

Review article.

Symbiotic Interactions in the Entomopathogenic Nematodes

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Abstract

The entomopathogenic nematodes are a neglected subject in the mainstream symbiosis literature. This article attempts to redress the balance by comparing the association with more well-known symbioses. The transmission strategies and nutritional interactions employed in the association display remarkable similarities to those found in the symbioses between insects and filamentous fungi. Consequently we conclude that the relationship between the entomopathogenic nematodes and their symbiotic bacteria should be considered an ectosymbiosis.

Keywords: Symbiosis, entomopathogenic nematodes, bacteria, specificity, recognition, transmission, nutritional interactions, ectosymbiosis

1. Introduction

Entomopathogenic nematodes and their symbiotic bacteria represent a current focus for research because of their commercial use in insect pest control. However, they do not feature prominently in the mainstream symbiosis literature, largely because the nature of the symbiosis has never been classified

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in the context of other, more well-known, associations. In this paper the symbiosis in *Heterorhabditis* and *Steinernema* nematodes is compared with symbioses which have a similar biology. The discussion is limited to associations in which the host organisms are invertebrates and the symbionts are various microorganisms (bacteria, yeasts or algae). More detailed discussions of symbioses in general may be found in Boucher (1985), Smith and Douglas (1988) and Douglas (1994). The characteristics which are important in the description of a symbiosis are detailed in Starr (1975) and Lewis (1985), and a broader discussion of the ecology of symbiotic microorganisms is given by Douglas (1995).

2. Specificity, Recognition and Transmission of Symbionts

The specificity of a symbiosis refers to the range of taxonomically distinct partners with which an organism can form an association. During the establishment of an association a number of processes may be involved to select or discriminate against various organisms. These processes are collectively termed recognition and may include specific chemical interactions, tolerance or suppression of host defences, and metabolic, morphological or behavioural interactions (Smith, 1981).

Specificity and recognition are closely linked. Recognition mechanisms determine the specificity of an association, and consequently they play an important role in determining how an association is maintained from one generation to the next. However the degree of importance of specificity and recognition per se depends on the source from which symbionts are recruited to the host at each generation. For instance, when symbionts are acquired from the free-living condition the mechanisms of specificity and recognition have to be particularly stringent, especially if there is the possibility of selection between several different partners.

Symbionts that are acquired from the free-living condition

There is some evidence that preferences for particular partners do occur. For example, the acoel turbellarian *Convoluta roscoffensis* forms an association with algal symbionts of the genus *Tetraselmis*. The flatworm is found on the coast of France and the Channel Islands (Holligan and Gooday, 1975). Juvenile animals, which hatch from sexually-produced eggs, are alga-free, and acquire their symbionts after feeding on the appropriate algae. In the laboratory, *C. roscoffensis* can form an association with any one of a variety of algae present in the growing medium, but in the field, the majority of individuals form an

association with just one species called *T. convolutae*, despite the presence of alternative *Tetraselmis* species in the immediate vicinity. Experimental studies have shown that *C. roscoffensis* actually prefers *T. convolutae* to other *Tetraselmis* species (Table 1). Although *C. roscoffensis* can form an association with both *T. convolutae* and *T. marinus*, when the animals are exposed to both species, either sequentially or simultaneously, only *T. convolutae* persists (Provasoli et al., 1968; Douglas, 1983 and 1994). By specificity and recognition mechanisms which are not fully understood, *T. marinus* is discriminated against and lost when both algae are present in the tissues of *C. roscoffensis*.

