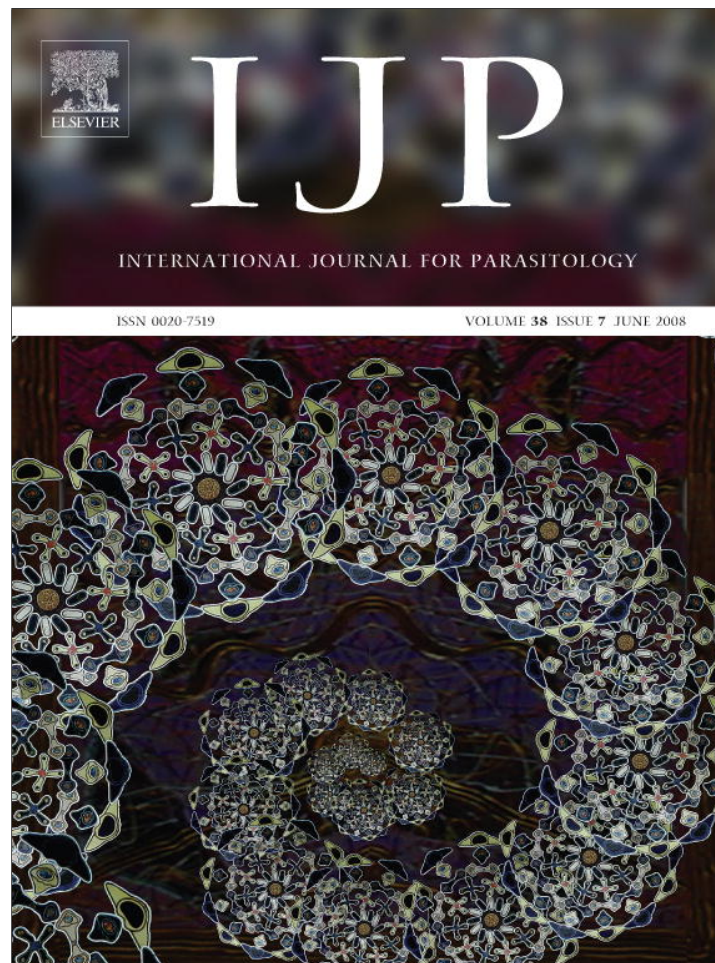


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# Inter- and intra-specific cuticle variation between amphimictic and parthenogenetic species of root-knot nematode (*Meloidogyne* spp.) as revealed by a bacterial parasite (*Pasteuria penetrans*)

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## Abstract

Specific host–parasite interactions exist between species and strains of plant parasitic root-knot nematodes and the Gram-positive bacterial hyperparasite *Pasteuria penetrans*. This bacterium produces endospores that adhere to the cuticle of migrating juveniles, germinate and colonise the developing female within roots. Endospore attachment of *P. penetrans* populations to second-stage juveniles of the root-knot nematode species *Meloidogyne incognita* and *Meloidogyne hapla* showed there were interactive differences between bacterial populations and nematode species. Infected females of *M. incognita* produced a few progeny which were used to establish two nematode lines from single infective juveniles encumbered with either three or 26 endospores. Single juvenile descent lines of each nematode species were produced to test whether cuticle variation was greater within *M. hapla* lines that reproduce by facultative meiotic parthenogenesis than within lines of *M. incognita*, which reproduces by obligate parthenogenesis. Assays revealed variability between broods of individual females derived from single second-stage juvenile descent lines of both *M. incognita* and *M. hapla* suggesting that progeny derived from a single individual can differ in spore adhesion in both sexual and asexual nematode species. These results suggest that special mechanisms that produced these functional differences in the cuticle surface may have evolved in both sexually and asexually reproducing nematodes as a strategy to circumvent infection by this specialised hyperparasite.

