CHAPTER 9

Understanding the Interaction Between an Obligate Hyperparasitic Bacterium, Pasteuria penetrans and its Obligate Plant-Parasitic Nematode Host, *Meloidogyne* spp.

Keith G. Davies

Contents

9.1. Introduction 212
9.2. Plant-Parasitic Nematodes, Biological Control and *Pasteuria Penetrans* 213
9.2.1. Nematodes as crop pests 213
9.2.2. *Pasteuria penetrans* as a hyperparasite 214
9.2.3. The life cycle of *Pasteuria penetrans* in relationship to root-knot nematodes 215
9.3. Exploiting Genomics to Understand the *P. Penetrans* Root-Knot Nematode Biology 217
9.3.1. Sequencing plant-parasitic nematodes and *Pasteuria* 217
9.3.2. Genomic insights for *in vitro* mass production of *P. penetrans* 217
9.3.3. Endospore attachment to the nematode cuticle 219
9.3.4. Endospore specificity and the Velcro-like model for attachment 226

Plant Pathology and Microbiology, Rothamsted Research, Harpenden, United Kingdom

Advances in Parasitology, Volume 68
ISSN 0065-308X, DOI: 10.1016/S0065-308X(08)00609-X

© 2009 Elsevier Ltd.
All rights reserved.

9.4. Building Cohesion Between Molecules and Populations 230
9.4.1. Endospore heterogeneity and the density necessary for nematode suppression 230
9.4.2. Learning from *Daphnia–P. ramosa* interactions 231
9.5. A Molecular Approach to Infection and Virulence from an Evolutionary Perspective 233
9.6. Summary, Conclusions and Implications for Biological Control 235
Acknowledgments 236
References 236

Abstract

Pasteuria penetrans is an endospore-forming bacterium, which is a hyperparasite of root-knot nematodes *Meloidogyne* spp. that are economically important pests of a wide range of crops. The life cycle of the bacterium and nematode are described with emphasis on the bacterium's potential as a biocontrol agent. Two aspects that currently prohibit the commercial development of the bacterium as a biocontrol agent are the inability to culture it outside its host and its host specificity. Vegetative growth of the bacterium is possible *in vitro*; however, getting the vegetative stages of the bacterium to enter sporogenesis has been problematic. Insights from genomic survey sequences regarding the role of cation concentration and the phosphorylation of Spo0F have proved useful in inducing vegetative bacteria to sporulate. Similarly, genomic data have also proved useful in understanding the attachment of endospores to the cuticle of infective nematode juveniles, and a Velcro-like model of spore attachment is proposed that involves collagen-like fibres on the surface of the endospore interacting with mucins on the nematode cuticle. Ecological studies of the interactions between *Daphnia* and *Pasteuria ramosa* are examined and similarities are drawn between the co-evolution of virulence in the *Daphnia* system and that of plant-parasitic nematodes.

9.1. INTRODUCTION

Over the course of evolution, natural selection has produced marvellous adaptations in which organisms have co-evolved through exquisitely subtle interactions. These adaptations can be observed and studied at different organisational levels that range from the biochemical and molecular, through the cellular and organismic, to the population and ecosystem. Each of these levels of organisation form a part of a nested hierarchy, or holarchy, each of which is complex and necessarily punctuated by a

certain degree of stratified stability if the holarchical structure is not going to collapse to extinction (Bronowski, 1977). Because of the subtle interactions that occur between a host and its parasite, they form a particularly interesting model on which to take a holistic approach and build a coherent understanding of the interactions that integrate the various organisational levels. To survive over evolutionary time, each of these organisational levels within a host–parasitic interaction is complex and has to remain creatively dynamic. This manuscript aims to describe the interactions between the obligate bacterial parasite *Pasteuria penetrans* and its obligate plant-parasitic nematode host, *Meloidogyne incognita*, and integrate our understanding from the biochemical and molecular through to the population and ecosystem level. This will be discussed from the perspective of a scientist involved in developing *Pasteuria* as a biological control agent to control plant-parasitic nematodes.

9.2. PLANT-PARASITIC NEMATODES, BIOLOGICAL CONTROL AND *PASTEURIA PENETRANS*

9.2.1. Nematodes as crop pests

Nematodes are the most abundant metazoans. The group as a whole is cosmopolitan and they are found in most environments. They can be broadly classified into animal parasites, plant parasites and free-living forms. *Caenorhabditis elegans*, a bacterial feeding, free-living nematode, is arguably the most well known and intensively studied nematode and was the first animal to have its genome completely sequenced (Herman, 2004). The plant-parasitic nematodes have been further sub-divided into five groups according to their habitat: migratory ectoparasites, migratory endoparasites, sedentary semi-endoparasites, sedentary endoparasites and above-ground parasites (Winslow, 1960). Plant-parasitic nematodes form part of the community of soil-dwelling nematodes (Yeates *et al*., 1993), and are not of monophyletic origin as their ability to parasitise plants is thought to have occurred on more than one occasion (Baldwin, *et al*., 2004; Blaxter *et al*., 1998; Holterman *et al*., 2006). The most economically important nematodes in agriculture are the sedentary endoparasites such as the root-knot nematodes, *Meloidogyne* spp., and the cyst nematodes, *Heterodera* spp. These nematodes, which affect the majority of arable and vegetable crops, are likely to become increasingly important pests in the context of climate change as they disrupt the ability of plant roots to take up water and nutrients from the soil. Historically, agriculture has developed a range of methods to control these pests that range from cultural methods, involving techniques such as crop rotation and solarisation, to the use of resistant varieties and chemical pesticides. Although chemical pesticides have proved useful in protecting crops from plant-parasitic nematodes, and are

likely to remain important into the foreseeable future, they are among some of the most toxic compounds used in agriculture and alternative approaches are being sought (Rich *et al.*, 2004).

The use of microbial enemies to control plant-parasitic nematodes has a long history (Stirling, 1991) but the concept of a soil being suppressive to nematode pests only really became a focus of intensive study since the latter part of the last century and the problem of cereal cyst nematode. It has long been known that cereals such as wheat and barley are susceptible to the cereal cyst nematode, *Heterodera avenae*, and that this nematode reduces yields considerably. However, during intensive cropping, when it might be expected that nematode populations would devastate yields, it has been recognised that after 4–5 years of continuous cereal cropping the soil becomes suppressive and the nematode population declines (Gair *et al.*, 1969). Intensive study, over the last 40 years has shown that nematode suppressiveness is related to a number of microbial parasites that are present in the rhizosphere and that key organisms could possibly be exploited to develop into a method for the control of nematode pests (Kerry, 2000). One such group of bacteria, the *Pasteuria* group, has potential to be developed into biological control agents (Stirling, 1991).

9.2.2. *Pasteuria penetrans* as a hyperparasite

The most studied of these bacteria is *Pasteuria penetrans*, a Gram-positive hyperparasitic bacterium of root-knot nematodes, *Meloidogyne* spp. The *Pasteuria* group of bacteria are endospore forming and are hyperparasites of plant-parasitic nematodes and water fleas (*Daphnia* spp.; Cladocera: Anomopoda). The fact that they are parasites of such two diverse groups of invertebrates, the Nematoda and the Anomopoda, suggests that there may be other undiscovered *Pasteuria* spp. that infect other known and unknown invertebrates. The detailed taxonomy of this group of bacteria remains unclear but the bacterium is a member of the *Bacillus*–*Clostridium* clade (Charles *et al.*, 2005; Preston *et al.*, 2003). Most studies have focused on two species of *Pasteuria*, *Pasteuria ramosa* that infect *Daphnia* spp. (Ebert, 2005; Ebert *et al.*, 1996) and *Pasteuria penetrans* that infect plant-parasitic nematodes, including *Meloidogyne* spp. All the economically important genera of plant-parasitic nematodes are parasitised by a *Pasteuria* species (Chen and Dickson, 1998), and to date, five species of *Pasteuria* have been described that differ in their host ranges and pathogenicity on plant-parasitic nematodes: (1) *P. penetrans* is parasitic on *Meloidogyne* spp. (Sayre and Starr, 1985); (2) *P. thornei* parasitises *Pratylenchus brachyurus* (Sayre *et al.*, 1988); (3) *P. nishizawae* parasitises cyst nematodes (Sayre *et al.*, 1991); (4) *Pasteuria usgae* is parasitic on the sting nematode *Belonolaimus longicaudatus* (Giblin-Davis *et al.*, 2003) and (5) *P. hartismeri* is parasitic on *M. ardenensis* (Bishop *et al.*, 2008).

Recently, variable-number tandem repeats (VNTRs) have been used as molecular markers for looking at biotypes (Mouton and Ebert, 2008; Mouton *et al*., 2007) and these will undoubtedly be useful for characterising *Pasteuria* populations parasitic on nematodes. As *P. penetrans* is a hyperparasite of root-knot nematodes, which account for 75% of crop losses due to nematodes, it has potential to be developed into a biological control agent (Stirling 1991) and the majority of studies have focused on the root-knot nematode–*P. penetrans* interaction.

9.2.3. The life cycle of *Pasteuria penetrans* in relationship to root-knot nematodes

Pasteuria penetrans is a member of the endospore forming group of Gram-positive bacteria and the initial stage of the infection process is when infective second-stage juvenile nematodes, migrating through the soil towards plant roots come into contact with endospores that lie dormant in the soil (Fig. 9.1A). Endospores are highly robust and can remain viable for many years (Giannakou *et al*., 1997). The endospores adhere to any part of the infective juvenile cuticle; although there is a slight preference for the anterior region (Davies, unpublished data). The numbers of endospores adhering to a particular juvenile can range from one to around 20 in field soils; however, in standardised attachment assays well over 100 spores per nematode have been observed in some instances. Infective juveniles encumbered with a large number of spores (>15) are less mobile and this reduces their ability to infect plant roots (Davies *et al*., 1988, 1991). Germination of the endospore, which involves a germination peg that breaches the juvenile cuticle (Fig. 9.1B), takes place in the period between the infective juvenile entering the plant root and established a feeding site and before the moult to a third-stage juvenile. However, in some other nematode species, such as *Heterodera avenae*, the endospore can germinate before the juvenile has entered the root (Davies *et al*., 1990) and this has been occasionally observed in root-knot nematodes (Davies unpublished). Recent cryo scanning microscopy observations on developing females infected with *P. penetrans* have revealed that following germination rhizoid structures grow out from the site of infection throughout the pseudoceolomic cavity (Fig. 9.1C) and granular masses of rod shaped Bacilli can be seen (Fig. 9.1D). Similar rod-shaped Bacilli have been observed growing in *in vitro* cultures of *P. penetrans* (Hewlett *et al*., 2004) and are likely to be the exponential growth phase of the bacterium. Electron microscopy has also revealed that some of these rod-shaped bacteria found in the pseudocoelom appear to have a single polar flagellum (Fig. 9.1E). Although it is likely that these rod-shaped bacteria are *Pasteuria*, it has not yet be shown unequivocally as there are reports that helper bacteria may be involved in the growth of *P. penetrans* (Duponnois

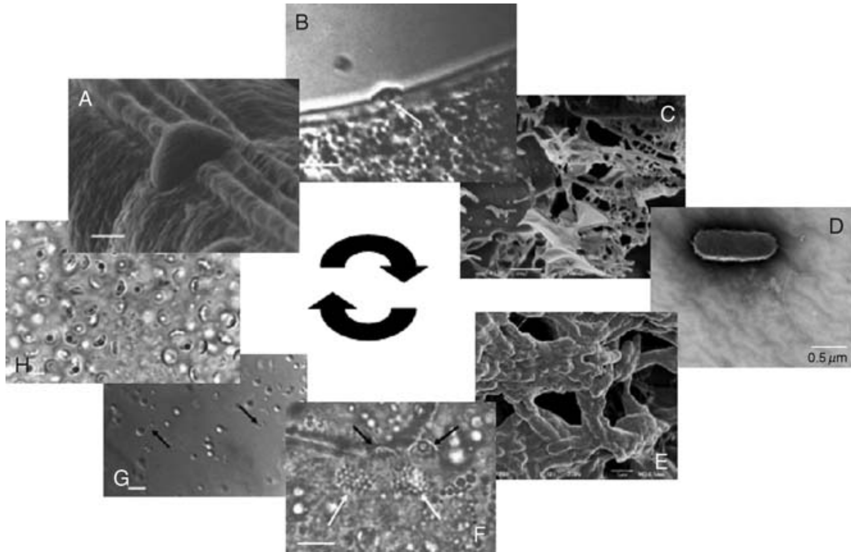

FIGURE 9.1 The life cycle of the parasite *Pasteuria penetrans* on its root-knot nematode host. (A) Scanning electron micrograph (SEM) of adhesion of endospore to the cuticle of an infective juvenile (bar = 1 μm); (B) light micrograph of infection peg (arrow) breeching the cuticle marking germination following formation of a feeding site by an infective juvenile (bar = 5 μm); (C) SEM showing rhizoids penetrating pseudocoelomic cavity (bar = 1 μm); transmission electron micrograph (TEM) of rod-shaped bacterium from within psuedocoelomic cavity with polar flagellum (bar = 0.5 μm); (E) SEM showing granular masses for bacterial rods within pseudocoelomic cavity (bar = 1 μm); (F) light micrograph of endospores (black arrows) on the infective juvenile cuticle surface and daughter microcolonies (white arrows) within pseudocoelomic cavity (bar = 5 μm); (G) light micrograph of fragmented microcolonies (doublets; black arrows) present in pseudocoelomic cavity (bar = 5 μm); (H) light micrograph of mature endospores from an adult infected female (bar = 4 μm).

et al., 1999; Gerber and White, 2001; Hewlett *et al.*, 2004). Root-knot nematode females infected with *P. penetrans* produce few, if any, progeny as their reproductive system quickly degenerates (Davies *et al.*, 2008). Sporogenesis begins when unidentified triggers, perhaps when certain key nutrients are limiting, with the production of microcolonies (Fig. 9.1F). These consist of clumps of dichotomously branching mycelial-like structures that subsequently fragment into quartets and doublets (Fig. 9.1G). This process continues until single, separate sporangia are produced, each containing a single endospore (Fig. 9.1H). An individual female that is infected can contain over 2×10^6 endospores and infected females after 6–8 weeks, often become larger than uninfected females (Davies *et al.*, 1988). This is similar to the situation in *P. ramosa* where infected *Daphnia* are larger than uninfected ones and gigantism has

been implicated (Ebert, 2005). The endospores are released back into the soil when infected nematodes and plant roots decay.