Table 1. Choice of algal symbionts (*Tetraselmis convolutae* or *T. marinus*) by the flatworm *Convoluta roscoffensis* (redrawn from Douglas, 1994)

Treatment of animals	Algal symbiont in animals at end of experiment
<i>T. convolutae</i> (2 days)	<i>T. convolutae</i>
<i>T. marinus</i> (2 days)	<i>T. marinus</i>
<i>T. convolutae</i> + <i>T. marinus</i> (mixed culture, 2 days)	<i>T. convolutae</i>
<i>T. convolutae</i> (days), then <i>T. marinus</i> (2 days)	<i>T. convolutae</i>
<i>T. marinus</i> (2 days), then <i>T. convolutae</i> (2 days)	<i>T. convolutae</i>

Juvenile animals were individually exposed to either *T. convolutae* or *T. marinus* for two days, or exposed to both algal species simultaneously or sequentially (two days for each species). The animals were washed free of exogenous algal cells and the symbiosis was allowed to develop for a further 30 days.

Vertical transmission

In many other associations, particularly endosymbioses, the mechanisms of specificity and recognition are less stringent since the symbiont population is transmitted from a monoculture within the parent directly to the offspring. This process, termed vertical transmission, is particularly prevalent in asexually reproducing animals but is also widespread in hosts which reproduce sexually. Vertical transmission has been particularly well studied in the symbiosis between insects and intracellular bacteria.

The association between insects and intracellular bacteria is known as the mycetocyte symbiosis, and occurs in a large number of species from across the insect orders (Buchner, 1965; Douglas, 1989). The association is particularly prevalent in the Homoptera, Dictyoptera, Coleoptera and Anoplura, and many

of these species, for example aphids, are of considerable economic importance. The bacteria are housed within specialized insect cells called mycetocytes (sometimes referred to as 'bacteriocytes') and the symbiosis generally has a nutritional basis – the insects all feed on nutritionally poor diets which are supplemented by the metabolic capabilities of their bacterial partner (Douglas, 1989 and 1992).

In all insect groups which have been investigated, the mycetocyte symbionts are transmitted from the maternal parent directly to the offspring by the passage of symbionts from the mycetocytes to the developing eggs in the ovary. After fertilization, the symbionts are retained within the developing embryo and are allocated to the mycetocyte cells as they differentiate. In cockroaches, whole mycetocytes migrate to the ovaries (Sacchi et al., 1985 and 1988) whereas in the human body louse *Pediculus humanus*, bacteria are released from the mycetocyte cells and they individually migrate through the haemolymph to the ovaries (Eberle and McLean, 1982, 1983).

Vertical transmission has interesting consequences for the evolutionary history of symbiotic associations. For instance, the phylogenetic relationships between eleven species of aphid and their endosymbiotic partners have recently been investigated using 16S rDNA sequence analysis (Fig. 1; see also Munson et al., 1991; Moran et al., 1993; Moran and Baumann, 1994). The phylogenetic relationships between the *Buchnera aphidicola* bacterial complex and their aphid hosts are closely matched. This is a direct result of vertical transmission; the association probably evolved in the common ancestor of the superfamily Aphidoidea (to which all the aphid species belong) during the Permian period (Wootton, 1981), and the bacteria have been passed from mother to offspring ever since. As the Aphidoidea diversified, each aphid lineage carried its own complement of bacteria with it, and as a result the symbionts diversified with the aphids.

Symbiont transmission in entomopathogenic nematodes

Symbiont transmission is indirect in the entomopathogenic nematodes since juveniles acquire bacteria from their immediate environment, i.e. the insect cadaver. Given the evidence from *Convolvulae roscoffensis* above, one might infer that specificity and recognition mechanisms would be vital to ensure the transmission of the correct symbiont from one generation to the next. However, the metabolic activities of the symbiont, particularly the production of antibiotic compounds which inhibit the growth of other microorganisms, result in a transmission strategy which is functionally analogous to vertical transmission, and therefore specificity and recognition may not be important.