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**Keywords:** Immunity; Mode of reproduction; Surface coat; Plant parasitic nematode; Pathogenesis

## 1. Introduction

One widely proposed benefit of sexual reproduction in nematodes is that it generates genetically diverse offspring to facilitate their escape from parasites and potential pathogens (Hurst and Peck, 1996). The occurrence and maintenance of asexual lineages over time is therefore intriguing as this mode of reproduction is predicted to reduce such diversity (Judson and Normark, 1996; Normark et al., 2003). Nematodes represent a group of metazoans with mechanisms of reproduction ranging from

amphimixis to obligate mitotic parthenogenesis (Evans, 1998), thus providing an opportunity to investigate the role of sexual reproduction in facilitating survival of parasite infection. Over the last several years a number of studies have investigated the interaction between root-knot nematodes (*Meloidogyne* spp.) and the Gram-positive bacterium *Pasteuria penetrans*. This obligate parasitic bacterium exhibits host specificity at two stages in the nematode life cycle; firstly, endospore isolates differ in their ability to encumber the second-stage juveniles (J2s) of particular species and strains of root-knot nematodes (Davies et al., 1988, 2001; Davies, 2005) and second, endospore populations differ in adhesion to different life stages of the same strain as exemplified by differential attachment to males (Davies and Williamson, 2006).

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The *Pasteuria* group of Gram-positive endospore forming bacteria are hyperparasites of plant parasitic nematodes and water fleas (*Daphnia* spp.; Cladocera: Anomopoda). 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). All the economically important genera of plant parasitic nematodes are parasitised by *Pasteuria*. To date five species of *Pasteuria* have been described that differ in their host ranges and pathogenicity on nematodes: (i) *P. penetrans* is parasitic on *Meloidogyne* spp., (Sayre and Starr, 1985); (ii) *Pasteuria thornei* parasitises *Pratylenchus brachyurus* (Sayre et al., 1988); (iii) *Pasteuria nishizawae* parasitises cyst nematodes (Sayre et al., 1991); (iv) *Pasteuria usgae* is parasitic on the sting nematode *Belonolaimus longicaudatus* (Giblin-Davis et al., 2003) and (v) *Pasteuria hartismeri* is parasitic on *Meloidogyne ardenensis* (Bishop et al., 2007). These bacteria initiate their infection by adhering to the cuticle of infective juveniles, then penetrate the body wall to enter the host root, usually after the nematode has set up a feeding site (Chen et al., 1996, 1997). Parasitised root-knot nematode females produce few, if any, progeny; therefore this bacterium has potential for its development as a biological control agent of these economically important crop pests (Stirling, 1991). However, the inability to mass culture the bacterium in vitro and its restricted host range are two constraints to commercial development.

The nematode cuticle is an essential structure for maintaining the hydrostatic skeleton and for locomotion; it also provides a barrier against the environment, including potentially damaging micro-organisms. It is a complex multilayered structure in which collagens play a major role and above which is a lipid-rich membrane-like epicuticular layer that can undergo biochemical transformation upon infection of the host (Proudfoot et al., 1990). In animal parasitic nematodes the surface of the cuticle, the glycocalyx, is rich in carbohydrate, plays a pivotal role as the interface between the nematode and its host, and is important in recognition of the nematode by the host immune system (Blaxter et al., 1992; Politz and Philipp, 1992; Maizels et al., 1993). There is increasing evidence that novel glycans are positioned to play an important role in the parasite's interaction with its host (Haslam et al., 2000; Appleton and Romaris, 2001). The surface coat of plant parasitic nematodes also has a glycocalyx that may have a similar role in the resistance of plants to nematodes (Kaplan and Davis, 1987). The glycocalyx is also the site of attachment for various fungi and bacteria that are parasites of nematodes (Timper and Davies, 2004). However, of the many groups of micro-organisms that inhabit the soil, very few have the ability to adhere to the cuticle and infect plant parasitic nematodes.

The most damaging root-knot nematodes, *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica*, have varying degrees of polyploidy and aneuploidy (Triantaphyllou, 1985; Trudgill and Blok, 2001; Castagnone-Sereno, 2006) and reproduce by mitotic parthenogenesis,

which is predicted to give rise to clonal progeny (Trudgill, 1997). This mode of reproduction should, therefore, result in clonal lines uniformly susceptible to an individual isolate of a bacterial parasite. However, attachment studies of isolates of *Pasteuria* to different populations and strains of root-knot nematodes showed a high degree of variation (Davies et al., 1988; Espanol et al., 1997; Mendoza de Gives et al., 1999b). In a study comparing adhesion of six endospore populations to the cuticles of J2s of a wide range of root-knot nematode species, it was found that adhesion was not linked to the phylogeny of the nematode (Davies et al., 2001). The surface of the J2 cuticle must be a key determinant in endospore adhesion, and therefore this finding begs the question of how nematodes that reproduce by mitotic parthenogenesis maintain cuticle variability to avoid extinction in the face of such an aggressive hyperparasite. Parthenogenic root-knot nematodes can display within-species differences in host range and in virulence on different cultivars of a host plant species (Roberts, 1995) as well as variability in *Pasteuria* susceptibility. The nematode genes involved in maintaining this variability have not been identified, due in part to the lack of a tractable genetic system to investigate their inheritance.