9.3. EXPLOITING GENOMICS TO UNDERSTAND THE *P. PENETRANS* ROOT-KNOT NEMATODE BIOLOGY

9.3.1. Sequencing plant-parasitic nematodes and *Pasteuria*

Over the last decade, the sequencing of eukaryotic and prokaryotic organisms has become routine. Comparing the genomes of different organisms can often lead to insights into their evolutionary history and help to answer questions regarding how organisms with similar developmental processes and genetics have very different life forms, and conversely, how very similar life forms can have very dissimilar developmental processes and genetics (Cañestro *et al.*, 2007; Frutos *et al.*, 2006). Computer software is being developed to make the comparisons between nematode species (Harris, 2003) and bacterial species (Field *et al.*, 2005) easy and accessible. Several plant-parasitic nematodes are currently being sequenced and these include *Meloidogyne incognita*, *M. hapla*, *Heterodera glycines* and *Globodera pallida*. In addition, there are a whole series of ESTs present within the public databases at GenBank and EMBL. The same is also true for *Pasteuria* and from a survey of the genome (Bird *et al.*, 2003) nearly 4,000 nucleotide sequences are available through GenBank and EMBL. Although at present there is no completed *Pasteuria* spp. genome available, at least one is very close to completion and new sequences are being deposited on a monthly basis. Even without having a completely sequenced genome, it is possible to start making comparisons between closely related species and gain an understanding into key biological processes. As *P. penetrans* has potential for being developed into a biological control agent, understanding of this particular host–parasite interaction is essential if it is ever going to be developed into a commercial control agent. There are two aspects that are currently prohibiting its commercial development: the inability to mass culture the bacterium *in vitro* and its restricted host range. Focusing on these two fundamental problems, it is therefore possible to gain insights from genomic comparisons of the host–parasite interactions that may help development of the bacterium as a biological control agent.

9.3.2. Genomic insights for *in vitro* mass production of *P. penetrans*

Up until very recently, the mass production of *P. penetrans* for the control of plant-parasitic nematodes has had to rely on *in vivo* culturing methods. The majority of these methods are adaptations of the method developed

by Stirling and Wachtel (1980). Briefly, females infected with *P. penetrans* spores are collected and an endospore suspension is made by homogenising infected females in water. Infective root-knot nematode juveniles are then exposed to these spores so that each juvenile is encumbered with 5–10 endospores. These encumbered second-stage juveniles are then placed around the roots of a tomato plant. After 6–8 weeks the nematode-infected roots containing infected nematodes are washed free of soil and air dried. The roots are then milled and can be used as inoculum for application to soil. Such milled tomato root powder can contain as many as 1.3×10^9 endospores per gram of root powder but the number of spores in each batch is highly variable (Pembroke and Gowen, personal communication). Although this is enough for application for small-scale growers, large-scale growers will require levels of mass production that would be better suited to an *in vitro* culturing method. Early attempts to grow *Pasteuria in vitro* (Bishop and Ellar, 1991; Williams *et al*., 1989) produced very limited success. Bishop and Ellar produced two media, one of which would sustain vegetative growth and another led to the production of endospores, but because at no point did the bacteria grow exponentially they were never able to produce enough for commercial application. More recently, Pasteuria Bioscience LLC, Florida, has developed media in which it is possible to grow vegetative stages of *Pasteuria* (Hewlett *et al*., 2004).

Both Bishop and Ellar and, more recently, Pasteuria Bioscience were unable to provide conditions in which vegetatively growing cells changed their growth form and entered sporogenesis. The initiation of sporulation in *Bacillus subtilis* has been extensively studied and is dependent on a phosphorelay pathway (Burbulys *et al*., 1991). In this pathway, a phosphoryl group is transferred to the regulator Spo0F through a group of five kinases that are under environmental regulation. This phosphoryl group is then transferred to the phosphotransferase Spo0B, which in turn passes it onto the regulator/transcription factor Spo0A. Phosphorylation of Spo0A enhances the activation and repression of approximately 500 stationary phase and sporulation genes (Fawcett *et al*., 2000). Like all known regulators, Spo0F requires a divalent metal ion to be present in the conserved aspartic acid pocket in order for phosphorylation to occur (Grimshaw *et al*., 1998) and magnesium has been shown to be important (Zapf *et al*., 1996). More recently, it has been suggested that metal cations other than Mg^{2+} may play a role in the structure and function of Spo0F and its involvement in the initiation of sporulation (Mukhopadhyay *et al*., 2004). Investigations of the effects of the divalent cations Ca^{2+}, Cu^{2+}, Mg^{2+}, and Mn^{2+} on the structure and function Spo0F of *B. subtilis* showed that they bound to the aspartic acid pocket and that while Mg^{2+} supports phosphotransfer from the kinase KinA to Spo0F the copper cation Cu^{2+} inhibited their phosphotransfer (Kojetin *et al*., 2005).

Interrogation of the *Pasteuria* survey sequence (using BlastP) revealed a large number of genes (approximately 6%) that had a high degree of similarity to genes involved in sporulation (Bird *et al.*, 2003) and this included Spo0F. Alignment of Spo0F between *B. subtilis*, *B. anthracis*, *B. thuringiensis* and *P. penetrans* showed that key amino acids that form the aspartic acid pocket are conserved across these groups. From the results discussed above it was hypothesised that the presence of Cu^{2+}, at non-lethal concentrations in the sporulation media for *B. subtilis* and the related bacterium *P. penetrans*, might inhibit endospore formation while continuing to permit vegetative growth. Indeed, subsequent experiments revealed that the absence of Cu^{2+} in the media showed an increased number of sporulating cells (Kojetin *et al.*, 2005). This result suggests that the availability of Cu^{2+} could be used to induce vegetative cells to enter sporulation.

9.3.3. Endospore attachment to the nematode cuticle

9.3.3.1. Host specificity in *Pasteuria penetrans*

The initial infection of infective root-knot juveniles by *P. penetrans* endospores is determined by the ability of viable endospores to adhere to the cuticle of migrating nematodes in search of a host plant root. Therefore, the attachment is the primary and, arguably, the most fundamental step in the infection process. There is a large number of studies (Channer and Gowen, 1992; Davies *et al.*, 1988, 1990; Espanol *et al.*, 1997; Mendoza de Gives *et al.*, 1999b; Sharma and Davies, 1996; Stirling, 1985; Wishart *et al.*, 2004) that show that endospores from individual isolates of the bacterium do not adhere to or recognise all populations of nematodes and exhibit host attachment specificity. Indeed, it has been shown that cuticle heterogeneity as exhibited by endospore attachment is not linked in any simple way to the phylogeny of the nematode (Davies *et al.*, 2001) and, in addition, in standard attachment assays differences can also be found between different stages of the same nematode population (Davies and Williamson, 2006). Perhaps more intriguing is the observation that inter- and intra-specific functional variation as measured again by *Pasteuria* spore attachment assays showed an equal amount of variation between amphimictic and parthenogenetically reproducing species of root-knot nematodes (Davies *et al.*, 2008). It is important to understand the mechanism that determines host specificity in the bacterium in order to identify suitable populations of *Pasteuria* to control specific nematode pests and possibly identify bacterial strains with a wide host range.

9.3.3.2. The hair-like fibrils of *Pasteuria penetrans* endospores

The structure and function of bacterial endospores has recently been reviewed (Henriques and Moran, 2007). In many species, the endospore coat is the outermost layer, however, in other species the spore is

surrounded by an additional layer called the exosporium. *P. penetrans* possesses an exosporium, which in other species provides it with resistance to chemical and enzymic treatments and gives the spore its adhesive properties (Kozuka and Tochibuko, 1985; Takumi, *et al*., 1979). Fibrils are known to be important in the attachment of many bacteria to host surfaces and their decoration with sugars has been observed to confer host specificity (Benz and Schmidt, 2002; Power and Jennings, 2003; Takeuchi *et al*., 2003). The exposure of endospores to hydrochloric acid removes its central body to reveal a structure containing fibrils (Persidis *et al*., 1991) and scanning electron microscope (SEM) studies on intact endospores have revealed that the parasporal fibres are positioned in such a way around the central body of the endospore to produce a skirt-like structure in which the under-surface of the endospore is in intimate contact with the nematode cuticle (Fig. 9.2A).

On the processing of endospores for SEM, occasionally the central body of the spore falls away from the nematode revealing a circular membrane adhering to the nematode cuticle (Fig. 9.2B) that was an integral part of the parasporal fibril, skirt-like structure. Transmission electron micrographs (TEMs) of the parasporal fibres covered with fine fibres both on the upper and lower surface (Fig. 9.2B) and further observations have shown the fibres on the concave surface of the endospore are more densely distributed than on the upper surface (Fig. 9.2B). It is difficult not to conclude that these fibres are involved in attachment of the mature endospore to the nematode cuticle.

In species of other closely related bacteria, *B. cereus*, *B. thuringiensis* and *B. anthracis*, for example, the structure of the exosporium is species and strain specific (Plomp *et al*., 2005a,b) and on the outer surface of which is an external hair-like nap (DesRosier and Lara, 1981; Wehrli *et al*., 1980). This is similar in *P. penetrans*. In *B. anthracis* the hair-like nap appears to be formed by a single collagen-like protein BclA in which the length of the filaments is related to the number of G-X-Y repeats (Boydston *et al*., 2005; Sylvestre *et al*., 2002, 2003, 2005). Homologous genes to *bclA* have been identified in other *Bacillus* spp., and they reside in a rhamnose cluster operon that contains a number of glycosyltransferases forming an exosporium island (Charlon *et al*., 1999; Steichen *et al*., 2003; Todd *et al*., 2003). In a genomic survey of *P. penetrans* (Bird *et al*., 2003) collagen-like sequences were identified using BlastP against *B. anthracis*, *B. cereus* and *B. thuringiensis*, that they were phylogenetically closer in structure to bacterial collagens (Davies and Opperman, 2006). Three contiguous gene sequences were identified each containing 28, 36 and 87, collagen-like G-X-Y repeats from which it was possible to predict that the *P. penetrans* hair-like nap would be made up of filaments with lengths ranging from 56 to over 200 nm in length (Davies and Opperman, 2006). TEM studies of *P. penetrans* endospores have so far not provided evidence

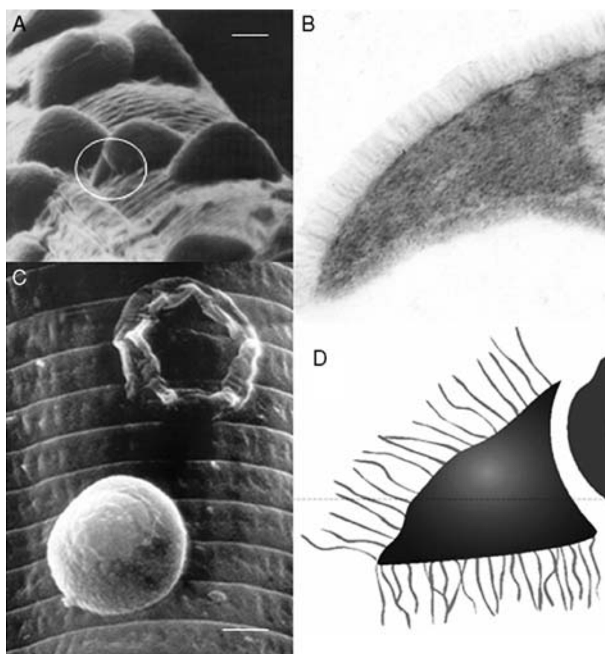

FIGURE 9.2 Endospore attachment (A) Scanning electron micrograph (SEM) of endospores adhering to the surface of an infective juvenile, ringed area reveals a point where the skirt-like structure that surrounds the central body of the endospore has broken away (bar = 1 μm); (B) transmission electron micrograph (TEM) of cross-section through the skirt-like structure that surrounds the central body of the endospore showing it to be covered in a hair-like nap the underside of which is the more dense than the upper surface; (C) SEM of an endospore and above a membrane-like structure that remains attached to the infective juvenile when the central body of the endospore has broken away during processing (bar = 1 μm); (D) cartoon (not to scale) of the skirt-like structure that surrounds the central body of the endospore and is covered with a fibrous nap.

of fibres with a length significantly greater than 100 nm but exosporial filaments ranging in length from 20 to over 100 nm have been identified (van de Meene *et al*., unpublished data). Evidence that these fibres on the surface of the endospore are collagen-like come from the facts that endospores incubated in collagenase are reduced in their ability to attach to the nematode juvenile cuticle (Davies and Danks, 1993), and that endospores pre-treated with either fibronectin or, perhaps more significantly, the collagen-binding domain of fibronectin, are also inhibited in their ability to attach to the juvenile cuticle (Davies and Redden, 1997; Mohan *et al*., 2001). However, confirmation of their structure will require further investigation.