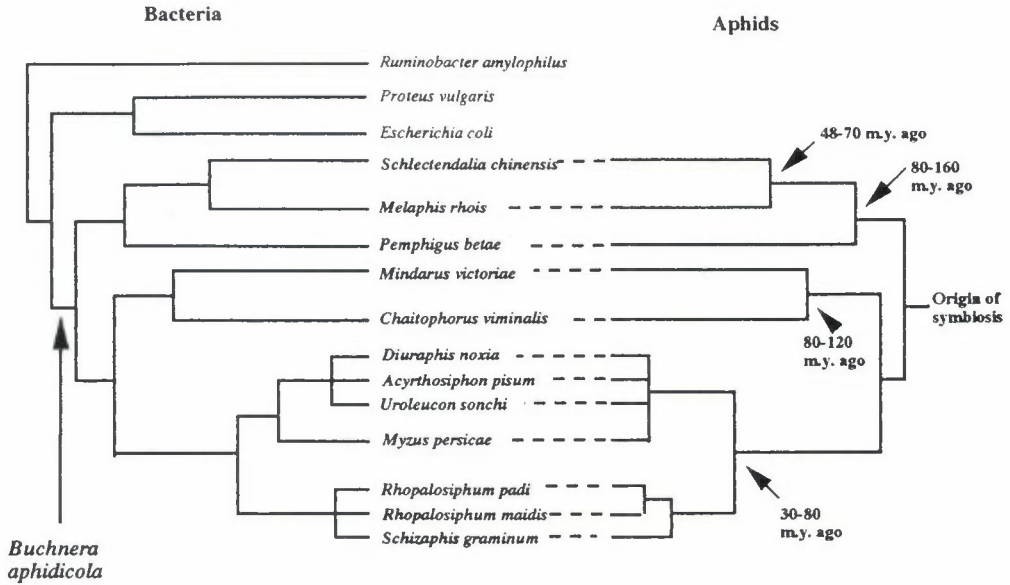


Figure 1. Phylogeny of the bacterial symbionts of aphids (the *Buchnera aphidicola* complex) and their corresponding aphid hosts (redrawn from Moran and Baumann, 1994). Dashed lines indicate associations. Taxa within *Buchnera* are represented by their aphid hosts.

There is some evidence that host-symbiont specificity is relatively low in the Steinernematidae. For example, Ehlers et al. (1990) have shown that diets comprising yeast, peptone and cholesterol sustain growth and reproduction of *Steinernema carpocapse* when supplemented with cells of *Escherichia coli*. Furthermore, Aguillera et al. (1993) have isolated a variety of different bacteria from *S. scapterisci*, including *Ochrobactrum anthropi*, *Paracoccus denitrificans*, and *Xanthomonas maltophili* in addition to members of the genus *Xenorhabdus*. However, specificity appears to be more significant in the Heterorhabditidae. Although various isolates of *Heterorhabditis* spp. have been cultured on diets comprising bacteria derived from other heterorhabditid nematodes, many experimental recombinations are unsuccessful (Han et al., 1991; Gerritsen and Smits, 1993). In some cases even where growth and reproduction does occur, the infective dauer juvenile stage fails to retain the bacteria and the symbiosis is degenerate (Gerritsen and Smits, 1993). Thus the ability to colonise the intestine of the host may be an important determinant of symbiont persistence through successive generations (Akhurst and Boemare, 1990).

3. Nutritional Interactions

Nutritional interactions between entomopathogenic nematodes and their symbiotic bacteria only occur during the colonisation and utilization of an insect host. At this stage the symbionts are external to the body of the nematode, i.e. the association is ectosymbiotic. Nutritional interactions in ectosymbioses are particularly well documented in associations between filamentous fungi and insects (Table 2), which rely on enzymes of fungal origin to digest and prepare the feeding substrate prior to assimilation by the insect (Martin, 1987). Before discussing the striking similarities between these associations and the entomopathogenic nematodes, two examples of ectosymbioses in insects will be considered in detail.

