

EVOLUTION OF INSECT PARASITISM IN RHABDITID AND DIPLOGASTRID NEMATODES

Walter Sudhaus

*Institut für Biologie/Zoologie, AG Evolutionsbiologie,
Freie Universität, D-14195 Berlin, Germany*

Abstract — Insect-parasitic nematodes, which obtain nutrients from a living host, arose independently in more than 20 lineages and in different geological periods since Silurian times. Except for Mermithidae, all these are Secernentea, which in various lineages exhibit different degrees between the first steps towards parasitism and obligate parasitism. Only in some tylenchids the transition passed through an ectoparasitic phase. From knowledge about the life cycles of parasitic and closely related free-living species of “Rhabditidae” and Diplogastridae associated with insects, hypotheses are formulated about how obligate parasites could have evolved. Prerequisites include the evolution of a dauer juvenile (J3) in the ancestral lineage of Secernentea and special adaptations as inhabitants of patchily distributed and rapidly changing saprobious habitats on land formed by decaying material, including phoretic behavior on insects. The preadaptation concept, i.e., that several problems for life as a parasitic nematode are “solved” by adaptations to a saprobiontic life in ephemeral habitats, requires further research in more detail. Several steps are included in this preadaptation hypothesis, including the transformation of a dauer stage, the association with an insect, the acquisition of special behavioral traits and recognition mechanisms of the J3, the ability to invade animal tissue, the reaction to triggers from organisms and habitats, etc. In particular, endo-phoretic behavior (transportation inside the carrier) was one of the key innovations facilitating the transition to different types of associations like entoecy, necromeny, entomopathogeny (shown to be a special case of parasitoidy), and larval parasitism. The different associations are classified, and the gliding transitions between, e.g., necromeny and entomopathogeny, facultative and obligate parasitism, or uncontrollable relationships called “facultative parasitoidy” are highlighted. Multiple pathways occurred from free-living bacterial feeding nematodes to insect-parasitic ones. Nevertheless, the infective stage always emerged from the third-stage juvenile, constrained by the existence of a dauerlarva which changed its behavior. The physiological and reproductive preadaptations and required adaptations for the transitions between different types of intimate associations with insects are worked out. A diagram depicts the reconstructed evolutionary steps towards different modes of parasitic

life of nematodes in insects: larval parasitism, adult parasitism, and a monoxenous or a heteroxenous parasitic life cycle. Three possibilities for the evolution of a heteroxenous life cycle of vertebrate-parasitizing nematodes that utilizes insects as paratenic or intermediate host are discussed, thus placing the J3 in the food chain of the definitive host, are discussed.

Key words: Dauerlarva, endo-phoresy, entoecy, entomopathogeny, ephemeral habitat, necromeny, parasitism, parasitoidy, phoresy, preadaptation

INTRODUCTION

The Secernentea is a monophyletic group within the nematodes, characterized by several radiations in terrestrial habitats which gave rise to the great diversity of taxa we have today. Many of these lineages became obligate parasites in various animal groups independently and at different times during evolution of the biosphere. The idea of independent (polyphyletic) origin of parasitism had already been predicted by Cobb (1898: 454) before it was substantiated by systematic work of nematologists (Baylis and Daubney, 1926; Chitwood, 1950; Dougherty, 1951; Osche, 1962, 1963, 1966; Maggenti, 1981; Malakhov, 1994). Blaxer et al. (1998) showed that at least three parasitic lineages evolved within the Secernentea and – in accordance with Malakhov (1994) – that the first offshoot in the secernentean tree (their clade III) changed to a parasitic mode of life, radiating to Ascaridida, Oxyurida, Rhigonematida and Spirurida. To judge from the phylogenetic relationships between these taxa and the lack of any free-living species within clade III, the above monophyla all have endoparasitic sister taxa, and – as Oxyurida and Rhigonematida apparently are not sister taxa – the stem species of clade III lived like Thelastomatidae (Oxyurida) and Rhigonematida in the hindgut of terrestrial arthropods. Therefore the vertebrate parasitic Spirurida and Subuluroidea, primarily utilizing insects as intermediate hosts, must have derived from ancestors exhibiting such a mode of life. In view to the multiple shifts to a parasitic mode of life in nematodes, it is plausible that the life cycles of parasitic taxa like Allantonematidae, Ascaridida, Dioctophymatoidea, Muspiceoidea, Rhabdiasidae, Strongyloidea, Strongylida, Trichinelloidea, etc. are just as diverse and cannot be derived from each other, although by far the majority of animal parasitic nematode taxa belong to the Secernentea.

The aim of this paper is to generate hypotheses on how insect parasitic nematodes could have evolved by using “models” within the paraphyletic “Rhabditidae” and the monophyletic Diplogastridae. (The many entomoparasitic taxa of Tylenchoidea and Mermithida are excluded.) Contrary to a common misconception, fossils are not a necessary prerequisite to deal with such an issue, there being enough information in extant nematodes to reconstruct their phylogeny and establish scenarios for the evolution of special life cycles. From knowledge of the life cycles in parasitic nematodes and their closely related free-living relatives that are associated with insects, hypotheses can be formulated on how obligate endoparasites in insects have evolved. This paper is based on a talk delivered as part of a symposium entitled

“Evolution of Parasitism in Nematoda” at the 40th Annual Meeting of the Society of Nematologists, 24–29 August 2001, Salt Lake City, UT, USA (Sudhaus, 2001).