9.3.2.2. The nematode cuticle and microbial adherence

The complex structure of the nematode cuticle reflects the multiple roles it has to perform, from protection of the nematode against the external environment, through to being important for nutrition and excretion, and to acting against the hydrostatic skeleton for locomotion (Wright, 1987). The cuticle of *C. elegans* is the most studied and best understood (Blaxter and Bird, 1997; Kramer, 1997; Politz and Philipp, 1992) and can therefore be used as a model. However, it should be remembered that the structure of the cuticle is highly variable among different groups and growth stages of nematodes (Malakhov, 1994). The nematode cuticle has consistently been divided into three easily definable layers, basal, medial and cortical layers, each of which is readily identifiable and easily observed by microscopy (Baldwin and Perry, 2004; Bird and Bird, 1991). The basal layer is typically striated and collagen is thought to be an important component; the medial layer is usually highly variable between species and frequently possesses struts, also made up of collagen, and upon which is the cortical layer. This outer layer appears to have a number of different specialised features, such as annulations and has been shown to contain cuticlins, lipids, surface-associated proteins and carbohydrates (Blaxter and Robertson, 1998; Cox *et al*., 1981a,b; Himmelhoch and Zuckerman, 1978; Zuckerman *et al*., 1979) and the binding of antibodies to this outer layer, the surface coat, affects nematode movement and behaviour (Sharon *et al*., 2002). This surface coat differs fundamentally from the lower collagenous layers and the epicuticle, in that ethanol is sufficient to extract it (Page *et al*., 1992). Because many standard electron microscopy techniques employ ethanol for dehydration, this means it is very difficult to observe.

Biochemical and immunological approaches have been used to characterise the surface coat of many animal-parasitic nematodes, and it is known that rapid changes in the surface coat can occur between pre-parasitic and post-parasitic forms (Proudfoot *et al*., 1993). Hence, seen from this perspective, *C. elegans* is a poor model as it is not a parasite and until relatively recently very little was known about its surface coat. New information is now being obtained through genetic studies, which is much more advanced in *C. elegans* than in other nematodes. Recent genetic studies have revealed genes which, when mutated, have produced defects in the surface coat (Grenache *et al*., 1996; Hemmer *et al*., 1991; Link *et al*., 1992; Politz *et al*., 1990). These changes to the surface coat have also been associated with the ability of pathogens to adhere to the cuticle and set up microbial infections (Gravato-Nobre *et al*., 2005; Hoflich *et al*., 2004; Mendoza de Gives *et al*., 1999a). In addition, wild-type *C. elegans* are susceptible to the formation of bacterial biofilms around the head of the nematode that prohibit feeding, and mutation experiments have identified a number of surface mutants in which the biofilm is absent

on the head (Darby *et al*., 2002, 2007). This suggests that surface coat properties are under direct genetic control and are important in the adherence of pathogenic and non-pathogenic bacteria.

Many of the genes identified by mutagenesis experiments that are associated with the bacterial adherence have a role in glycosylation pathways. Glycosylation occurs at the Golgi apparatus or endoplasmic reticulum where proteins can be decorated with sugars, a process that requires glycosyltransferases. Many of the mutants that have been identified with alterations in bacterial adherence encode genes involved with nucleotide sugar transporters and glycosyltransferases (Darby *et al*., 2007; Hoflich *et al*., 2004; Yook and Hodgkin, 2007). Mucin-like glycoproteins are important molecules that appear to be involved in host–parasite interactions and have been found in a variety of nematode species (Gems and Maizels, 1996; Loukas *et al*., 2000; Tetteh *et al*., 1999; Theodoropoulos *et al*., 2001). Mucins are a family of polypeptides associated with both the innate and adapted immune systems and can be secreted or membrane bound to form a protective barrier that covers epithelial surfaces (Strous and Dekker, 1992). Mucins possess a polypeptide backbone, parts of which are highly glycosylated with sugar side chains making up 85% of the molecule's weight. Glycosylation is predominantly *O*-linked through *N*-acetylgalactosamine (GalNAc) to serine and threonine residues within a VNTR region of the polypeptide core (Hicks *et al*., 2000; Theodoropoulos *et al*., 2001). Although there is no information on the role of mucins in plant-parasitic nematodes, it is interesting that the genes *muc-2*, *muc-3* and *muc-4* (which are members of the TES-120 family of proteins present in *Toxocara canis* (Tetteh *et al*., 1999) and are responsible for surface coat variation and have homologues in *C. elegans*) are also all present in various species of plant-parasitic nematodes (Table 9.1).

9.3.2.3. Microvilli and plant-parasitic nematode cuticles

The cuticle of plant-parasitic nematodes is broadly similar in structure to that discussed above; it can be divided into three layers, the outer most of which, the cortical layer, contains an epicuticle that is covered with the surface coat. The surface coat has a fuzzy appearance (Wright, 1987) and is composed mainly of proteins, carbohydrates and lipids (Blaxter and Robertson, 1998; Spiegel and McClure, 1995). This outermost layer is an important structure in that it provides a barrier between plant-parasitic nematodes and their environment. The second-stage juvenile is the infective stage of root-knot nematodes and thus the surface coat is exposed to two very different environments: a) the soil, as juveniles migrate in search of a plant root and b) the plant root, which they enter and through which they migrate to find a position to establish a feeding site. In the soil the infective juvenile will need to defend itself against microbial pathogens (Davies, 2005) and when it enters a host root it will need to remain

TABLE 9.1 TBlastN[1] of *Toxocara canis* surface coat glycoproteins (mucins) to top hit to plant-parasitic nematodes (E-value $<1e^{-10}$)

Toxocara mucin	Plant-parasitic nematode spp.	Score	E-value	% Similarity	Account number
T-120 (U39815)	*G. rostochiensis*	204	$2.6e^{-15}$	55	BM354635
	H. glycines	187	$1.6e^{-13}$	69	BI749309
	H. schachtii	185	$2.7e^{-13}$	65	CF100132
	M. hapla	170	$1.3e^{-11}$	81	BM901418
	G. pallida	169	$2.3e^{-11}$	52	BM416491
Muc-2 (AF167707)	*M. javanica*	208	$8.0e^{-16}$	53	CF350929
	M. chitwoodi	197	$1.5e^{-14}$	45	CD420128
	G. rostochiensis	189	$1.0e^{-13}$	59	BM354635
	M. hapla	187	$1.2e^{-13}$	49	CN194142
	H. glycines	160	$1.2e^{-10}$	67	BI749309
Muc-3 (AF167708)	*M. javanica*	273	$1.0e^{-22}$	49	CF350929
	M. chitwoodi	246	$9.5e^{-20}$	51	CD420128
	M. hapla	232	$3.1e^{-17}$	58	CN194142
	H. glycines	206	$1.6e^{-15}$	66	BI749309
	G. rostochiensis	192	$4.3e^{-14}$	56	BM355263
Muc-4 (AF167709)	*M. hapla*	236	$1.2e^{-18}$	60	CN194523
	H. glycines	227	$9.5e^{-18}$	67	BI749309
	M. chitwoodi	229	$6.0e^{-18}$	48	CD420128
	G. rostochiensis	226	$1.1e^{-17}$	64	BM355263
	M. javanica	221	$3.3e^{-17}$	54	CF350929
	H. schachtii	210	$6.1e^{-16}$	70	CF100132

Note:[1] complexity filter removed due to repetitive Ser and Thr repeats in core polypeptide. BLAST searches undertaken at: http://www.nematode.net/BLAST/.

undetected by the plant (Jones and Robertson, 1997; Kaplan and Davis, 1987). An important property of the surface coat is its lability, and the fact that the infective juvenile has to contend with two very different environments, the soil and the plant root, suggests that its ability to shed and regenerate its surface (Bird and Zuckerman, 1989; Gravato-Nobre *et al.*, 1995, 1999, 2001; Lin and McClure, 1996) is important to its survival. Therefore, as in animal parasitic nematodes (Almond and Parkhouse, 1985; Blaxter *et al.*, 1992), the surface coat plays an important role in actively evading infectious microbial agents and the immunity of the host plant.

Early studies using the outer layer of the cortex of some insect parasitic nematodes have been studied using TEM and have revealed finger-like projections or microvilli (Riding, 1970). Subsequent studies have suggested that the presence of two other types of cuticle, one where the secretory activity of the hypodermis had produced a covering or surface coat under which was a layer of microvilli, and another where the microvilli were fused and interwoven with a secreted surface coat (Subbotin *et al.*, 1993, 1994). TEM studies of infective juveniles of the plant-parasitic nematodes *Globodera rostochiensis*, *Meloidogyne incognita* and *Heterodera glycines* have also revealed fibrillar and/or microvilli like projections that were thought to be secreted (Endo, 1993; Forrest *et al.*, 1989; Fig. 9.3). The fixation of nematodes for electron microscopy is considered difficult and the use of alcohol in the preparation process can have very detrimental effects on surface structures. New techniques employing cryofixation and high-pressure freezing of *C. elegans* has produced images where microfibrial structures are observed on top of which is a surface coat that varies in thickness (Müller-Reichert *et al.*, 2003). Endospores of

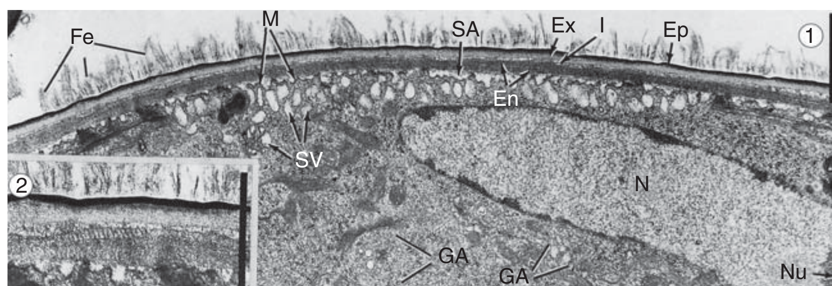

FIGURE 9.3 Longitudinal section of J2 at 2 days after inoculation. Figure shows fibrillar exudates (*fe*) on cuticle surface near stylet region. Section through hypodermal cord shows elongated nucleus (*N*) and extensive accumulation of secretory vesicles (*SV*) adjacent to apical membrane (*M*) of hypodermis (*H*). *En*, endocuticle; *Ep*, epicuticle; *Ex*, exocuticle; *GA*, Golgi apparatus; *I*, intermediate zone; *Nu*, nucleolus; *SA*, secretion accumulation zone. Bar = 1.0 μm (adapted from Endo, 1998; Figs. 162 and 163).

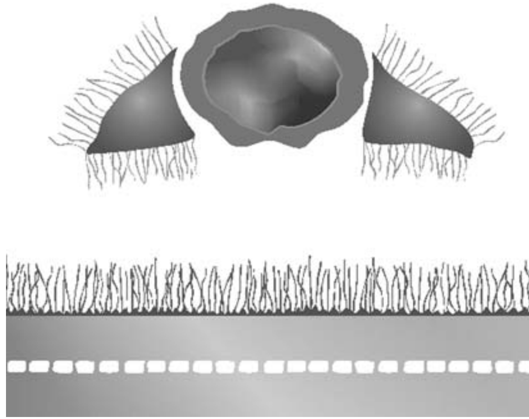

FIGURE 9.4 Cartoon (not to scale) showing endospore of *Pasteuria.* The skirt-like structure that surrounds the central body of the endospore is covered in a fibrous hair-like nap that interacts with fibrous structures present on the surface of the nematode cuticle. It is proposed that endospores attach to the nematode by a Velcro-like mechanism (see text Section 9.3.3).