Table 2. Ectosymbioses in insects (see also review by Batra, 1979)

Insect host		Fungal symbiont	Location of symbiosis
Isoptera –	Higher termites Macrotermitinae	<i>Termitomyces</i>	Gardens in nests
Hymenoptera –	Attine ants <i>Acromyrmex</i> , <i>Atta</i>	<i>Attamyces</i>	Gardens in nests
	Siricid wood wasps Siricidae	<i>Amylosterum</i> and others	Galleries in wood
Coleoptera –	Ambrosia beetles Scolytidae and Platypodidae	Various	Galleries in wood

Siricid woodwasps

The wood-feeding larvae of the siricid woodwasps are dependent on their external fungal symbionts for normal growth and reproduction. The larvae acquire essential digestive enzymes (which contribute to lignin, cellulose and hemicellulose digestion) when they ingest their fungal symbiont (Kukor and Martin, 1983), but larvae deprived of their symbionts do not survive (Stilwell, 1966 and 1967). Transmission of the symbionts from adult to offspring is effected in an extraordinary manner. Adult females carry fragments of fungal mycelium, suspended in mucus, within two pear-shaped structures (the intersegmental

pockets) at the base of the egg-laying apparatus (Buchner, 1965). These fragments, or oidia, are implanted in the wood with each egg during oviposition and produce vegetative hyphae which colonise the wood surrounding the oviposition hole (Francke-Grossman, 1939). As the larvae feed, lumps of fungal mycelium accumulate in a pair of deep folds, present only in the female, between the 1st and 2nd abdominal segments. These mycelia remain viable throughout the pupal stage and eventually inoculate the intersegmental pockets of the emerging adult.

Leaf-cutting ants

The well-known fungus gardens of the advanced attine ants, the so-called leaf-cutting ants, are monocultures of an obligate symbiotic basidiomycete, generally described as *Attamyces bromatificus*, which has lost the complex of sporulating sexual and asexual reproductive stages characteristic of free-living basidiomycetes (see review of Cherrett et al., 1989). The ants carry freshly cut young leaf material to the nest which is then extensively prepared prior to inoculation with fungal hyphae. The role of the ants in the degradation of the food substrate is mainly mechanical (see Bass and Cherrett, 1994). The attine workers lick and chew the substrate to remove inhibitory surface waxes and aid hyphal penetration, and apply liquid faeces which contain significant enzyme activity, particularly proteases (Martin and Martin, 1970), α -amylase and chitinase (Martin et al., 1973). These enzymes, which are of fungal origin and are not secreted by the gut (Boyd and Martin, 1975), appear to accelerate the digestion of the fresh substrate by initiating cell wall breakdown. The fungal contribution is biochemical and is largely concerned with the denaturing of plant defensive compounds (Powell, 1984) and the conversion of the low protein substrate into high protein gongylidia which are fed by the workers to the developing larvae.

The ectosymbiotic associations in siricid woodwasps and leaf-cutting ants bear considerable similarities to the association between entomopathogenic nematodes and bacteria (Fig. 2). Each association has two phases; (i) a stable ectosymbiotic phase, and (ii) a mobile phase in which the symbiont is transported by the host.

During the initial stages of infection, the bacteria released by entomopathogenic nematodes contribute significantly to the pathology of the infected insect (Dunphy and Webster, 1988; 1991). Similarly, the fungi carried by the siricid woodwasps (and also by the ambrosia beetles, see Table 2) cause pathology of the host tree and contribute to the eventual breakdown of host defences (Wood, 1972). Subsequently, during the stable phase of the association, the bacteria confer novel metabolic capabilities on the nematode