1) Phylogeny of insect parasitic nematode taxa

We may assume that, since Silurian times (ca. 430 m.y.B.P.), nematode lineages occupied terrestrial habitats. The most important of these lineages became the Secernentea. In this period terrestrial arthropods were present. Scorpions (†*Palaeophonus*) are documented in the fossil record, and because scorpions live off other terrestrial arthropods, mainly insects, these should have existed at the same time. Insects of the crown group are known from the Devonian period onward. We can suppose that at the end of Devonian (ca. 350 m.y.B.P.) at the latest, the first insect-parasitic nematode lineages had evolved, though this cannot be substantiated by fossil documents. Since that time insect-parasitic nematodes originated independently in about 20 or more lineages. Inasmuch as the evolution of a parasitic mode of life in insects occurred all the time, we should still find cases in which nematodes are taking the first steps towards parasitism, namely nematodes living on the borderline between being saprobiontic and parasitic. Such species provide the models we can use to elucidate events in the past. These models serve to develop scenarios for how a change from a saprobiontic way of life as bacterial feeders or fungal feeders towards parasitism was possible in the 20 independent lineages to extant insect-parasitic nematode taxa.

If in a monophyletic group of nematodes all species are insect parasites, we may conclude with much certainty that the stem species of this monophylum already was parasitic in insects and that the radiation took place in this ecozone (e.g., the Diplogastridae *Cephalobium*, *Mehdinema*, and *Parasitodiplogaster*; the rhabditid *Oryctonema*). In some cases, we can also conclude for good reasons that the stem species of a monophylum used insects as hosts, even if only some species are insect-parasitic and others are parasitic in different taxa (e.g., the Oxyurida; Anderson, 2000). If the sister taxon of such a primarily insect-parasitic monophylum is free-living, we can be certain that the parasitic mode of life was established in the ancestral lineage of this monophylum because it is highly unlikely that obligate parasites could evolve into free-living species. This ancestral lineage could be a single species, but it is reasonable to assume that it is composed of a pair of species whose sister lineages (species or monophyla, side branches of the ancestral lineage) became extinct, if many apomorphic changes distinguish a given monophylum.

2) Problems in the switch to endoparasitism by free-living nematodes, and the preadaptation concept

We can claim that the terrestrial progenitors of insects and most likely also the stem species of the insect crown group were free of parasitic nematodes. As was first realized by Günther Osche, nearly no parasitic nematodes originated in the marine environment, and the thousands of gastropods, polychaete worms, crusta-

ceans, etc. are nearly free of parasitic adenophorean nematodes. There is a "paucity of parasitic marine Adenophorea" (Nicholas, 1984: 6), a "void in the sea" as Osche (1966: 19) called it. The explanation of this deficiency is part of Osche's (1956, 1962) preadaptation concept of parasitism in nematodes. Unpredictable ephemeral habitats, where nematodes can evolve preadaptations for parasitism, and the crucial capability to be transported with mobile inhabitants of such patchily distributed habitats, are nearly nonexistent in the marine environment. Therefore, the preadaptive plateau for parasitism is absent for marine "Adenophorea" (quotation marks are used here to distinguish the group as paraphyletic). "All nematode parasitism had its developmental origin terrestrially" (Maggenti, 1981: 249). Parasitic nematodes in marine animals are "essentially an extension of terrestrial parasitism into the ocean" (Christie, 1960: 10). Remarkable exceptions are Marimermithida in echinoderms, priapulids, and other marine invertebrates (Rubtzov and Platanova, 1974) and a few specialists like the monhysterid *Theristus polychaetophilus* (Hopper, 1966). Other adenophorean parasites "appear to be most closely related to the soil inhabiting dorylaims" (Maggenti, 1981: 249); in that paraphylum, parasitism has also evolved in terrestrial nematodes, as in Secernentea.

Because the terrestrial arthropods in the ancestral group (stem lineage) of insects had no parasitic nematodes, they would have offered an "ecological license" (as we call this in evolutionary ecology) for worms to adopt an endoparasitic mode of life. At this very first stage, there was no competition that would render these first steps of parasitism more difficult. The first invaders of secernentean nematodes had to cope only with all the problems of a parasitic life cycle and with all the defending mechanisms of the host. Nematodes or their eggs that accidentally were ingested by an insect with its food were digested or at most lived for a while without any ability to propagate. The same can be observed today with all the nematodes living in soil and on plants. The possibility to start a parasitic life was clearly improved by a set of preadaptations or preconditions that evolved as a complex of adaptations to a saprobiontic life in short-lived substrates formed by decaying material (Osche, 1956; 1962; Maggenti, 1981: 258 f.).

This "preadaptation concept" can be best explained by discussing some of the problems of becoming an endoparasite. A parasite egg in the intestine must tolerate or overcome (1) little partial pressure of oxygen, (2) strong osmotic fluctuations, (3) the activities of digesting enzymes, (4) the difficulties to find and infect a new host, and (5) the requirement of an infective stage.

The first three problems are evolutionarily solved by special adaptations to a life in habitats formed by decaying organic matter that are short-lived and patchily distributed, as we find in saprobiontic nematodes.

(1) In decomposing situations there is often a shortage of oxygen. Nematodes adapted to these habitats can also survive anaerobic conditions.

(2) Saprobiontic nematodes possess well-developed regulatory mechanisms to accommodate changing osmotic pressure in their environment.

(3) A rich bacterial flora exists on decaying organic substances. The bacteria

release their enzymes into the substratum. The nematode cuticle has evolved resistance against these exo-enzymes.

All these adaptations of saprobiontic nematodes at the same time are preadaptations with regard to a parasitic life, particularly in the intestine of insects. But what about the two remaining problems?