Pasteuria are known to attach to the surface of infective juveniles of *H. glycines* (Atibalentja *et al*., 2004) and it is reasonable to suggest that the collagen fibrils present on the surface of the endospore, as discussed above, are either directly or indirectly interacting, with the microvilli present on the surface of the infective juvenile using what can be described as a Velcro-like attachment mechanism (Fig. 9.4). As *Pasteuria* endospores have been seen adhering to a large number of plant-parasitic nematodes (Chen and Dickson, 1998) this would suggest that the presence of microvilli on the surface of nematodes is likely to be a more common phenomenon than is currently appreciated. However, the fact that a population of endospores will not attach to all plant-parasitic nematodes (Davies *et al*., 2001) suggests that the surface coat has some system of generating variability that produces the observed host specificity.

9.3.4. Endospore specificity and the Velcro-like model for attachment

The Velcro-like mechanism of endospore attachment described above suggests that collagen-like fibres similar in structure to BclA in *B. anthracis* and *B. thuringiensis* are between 20 and 120 nm in length, and as migrating infective juveniles come into contact with endospores lying dormant in the soil it is likely that the *Pasteuria* collagen-like fibres will bind to the microvilli that form a part of the nematode's surface coat. Mucins are molecules that have a high degree of glycosylation, and are reported to

have an important role in host–nematode interactions (Hicks *et al.*, 2000; Theodoropoulos *et al.*, 2001). As reported above, mucins present in *T. canis* have homologues present in plant-parasitic nematodes and, arguably, it can be hypothesised that it is likely that similar mucin-like molecules may be important in the attachment of endospores to the nematode cuticle. Attachment of endospores to the infective juvenile cuticle is highly specific (Channer and Gowen, 1992; Davies *et al.*, 1988, 1990; Espanol *et al.*, 1997; Mendoza de Gives *et al.*, 1999b; Sharma and Davies, 1996; Stirling, 1985; Wishart *et al.*, 2004) and monoclonal and polyclonal antibodies raised to the surface of a population of *Pasteuria* endospores showed the surface characteristics of these spores to be diverse (i.e., different subpopulations of an initial population of *Pasteuria* would adhere to different populations of root-knot nematode, revealing a diversity of the surface coat of the cuticle to which the endospores were adhering) (Costa *et al.*, 2006; Davies and Redden, 1997; Davies *et al.*, 1994). This raises the question of the molecular nature of host specificity and the genetics that defines it.

9.3.3.1. Importance of carbohydrates in *Pasteuria*–nematode interactions

Mucins are polypeptides that are highly decorated with sugars (Hicks *et al.*, 2000; Theodoropoulos *et al.*, 2001), the addition of which can have both a physio-chemical and a biological function. The addition of carbohydrate domains to protein can modify solubility, electrical charge, mass and viscosity, and can control protein folding and three-dimensional stability, as well as protect it from enzymatic digestion (Lis and Sharon, 1993). Biologically, glycosylation can determine the lifetime of a protein and regulates its movement and position in the cell; it is also highly important in cellular interactions where it can be important in cellular recognition and determine a cell's antigenic characteristics (Lis and Sharon, 1993). Glycosylation is ubiquitous in eukaryotes and recently there have been increasing reports of glycosylation pathways in bacteria, particularly among mucosal-associated pathogens (Szymanski and Wren, 2005). To investigate the role that carbohydrates may play in endospore attachment, infective juvenile nematodes and endospores were pre-treated with a series of glycolytic enzymes and their effect on endospore attachment quantified (Davies and Danks, 1993). Pre-treatment of infective juveniles had a much greater effect on reducing endospore attachment than the pre-treatment of endospores, suggesting the importance of carbohydrates on the second-stage juvenile cuticle. Interestingly, in the same study, the pre-treatment of endospores with periodic acid, a treatment that breaks the hexose sugar ring and therefore disrupts the epitope, but does not break the saccharide chain (Maizels *et al.*, 1991), had a greater effect in reducing attachment than that of pre-treatment of cuticle (Davies

and Danks, 1993). This result suggested that a carbohydrate–protein interaction was responsible for the specificity of the adhesion. However, recently atomic force microscopy has shown that equally strong adhesive forces between two glycan molecules can be obtained, as between proteins in antibody–antigen interactions (Bucior *et al.*, 2004). This report leaves open the possibility that carbohydrate–carbohydrate interactions may play a major role in regulating the specificity of endospore attachment to the nematode cuticle. Some sugars have been shown to inhibit endospore attachment; pre-treatment of the infective juvenile cuticle of *M. incognita* with *L*-fucose, *N*-acetylglucosamine and *D*-xylose all reduced endospore attachment by 50% or more, whereas other sugars, *D*-glucose, *N*-acetylneuraminic acid, *D*-galactose, *D*-mannose and *N*-acetylgalactosamine had no or very little effect (Davies and Danks, 1993).

9.3.3.2. Molecular mimicry and fucose

In an investigation of the role of the surface coat in nematode–plant interactions, it was shown that a monoclonal antibody raised specifically against the outer surface of infective juveniles of *M. incognita* recognised a fucosyl-bearing glycoprotein and that this glycoprotein was sloughed off and deposited along the migratory track of the nematode as it migrated within the plant root (Gravato-Nobre *et al.*, 1999). It has been suggested that plant-parasitic nematodes share antigens with their host plant (McClure *et al.*, 1973) and indeed the nematode surface coat appears to share a fucosyl-bearing epitope with the phloem elements of nematode-infected roots (Gravato-Nobre *et al.*, 1999). However, fucose is present in the side chains of xyloglucan, a component of the plant cell wall (Masuda *et al.*, 1989), but the anti-fucose antibody did not recognise the fucosyl-moieties in roots that were not infected by nematodes. This suggests that either the fucose is of nematode origin, or, if of plant origin, it only becomes available for labelling as a result of infection of the roots by nematodes. If the antigen is of nematode origin can be hypothesised that the secreted antigen is involved in antigenic mimicry as a form of molecular camouflage and/or it is a signalling molecule where it may be responsible for the induction of the host response (Gravato-Nobre *et al.*, 1999).

Following on from the published evidence reviewed above, it could be hypothesised that the carbohydrates involved in the decoration of collagen-like fibrils on the surface of the endospore, and the glycosylation of the core mucin peptides of the nematode surface coat are responsible for the host specificity observed between *Pasteuria* endospores and infective juvenile nematodes. In addition, these same decorated mucins of the nematode surface coat may also be important in the regulation of specificity between the nematode and its plant host. In an investigation of glycosylation in Gram-negative bacteria representing a wide evolutionary

distance (Power and Jennings, 2003), it was shown that there were a number of conserved features: 1) where the protein target was known the genes responsible for glycosylation are adjacent to the protein they are to glycosylate, 2) there is a common arrangement of the biosynthetic genes necessary for glycosylation that suggests a common evolutionary origin, 3) the occurrence of acyl carrier proteins occur in groups of genes where proteins need to be glycosylated and therefore suggests they have a role, and 4) a large number of polymorphisms and the presence of mechanisms for phase-variable expression of glycosylation genes within a strain suggests periodic immune or functional selection for variation in glycan structure. These common features suggest the genes involved with glycosylation are within islands that can generate variable polymorphisms that are the consequence of an on-going host–parasite arms race (Dawkins and Krebs, 1979). Therefore, these conserved features might be expected to be present in the genomes of *Pasteuria* and plant-parasitic nematodes and with the publication of the root-knot nematode sequence (Opperman *et al*., 2008) and imminent publication of the *Pasteuria*-sequencing project this hypothesis will be open for rigorous testing.

9.3.3.3. Evidence of molecular diversity to produce polymorphism

The attachment of endospores to the cuticle of infective juveniles is a key interaction that will have co-evolved in the context of a host–parasite molecular arms race to produce polymorphism and molecular diversity. The evidence that this has occurred can be seen in the host specificity observed in endospore attachment studies and where this has been investigated further using immunological approaches.

Monoclonal antibodies (Mabs) raised to endospores from a single host female of *Pasteuria* endospores, strain PP1, produced five Mabs that showed that there was a diversity of surface types as different subpopulations of the endospore of strain PP1 were recognised by each of the five different Mabs (Davies *et al*., 1994). Baiting the PP1 population of endospores with different species and races of root-knot nematode and using the Mabs to characterise the endospores that were adhering to each of the different populations of nematode, showed that different subpopulations of the endospores were adhering to the different nematode populations. This indicates that immunological heterogeneity in the surface of the endospore was related to heterogeneity present in the outer surface coat of the different nematode populations (Davies *et al*., 1994). Similar differences were also observed in the recognition of the surface of endospores between isolates of *Pasteuria* from different geographical regions by the different Mabs. One particular Mab, PP1/117, appeared to recognise the concave surface of the endospore to a greater extent than the upper surface, revealing that the density of the antigen was greater on the concave surface; pre-treatment of these endospores with sugars or

glycolytic enzymes reduced the ability of the Mab to bind suggesting the Mab was recognising a carbohydrate epitope (Davies and Redden, 1997). Interestingly, the greatest reduction in recognition by pre-incubation of the spore in a carbohydrate by Mab PP1/117 was by fucose (Davies and Redden, 1997), which, as discussed above, may be involved in molecular camouflage of the infective juvenile to evade a plant host response. Other pre-treatments of the endospore that had a significant effect (>70%) on Mab PP1/117 recognition were proteinase K, fibronectin, wheat germ agglutinin and *n*-acetylglucosamine (Davies and Redden, 1997).

In Section 9.2.3 above, a Velcro-like molecular model is proposed that involves glycosylated collagen-like fibres on the surface of the endospore interacting with mucin-like peptides present on the outer surface coat of the infective juvenile. The evidence above points to the fact that glycosylation of the core collagen and mucin peptides that are present on the endospore and nematode cuticle, respectively, may be a source of polymorphism that determines the specificity of attachment. There may be other aspects of this molecular interaction that may also be important in maintaining the polymorphism. *P. penetrans* has been shown to have genes that encode for different lengths of the collagen-like protein (Davies and Opperman, 2006). The lengths of these collagen-like endosporal fibres in *Pasteuria* may well also be strain specific and play a role in attachment specificity. Thus, it is likely that collagen length and structure, core mucin length and structure, together with glycosylation, combine together to account for host range and specificity observed between endospores and nematode cuticle.

9.4. BUILDING COHESION BETWEEN MOLECULES AND POPULATIONS

9.4.1. Endospore heterogeneity and the density necessary for nematode suppression

P. penetrans has been identified as a key organism contributing to the suppression of plant-parasitic nematodes in a number of situations (Davies *et al*., 1990; Giblin-Davis *et al*., 1990; Mankau, 1975; Minton and Sayre, 1989; Oostendorp *et al*., 1991; Weibelzahl-Fulton *et al*., 1996). It has been argued that it has potential to be developed into a biological control agent and there are a number of worked examples where this has been achieved on a wide number of crops in different situations (Bhattacharya and Swarup, 1988; Chen *et al*., 1997; Chen *et al*., 1996; Chen and Dickson, 1998; Stirling, 1984; Trudgill *et al*., 2000). Nevertheless, there are many anecdotal reports where *P. penetrans* has been applied but has not successfully controlled the nematode population. Clearly, the ability to attach

to and subsequently infect the nematode target is fundamental. It has been estimated that for a soil to become suppressive, or for nematode control to take place, 10^4 endospores per gram of soil are required (Davies *et al*., 1990; Stirling, 1991). However, these estimates do not take account of the fact, as reviewed above (Section 9.2.2.), that there is a large amount of surface heterogeneity present within each endospore population, which could be potentially important in determining endospore adhesion. Therefore, for any particular nematode population there will only be a sub-population of the 10^4 endospores per gram of soil that will indeed attach to and infect any particular nematode population.

From the perspective of biological control, the amount of inoculum required is a key constraint, and it could be argued that 10^4 endospores per gram of soil is an overestimate of the number of spores necessary to suppress nematode populations. This is because what will be important in understanding this interaction at the population level is not the total numbers of nematodes or endospores, but the relative proportions of compatible and non-compatible nematode–*Pasteuria* interactions and the underlying genetics responsible for the interaction. Both immunological and DNA-based methods have been developed to characterise and quantify *P. penetrans* (Costa *et al*., 2006; Davies *et al*., 1994; Davies and Redden, 1997; Duan *et al*., 2003; Preston *et al*., 2003; Schmidt *et al*., 2004; Sturhan *et al*., 2005) and root-knot nematodes (Davies and Carter, 1995; Davies and Lander, 1992; Davies *et al*., 1996; Powers, 2004; Tigano *et al*., 2005) in soil, but relating this intra-specific variation to host–parasite compatibility and understanding the genetics involved has so far remained elusive. However, the co-evolutionary interaction between *Daphnia* and *P. ramosa* has been an active area of study and perhaps this will provide insight into the plant-parasitic nematode/*Pasteuria* interaction.