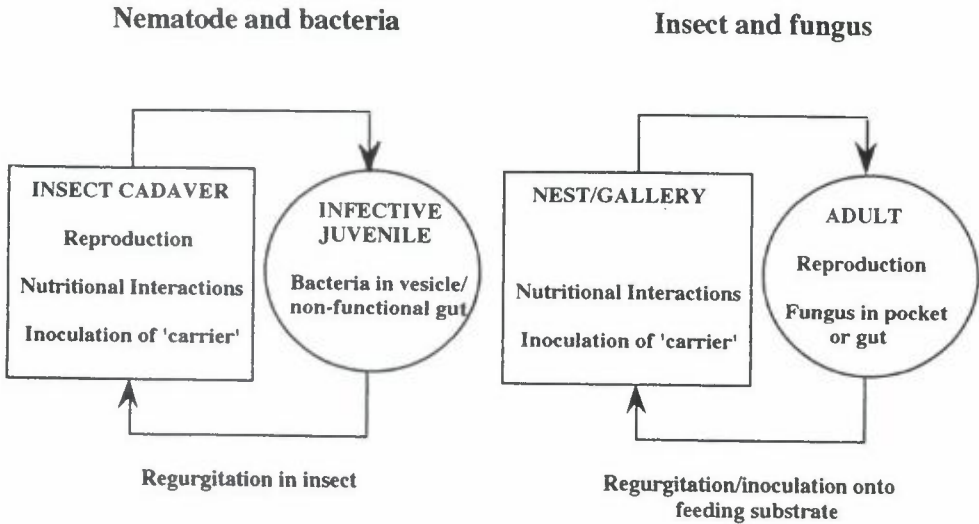


Figure 2. Ectosymbiotic associations in insects and entomopathogenic nematodes. The 'stable' and 'mobile' phases are represented by the box and circle, respectively (see text for further details).

and improve the nutritional quality of the substrate (e.g. Ehlers et al., 1990). Also in this environment, the inoculation of the transmission or 'carrier' stage occurs which ensures the continuance of the association in the next generation, e.g. the ingestion of bacteria into the vesicle or non-functional gut by the nematodes, and the acquisition of fungal mycelia into the intersegmental pockets of the woodwasps, or the ingestion of mycelia by the emerging ant queens.

The maintenance of a bacterial population within the gut or vesicle during the infective dauer juvenile stage must represent a significant cost to the host organism, in terms of energy and nutrients, which could limit the longevity and infective capability of the nematode. Since the dauer juvenile is a non-feeding stage, it is unlikely that the bacteria have any metabolic importance; most probably the bacteria are dormant or have a much lower growth rate than free-living species (many intracellular symbionts are regulated in this manner; see Douglas, 1994). The characteristics of bacterial phase shift that appear to typify members of the genera *Photorhadus* and *Xenorhabdus* (Akhurst and Boemare, 1990) may be important in this respect (Krasomil-Osterfeld, 1993).

4. Conclusions

The association between entomopathogenic nematodes and their symbiotic bacteria should be regarded as an ectosymbiosis, and as such bears many similarities to ectosymbioses between insects and filamentous fungi. The wealth of information concerned with the manipulation of the association, particularly the culture of both partners in isolation and the recombination of the symbiosis with different bacterial partners, will enable future research to address some fundamental issues in symbiosis which remain elusive in other, more intractable, associations.