(4 and 5) For small organisms, life in patchily distributed ephemeral substrates requires a strategy for colonizing new habitats. For nematodes the strategy is to get in contact with emerging insects of the same habitat that overcome the distance between such "islands" by flight and have sense organs to find a new (fresh) habitat. This association for transport is called phoresy. The characterization of phoresy is that an individual of a different animal species serves only as a means of transport. The phoretic stage in Secernetea is the dauerlarva.

The dauerlarva is the non-feeding, resistant third stage juvenile, which exhibits a closed stoma, has stored fat deposits, and usually is enclosed (ensheathed) in the cuticle of the preceding J2-stage. This dauer juvenile is an evolutionary novelty of the Secernetea, an adaptation to life in a terrestrial environment that sometimes requires the animal to outlive temporary adverse conditions (Sudhaus, 1987: 149). In all known cases of phoresy in "Rhabditidae" and Diplogastridae, the dauerlarva became the phoretic stage. In most parasitic taxa of Secernetea, it was transformed into the infective stage.

Establishing a parasitic life cycle in particular requires mechanisms to find and select a new host as well as a high rate of reproduction with thousands or millions of eggs to compensate for the great loss of progeny that fail to infect a new host. Host (carrier) selection is also required in phoresy. Thus, mechanisms to detect and use an insect for transport were established. All the adaptations of the dauerlarva in a phoretic association with an insect were preadaptations to the evolution of an infective stage when becoming parasitic. An increased reproductive capacity with hundreds of offspring is also an adaptation of saprobiontic nematodes of ephemeral habitats, as can be shown in comparison with soil-inhabiting nematodes, which exhibit more the K- than the r-strategy. Under optimal conditions in culture, saprobiontic nematodes can even produce many more than ~200 eggs or juveniles (Sudhaus, 1976; Nicholas, 1984). This could indicate that the genetic prerequisite already exists in such species for an increase in reproduction under ideal nourishment conditions, as in a host. In such a scenario, the increase to nearly one thousand offspring does not need an extra evolutionary step for a parasitic nematode in *statu nascendi*, but is merely an automatic "compensation reaction" of an organism that colonizes a favorable environment.

If all the progeny would complete their life cycles and reproduce within the host, the parasites would overwhelm the host and cause its death very soon. So the parasite would destroy its environment, leading to demise of the parasite itself. There are so-called "entomopathogenic" nematodes like *Steinernema* and *Heterorhabditis* that have such a strategy to develop parasitically in the insect host and then, over several generations, kill it. They are therefore welcomed as biological control agents

against insects. However, this strategy is not an advantageous step towards becoming a parasite. Parasites do indeed develop at the expense of the host, but they do not kill it. Thus, a restriction for a parasite would be that it should pass only one generation within the host, and development should cease after reaching the infective stage. In this regard, it is remarkable that there exist certain saprobiontic nematodes [on carrion [(*Choriorhabditis maxima*) and on dung (*Fictor levidentus*, *Pelodera coarctata*, *P. tretzeli*, *Choriorhabditis dubia*, and others), called "fugitive species"] that pass only one generation in the substratum, even though the resource is by no means exhausted (Sachs, 1950; Sudhaus, 1981; Sudhaus and Kühne, 1990; and unpublished observations). They have evolved mechanisms to stop progression to the next generation and arrest at the dauerlarva stage.

The main point of this chapter is that several problems for life as a parasitic nematode are "solved" by adaptations to a saprobiontic life in ephemeral habitats. The preadaptation concept implies that the opening of a new ecozone which presents many simultaneous problems can be managed when some of the problems are already solved by adaptations to a special mode of life in a transitional zone (Sudhaus and Rehfeld, 1992; Sudhaus, 2002). A set of preadaptations has to be linked by apomorphic key characters (Sudhaus, 2004). Life forms of the transitional zone provided a "preadaptive plateau" for the evolution of different lineages of parasitic Secernentea again and again. This means that the special transitional zone must be elucidated from different sources of data in order to reconstruct the switch to parasitism for a certain lineage of nematodes.

3) Clarifying some terms: different types of associations between nematodes and insects

When we try to reconstruct a scenario for the evolution of insect parasitic nematodes, it is necessary to consider the different types of associations between insects and non-parasitic nematodes. When classifying these associations, we must be aware that the terms cannot be precisely defined, but can only be characterized injunctively by presenting typical examples and pointing to the fuzzy boundaries (for an explanation of the term "injunction", see Sudhaus and Rehfeld, 1992: 9). The categories "are arbitrary and without exact boundaries" because there "is a continuous spectrum of relationships linked by intermediates" (Maggenti, 1981: 246). It is for this reason that a special type of association can easily turn into another one.

▷ As we saw above, phoresy is a prerequisite for nematodes to colonize patchily distributed ephemeral substrates. **Phoresy** means the use of an insect (as a carrier) by a special stage in the life cycle of a nematode for transportation only in order to reach a new habitat. Examples are provided by many rhabditids and diplogastrids living in droppings of big mammals, on carcasses, in frass of wood-inhabiting insects, in sap exudate of trees, in rotting cactus, and in other disjunct "biochores" (Osche, 1963). Their dauerlarvae exhibit the special behavior of being carried by beetles and some diptera, which involves the ability to respond to dif-

ferent stimuli of the environment and use them as behaviour-regulating signals prompting the dauerlarvae to embark upon an insect, search for a good place for transport, and leave it in a fresh substratum to continue their development (Sachs, 1950; Völk, 1950; Körner, 1954; Rühm, 1956; Sudhaus, 1976; Kiontke, 1999; etc.). A well-known example is *Pelodera coarctata*, whose cyst-like dauerlarvae attach their anterior ends by a certain secretion to the legs of *Aphodius* dung beetles in order to leave the dropping and reach a new one to complete their life cycle (Sudhaus, 1976). For the same purpose, dauerlarvae of *Choriorhabditis dubia* coil around the abdomen in the intersegmental furrows of moth-flies (*Psychoda* sp.) (Sudhaus and Kühne, 1990).