9.4.2. Learning from *Daphnia*–*P. ramosa* interactions

9.4.2.1. *Daphnia* and its parasitism by *P. ramosa*

Daphnia spp. are small transparent crustaceans found in most freshwater ponds and lakes that feed on plankton that can reproduce sexually and asexually but under normal conditions females reproduce by apomictic parthenogenesis (under unfavourable conditions a female will produce haploid eggs that need fertilisation by asexually produced sons). *Daphnia* can live for 2–3 months with the first eggs being formed after 7–15 days at 20 °C and can easily be maintained in the laboratory (Ebert, 2005). *P. ramosa* is a parasite of *Daphnia* that produces gigantism and sterilises the host shortly after infection, in a similar manner to *P. penetrans*, but whereas the route of infection is well understood in nematodes, it is not well understood in *Daphnia*. In *Daphnia*, it has been demonstrated that a strong host genotype–parasite genotype interaction exists (Carius *et al*.,

2001; Ebert, 1994; Refardt and Ebert, 2007), and as with *P. penetrans* there are no *Pasteuria* isolates that are able to infect all host genotypes (Carius *et al.*, 2001) and even from within the same infected host isolates of *P. ramosa* could be obtained that exhibited different amounts of virulence (Little *et al.*, 2008).

Epidemiological models usually assume that the mass action model applies and that the number of susceptible individuals that become infected is a result of the density of the host and the concentration of the parasite (Regoes *et al.*, 2003). Recently significant deviations from this basic model have been observed and attributed to biotic and abiotic factors such as seasonality, temperature, spatial structure and non-genetic host heterogeneity with respect to immunity and susceptibility (Ben-Ami *et al.*, 2008; Vale *et al.*, 2008). However, taken as a whole, none of these non-genetic factors were as strong as the genetic effects, and only genetic effects have been shown to explain variation in resistance under natural conditions (Ebert, 2008; Little and Ebert, 2000). Interestingly, in a comparison of *P. penetrans* endospore attachment to single juvenile descent lines of sexual and asexual reproducing root-knot nematodes, even within clonal lines there were significant differences in endospore attachment, suggesting some special mechanism was operating that produced functional differences in the cuticle surface that affected endospore attachment (Davies *et al.*, 2008). Therefore, similar to the *Daphnia* system, where the variation in the interaction is not all accounted for by genetics, perhaps this is also true for the root-knot nematodes, where some, as yet, unspecified non-genetic mechanism is contributing to host heterogeneity.

9.4.2.2. 'Arms races' and the 'Red Queen' hypothesis

It is difficult to study from a co-evolutionary perspective the reciprocal interactions that occur between hosts and parasites in a natural system because of the many generations needed. Recently the *Daphnia–Pasteuria* system was shown to offer a rare opportunity because lake sediments contain a unique archive due to the fact that both *Daphnia* and *Pasteuria* produce dormant propagule banks. Samples from different age strata in the sediments can be revived and then accessed using infectivity and virulence assays between different populations from the age-stratified sediments to assess 'Red Queen' co-evolutionary dynamics (Decaestecker *et al.*, 2007). The 'Red Queen' hypothesis (Van Valen, 1973) is based on the concept that within an antagonistic 'arms race' (e.g., between a predator and its prey, or a host and its parasite), where an increase in fitness of the host (e.g., genes for resistance against a parasite) will lead to a reciprocal increase in fitness in the parasite (e.g., new virulence against its host). This naturally leads to what has been regarded as an 'arms race' between a host and its parasite. Although the results from the *Daphnia–P. ramosa* system did not show any change in

parasite infectivity over time, there was a continued decrease in fecundity of the host and an increase in *Pasteuria* endospore production; these results were interpreted as an increase in the virulence of *P. ramosa* (Decaestecker *et al*., 2007). These results clearly show that infection needs to be separated from virulence, and, interestingly, different life-cycle strategies can be observed between different species of *Pasteuria* that infect different host nematodes.

The spores of *P. penetrans* that infect root-knot nematodes do not germinate until the nematode has entered the plant and set up a feeding site. An individual nematode can produce over 2×10^6 endospores (Davies *et al*., 1988), which greatly contrasts with the *Pasteuria* population that infected *Heterodera avenae* where spores attached to infective juveniles germinate immediately, kill their host rapidly and stop infected individuals from migrating; these infected individuals produce fewer than 1,000 endospores per infected individual (Davies *et al*., 1990). These observations, in light of the results reported by Decaestecker *et al*. (2007), suggest that there may be a co-evolutionary development from a necrotrophic lifestyle to a more biotrophic one, where it could be argued that two contrasting strategies, which probably form two extremes of a continuum, have evolved: 1) where the host is killed rapidly and few endospores are produced, with another, 2) where the nematode's life span is maintained or even extended together with the production of a large number of endospores. Indeed, there is now growing evidence in the *Daphnia–P. ramosa* system that the 'trade-off' hypothesis is at work, and that the evolution of virulence has led to a situation in which the production of endospores is balanced with the exploitation of the host in such a way that lifetime transmission success, production of endospores, is maximised (Jensen *et al*., 2006).

9.5. A MOLECULAR APPROACH TO INFECTION AND VIRULENCE FROM AN EVOLUTIONARY PERSPECTIVE

As can be seen above, there is a growing literature on understanding the interaction between *Pasteuria* spp. and their respective hosts, both at the molecular level and at the population level and this knowledge needs to be brought together. Molecular biologists might argue that phenomena at the population level can be explained by the facts at the lower level, a 'bottom up', or reductionist, approach; while the ecologist might argue that phenomena at the population level cannot be explained from knowledge of the parts, and must be studied directly, a 'top down', or holistic approach (Maynard-Smith, 1986). Therefore, the challenge for the 'pure' scientist is to bring the knowledge of these two approaches together and integrate them, while the challenge for the applied scientist is to utilise

this knowledge and apply it to solve problems. Many polymorphisms are maintained by the interactions of hosts with their parasites (Haldane, 1949). This idea was developed by plant pathologists and formalised within what is known as the gene-for-gene hypothesis (Flor, 1956, 1971). The concept of gene-for-gene co-evolution has been brought into question as it has been suggested that it is an artefact of studies undertaken using agricultural examples (Thompson and Burdon, 1992). This is a consequence of studies using spatially, or in the case of potato cyst nematode (*Globodera* spp.) in the United Kingdom, geographically isolated populations, and not natural populations. Nevertheless, the gene-for-gene model, along with a number of other conceptual models such as 'matching-genotypes', 'quantitative-trait', 'multiplicative matching-alleles' and 'additive matching-alleles' models have been found to be useful in developing our understanding (Nuismer and Otto, 2004; Otto and Nuismer, 2004). Mathematics has proved useful in understanding host–parasite interactions in relation to the Red Queen hypothesis and the evolution of sex and different levels of ploidy. Within this context, it is interesting that *H. avenae*, parasitised by a *Pasteuria* sp. that germinates and reproduces in the infective juvenile, is diploid and amphimictic, while *M. incognita* where *Pasteuria* normally germinates in the developing females causing gigantism, is polyploid and mitotically parthenogenetic. Until more comparative life-cycle studies have been undertaken investigating other nematode–*Pasteuria* associations and their genetics, this just remains an interesting observation.

Biochemical recognition systems are important in determining which matching host and parasite genotypes result in controlling resistance and susceptibility (Frank, 1994). The polymorphic diversity revealed by endospore attachment, where this manuscript proposes a form of Velcro-like mechanism of attachment, probably involving nematode mucins on the cuticle and *Pasteuria* collagen-like fibres that are glycosylated on the endospore, is likely to be under direct genetic control. However, whether or not this is directional and the result of a Red Queen arms race remains to be ascertained because other methods of generating variation, such as gene rearrangement and reading frame shifts (de Vries *et al*., 2002; Weiser, 2000), error prone DNA polymerase (Rattray and Strathern, 2003) and post-translational modification (Hicks *et al*., 2000; Theodoropoulos *et al*., 2001) can also produce phenotypic variation. The importance of the genetics that underpins these interactions will only be clarified when the biochemical and molecular mechanism of the attachment process is fully understood. Endospore attachment, however, only represents the first stage in the infection process. The germination of endospores is also important and differs between the *Pasteuria* parasitising different hosts. This suggests different signalling processes are probably operational at different stages in the infection process and it is likely that natural

selection will have produced another level of specificity. Finally, once infection has successfully taken place, the virulence of the bacterium is also important and it has been observed that *P. ramosa* strains isolated from the same host have been shown to have very different levels of virulence against an isolate of *Daphnia* (Jensen *et al*., 2006). Attachment, germination and virulence are all likely to be important in the co-evolved adaptations of *Pasteuria* to its nematode host. It will, therefore, be the subtleties of these interactions that will determine the outcomes of different *Pasteuria* spp. relationships with their nematode hosts.

9.6. SUMMARY, CONCLUSIONS AND IMPLICATIONS FOR BIOLOGICAL CONTROL

It is only relatively recently with the development of industrialised agriculture that plant-parasitic nematodes have been recognised as an important constraint on crop production. For the majority of their evolutionary history, plant-parasitic nematodes have been part of a multi-trophic interaction between their plant host, and their natural enemies. The biotic constraints to their population growth will be through several mechanisms: 1) top-down control, exerted by their natural enemies; 2) horizontal control, exerted by inter- and intra-specific competition among the nematodes, and 3) bottom-up control exerted by their host plant (Van der Putten *et al*., 2006).

This manuscript concentrates on top-down control by the obligate hyperparasitic bacterial group of natural enemies that are from the endospore-forming genus *Pasteuria*. The life cycle of the bacterium is described in which migrating infective juvenile nematodes become encumbered with *Pasteuria* endospores that infect the nematode and prohibit it from reproducing. There are two problems in developing *Pasteuria* as a biological control agent: 1) its obligate nature and the inability to mass culture it and 2) its host specificity.

Genomic approaches have been useful in gaining insights into both *in vitro* culture and host specificity. Knowledge of the sporulation pathway of closely related *Bacillus* spp. is well developed and highly conserved across different groups of endospore-forming bacteria. This has provided useful insights into the cation concentration required in growth media that are conducive for sporogenesis. Genomics has also been used to understand endospore specificity.

The first stage of the infection process is the attachment of endospores to the cuticle of the infective juvenile and this is highly host specific. Reviewing the literature and gaining insights from sequencing data suggests a Velcro-like mechanism of spore attachment. A molecular model is proposed in which glycosylated collagen-like fibres on the surface of the

endospore interact with fibrous mucins present in the surface coat of the infective juvenile. It is this process where the collagen and mucin fibres act as the primary architectural structures for attachment and where glycosylation is likely to be involved in determining host specificity.

The *Daphnia*–*Pasteuria* model is then drawn upon to show that even from within the same *Daphnia* host isolate, different levels of virulence can be observed in different *Pasteuria* strains and that there is an observable relationship between the life spans of infected hosts and the number of endospores produced that suggests that there is a co-evolutionary development. A similar difference in life-cycle strategies can also be seen in plant-parasitic nematodes infected with *Pasteuria*.

The development of knowledge about the *Pasteuria*–nematode interaction has provided insights into two problems that prohibit *Pasteuria* from being developed into a commercial control agent, culturing and host specificity. As more is learnt through genomic approaches, and our understanding of the co-evolutionary processes that have shaped different life-cycle strategies between different strains of *Pasteuria* grow, this knowledge will help in the development of novel and new strategies of plant-parasitic nematode control.

ACKNOWLEDGMENTS

KGD would like to thank Professor R. N. Perry for his comments on the manuscript. Rothamsted Research receives grant aided from the Biotechnological and Biological Sciences Research Council of the United Kingdom.

REFERENCES

Almond, N. M., and Parkhouse, R. M. E. (1985). Nematode antigens. *Curr. Topics Microbiol. Immunol.* **120,** 173–203.

Atibalentja, N., Jakstys, B. P., and Noel, G. R. (2004). Life cycle, ultrastructure and host specificity of the North American isolate of *Pasteuria* that parasitizes the soybeancyst nematode *Heterodera glycines*. *J. Nematol.* **36,** 171–180.

Baldwin, J. G., and Perry, R. N. (2004). Nematode morphology, sensory structure and function. *In* ''Nematology Advances and Perspectives Volume I: Nematode Morphology, Physiology and Ecology.'' (Chen, Chen, and Dickson, eds.) pp. 175–257. CABI Publishing, Wallingford, United Kingdom.

Baldwin, J. G., Nadler, S. A., and Adams, B. J. (2004). Evolution of plant parasitism among nematodes. *Ann. Rev. Phytopathol.* **42,** 83–105.

Ben-Ami, F., Regoes, R. R., and Ebert, D. (2008). A quantitative test of the relationship between parasite does and infection probability across different host–parasite combinations. *Proc. R. Soc. B* **275,** 853–859.

Benz, Z., and Schmidt, M. A. (2002). Never say never again: Protein glycosylation in pathogenic bacteria. *Mol. Microbiol.* **45,** 267–276.