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REFERENCES

- Akhurst, R.J. and Boemare, N.E. 1990. Biology and taxonomy of *Xenorhabdus*. In: *Entomopathogenic Nematodes in Biological Control*. Gaugler, R. and Kaya, H. eds. CRC Press, FL, USA. pp. 75–90.
- Bass, M. and Cherrett, J.M. 1994. The role of leaf-cutting ant workers (Hymenoptera: Formicidae) in fungus garden maintenance. *Journal of Economic Entomology* **19**: 215–220.
- Batra, L.R., ed. 1979. *Insect-Fungus Symbiosis. Nutrition, Mutualism, and Commensalism*. John Wiley, New York. 276 pp.
- Boucher, D.H., ed. 1985. *The Biology of Mutualism: Ecology and Evolution*. Croom-Helm, London. 358 pp.
- Boyd, N.D. and Martin, M.M. 1975. Faecal proteinases of the fungus-growing ant, *Atta texana* (Hym. Formicidae): their fungal origin and ecological significance. *Journal of Insect Physiology* **21**: 1815–1820.
- Buchner, P. 1966. *Endosymbioses of Animals with Plant Microorganisms*. John Wiley, New York, 909 pp.
- Cherrett, J.M., Powell, J., and Stradling, D.J. 1989. The mutualism between leaf cutting ants and their fungus. In: *Insect-Fungus Interactions*. N. Wilding, N.M. Collins, P.M. Hammond, and J.F. Webber, eds. R.E.S. Symp. 14. Academic Press, pp. 93–120.
- Douglas, A.E. 1983. Establishment of the symbiosis in *Convolutae roscoffensis*. *Journal of the Marine Biological Association, UK* **63**: 419–434.
- Douglas, A.E. 1989. Mycetocyte symbiosis in insects. *Biological Reviews* **64**: 409–434.
- Douglas, A.E. 1992. Microbial brokers of insect-plant interactions. *Proceedings of the 8th International Symposium Insect-Plant Relationships*. S.B.J. Menken, J.H. Visser, and P. Harrewijn, eds. Kluwer Academic Press, The Netherlands.
- Douglas, A.E. 1994. *Symbiotic Interactions*. Blackwells, UK.

- Douglas, A.E. 1995. The ecology of symbiotic microorganisms. *Advances in Ecological Research* **26**: 69–103.
- Dunphy, G.B. and Webster, J. 1988. Lipopolysaccharides of *Xenorhabdus nematophilus* (Enterobacteriaceae) and their haemocyte toxicity in nonimmune *Galleria mellonella* (Insecta: Lepidoptera) larvae. *Journal of General Microbiology* **134**: 1017–1028.
- Dunphy, G.B. and Webster, J. 1991. Antihemocytic surface components of *Xenorhabdus nematophilus* var. *dutki* and their modification by serum of nonimmune larvae of *Galleria mellonella*. *Journal of Invertebrate Pathology* **58**: 40–51.
- Eberle, M.W. and McLean, D.L. 1982. Initiation and orientation of the symbiote migration in the human body louse *Pediculus humanus*. *Journal of Insect Physiology* **28**: 417–422.
- Eberle, M.W. and McLean, D.L. 1983. Observations of symbiote migration in human body lice with scanning and transmission electron microscopy. *Canadian Journal of Microbiology* **29**: 755–762.
- Ehlers, R.U., Stoessel, S. and Wyss, U. 1990. The influence of phase variants of *Xenorhabdus* spp. and *Escherichia coli* (Enterobacteriaceae) on the propagation of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis*. *Revue Nématologie* **13**: 417–424.
- Farmer, J.J., Davis, B.R., Hickman-Brenner, F.W. et al. 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. *Journal of Clinical Microbiology* **21**: 46–76.
- Francke-Grossman, H. 1939. Ueber das Zusammenleben von Holzwespen (Siricinae) mit Pilzen. *Zeitschrift für Angewandte Entomologie* **25**: 647–680.
- Gerritsen, L.J.M. and Smits, P.H. 1993. Variation in pathogenicity of recombinations of *Heterorhabditis* and *Xenorhabdus luminescens* strains. *Fundamentals of Applied Nematology* **16**: 367–373.
- Han, R., Wouts, W.M., and Liying, L. 1991. Development and virulence of *Heterorhabditis* spp. strains associated with different *Xenorhabdus luminescens* isolates. *Journal of Invertebrate Pathology* **58**: 27–32.
- Holligan, P.M. and Gooday, G.W. 1975. Symbiosis in *Convolvutae roscoffensis*. *Symposia of the Society for Experimental Biology* **29**: 205–227.
- Krasomil-Osterfeld, K. 1994. Phase variation in *Photorhabdus*, *Xenorhabdus* and other bacteria. *Proceedings of the COST Workshop on Entomopathogenic Nematodes*, Maynooth, Ireland, August 1993 (in press).
- Kukor, J.J. and Martin, M.M. 1983. Acquisition of digestive enzymes by siricid woodwasps from their fungal symbiont. *Science (Washington, DC)* **220**: 1161–1163.
- Lewis, D.H. 1985. Symbiosis and mutualism: crisp concepts and soggy semantics. In: *The Biology of Mutualism: Ecology and Evolution*, D.H. Boucher, ed. Croom-Helm, London, pp. 29–39.
- Martin, M.M. 1987. *Invertebrate-Microbial Interactions; Ingested Fungal Enzymes in Arthropod Biology*. Cornell University Press, USA. 148 pp.
- Martin, J.S. and Martin, M.M. 1970. The presence of protease activity in the rectal fluid of attine ants. *Journal of Insect Physiology* **16**: 227–232.
- Martin, M.M., Gieselmann, M.J., and Martin, J.S. 1973. Rectal enzymes of attine ants: alpha-amylase and chitinase. *Journal of Insect Physiology* **19**: 1409–1416.