▷ If the phoretic nematodes are inside the insect and thus protected we may call this “internal phoresy” or **endo-phoresy**. Dauerlarvae of rhabditids and diplogastrids often enter the genital chamber, the rectum, pharyngeal glands, or other protected cavities of the insects. The dauerlarvae of *Rhabditoides stammeri* enter the hindgut of carrion beetle larvae (*Nicrophorus* spp.) via the anus, before the beetles leave the carrion (Richter, 1993). Dauerlarvae of *Caenorhabditis drosophilae* are carried inside a pouch on the head of *Drosophila nigrospiracula* which is formed by the retracted ptilinum (Kiontke, 1997; Kiontke and Sudhaus, 2006). Dauerlarvae of *Koerneria hirschmannae* and related species are located in the genital chamber of *Geotrupes* beetles (Kühne, 1994) and those of *Paroigolaimella coprophila* in pouches of the reproductive system of *Sepsis* flies (Kiontke, 1996). Phoretic dauerlarvae of *Rhapanus ossiculum* and *Oigolaimella attenuata* invade the preoral cavities of termites (Massey, 1971; Fürst von Lieven and Sudhaus, 2008); those of *Diploscapter lycostoma*, *Oscheius dolichurus*, and *Koerneria histophora* the pharyngeal glands of ants (Wahab, 1962); and those of *Neodiplogaster wacheki* and *N. acaloleptae* the tracheal system of cerambycid beetles (Körner, 1954; Kanzaki et al., 2008).

▷ It is only a small step from internal transport to a different mode of life, starting with a change in behavior of the dauerlarva. Thus, endo-phoresy is a preadaptation for other associations like entoecy, necromeny, entomopathogeny, and larval parasitism. We call an association **entoecy** if commensal nematodes complete their life cycles in hollow spaces of the host without (as far as we know) obtaining nutrients from the host. Mostly they feed on bacteria or fungi. Examples include *Rhabditis adenobia*, which lives and reproduces in accessory glands of the female genital apparatus of dynastid beetles (*Oryctes* spp.) (Poinar, 1971) and some species of *Crustorhabditis* living in the gill chambers of ghost crabs (*Ocypode* spp.) (Sudhaus, 1976).

▷ **Necromeny** means “waiting inside the body for the cadaver to decompose”. For example, this special association has been long known to occur between nematodes and earthworms. The dauerlarvae enter the host or are ingested by it, but never leave it (as in endo-phoresy) or reach maturity and complete their life cycles while the host is alive. Instead, they wait until the host dies to continue development, mature, and reproduce, feeding on bacteria of the decaying carcass. It is only a small evolutionary step from endo-phoresy to necromeny. Dauerlarvae achieve

this step by extending their time on the carrier and never leaving it until the host is dead and itself produces the habitat for the nematode to complete its life cycle. For instance, the dauerlarvae of *Oscheius necromenus* in Australia are ingested by native millipedes with leaf litter as their food, penetrate through the gut wall, and get encapsulated in the body cavity (Schulte, 1990). The dauer stage recovers after the death of the millipede, and a new population develops on the carcass. Dauerlarvae are generated which disperse in the surrounding soil and leaf litter. Similar, but rare, examples are found in insects. Necromenic behavior was described for *Diplogasteroides nasuensis* associated with cockchafers (*Melolontha hippocastani*) (Manegold and Kiontke, 2001). Some other diplogastrids (Poinar, 1972; Herrmann et al., 2006) and *Oscheius insectivorus* are what Kiontke and Sudhaus (2006) called “facultatively necromenic”: usually they are phoretic; however, they are able to develop on the cadaver of the carrier.

▷ The term **entomopathogeny** is used for that kind of association where the dauerlarvae introduce insect-pathogenic bacteria into the body cavity of an insect, and after living as a parasite for a while, both vector and bacteria kill the host in the end. It appears to be a small step from necromeny to entomopathogeny, as demonstrated by *Oscheius necromenus* in the neobiotic millipede *Ommatoiulus moreletii*, which is not coadapted with this nematode (Schulte, 1990). When *O. necromenus* dauerlarvae penetrate the wall of the gut, bacteria from the intestine (which are presumably attached to the surface of the nematodes) reach the millipede's body cavity. These bacteria (Enterobacteriaceae) are lethal for this millipede. As a result, the mentioned nematode was considered a potential biological control agent for this introduced Portuguese millipede, which was a nuisance in gardens and bungalows of the Adelaide region. If such a nematode were to gain a more specific bacterium, the next evolutionary stage would be reached, i.e., that of a mutualistic association and an entomopathogeny. Up to this point, examples include only *Steinernema* and *Heterorhabditis*, which live mutualistically with different bacteria (*Xenorhabdus* and *Photorhabdus*, respectively). Their dauerlarvae carry these specific bacteria in their intestine and release them in the body cavity of the host. The developing nematodes later feed on the proliferating bacteria when the host is still alive and also when it is already dead (summarized by Griffin et al., 2005).