Bhattacharya, D., and Swarup, G. (1988). *Pasteuria penetrans*, a pathogen of the genus *Heterodera*, its effect on nematode biology and control. *Indian J. Nematol.* **18,** 61–70.

Bird, A. F., and Bird, J. (1991). ''The Structure of Nematodes.'' Academic Press, San Diego.

Bird, A. F., and Zuckerman, B. M. (1989). Studies on the surface coat (glycocalyx) of the daur larva of *Anguina agrostis*. *Int. J. Parasitol.* **19,** 235–240.

Bird, D. M., Opperman, C. H., and Davies, K. G. (2003). Interactions between bacteria and plant parasitic nematodes: now and then. *Int. J. Parasitol.* **33,** 1269–1276.

Bishop, A. H., Gowen, S. R., Pembroke, B., and Trotter, J. R. (2008). Morphological and molecular characteristics of a new species of *Pasteuria* parasitic on *Meloidogyne ardenensis*. *J. Invert. Pathol.* **96,** 28–33.

Bishop, A. H., and Ellar, D. J. (1991). Attempts to culture *Pasteuria penetrans* in vitro. *Biocon. Sci. Technol.* **1,** 101–114.

Bishop, A. H., Gowen, S. R., Pembroke, B., and Trotter, J. R. (2007). Morphological and molecular characteristics of a new species of *Pasteuria* parasitic on *Meloidogyne ardenensis*. *Journal of Invertebrate Pathology* **96,** 28–33.

Blaxter, M. L., and Bird, D. (1997). Parasitic nematodes. *In* ''*C. elegans* II.'' (Riddle, Blumenthal, Meyer, and Priess, eds.) pp. 851–878. Cold Spring Harbor Laboratory Press, New York.

Blaxter, M. L., and Robertson, W. M. (1998). The cuticle. *In* ''The Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes.'' (Perry and Wright, eds.), pp. 25–48. CABI Publishing, Wallingford, United Kingdom.

Blaxter, M. L., De Lay, P., and Garey, J. R. (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature* **392,** 71–75.

Blaxter, M. L., Page, A. P., Rudin, W., and Maizels, R. M. (1992). Nematode surface coats: Actively evading immunity. *Parasitol. Today* **8,** 243–247.

Boydston, J. A., Chen, P., Steichen, C. T., and Turnbough, C. L., Jr. (2005). Orientation within the exosporium and the structural stability of the collagen-like glycoprotein BclA of *Bacillus anthracis*. *J. Bacteriol.* **187,** 5310–5317.

Bronowski, J. (1977). New concepts in evolutionary complexity. *In* ''A Sense of the Future.'' (Bronowski, ed.), pp. 175–195. MIT Press, Boston.

Bucior, I., Scheuring, S., Engel, A., and Burger, M. M. (2004). Carbohydrate-carbohydrate interaction provides adhesion force and specificity for cellular recognition. *J. Cell Biol.* **165,** 529–537.

Burbulys, D., Trach, K. A., and Hoch, J. A. (1991). Initiation of sporulation in *B. subtilis* is controlled by a multicomponent phosphorelay. *Cell* **64,** 545–552.

Carius, H. J., Little, T. J., and Ebert, D. (2001). Genetic variation in a host–parasite association: Potential for coevolution and frequency-dependent selection. *Evolution* **55,** 1136–1145.

Cañestro, C., Yokoi, H., and Postlethwait, J. H. (2007). Evolutionary developmental biology and genomics. *Nat. Rev. Genet.* **8,** 932–942.

Channer, A. G., and Gowen, S. R. (1992). Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne-Pasteuria penetrans* interaction. *Fund. Appl. Nematol.* **15,** 331–339.

Charles, L., Carbonne, I., Davies, K. G., Bird, D., Burke, M., Kerry, B. R., and Opperman, C. H. (2005). Phylogenetic analysis of *Pasteuria penetrans* using multiple genetic loci. *J. Bacteriol.* **187,** 5700–5708.

Charlon, S., Moir, A. J. G., Baillie, L., and Moir, A. (1999). Characterisation of the exosporium of *Bacillus cereus*. *J. Appl. Microbiol.* **87,** 241–245.

Chen, Z. X., and Dickson, D. W. (1998). A review of *Pasteuria penetrans*: Biology, ecology and biological control potential. *J. Nematol.* **30,** 313–340.

Chen, Z. X., Dickson, D. W., and Mitchell, D. J. (1997). Suppression mechanisms of *Meloidogyne arenaria* race 1 by *Pasteuria penetrans*. *J. Nematol.* **29,** 1–8.

Chen, Z. X., Dickson, D. W., McSorley, R., Mitchell, D. J., and Hewlett, T. E. (1996). Suppression of *Meloidogyne arenaria* race 1 by soil application of endospores of *Pasteuria penetrans*. *J. Nematol.* **28,** 159–168.

Costa, S. R., Kerry, B. R., Bardgett, R., and Davies, K. G. (2006). Exploitation of immunofluorescence for the quantification and characterisation of small numbers of Pasteuria endospores. *FEMS Microbiol. Ecol.* **58,** 593–600.

Cox, G. N., Kusch, M., and Edgar, R. S. (1981a). Cuticle of *Caenorhabditis elegans*: Its isolation and partial characterisation. *J. Cell Biol.* **90,** 7–17.

Cox, G. N., Staprans, S., and Edgar, R. S. (1981b). The cuticle of *Caenorhabditis elegans* II. Stage-specific changes in ultrastructure and protein composition during post embryonic development. *Dev. Biol.* **86,** 456–470.

Darby, C., Chakraborti, A., Politz, S. M., Daniels, C. C., Tan, L., and Drace, K. (2007). *Caenorhabditis elegans* mutants resistant to attachment of Yersinia biofilms. *Genetics* **176,** 221–230.

Darby, C., Hsu, J. W., Ghori, N., and Falkow, S. (2002). *Caenorhabditis elegans*: Plague bacteria biofilm blocks food intake. *Nature* **417,** 243–244.

Davies, K. G. (2005). Interactions between nematodes and microorganisms: Bridging ecological and molecular approaches. *Adv. Appl. Microbiol.* **57,** 53–78.

Davies, K. G., and Carter, B. (1995). Comparison of immunoassays for the quantification of root-knot nematodes extracted from soil. *EPPO Bull.* **25,** 367–375.

Davies, K. G., and Danks, C. (1993). Carbohydrate/protein interactions between the cuticle of infective juveniles of *Meloidogyne incognita* and spores of the obligate hyperparasite *Pasteuria penetrans*. *Nematologica* **39,** 54–64.

Davies, K. G., and Lander, E. B. (1992). Immunological differentiation of root-knot nematodes (*Meloidogyne* spp.) using monoclonal and polyclonal antibodies. *Nematologica* **38,** 353–366.

Davies, K. G., and Opperman, C. H. (2006). A potential role for collagen in the attachment of *Pasteuria penetrans* to nematode cuticle. *In* ''Multitrophic Interactions in the Soil and Integrated Control.'' (Raaijmakers and Sikora, eds.), IOBC WPRS Bull. **29,** pp. 11–15.

Davies, K. G., and Redden, M. (1997). Diversity and partial characterisation of putative virulence determinants in *Pasteuria penetrans*, the hyperparasite of root-knot nematodes. *J. Appl. Microbiol.* **83,** 227–235.

Davies, K. G., and Williamson, V. M. (2006). Host-specificity exhibited by populations of endospores of *Pasteuria penetrans* to the juvenile and male cuticles of *Meloidogyne hapla*. *Nematology* **8,** 475–476.

Davies, K. G., Curtis, R. H., and Evans, K. (1996). Serologically based diagnostic and quantification tests for nematodes. *Pest. Sci.* **47,** 81–87.

Davies, K. G., Fargette, M., Balla, G., Daudi, A., Duponnois, R., Gowen, S. R., Mateille, T., Phillips, M. S., Sawadogo, A., Trivino, C., Vouyoukalou, E., and Trudgill, D. L. (2001). Cuticle heterogeneity as exhibited by *Pasteuria* spore attachment is not linked to the phylogeny of parthenogenetic root-knot nematodes (*Meloidogyne* spp.). *Parasitology* **122,** 111–120.

Davies, K. G., Flynn, C. A., Laird, V., and Kerry, B. R. (1990). The life-cycle, population dynamics and host specificity of a parasite of *Heterodera avenae*, similar to *Pasteuria penetrans*. *Rev. Nematol.* **13,** 303–309.

Davies, K. G., Kerry, B. R., and Flynn, C. A. (1988). Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. *Ann. Appl. Biol.* **112,** 1491–501.

Davies, K. G., Laird, V., and Kerry, B. R. (1991). The motility, development and infection of *Meloidogyne incognita* encumbered with spores of the obligate hyperparasite *Pasteuria penetrans*. *Rev. Nematol.* **14,** 611–618.

Davies, K. G., Redden, M., and Pearson, T. K. (1994). Endospore heterogeneity in *Pasteuria penetrans* related to attachment to plant-parasitic nematodes. *Lett. Appl. Microbiol.* **19,** 370–373.

Davies, K. G., Rowe, J., and Williamson, V. M. (2008). Cuticle variation amongst amphimictic and parthenogenetic populations of nematode (*Meloidogyne* spp.) as exhibited by a bacterial parasite (*Pasteuria penetrans*). *Int. J. Parasitol.* **38,** 851–859.

Decaestecker, E., Gaba, S., Raeymaekers, J. A. M., Stoks, R., Kerckhoven, L., Van, Ebert, D., and de Meester, L. (2007). Host–parasite 'Red Queen' dynamics archived in pond sediment. *Nature* **450,** 870–873.

DesRosier, J. P., and Lara, J. C. (1981). Isolation and properties of pili from spores of *Bacillus cereus*. *J. Bacteriol.* **145,** 613–619.

de Vries, N., Duinsbergen, D., Kuipers, E. J., Pot, R. G. J., Wiesenekker, P., Penn, C. W., van Vliet, A. H. M., andenbroucke-Grauls, C. M. J. E., and Kusters, J. G. (2002). Transcriptional phase variation of a type III restriction-modification system in Helicobacter pylori. *J. Bacteriol.* **184,** 6615–6623.

Duponnois, R., Ba, A. M., and Mateille, T. (1999). Beneficial effects of *Enterobacter cloacae* and *Pseudomonas mendocina* for the biocontrol of *Meloidogyne incognita* with the endospore-forming bacterium *Pasteuria penetrans*. *Nematology* **1,** 95–101.

Davies, K. G., and Redden, M. (1997). Diversity and partial characterisation of putative virulence determinants in *Pasteuria penetrans*, the hyperparasite of root-knot nematodes. *J. Appl. Microbiol.* **83**, 227–235.

Dawkins, R., and Krebs, J. R. (1979). Arms races between and within species. *Proceedings of the Royal Society of London* series B **205,** 489–511.

Ebert, D. (1994). Virulence and local adaption of a horizontally transmitted parasite. *Science* **265,** 1084–1086.

Ebert, D. (2005). "Ecology, Epidemiology and Evolution of Parasitism in Daphnia." National Library of Medicine (United States), National Center for Biotechnology Information, Bethesda.

Ebert, D., Rainey, P., Embley, T. M., and Scholz, D. (1996). Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Phil. Trans. R. Soc. Lond. B* **351,** 1689–1701.

Ebert, D. (2008). Host-parasite coevolution: Insights from the Daphnia-parasite model system. *Curr. Opin. Microbiol.* **11,** 290–301.

Endo, B. Y. (1993). Ultrastructure of cuticular exudates and related cuticular changes on juveniles in *Heterodera glycines*. *J. Helminthol. Soc. Washington* **60,** 76–88.

Endo, B. Y. (1998). ''Atlas on Ultrastructure of Infective Juveniles of the Soybean Cyst Nematode, Heterodera glycines." United States Department of Agriculture, Agricultural Research Service, Agriculture Handbook 711, Washington.

Espanol, M., Verdejo-Lucas, S., Davies, K. G., and Kerry, B. R. (1997). Compatibility between *Pasteuria penetrans* and *Meloidogyne* populations from Spain. *Biocontrol Sci. Technol.* **7,** 219–230.

Fawcett, P., Eichenberger, P., Losick, R., and Youngman, P. (2000). The transcriptional profile of early to middle sporulation in *Bacillus subtilis*. *Proc. Natl Acad. Sci. USA* **97,** 8063–8068.

Field, D., Feil, E. J., and Wilson, G. A. (2005). Databases and software for the comparison of prokaryotic genomes. *Microbiology* **151,** 2125–2132.

Flor, H. H. (1956). The complementary genic systems in flax and flax rust. *Adv. Genet.* **8,** 29–54.

Flor, H. H. (1971). Current status of the gene-forgene concept. *Ann. Rev. Phytopathol.* **32,** 653–669.

Forrest, J. M. S., Robertson, W. M., and Milne, E. W. (1989). Observations on the cuticle surface of second-stage juveniles of *Globodera rostochiensis* and *Meloidogyne incognita*. *Rev. Nematol.* **12,** 337–341.