- Moran, N.A., Munson, M.A., Baumann, P., and Ishikawa, H. 1993. A molecular clock in endosymbiont bacteria is calibrated using the insect hosts. *Proceedings of the Royal Society of London Series B* **253**: 167–171.
- Moran, N.A. and Baumann, P. 1994. Phylogenetics of cytoplasmically inherited microorganisms of arthropods. *Trends in Ecology and Evolution* **9**: 15–20.
- Munson, M.A., Baumann, P., and Kinsey, M.G. 1991. *Buchnera* gen. nov. and *Buchnera aphidicola* sp. nov., a taxon consisting of the mycetocyte associated primary endosymbionts of aphids. *International Journal of Systematic Bacteriology* **41**: 566–568.
- Powell, R.J. 1984. The influence of substrate quality on fungus cultivation by some attine ants. Ph.D. thesis, University of Exeter, UK (unpublished).
- Provasoli, L., Yamasu, T., and Manton, I. 1968. Experiments on the resynthesis of symbiosis in *Convolutae roscoffensis* with different flagellate cultures. *Journal of the Marine Biological Association, UK* **48**: 465–479.
- Sacchi, L., Grigolo, A., Laudani, U., Ricevuti, G., and Dealessi, F. 1985. Behaviour of symbionts during oogenesis and early stages of development in the German cockroach *Blattella germanica* (Blattodea). *Journal of Invertebrate Pathology* **46**: 139–152.
- Sacchi, L., Grigolo, A., Mazzini, M., Bigliardi, E., Baccetti, B., and Laudani, U. 1988. Symbionts in the oocytes of *Blattella germanica* (Blattodea): their mode of transmission. *International Journal of Insect Morphology and Embryology* **17**: 437–446.
- Smith, D.C. 1981. The role of nutrient exchange in recognition between symbionts. *Berichte Deutsche Botanische Gesellschaft* **94**: Suppl. 517–528.
- Smith, D.C. and Douglas, A.E. 1987. *The Biology of Symbiosis*. Edward Arnold, London. 302 pp.
- Starr, M.B. 1975. A generalized scheme for classifying organismic associations. *Symposia of the Society for Experimental Biology* **29**: 1–20.
- Stillwell, M.A. 1966. Woodwasps (Siricidae) in conifers and the associated fungus, *Stereum chailletii*, in Eastern Canada. *Forestry Science* **12**: 121–128.
- Stillwell, M.A. 1967. The pigeon tremex, *Tremex columba* (Hymenoptera: Siricidae), in New Brunswick. *Canadian Entomologist* **99**: 685–689.
- Wood, D.L. 1972. Selection and colonisation of ponderosa pine by bark beetles. In: *Insect-Plant Relationships*, Van Emden, H.F., ed. Blackwells, UK. pp. 101–107.
- Wooton, R.A. 1981. Palaeozoic insects. *Annual Review of Entomology* **26**: 319–344.