As these nematodes ultimately kill their host after a parasitic phase, they are comparable with **parasitoids**, which are well-known in different groups of insects (Hymenoptera, Diptera, and Coleoptera). Death of the host is an inevitable consequence of a parasitoid life-style. Another example among nematodes is observed in the case the Mermithidae, which are larval parasites mostly in insects and during that phase accumulate all the substances they need to complete their life cycle. But when they leave the host, they kill it. There is no reason not to classify this type of nematode as parasitoid, as was already done by Kaiser (1986). The question as to whether parasitoidy could possibly have also derived from larval parasitism cannot be answered at the moment.

▷ The last type of relationship that can be derived from endo-phoresy of a

dauerlarva is **larval parasitism**, where only the juvenile stage obtains nutrients or energy at the expense of the living host, irrespective of how severe the effect is on the host. The parasite reduces the fitness of the host, but only accidentally kills it. Larval parasitic nematodes are known in *Parasitorhabditis*. Most of the species are associated with bark beetle (Scolytidae) larvae and live and propagate in the frass of their tunnels. Dauerlarvae enter the anus and the rectum of a beetle to be transported. That this endo-phoresy was an intermediate step to larval parasitism is obvious, as some species (like *P. piniperdae* and *P. proximi*) take nutrients from the host and thus became larval-parasitic in the gut, the malpighian tubules, or even the body cavity (Rühm, 1956; Lazarevskaya in Poinar, 1972). The same was claimed for the rhabditids *Diploscapter lycostoma* and *Oscheius dolichurus* in pharyngeal glands in the heads of ants (Wahab, 1962) and similarly for *Rhabpanus ossiculum* from termites (Massey, 1971). It is not known in any of these species if the incorporation of substances from the host is essential for completion of the life cycle. The diplogastrids *Diplogastrellus secundus* and *Mononchoides aphodii* are larval-parasitic in the body cavity of *Aphodius* dung beetles (Bovien, 1937; Poinar et al., 1976).

4) Classification of entomoparasitic nematodes

Only a short overview is here presented on the different modes of insect-parasitic life-styles in Rhabditida and how they could have evolved (Fig. 1). For a hypothesis on the evolution of obligate parasites, we have to compare the parasitic taxa with closely related taxa comprising free-living or sometimes facultatively parasitic species.

(a) Intermediate life-style between entoecy and parasitism

Oxyurida (like Thelastomatidae in insects) live in the rectum of animals, where the feces are produced and a rich flora of bacteria and fungi grows. These nematodes "are saprophagous rather than parasitic" (Chabaud, 1971: 219). They retained the rhabditoid pharynx with a valvular apparatus or grinder, which in rhabditid nematodes is used to grind bacteria. So we can conclude that Thelastomatidae feed on the intestinal microflora, whereas their nourishment might consist additionally of partially digested food of the host. The conditions inside a host's hindgut are not very different from a saprobious substratum like feces outside. It is therefore possible to maintain thelastomatids and rhigonematids alive on diplopods' hindgut contents in Petri dishes for days or weeks (Osche, 1963; personal observation). The colonization of the hindgut in millipedes and insects that live as scavengers (like Blattidae) or have saprophagous larvae (like Scarabaeidae, Lucanidae, and Cerambycidae) appears to have occurred easily (Leibersperger, 1960). The life cycle is comparatively simple: eggs pass out of the host with the feces and need oxygen to develop. Two moults occur within the egg, which is apomorphic in Oxyurida. Thus, other free-living stages are suppressed. If an infective egg enclosing a third stage juvenile (J3) is ingested with contaminated food by an appropriate insect, the J3 hatches in the posterior part of the gut and starts its specialized entoec or partial parasitic life.