Frank, S. A. (1994). Recognition and polymorphism in host–parasite genetics. *Phil. Trans. R. Soc. Lond. B* **346,** 283–293.

Frutos, R, Viari, A., Ferraz, C., Morgat, A., Eychenié, S., Kandassamy, Y., Chantal, I., Bensaid, A., Coissac, E., Vachiery, N., Demaille, J., and Martinez, D. (2006). Comparative genomic analysis of three strains of *Ehrlichia ruminantium* reveals an active process of genome size plasticity. *J. Bacteriol.* **188,** 2533–2542.

Gair, R., Mathias, P. L., and Harvey, P. N. (1969). Studies of cereal nematode populations and cereal yields under continuous or intensive culture. *Ann. Appl. Biol.* **63,** 503–512.

Gems, D., and Maizels, R. M. (1996). An abundantly expressed mucin-like protein from *Toxocara canis* infective larvae: The precursor of the larval surface coat glycoprotein. *Proc. Natl Acad. Sci. USA* **93,** 1665–1670.

Gerber, J. F., and White, J. H. (2001). Materials and methods for the efficient production of *Pasteuria.* International patent application EP20000953963.

Giannakou, I. O., Pembroke, B., Gowen, S. R., and Davies, K. G. (1997). Effects of long term storage and above normal temperatures on spore adhesion of *Pasteuria penetrans* and infection of root-knot nematode *Meloidogyne javanica*. *Nematologica* **43,** 185–192.

Giblin-Davis, R. M., McDaniel, L. L., and Bilz, F. G. (1990). Isolates of the *Pasteuria penetrans* group from phytoparasitic nematodes in Bermudagrass turf. *J. Nematol.* **22,** 750–762.

Giblin-Davis, R. M., Williams, D. S., Bekal, S., Dickson, D. W., Brito, J. A., Becker, J. O., and Preston, J. F. (2003). '*Candidatus Pasteuria usgae*' sp nov., an obligate endoparasite of the phytoparasitic nematode *Belonolaimus longicaudatus*. *Int. J. Sys. Evol. Microbiol.* **53,** 197–200.

Gravato-Nobre, M. J., Davies, K. G., von Mende, N., and Evans, K. (2001). The identification of cuticular and ES antigens conserved across some groups of plant parasitic and free living nematodes. *Int. J. Nematol.* **11,** 157–167.

Gravato-Nobre, M. J., McClure, M. A., Dolan, L., Calder, G., Davies, K. G., Mulligan, B., Evans, K., and von Mende, N. (1999). *Meloidogyne incognita* surface antigen epitopes in infected *Arabidopsis* roots. *J. Nematol.* **31,** 212–223.

Gravato-Nobre, M. J., Nicholas, H. R., Nijland, R., O'Rouke, D., Whittington, D. E., Yook, K. J., and Hodgkin, J. (2005). Multiple genes affect sensitivity of *Caenorhabditis elegans* to the bacterial pathogen *Microbacterium nematophilum*. *Genetics* **171,** 1033–1045.

Gravato-Nobre, M. J., von Mende, N., Dolan, L., Schmidt, K. P., Evans, K., and Mulligan, B. (1995). Immunolabelling of the cell surfaces of *Arabidopsis thaliana* roots following infection by *Meloidogyne incognita* (Nematoda). *J. Exp. Botany* **46,** 1711–1720.

Grenache, D. G., Caldicott, I., Albert, P. S., Riddle, D. L., and Politz, S. M. (1996). Environmental induction and genetic control of surface antigen switching in the nematode *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **93,** 12388–12393.

Grimshaw, C. E., Huang, S., Hanstein, C. G., Strauch, M. A., Burbulys, D., Wang, L., Hoch, J. A., and Whiteley, J. M. (1998). Synergistic kinetic interactions between components of the phosphorelay controlling sporulation in *Bacillus subtilis*. *Biochemistry* **37,** 1365–1375.

Haldane, J. B. S. (1949). Disease and evolution. *Ricerca Sci. Suppl.* **19,** 1–11.

Harris, T. W., Lee, R., Schwarz, E., Bradnam, K., Lawson, D., Chen, W., Blasier, D., Kenny, E., Cunningham, F., Kishore, R., Chan, J., Muller, H. -M., *et al*. (2003). WormBase: A cross-species database for comparative genomics. *Nucleic Acids Res.* **31,** 133–137.

Hemmer, R. M., Donkin, S. G., Chin, K. J., Grenache, D. G., Bhatt, H., and Politz, S. M. (1991). Altered expression of an L1-specific, O-linked cuticle surface glycoprotein in mutants of the nematode *Caenorhabditis elegans*. *J. Cell Biol.* **115,** 1237–1247.

Henriques, A. P., and Moran, C. P., Jr. (2007). Structure, assembly, function of the spore surface layers. *Ann. Rev. Microbiol.* **61,** 555–588.

Herman, R. K. (2004). The tale behind the worm. *Science* **303,** 42.

Hewlett, T. E., Gerber, J. F., and Smith, K. S. (2004). *In vitro* culture of *Pasteuria penetrans*. *In* ''Nematology Monographs and Perspectives.'' (Cook and Hunt, eds.), Vol. 2, pp. 175–185. *Proc. Fourth Int. Congr. Nematol.*

Hicks, S. J., Theodoropoulos, G., Carrington, S. D., and Corfield, A. P. (2000). The Role of mucins in host–parasite interactions. Part I—protozoan parasites. *Parasitol. Today* **16,** 476–481.

Himmelhoch, S., and Zuckerman, B. M. (1978). *Caenorhabditis briggsae* aging and the structural turnover of the outer cuticle surface and the intestine. *Exp. Parasitol.* **45,** 208–214.

Hoflich, J., Berninsone, P., Gobel, M. J., Gravato-Nobre, M. J., Libby, B. J., Darby, C., Politz, S. M., Hodgkin, J., Hirschberg, C. B., and Baumeister, R. (2004). Loss of a *srf-3*-encoded nucleotide sugar transporter activity in *Caenorhabditis elegans* alters surface antigenicity and prevents bacterial adherence. *J. Biol. Chem.* **279,** 30440–30448.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., and Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol. Biol. Evol.* **23,** 1792–1800.

Jensen, K. H., Little, T., Skorping, A., and Ebert, D. (2006). Empirical support for optimal virulence in a castrating parasite. *POLS Biol.* **4,** 1265–1269.

Jones, J. T., and Robertson, W. M. (1997). Nematode secretions. *In* ''Cellular and Molecular Aspects of Plant–Nematode Interactions.'' (Fenoll, Grundler, and Ohl, eds.) pp. 98–106. Kluwer Academic Publishers, Dordrecht.

Kaplan, D. T., and Davis, E. L. (1987). Mechanisms of plant incompatibility with nematodes. *In* ''Vistas on Nematology.'' (Veech and Dickson, eds.), pp. 267–276. Society of Nematologists, Inc., Hyattsville, MC.

Kerry, B. R. (2000). Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant parasitic nematodes. *Ann. Rev. Phytopathol.* **38,** 423–441.

Kojetin, D. J., Thompson, R. J., Benson, L. M., Naylor, S., Waterman, J., Davies, K. G., Opperman, C. H., Stephenson, K., Hoch, J. A., and Cavanagh, J. (2005). Structural analysis of divalent metals binding to the *bacillus subtilis* response regulator Spo0F: The possibility for *in vitro* metalloregulation in the initiation of sporulation. *Biometals* **18,** 449–466.

Kramer, J. M. (1997). Extracellular matrix. *In* ''C. elegans II.'' (Riddle, Blumenthal, Meyer, and Priess, eds.) pp. 417–500. Cold Spring Harbor Laboratory Press, New York.

Kozuka, S., and Tochibuko, K. (1985). Properties and origin of filamentous appendages on spores of *Bacillus cereus. Microbiol. Immunol.* **29,** 21–37.

Link, C. D., Silverman, M. A., Breen, M., Watt, K. E., and Dames, S. A. (1992). Characterisation of *Caenorhabditis elegans* lectin-binding mutants. *Genetics* **131,** 867–881.

Lin, H. J., and McClure, M. A. (1996). Surface Coat of *Meloidogyne incognita*. *J. Nematol.* **28,** 216–224.

Lis, H., and Sharon, N. (1993). Protein glycosylation: Structural and functional aspects. *Eur. J. Biochem.* **218,** 1–27.

Little, T. J., and Ebert, D. (2000). The cause of parasitic infection in natural populations of *Daphnia* (Crustacea: Cladocera): The role of genetics. *Proc. R. Soc. B* **267,** 2037–2042.

Little, T. J., Chadwick, W., and Watt, K. (2008). Parasite variation and the evolution of virulence in a *Daphnia*–microparasite system. *Parasitology* **135,** 303–308.

Loukas, A., Hintz, M., Linder, D., Mullin, N. P., Parkinson, J., Tetteh, K. K., and Maizels, R. M. (2000). A family of secreted mucins from the parasitic nematode *Toxacara canis* bears diverse mucin domains but shares similar flanking six-cysteine repeat motifs. *J. Biochem.* **275,** 39600–39607.

Maizels, R. M., Blaxter, M. L., Robertson, B. D., and Selkirk, M. E. (1991). "Parasite Antigens, Parasite Genes: A Laboratory Manual for Molecular Parasitology." Cambridge University Press, Cambridge.

Malakhov, V. V. (1994). "Nematodes: Structure, Development, Classification and Phylogeny." Smithsonian Institute Press, Washington.

Mankau, R. (1975). *Bacillus penetrans* n. comb. Causing a virulent disease of plant parasitic nematodes. *J. Invert. Pathol.* **26,** 333–339.

Maynard Smith, J. (1986). "The Problems of Biology." Oxford University Press, Oxford.

Masuda, Y., Hoson, T., Yamamoto, R., and Inouhe, M. (1989). Hormone-regulated modifications to cell wall polysaccharides: Relevence to cell separation. *In* ''Cell separation in plants" (Osborne, D. J. and Jackson, M. B. eds.), pp. 139–144. NATO ASI series, Vol. H25. Berlin: Springer-Verlag.

McClure, M. A., Misaghi, I., and Nigh, E. L. (1973). Shared antigens of parasitic nematodes and host plants. *Nature* **244,** 306–307.

Mendoza de Gives, P., Davies, K. G., Clark, S. J., and Behnke, J. M. (1999a). Predatory behaviour of trapping fungi against *srf* mutants of *Caenorhabditis elegans* and different plant and animal parasitic nematodes. *Parasitology* **119,** 95–104.

Mendoza de Gives, P., Davies, K. G., Morgan, M., and Behnke, J. M. (1999b). Attachment tests of *Pasteuria penetrans* to the cuticle of plant and animal parasitic nematodes, free living nematodes and *srf* mutants of *Caenorhabditis elegans*. *J. Helminthol.* **73,** 67–71.

Minton, N. A., and Sayre, R. M. (1989). Suppressive influence of *Pasteuria penetrans* in Georgia soil on reproduction of *Meloidogyne arenaria*. *J. Nematol.* **21,** 574–575.

Mohan, S., Fould, S., and Davies, K. G. (2001). The interaction between the gelatine binding domain of fibronectin and the attachment of *Pasteuria penetrans* endospores to nematode cuticle. *Parasitology* **123,** 271–276.

Mouton, L., and Ebert, D. (2008). Variable-number-of-tandem-repeats analysis of genetic diversity in *Pasteuria ramosa*. *Curr. Microbiol.* **56,** 447–452.

Mouton, L., Nong, G., Preston, J. F., and Ebert, D. (2007). Variable-number tandem repeats as molecular markers for biotypes of *Pasteuria ramosa* in *Daphnia* spp. *Appl. Environ. Microbiol.* **73,** 3715–3718.

Mukhopadhyay, D., Sen, U., Zapf, J., and Varughese, K. I. (2004). Metals in the sporulation phosphorelay: Manganese binding by the response regulator Spo0F. *Acta Crystallogr. D Biol. Crystallogr.* **60,** 638–645.

Müller-Reichert, T., Hohenberg, H., O'Toole, E. T., and McDonald, K. (2003). Cryoimmobilization and three-dimensional visualization of *C. elegans* ultrastructure. *J. Microscopy* **212,** 71–80.

Nuismer, S. L., and Otto, S. P. (2004). Host–parasite interactions and the evolution of ploidy. *Proc. Natl Acad. Sci. USA* **101,** 11036–11039.

Oostendorp, M., Dickson, D. W., and Mitchell, D. J. (1991). Population development of *Pasteuria penetrans* on *Meloidogyne arenaria*. *J. Nematol.* **23,** 58–64.

Opperman, C. H., Bird, D. M., Williamson, V. M., Rokhsar, D. S., Burke, M., Cohn, J., Cromer, J., Diener, S., Gajan, J., Graham, S., Houfek, T. D., Liu, Q., *et al*. (2008). Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. *Proc. Natl Acad. Sci. USA* **105,** 14802–14807.

Otto, S. P., and Nuismer, S. L. (2004). Species interactions and the evolution of sex. *Science* **304,** 1018–1020.