While *M. hapla* Race B reproduces by mitotic parthenogenesis, the majority of isolates of this species belong to Race A, which undergoes meiosis and reproduces by facultative meiotic parthenogenesis (Triantaphyllou, 1966, 1985; Van der Beek et al., 1998). Meiosis occurs in Race A females and, if males are present and there are sperm in the spermatheca, the sperm nucleus will fuse with the haploid egg pronucleus to form a sexual diploid embryo. However, if males are absent the sister products of meiosis II will fuse. Due to fusion of sister nuclei, meiotic parthenogenesis results in rapid genomic homozygosity (Liu et al., 2007).

This present study investigates within-strain variability in endospore adhesion by utilising single J2 descent lines of root-knot nematodes of mitotic, obligately parthenogenetic and meiotic, facultatively parthenogenetic species. We make use of the life cycle of root-knot nematodes in which the progeny of each female comprising several hundred eggs is deposited into a single, gelatinous egg mass as well, as our ability to establish lines descended from a single individual, to examine variability within broods of a single female and in single-nematode (J2) descent lines. The hypothesis that within-strain cuticle variation will differ between root-knot nematode species that have different modes of reproduction is tested.

## 2. Materials and methods

### 2.1. Nematode strains and lines

*Meloidogyne incognita* strain 020804 was derived from a single egg mass isolated on 02 August 2004. Race A *M. hapla* strains VW8 and VW9 were developed by inbreeding isolates for 18 generations by sequentially transferring a

single egg mass onto tomato plants (Liu and Williamson, 2006). These strains were maintained in the glasshouse at Rothamsted Research at 20 °C on tomato, cultivar Pixie, was grown in peat/sand (1:1, v/v) with a 16 h day 8 h night periodicity. Single juvenile lines of descent were established by placing individual J2s on small tomato seedlings and maintaining those at 20 °C. Nematodes were hatched from egg masses dissected from root material and placed on a small sieve in a tray of water at room temperature (Hooper, 1986). Each egg mass, representing the brood of a single female, was hatched in a separate tray.

## 2.2. *Pasteuria* populations

Endospores of *Pasteuria* populations RES147 and PP3 were cultured on *M. incognita*, strain 020804, using an adapted method of Stirling and Wachtel (1980) in which, instead of allowing the roots to dry, infected female nematodes were dissected from root galls, collected in a small volume of water and crushed with a small tissue grinder. Endospores were counted using a haemocytometer slide; their concentrations were adjusted to  $10^6$  spores  $\text{ml}^{-1}$  and frozen (−20 °C) until needed.

## 2.3. Endospore attachment bioassay

A 100  $\mu\text{l}$  sample of a suspension of freshly hatched juveniles (1,000 J2  $\text{ml}^{-1}$ ) was placed in a silicone-coated microtube (0.5 ml) together with a 100  $\mu\text{l}$  sample of a *Pasteuria* endospore suspension and centrifuged together for 5 min at 10,000g (Hewlett and Dickson, 1993). After centrifugation the nematodes were removed from the microtube and examined using an inverted microscope (200 $\times$ ). Endospores adhering to the cuticle of a minimum of 20 individuals (Chen et al., 1997) were counted.

## 2.4. Data analysis

Means and standard errors were calculated and ANOVA was performed using either Microsoft Excel 2002 for Windows or GENSTAT version 10.0. A  $P$  value < 0.05 was considered significant.