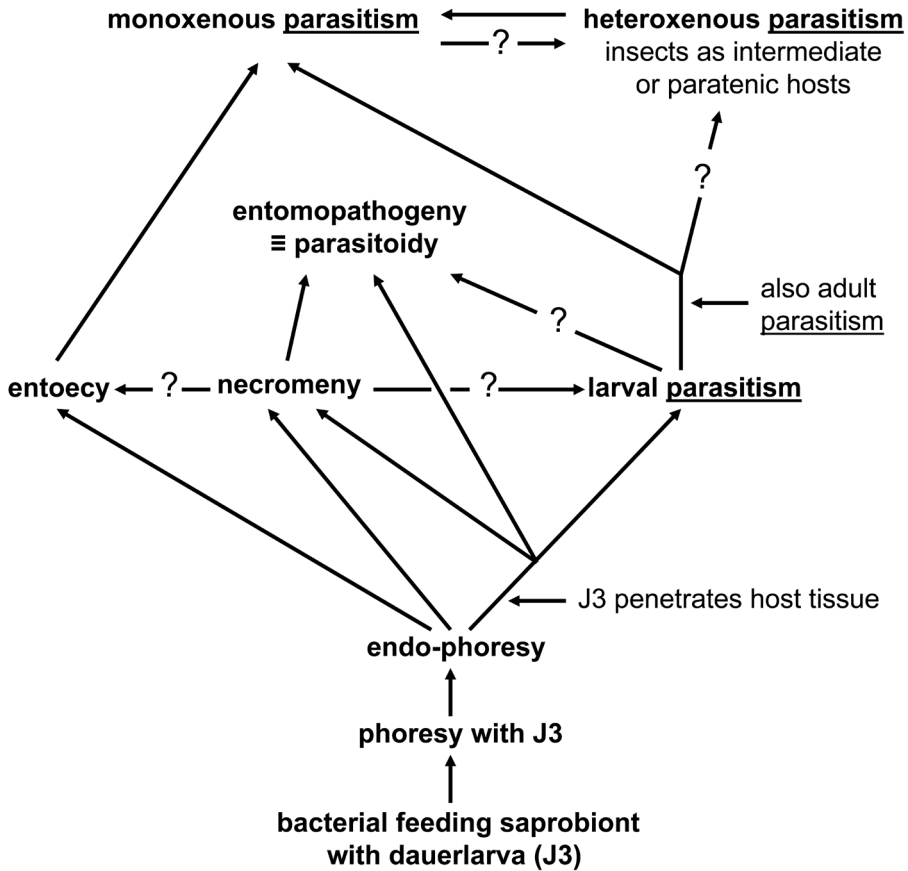


Fig. 1. Origin of insect parasitism in rhabditoid Secernentea.

Another example is provided by *Oryctonema*, which lives and reproduces in the bursa copulatrix of scarabaeid beetles and is transmitted to a new host during mating of the beetles (Poinar, 1983). An evolutionary model forming a bridge between a free-living species and a taxon like *Oryctonema* (obligately associated with beetles) is *Rhabditis adenobia* (discussed above as an example for entoeocy), which is also transmitted by copulation of the “hosts”, but can be easily cultured on agar plates (Poinar, 1971). Such examples demonstrate that there is only a small step from entoeocy in the rectum or a genital pouch to parasitism in the intestine or a genital gland.

(b) Only larval stages (J3) are parasitic

As previously mentioned, it is a small evolutionary step from endo-phoresy (only for the sake of being transported) to an endo-phoretic phase (where the stowaway takes up some nutritive substance from the site chosen for transport in the carrier) and finally to an obligate larval parasitic mode of life. During this transitional phase, the former carrier gradually becomes a true host. As long as dauerlarvae do not feed,

the uptake of substances is only possible through the cuticle and the body surface (parenteral). *Parasitorhabditis* was already mentioned in this context, where endophoretic dauerlarvae of some species are described as partially parasitic even in the body cavity (Rühm, 1956). This marks a new step, where the dauerlarva penetrates the wall of the intestine to get into the body cavity of the host and, after a larval parasitic phase, crawls back into the alimentary tract and to the outside. The life cycle is completed in a saprobious habitat created by the temporary host. Thus, the life cycle alternates between free-living stages and a larval/parasitic stage.

Typical examples of larval parasitism are provided by the diplogastrids *Diplogastrellus secundus* and *Mononchoides aphodii* (Bovien, 1937). Their larval-parasitic mode of life in the body cavity of *Aphodius* dung beetles must have evolved independently, as they are not closely related and the sister species of each of them is completely free-living. In *Mononchoides aphodii*, third stage juveniles (J3's) enter the mouth of a feeding *Aphodius*-larva, bore through the midgut, and enter the body cavity, where they are carried through metamorphosis and become swollen by parenteral uptake. During mating and oviposition of the beetle, the J3's leave their hosts (probably via the alimentary and reproductive tract), after which they develop to adulthood and reproduce in dung, where they pass through several successive generations (Poinar et al., 1976).

(c) *Parasitism in later stages, including the adults*

Though an initial step of larval parasitism of the J3 stage is not necessary to evolve parasitism in the adult stage (as illustrated by different tylenchids, where only the infecting fertilized females and the first two stages of their offspring are parasitic), adult parasitism definitely *can* evolve from larval parasitism by extending the parasitic phase in the same host. This requires that dauerlarvae of a larval parasitic species respond to stimuli within the host to terminate the J3 stage and continue its development and life cycle. After ecdysis, substances are ingested through the stoma. Furthermore, the possibility to reproduce within the host must be established. This implies that several parasitic specimens of a species (and both sexes) develop within the same host individual, without damaging it considerably or even killing it. Attraction of sexual partners within the host and mating appear not to be a problem. Deposited eggs leave the host passively with the feces if the parasites live in the intestine. Reproduction of parasites in other organs must be more advanced, as the transfer of eggs or juveniles into the alimentary tract requires the evolution of a special behavior.

Examples are provided by the diplogastrids *Cephalobium* and *Mehdinema* in the hindgut of crickets, and by the Tetradonematidae and the Thelastomatidae in different insects. In *Cephalobium*, eggs pass out of the host with the feces, and it appears that juveniles must be ingested (Ackert and Wadley, 1921). In *Mehdinema alii*, dauerlarvae are exclusively sexually transmitted (Luong et al., 2000). The two *Parasitorhabditis* species mentioned above as larval parasitic in the body cavity of scolytid beetles molt to the fourth stage before they leave the host via the intestine

to complete development in frass of the host. The same holds for *Parasitaphelenchus* (Rühm, 1956). In the rhabditid *Oscheius insectivorus*, the J3's enter the anus of a beetle larva (*Dorcus* or *Lucanus*, Lucanidae) and invade the body cavity via the intestine. There they grow to the adult stage and reproduce with a regulated low number of offspring in the pupa or young beetle during the insect's metamorphosis. The developing third stage juveniles leave the beetle and accumulate in the intersegmental furrows dorsally underneath the elytra (Körner, 1954). The question as to how they get there is still unanswered, as are the questions as to the occasion (oviposition?) which they leave the host and whether they are able to leave the male beetle at all. Obviously, *O. insectivorus* evolved lower virulence (Körner, 1954), as more virulent genotypes would kill their host, and therefore themselves too. As discussed above for *Rhabditis adenobia*, *O. insectivorus* can be grown easily on artificial media for years.