Page, A. P., Rudin, W., Fluri, M., Blaxter, M. L., and Maizels, R. M. (1992). *Toxocara canis*: A labile antigenic surface coat overlying the epicuticle of infective larvae. *Exp. Parasitol.* **75,** 72–86.

Persidis, A., Lay, J. G., Manousis, T., Bishop, A. H., and Ellar, D. J. (1991). Characterisation of potential adhesions of the bacterium *Pasteuria penetrans*, and of putative receptors on the cuticle of *Meloidogyne incognita*, a nematode host. *J. Cell Sci.* **100,** 613–622.

Plomp, M., Leighton, T. J., Wheeler, K. E., and Malkin, A. J. (2005a). The high-resolution architecture and structural dynamics of *Bacillus* spores. *Biophys. J.* **88,** 603–608.

Plomp, M., Leighton, T. J., Wheeler, K. E., and Malkin, A. J. (2005b). Architecture and high-resolution structure of *Bacillus thuringiensis* and *Bacillus cereus* spore coat surfaces. *Langmuir* **21,** 7892–7998.

Politz, S. M., and Philipp, M. (1992). *Caenorhabditis elegans* as a model for parasitic nematodes: A focus on the cuticle. *Parasitol. Today* **8,** 6–12.

Politz, S. M., Philipp, M., Estevez, P. J., O'Brian, P. J., and Chin, K. J. (1990). Genes that can be mutated to unmask hidden antigenic determinants in the cuticle of the nematode *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **87,** 2901–2905.

Power, P. M., and Jennings, M. P. (2003). The genetics of glycosylation in Gram-negative bacteria. *FEMS Microbiol. Lett.* **218,** 211–222.

Powers, T. (2004). Nematode molecular diagnostics: From bands to barcodes. *Ann. Rev. Phytopathol.* **42,** 367–383.

Preston, J. F., Dickson, D. W., Maruniak, J. E., Nong, G., Brito, J. A., Scmidt, L. M., and Giblin-Davis, R. M. (2003). *Pasteuria* spp.: Systematics and phylogeny of these bacterial parasites of phytopathogenic nematodes. *J. Nematol.* **35,** 198–207.

Proudfoot, L., Kusel, J. R., Smith, H. V., and Kennedy, M. W. (1993). Rapid changes in the surface of parasitic nematodes during transition from pre- to post-parasitic forms. *Parasitology* **107,** 107–117.

Rattray, A. J., and Strathern, J. N. (2003). Error-prone DNA polymerase: When making a mistake is the only way ahead. *Ann. Rev. Genet.* **37,** 31–66.

Refardt, D., and Ebert, D. (2007). Inference of a parasite local adaption using two different fitness components. *J. Evol. Biol.* **20,** 921–929.

Regoes, R. R., Hottinger, J. W., Sygnarski, L., and Ebert, D. (2003). The infection rate of *Daphnia magna* by *Pasteuria ramosa* conforms with the mass-action principle. *Epidemiol. Infect.* **131,** 957–966.

Rich, J. R., Dunn, R. A., and Noling, J. W. (2004). Nematicides: Past and present uses. *In* ''Nematology Advances and Perspectives Volume 2: Nematode Management and Utilisation.'' (Chen, Chen, and Dickson, eds.) pp. 1179–1200. CABI Publishing, Wallingford, United Kingdom.

Riding, I. L. (1970). Microvilli on the outside of a nematode. *Nature* **226,** 179–180.

Sayre, R. M., and Starr, M. P. (1985). *Pasteuria penetrans* (Ex Thorne, 1940). nom-rev, comb-n, sp-n, a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. *P. Helm. Soc. Wash.* **52,** 149–165.

Sayre, R. M., Starr, M. P., Golden, A. M., Wergin, W. P., and Endo, B. Y. (1988). Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a related mycelial and endospore-forming bacterial parasite from *Pratylenchus brachyurus*. *P. Helm. Soc. Wash.* **55,** 28–49.

Sayre, R. M., Wergin, W. P., Schmidt, J. M., and Starr, M. P. (1991). *Pasteuria nishizawae* sp-nov, a mycelial and endospore-forming bacterium parasitic on cyst nematodes of genera *Heterodera* and *Globodera*. *Res. Microbiol.* **142,** 551–564.

Schmidt, L. M., Preston, J. F., Nong, G., Dickson, D. W., and Aldrich, H. C. (2004). Detection of *Pasteuria penetrans* infection in *Meloidogyne arenaria* race 1 in planta by polymerase chain reaction. *FEMS Microbiol. Ecol.* **48,** 457–464.

Sharma, S. B., and Davies, K. G. (1996). Characterisation of *Pasteuria* isolated from *Heterodera cajani* using morphology, pathology and serology of endospores. *Syst. Appl. Microbiol.* **19,** 106–112.

Sharon, E., Spiegel, Y., Salomon, R., and Curtis, R. H. C. (2002). Characterisation of *Meloidogyne javanica* surface coat with antibodies and their effect on nematode behaviour. *Parasitology* **125,** 177–185.

Spiegel, Y., and McClure, M. A. (1995). The surface coat of plant-parasitic nematodes: Chemical composition, origin and biological role—a review. *J. Nematol.* **27,** 127–134.

Steichen, C. T., Chen, P., Kearney, J. F., and Turnbough, C. L., Jr. (2003). Identification of an immunodominant protein and other proteins of the *Bacillus anthracis* exosporium. *J. Bacteriol.* **185,** 1903–1910.

Stirling, G. R. (1984). Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. *Phytopathology* **74,** 55–60.

Stirling, G. R. (1985). Host specificity in *Pasteuria penetrans* within the genus *Meloidogyne*. *Nematologica* **31,** 203–209.

Stirling, G. R. (1991). "Biological Control of Plant Parasitic Nematodes: Progress Problems and Prospects." CABI Publishing, Wallingford, United Kingdom.

Stirling, G. R., and Wachtel, M. F. (1980). Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. *Nematologica* **26,** 308–312.
Strous, G. J., and Dekker, J. (1992). Mucin-type glycoproteins. *Crit. Rev. Biochem. Mol. Biol.* **27,** 57–92.
Sturhan, D., Shutova, T. S., Alimov, V. N., and Subbotin, S. A. (2005). Occurrence, hosts, morphology, and molecular characterisation of *Pasteuria* bacterial parasite in nematodes of the family Pectidae. *J. Invert. Pathol.* **88,** 17–26.
Subbotin, S. A., Chizhov, V. N., and Zakharenkova, N. N. (1993). Ultrastructure of the body wall of parasitic and infective females of *Skarbilovinema laumondi* (Tylenchida: Iotonchiidae). *Fund. Appl. Nematol.* **16,** 1–4.
Subbotin, S. A., Chizhov, V. N., and Zakharenkova, N. N. (1994). Ultrastructure of the integument of parasitic females in entomogenous tylenchids. 1. Two species of the genus *Wachekitylenchus, Allantonema mirabile* and *Bradynema rigidum*. *Russiona J. Nematol.* **2,** 105–112.
Sylvestre, P., Couture-Tosi, E., and Mock, M. (2002). A collagen-like surface glycoprotein is a structural component of the *Bacillus anthracis* exosporium. *Mol. Microbiol.* **45,** 169–178.
Sylvestre, P., Couture-Tosi, E., and Mock, M. (2003). Polymorphism in the collagen-like region of the *Bacillus anthracis* BclA protein leads to variation in length in the exosporium filament length. *J. Bacteriol.* **185,** 5155–5163.
Sylvestre, P., Couture-Tosi, E., and Mock, M. (2005). Contribution of ExsFA and ExsFB proteins to the localisation of BclA on the spore surface and to the stability of the *Bacillus anthracis* exosporium. *J. Bacteriol.* **187,** 5122–5128.
Szymanski, C. M., and Wren, B. W. (2005). Protein glycosylation in bacterial mucosal pathogens. *Nat. Rev. Microbiol.* **3,** 225–237.
Takeuchi, K., Taguchi, F., Inagaki, Y., Toyoda, K., Shiraishi, T., and Ichinose, Y. (2003). Flagellin glycosylation island in *Pseudomonas syringae* pv. Glycinea and its role in host specificity. *J. Bacteriol.* **185,** 6658–6665.
Takumi, K., Kinouchi, T., and Kawata, T. (1979). Isolation and partial characterisation of exosporium from spores of a highly sporogenic mutant of *Closteridium botulinum* Type-A. *Microbiol. Immunol.* **23,** 443–454.
Theodoropoulos, G., Hicks, S. J., Corfield, A. P., Miller, B. G., and Carrington, S. D. (2001). The role of mucins in host–parasite interactions: Part II—helminth parasites. *Trends Parasitol.* **17,** 130–135.
Tetteh, K. K. A., Loukas, A., Tripp, C., and Maizels, R. M. (1999). Identification of abundantly expressed novel and conserved genes from the infective larval stage of *Toxacara canis* by an expressed sequence tag strategy. *Infect. Immunol.* **67,** 4771–4779.
Thompson, I. J., and Burdon, J. J. (1992). Gene-for-gene coevolution between plants and parasites. *Nature* **360,** 121–125.
Tigano, M. S., Carneiro, R. M. D. G., Jeyaprakash, A., Dickson, D. W., and Adams, B. J. (2005). Phylogeny of Meloidogyne spp. based on 18S rDNA and the intergenic region of mitochondrial DNA sequences. *Nematology* **7,** 851–862.
Todd, S. L., Moir, A. J. G., Johnson, M. J., and Moir, A. (2003). Genes of *Bacillus cereus*, and *B. anthracis* encoding proteins for the exosporium. *J. Bacteriol.* **185,** 3373–3378.
Trudgill, D. L., Bala, G., Blok, V. C., Daudi, A., Davies, K. G., Fargette, M., Gowen, S. R., Madulu, J. D., Mateille, T., Mwageni, W., Netscher, C., Phillips, M. S., *et al.* (2000). The importance of tropical root-knot nematodes (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria penetrans* as a biocontrol agent. *Nematology* **2,** 823–845.
Vale, P. F., Stjernman, M., and Little, T. J. (2008). Temperature-dependent costs of parasitism and maintenance of polymorphism under genotype-by-environment interactions. *J. Evol. Biol.* **21,** 1418–1427.

Van der Putten, W. H., Cook, R., Costa, S., Davies, K. G., Fargette, M., Freitas, H., Hol, W. H. G., Kerry, B. R., Maher, N., Mateille, T., Moens, M., *et al.* (2006). Nematode interactions in nature: Models for sustainable control of nematode pests of crop plants. *Adv. Agronomy* **89,** 227–260.

Van Valen, L. (1973). A new evolutionary law. *Evol. Theor.* **1,** 1–30.

Wehrli, E., Scherrer, P., and Kubler, O. (1980). The crystalline layer in spores of *Bacillus cereus* and *Bacillus thuringiensis* studied by freeze-etching and high resolution electron microscopy. *Eur. J. Cell Biol.* **20,** 283–289.

Weibelzahl-Fulton, E., Dickson, D. W., and Whitty, E. B. (1996). Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in field soil. *J. Nematol.* **28,** 43–49.

Weiser, J. N. (2000). The generation of diversity in *Haemophilus influenzae*. *Trends Microbiol.* **8,** 433–435.

Winslow, R. D. (1960). Some aspects of the ecology of free-living and plant-parasitic nematodes. *In* ''Nematology, Fundamentals and Recent Advances with Emphasis on Plant-parasitic and Soil Forms.'' (Sasser and Jenkins, eds.), pp. 341–415. University of North Carolina Press, Chapel Hill.

Wishart, J., Blok, V. C., Phillips, M. S., and Davies, K. G. (2004). *Pasteuria penetrans* and *P. nischizawae* attachment to *Meloidogyne chitwoodi, M. fallax* and *M. hapla*. *Nematology* **6,** 507–510.

Wright, K. A. (1987). The nematode's cuticle—its surface and epidermis: Function, homology, analogy—a current consensus. *J. Parasitol.* **73,** 1077–1083.

Yeates, G. W., Bongers, T., De Goede, R. G. M., Freckman, D. W., and Georgieva, S. S. (1993). Feeding-habits in soil nematode families and genera—an outline for soil ecologists. *J. Nematol.* **25,** 315–331.

Yook, K., and Hodgkin, J. (2007). Mos1 mutagenesis reveals a diversity of mechanisms affecting the response of *Caenorhabditis elegans* to the bacterial pathogen *Microbacterium nematophilum*. *Genetics* **175,** 681–697.

Zapf, J., Hoch, J. A., and Whitely, J. M. (1996). A phosphotransferase activity of the *bacillus subtilis* sporulation protein Spo0F that employs phosphoramidate substrates. *Biochemistry* **35,** 2926–2933.

Zuckerman, B. M., Kahane, I., and Himmelhoch, S. (1979). *Caenorhabditis briggsae* and *C. elegans* partial characterization of the cuticle surface carbohydrates. *Exp. Parasitiol.* **47,** 419–424.