## 3. Results

### 3.1. Comparison of spore adhesion of two *Pasteuria* populations to *M. incognita* and *M. hapla* parental strains

The differences in attachment between endospores of *Pasteuria* populations RES147 and PP3 to the parental root-knot nematode strains were large ( $P < 0.001$ ; Fig. 1), and there was a statistically significant interaction between nematode strain and *Pasteuria* isolate ( $P = 0.003$ ). *Pasteuria* population RES147 encumbered the *M. incognita* strain Mi020804 with a mean of two endospores  $\text{J2}^{-1}$  and each of the *M. hapla* strains VW8 and VW9 with one or fewer endospore  $\text{J2}^{-1}$ . Whereas all of the nematode strains had more

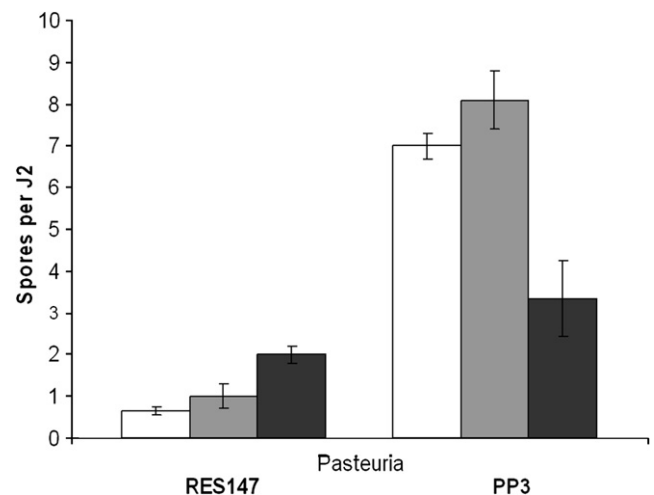


Fig. 1. Comparison between endospore adhesion to *Meloidogyne incognita* and *Meloidogyne hapla* strains. Mean number of *Pasteuria* spores of populations RES147 and PP3 attaching to second-stage juveniles of *M. hapla* strains VW8 (white), VW9 (grey) and *M. incognita* 020804 (black). SEM are shown. Using ANOVA,  $P < 0.001$ .

adhering endospores of *Pasteuria* population PP3 than of population RES147, J2s of the *M. hapla* parental strains had around seven endospores  $\text{J2}^{-1}$  compared with Mi020804 being encumbered with fewer than four endospores  $\text{J2}^{-1}$ .

### 3.2. Spore adhesion in single J2 descent lines of *M. incognita*

To test the hypothesis that the next generation resulting from J2s encumbered with very few spores (three) and progeny from J2s encumbered with over 26 spores would differ in affinity for endospores, two single J2 descent lines were established (Fig. 2A), one originating from an individual J2 encumbered with three spores (designated Mi020804-3S) and another originating from a J2 encumbered with 26 spores (designated Mi020804-26S). Preliminary experiments had shown that developing females infected by *Pasteuria* each produced a small number of eggs. Hatched J2s from these eggs were able to reinvade the root system to produce fully fertile females. This was exploited to obtain the two specific nematode lines derived from single juveniles encumbered with either three or 26 endospores. These individual juveniles represented the extreme high (26 endospores) and extreme low (three endospores) end of endospore attachment in a standard attachment assay. These two individual J2s of *M. incognita* encumbered with either three or 26 infective *Pasteuria* endospores (population RES147) were inoculated onto the roots of small tomato seedlings (3 weeks old) and maintained in the glasshouse at 25 °C. These single J2 descent lines were then carefully maintained and sub-cultured, taking care to avoid cross-contamination.

In attachment assays, the mean number of endospores of PP3 adhering to J2s of line Mi020804-3S was 15.6 endospores  $\text{J2}^{-1}$  compared with 20.5 endospores  $\text{J2}^{-1}$  for line