(d) *Insects as paratenic or intermediate hosts for vertebrate parasites*

A parasite lives at the expense of a host without causing serious damage to it or killing it. In case of a switch of hosts, the definitive host is that which harbors the mature parasite. Further hosts needed for development of the parasite are called intermediate hosts. There might be paratenic, intercalary, or transport hosts within which no development of the parasite takes place and which are not considered to be true intermediate hosts, as they only serve to infect the definitive host.

The question is how such heteroxenous life cycles that utilize insects as intermediate hosts could have evolved. Theoretically there are three possibilities:

- Larval-parasitic nematodes in insects with a free-living adult stage for reproduction might have adopted the parasitic mode of life from the formerly free-living adults in insect-eating vertebrates, but I know of no example of such a scenario.

- “In no case is there reason to believe that intermediate hosts of nematodes are ancestral hosts...” (Chandler et al., 1974: 268).

- Parasites in vertebrates incorporated insects as additional hosts during their evolution. At first, insects could have been used as paratenic hosts to infect the insect-eating definitive host. As definitive hosts are likely to evolve a behavior that reduces the risk of infection, they often avoid feeding among the sites of their droppings that harbor the infective stages of their parasites (e.g., cows avoid feeding near their strongylid-contaminated droppings). A solution for the parasite could be that dung-feeding insects carry parasite eggs away from the feces. Such paratenic hosts can become intermediate hosts if the parasite commences to develop and adopts a parasitic life-style in the insect. We have examples of such a scenario from Spirurida, Subuluridae, and Syngamidae (Anderson, 2000).

Syngamidae are on the way to evolve a heteroxenous life cycle. They are parasites mainly in the respiratory system of birds and mammals. The infection usually occurs through food contaminated with developed eggs. If such embryonated eggs are swallowed by various invertebrates, the juveniles hatch inside, penetrate the gut wall, and become encapsulated in the body cavity. *Syngamus trachea* can still

develop without an intermediate or a paratenic host, but it can also use such bridging hosts. This species is evidently undergoing evolution in its life cycle.

The incorporation of insects as true intermediate hosts that derived from transporting paratenic hosts can also be assumed for Spirurida, which are mainly stomach parasites of birds and mammals. Embryonated eggs escape with the feces and contaminate the food of insects (several beetles, Blattidae, Muscidae, Dermaptera, etc.). The first-stage juveniles hatch in the intestine and penetrate into the body cavity, where they live parasitically and undergo two molts. They then become encapsulated as third-stage juveniles, which is the infective stage for the definitive host. The vertebrate definitive hosts are infected when they feed on insects that are infested with encapsulated J3's. This life cycle is very similar to that of the ascaroids of Subuluridae, which are parasites in the intestines of birds and mammals. Their eggs are deposited with the feces of the vertebrate host and ingested by insects, which are used as intermediate hosts. In the insect intermediate host, the juveniles (J1) perforate the gut wall to reach the body cavity, where they grow to third stage infective juveniles.

5) Historical reconstruction of evolutionary pathways towards insect parasitism

In spite of the great diversity in the life cycles of separate insect-parasitic taxa within Secernentea, with the exception of tylenchids, the infective stage is the third-stage juvenile. Anderson (2000: 5 and 9) speaks of a "rule of the infective third stage". Of all four juvenile stages, the third is best suited for becoming the infective stage in Secernentea because it is the dauerlarva of free-living secernenteans and already exhibits many characters that qualify this stage as the most resistant and long-lived in the secernentean ontogeny (see Chapter 2). The examples used here to outline some crucial steps in the evolution of parasitic nematodes in this group underline the significance of behavioral changes as pacemakers in evolution.

For the following, compare Fig. 1.

(1) Except in tylenchids, which mostly developed from fungus feeders (Maggenti, 1981; Giblin – Davis et al., 2003), parasitic lineages in Secernetea always started from bacterial-feeding species dwelling in short-lived and patchily distributed saprobious biochores.

(2) A precondition for life in such ephemeral habitats was transformation of the dauerlarva into a phoretic stage that becomes associated with an insect and is able to respond to different stimuli, like those provided by the carrier and those provided by the fresh substratum of a new habitat after transportation. Some nematodes (*Choriorhabditis dubia*, *Paroigolaimella coprophila*, *Caenorhabditis drosophilae*) necessarily need unknown substances from the transporting insect as a trigger for exit from the dauer stage in order to continue development on the substratum (Sudhaus and Kühne, 1989; Kiontke, 1996, 1997).

(3) Different physiological and reproductive adaptations to saprobious habitats

served as preadaptations to facilitate different types of intimate associations with insects like entoecy, necromeny, entomopathogeny, and larval parasitism.

(4) A key innovation which facilitated the transition to all these associations and particularly to parasitism was endo-phoresy or invasion of the inside of the carrier for transportation.

(5) There were multiple pathways from endo-phoresy to parasitism in insects. One intermediate way of life appears to be entoecy or invasion of accessible ecto- and entodermal cavities of the insect body, the invader remaining a bacterial feeder, as exemplified by the Thelastomatidae. Entoecy possibly also could be reached by a detour through necromeny, which has not yet been mentioned. Whereas the dauerlarvae of the necromenous *Diplogasteroides nasuensis* are waiting for the death of their carrier (a scarabaeid beetle) within a pouch of the genital area, *Rhabditis adenobia* lives there entoecously. Under certain conditions, the latter species might also develop on the cadaver of the beetle (Poinar, 1971), which could be interpreted as an atavistic behavior. (Entoecy is believed to be derived from necromeny in this example.)

