myiasis in Man and Animals in the Old World*, Butterworth
- 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 Kerridge, A. *et al.* (2005) Anti-bacterial properties of larval secretions of the blowfly, *Lucilia sericata*. *Med. Vet. Entomol.* 19, 333–337
- 6 Donovan, S.E. *et al.* (2006) Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures. *Med. Vet. Entomol.* doi:10.1111/j.1365-2915.2006.00600.x (<http://www.blackwell-synergy.com/toc/mve/0/0.jsessionid=aXZ21wNp4H2a7Z34oL>)
- 7 Chabaudie, N. and Boulard, C. (1992) Effect of hypodermin A, an enzyme secreted by *Hypoderma lineatum* (Insect Oestridae) on the bovine immune system. *Vet. Immunol. Immunopathol.* 31, 167–177
- 8 Moiré, N. (1998) Hypodermin A and inhibition of lymphocyte proliferation. *Parasitol. Today* 14, 455–457
- 9 Stevens, J.R. (2003) The evolution of myiasis in blowflies (Calliphoridae). *Int. J. Parasitol.* 33, 1105–1113
- 10 Hall, M. and Wall, R. (1995) Myiasis of humans and domestic animals. *Adv. Parasitol.* 35, 257–334
- 11 French, N.P. *et al.* (1992) Prevalence, regional distribution and control of blowfly strike in England and Wales. *Vet. Rec.* 131, 337–342
- 12 Stevens, J. and Wall, R. (1997) The evolution of ectoparasitism in the genus *Lucilia* (Diptera: Calliphoridae). *Int. J. Parasitol.* 27, 51–59
- 13 Aubertin, D. (1933) Revision of the genus *Lucilia* R.-D. (Diptera, Calliphoridae). *Linnaean Soc. J. Zool.* 38, 389–463
- 14 Rognes, K. (1997) The Calliphoridae (blowflies) (Diptera: Oestroidea) are not a monophyletic group. *Cladistics* 13, 27–66
- 15 Wallman, J.F. *et al.* (2005) Molecular systematics of Australian carrion-breeding blowflies (Diptera: Calliphoridae) based on mitochondrial DNA. *Invert. Syst.* 19, 1–15
- 16 Papavero, N. (1977) *The World Oestridae (Diptera), Mammals and Continental Drift*. W. Junk
- 17 Pape, T. (2001) Phylogeny of Oestridae (Insecta: Diptera). *Syst. Entomol.* 26, 133–171

- 18 Stevens, J. and Wall, R. (1996) Classification of the genus *Lucilia* (Diptera: Calliphoridae): a preliminary parsimony analysis. *J. Nat. Hist.* 30, 1087–1094
- 19 Espmark, Y. (1968) Observations of defence reactions to osterid flies by semi-domestic forest reindeer (*Rangifer tarandi* L.) in Swedish Lapland. *Zool. Beitr.* 14, 155–167
- 20 Pape, T. (2006) Phylogeny and evolution of the bot flies. In *The Oestrid Flies: Biology, Host-Parasite Relationships, Impact and Management* (Colwell, D.D. et al., eds), CABI
- 21 Ronquist, F. (1996) DIVA version 1.1. Computer program and manual, Uppsala University (ftp.uu.se or ft.syst-bot.uu.se)
- 22 Smith, A.G. et al. (1994) *Atlas of Mesozoic and Cenozoic Coastlines*, Cambridge University Press
- 23 Michelsen, V. (2000) Oldest authentic record of a fossil calypterate fly (Diptera): a species of Anthomyiidae from early Coenozoic Baltic amber. *Studia Dipterol.* 7, 11–18
- 24 Amorim, D.S. and Silva, V.C. (2002) How far advanced was Diptera evolution in the Pangaea? *Ann. Soc. Entomol. Fr.* 38, 177–200
- 25 McAlpine, J.F. (1970) First record of calypterate flies in the Mesozoic Era (Diptera: Calliphoridae). *Can. Entomol.* 102, 342–346
- 26 Zherikhin, V.V. (2002) Ecological history of the terrestrial insects. In *History of Insects* (Rasnitsyn, A.P. and Quicke, D.L.J., eds), pp. 331–388, Kluwer
- 27 Weigelt, J. (1935) Was bezwecken die Hallenser Universitäts-Grabungen in der Braunkohle des Gieseltales? *Nat. Volk* 5, 347–356
- 28 Gautier, A. and Schumann, H. (1973) Puparia of the subantarctic or black blowfly *Protophormia terraenovae* (Robineau-Desvoidy, 1830) in a skull of a Late Eemian (?) bison at Zemst, Brabant (Belgium). *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 14, 119–125
- 29 Handlirsch, A. (1921) Palaeontologie. In *Handbuch der Entomologie* (Pt 3) (Schroeder, C.W.M., ed.), Jena
- 30 Evenhuis, N.L. (1994) *Catalogue of the Fossil Flies of the World (Insects: Diptera)*, Backhuys Publishers
- 31 Townsend, C.H.T. (1921) Some new muscoid genera ancient and recent. *Insector Inscitiae Menstruus* 9, 132–134
- 32 Wood, D.M. (1987) Oestridae. In *Manual of Nearctic Diptera* (Vol. 2) (McAlpine, J.F., ed.), pp. 1147–1158, Research Branch, Agriculture Canada
- 33 Raven, P.H. and Axelrod, D.I. (1972) Plate tectonics and Australasian paleobiogeography. *Science* 176, 1379–1386
- 34 Parrish, J.T. (1993) The palaeogeography of the opening South Atlantic. In *The Africa-South America Connection. Oxford Monographs on Biogeography* (George, W. and Lavocat, R., eds), pp. 8–27, Clarendon Press
- 35 Stevens, J. and Rambaut, A. (2001) Evolutionary rate differences in trypanosomes. *Infect. Genet. Evol.* 1, 143–150
- 36 Rambaut, A. and Bromham, L. (1998) Estimating divergence dates from molecular sequences. *Mol. Biol. Evol.* 15, 442–448
- 37 Gaunt, M.W. and Miles, M.A. (2002) An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. *Mol. Biol. Evol.* 19, 748–761
- 38 Buckland, P.C. et al. (1996) Bioarchaeological and climatological evidence for the fate of Norse farmers in medieval Greenland. *Antiquity* 70, 88–96
- 39 Zhang, X.D. and Li, C. (1993) *Zoonoses* Vol. 3, 338–339
- 40 Greenberg, B. (1984) Two cases of human myiasis caused by *Phaenicia sericata* (Diptera: Calliphoridae) in Chicago area hospitals. *J. Med. Entomol.* 21, 615
- 41 Sherman, R.A. et al. (2000) Medicinal maggots: an ancient remedy for some contemporary afflictions. *Annu. Rev. Entomol.* 45, 55–81
- 42 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
- 43 Brower, A.V.Z. (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl. Acad. Sci. U. S. A.* 91, 6491–6495
- 44 Stevens, J.R. and Otranto, D. (2003) Patterns of evolution in myiasis-causing calliphoridae and oestridae, and the evolution of parasitism. In *Proceedings of EU-COST Action 833: mange and myiasis of livestock, Bari, 19–22 September 2002*, pp. 199–203, European Commission
- 45 Otranto, D. and Stevens, J.R. (2002) Molecular approaches to the study of myiasis-causing larvae. *Intl J. Parasitol.* 32, 1345–1360

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