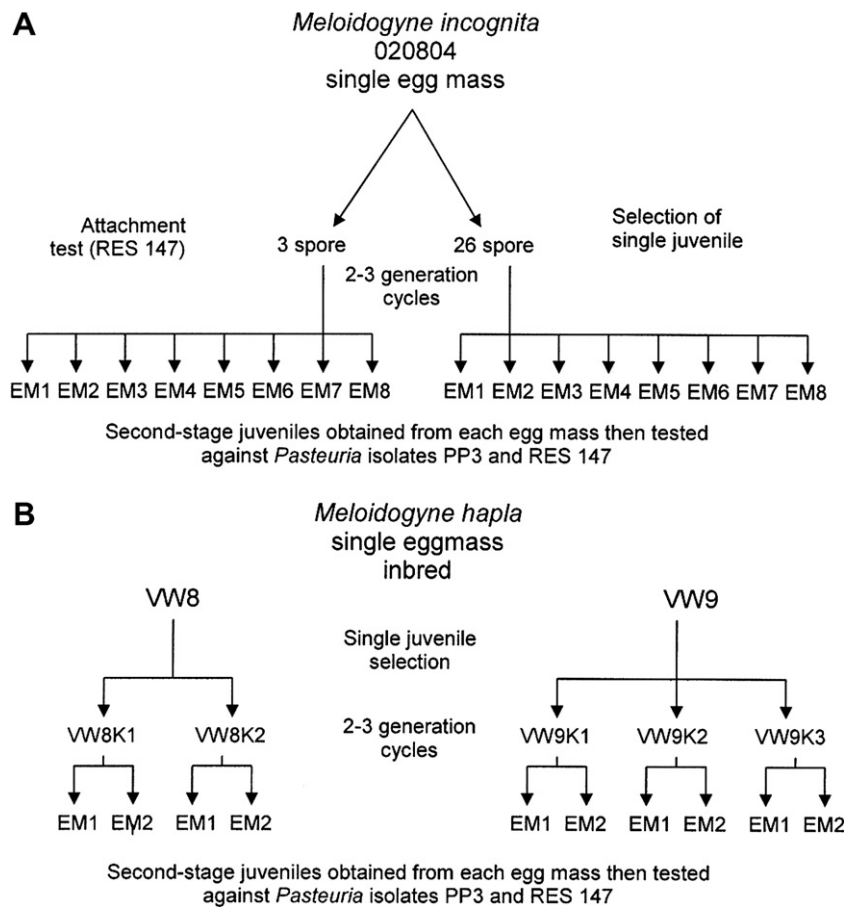


Fig. 2. Diagram of strategy for producing single second-stage juvenile (J2) descent lines. (A) Single J2 of *Meloidogyne incognita* 020804 encumbered with three endospores or 26 endospores of RES147 were used to infect tomato seedlings and establish lines 020804-3S and 020804-26S. After two to three generation cycles, eight egg masses were collected from each line and juveniles from these egg masses were used in attachment assays. (B) Single juveniles of *Meloidogyne hapla* strain VW8 were used to infect tomato seedlings and two single J2 descent lines were obtained, VW8K1 and VW8K2. Similarly, three single J2 descent lines were produced from *M. hapla* strain VW9. After two to three generation cycles, two egg masses were collected from each single descent line and juveniles from each were used to assess spore attachment. EM, egg mass.

Mi020804-26S (Fig. 3). Although this difference was statistically significant ( $P < 0.001$ ), a large amount of overlap was seen between the mean of spores attached to progeny from single egg masses of each of the two groups. Indeed, the differences between attachment to the individual single egg mass progeny derived from within the 3S line was not significant ( $P = 0.117$ ), whereas the differences between the progeny from individual single egg masses within the 26S line were significant ( $P < 0.005$ ). For *P. penetrans* population RES147, the number of endospores adhering to progeny of individual egg masses of the 3S and 26S lines ranged from a mean of less than 1 endospore  $J2^{-1}$  to just over 3.5 endospores  $J2^{-1}$  with a mean of 1.6 endospores  $J2^{-1}$  for the 3S line and 1.18 endospores  $J2^{-1}$  for the 26S line (Fig. 4). This small difference was statistically significant ( $P = 0.011$ ). The differences in the number of endospores attached to J2s from single egg masses from within the 3S and the 26S lines was also statistically significant for both groups ( $P < 0.001$ ). Again, a large amount of overlap in the number of attached endospores occurred between these two groups.

### 3.3. Spore adhesion to single J2 descent lines of *M. hapla*

There was no statistically significant difference in endospore attachment to parental lines of VW8 and VW9 (Fig. 1). Single juvenile descent lines of *M. hapla* VW8 and VW9 were produced by placing individual J2s of VW8 and VW9 on small tomato seedlings as described above and maintaining those at 20 °C. Two lines of *M. hapla* VW8 (VW8K1 and VW8K2) and three of VW9 (VW9K1, VW9K2 and VW9K3) were established (Fig. 2B).