(6) The ability of endo-phoretic dauer juveniles to invade the body cavity of a "host" by penetrating tissue opened up new evolutionary possibilities to adopt (endo-) necromenic, entomopathogenic, or larval-parasitic ways of life.

(7) Most necromenic nematode species wait encapsulated inside the body cavity or special organs of the potential cadaver. A transition from necromeny to larval parasitism appears possible, but is not yet exemplified in any known extant species. The possible step from necromeny to entoecy was outlined before.

(8) Invasion of the body cavity is a precondition for entomopathogeny, where special bacteria from the intestine are released. As long as there is an obligate mutualistic relationship with bacteria, entomopathogeny – as a special type of parasitoidy – appears to be a dead end with respect to the evolution of true parasitism.

(9) Species with dauerlarvae that entered special organs or the body cavity of a "host" are preadapted to become larval-parasitic by ingesting gland secretions or hemolymph. In the next step, the uptake of nutrients from a host can become essential to complete the life cycle. Later stages leave the host, and the adult stage and mating occur outside.

(10) If it would be an advantage to be parasitic also in later stages, such parasitism would evolve very quickly, and monoxenous parasites would result.

(11) I cannot answer the question if a heteroxenous parasitic life cycle with insects as intermediate hosts and vertebrates as definitive hosts evolved from larval parasites in insects. At the moment, no example supports this theoretical possibility. The known heteroxenous life cycles with insects as intermediate hosts evolved as an alteration of a parasitic life in vertebrates. Insects have been embraced when this proved to be a better way to infect the definitive host. Insects first became paratenic hosts to distribute the eggs of parasites from vertebrate feces. Then, by hatching within this host and receiving substances from it, the paratenic host became an

intermediate host.

(12) A monoxenous parasitic life cycle in insects could also be derived secondarily from heteroxenous parasites of vertebrates which abandoned the vertebrate host. Thus, some species of Spirurida and of Subuluridae can complete their development in a variety of insects (members of the Orthoptera, Dermaptera, Coleoptera).

6) Concluding remarks on different pathways towards parasitism in insects that took place during evolutionary periods

Secernentean nematodes, with their various lineages exhibiting different degrees from first steps towards parasitism to true parasitism, provide a rare opportunity to study all different associations that might serve as “models” to illuminate the various transitions to parasitism in the past (Osche, 1966). Most if not all of these lineages originated from inhabitants of patchily distributed saprobious habitats on land that existed continuously since Devonian times or earlier. Saprobiont nematodes evolved a complex of adaptations to live in such habitats, including associations with insects present in them. Phoretic and especially endo-phoretic behavior was one of the key innovations that opened the door for different types of associations with insects. In all cases, the phoretic stage became the infective stage, and with the exception of tylenchids, this stage was the dauerlarva. In retrospect, the different physiological adaptations to saprobious habitats - the formation of a dauer stage, the association with an insect, the ability to invade animal tissue, the reaction to triggers from organisms and habitats, etc. - were preadaptive for transition to a parasitic mode of life. From this “preadaptive plateau”, which was supplemented permanently by the radiations of saprobiontic taxa, facultative and obligate parasitic species evolved in several separate lineages and in different geological periods. The end of a typogenetic phase of a lineage between saprobiontic and parasitic can be precisely named: it is reached when an obligately parasitic species arose which now by speciation became the founder of an exclusively parasitic monophylum.

The beginning of parasitism is not as clear-cut, since there is a continuous transition from a free-living existence via different “preparasitic contacts” (Osche, 1966) to accidental and obligate parasitism. One encounters facultatively parasitic nematode species in insects within “Rhabditidae” and Diplogastridae. Some of them are potentially able to bring about insect mortality by entering the body cavity and developing to adulthood, as reported for various diplogastrids (Poinar, 1972) and *Pristionchus uniformis* (Fedorko and Stanuszek, 1971), which could not be confirmed by Herrmann et al. (2006). Sometimes *Oscheius insectivorus* brings about the death of lucanid beetle larvae (Körner, 1954). This relationship can be called “facultative parasitoidy”. These species might be of practical interest as biological control agents. They are also particularly interesting as evolutionary “experiments” to study early steps to parasitism in nematodes. Their ecological behavior can be variable within the species or it may depend on the host and its defense mechanisms, which can be best shown by necromenic behavior that gets out of control in a wrong host (described above for *Oscheius necromenus*). In the light of such examples, Schulte

(1990) suggested the origin of necromeny by selection of arthropods that were able to control the introduced bacteria and prevent the commencement of development of the third-stage nematode in their body cavity.

However, the stock of preadaptations is only one essential part of an evolutionary event. The other prerequisite is the ecological license provided by potential hosts (in this case different insects) and depends on the existence of pre-parasitic or already parasitic competitors. In general, unutilized ecological licenses or few exploited resources offer opportunities for a transformation of specific ecological niches. Unparasitized hosts could offer an incentive or even exercise an "ecological pull" (Gerd von Wahlert, pers. comm.) for preadapted nematodes. On the other hand, new invaders potentially could impose a selective pressure upon pre-parasitic species to accelerate the process of becoming truly parasitic (Dougherty, 1951). In the end, new invaders occupy the position of the true parasites previously established in the host, which then disappeared as serious competitors. From these considerations, it follows that if we observe today a species that is parasitic only as a juvenile stage, it is worthwhile to analyze different potential reasons that might prevent (!) a fully parasitic life cycle. This calls for a synecological approach to the study of parasitism.

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