The mean number of endospores of RES147 and PP3 attaching to the single J2 descent lines derived from VW8 were 1.85 and 6.7 endospores  $J2^{-1}$  (Fig. 5). Although there was no statistically significant difference in the numbers of endospores attaching to juveniles from each of the two egg masses descended from the same single J2 line, there were statistically significant interactions between the nematode lines and the *Pasteuria* isolate ( $P < 0.001$ ). The mean number of endospores adhering to each of the two *M. hapla* lines was 0.45 and 3.2 endospores  $J2^{-1}$  of RES147 adhering

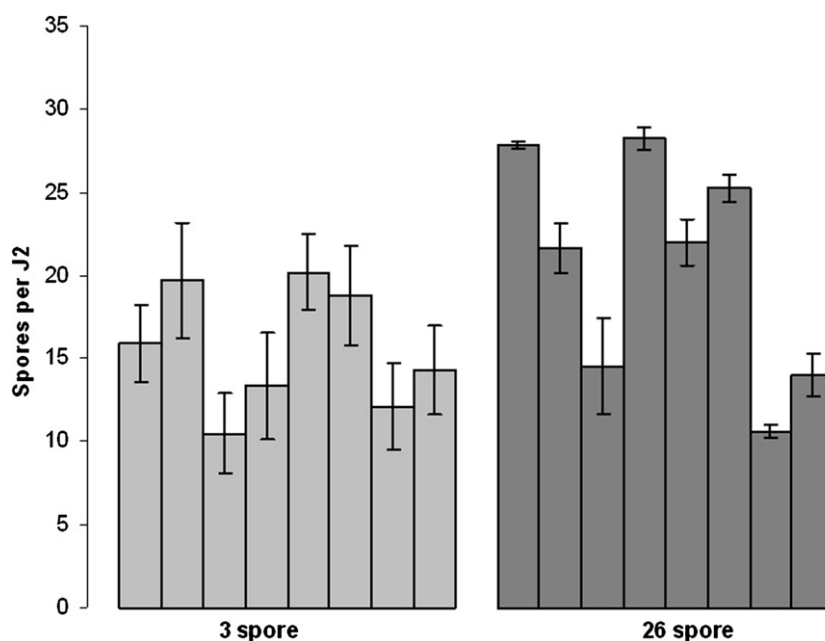


Fig. 3. Comparison between endospore attachment of *Pasteruria* population PP3 to single juvenile descendents. Mean endospore attachment to second-stage juveniles of *Meloidogyne incognita* population 020804 from eight individual egg masses produced from single juvenile populations S3 and 26S. SEM are shown. Using ANOVA,  $P < 0.001$ .

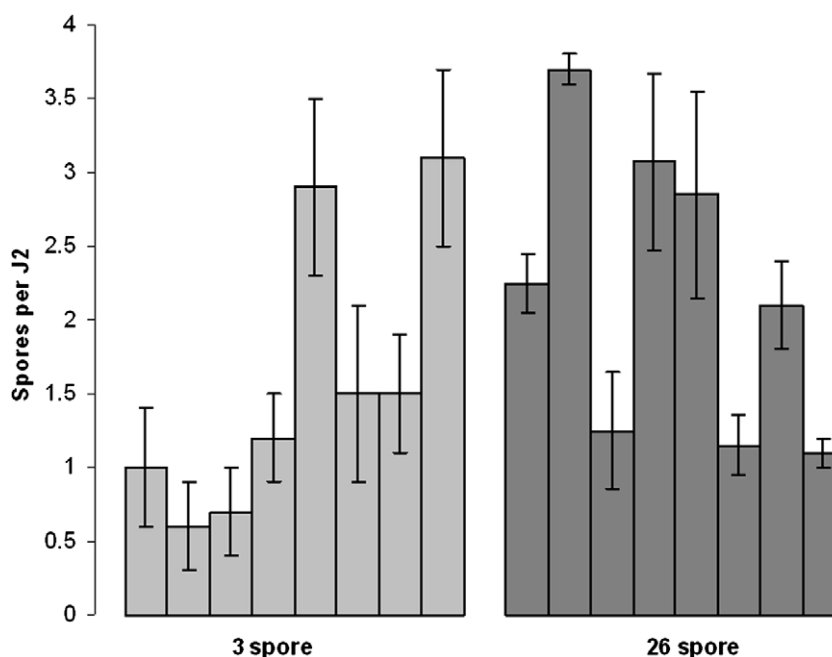


Fig. 4. Comparison between endospore attachment of *Pasteruria* population RES147 to single juvenile descendents. Mean endospore attachment to second-stage juveniles of *Meloidogyne incognita* population 020804 from eight single individual egg masses produced from single juvenile populations three spore and 26 spore. SEM are shown. Using ANOVA,  $P < 0.001$ .

to lines VW8K1 and VW8K2, respectively, and 10.4 and 1.75 endospores  $J2^{-1}$  of PP3 adhering in turn to VW8K1 and VW8K2. The mean number of endospores of RES147 and PP3 attaching to the three single  $J2$  descent lines VW9K1, VW9K2 and VW9K3, were 0.62 and 7.5, respectively (Fig. 6). Again, although there was no statistically significant difference in the number of endospores

attaching to juveniles between egg masses derived from the same line, there were statistically significant interactions ( $P = 0.008$ ). The mean numbers of endospores of RES147 adhering to lines VW9K1, VW9K2 and VW9K3 were 0.4, 1.5 and 0.4 endospores  $J2^{-1}$ , respectively, and 10.3, 4.4 and 7.5 endospores  $J2^{-1}$  of PP3 adhering to the same lines, respectively.

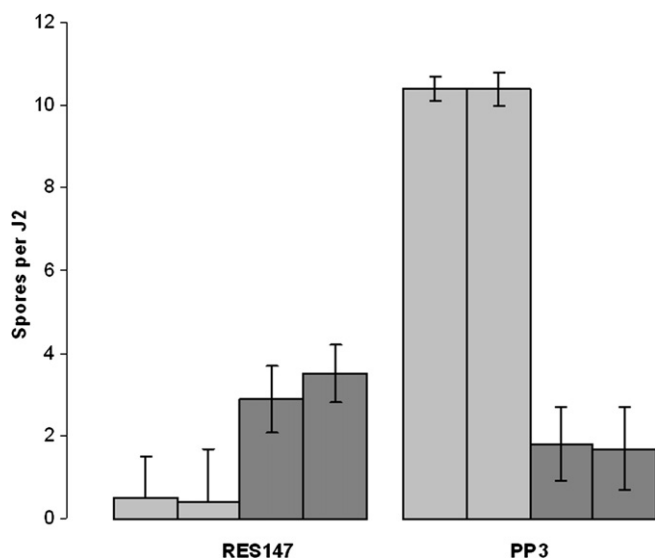


Fig. 5. Comparison between mean endospore attachment to second-stage juveniles from single juvenile descent lines. *Meloidogyne hapla* line VW8K1 (light grey) and VW8K2 (dark grey) were forced to reproduce through meiotic parthenogenesis and exposed to *Pasteuria* populations RES147 and PP3. SEM are shown. Using ANOVA,  $P < 0.001$ .

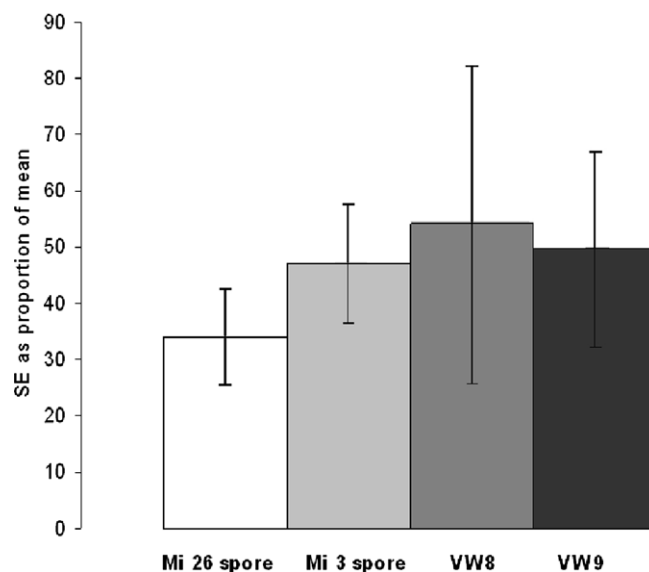


Fig. 7. Standard error as a proportion of the mean of the number of endospores of *Pasteuria* population PP3 adhering to juveniles from individual egg masses of single juvenile descent lines Mi 26 Spore, Mi 3 Spore, VW8 and VW9. SEM are shown. Using ANOVA,  $P =$  not significant.

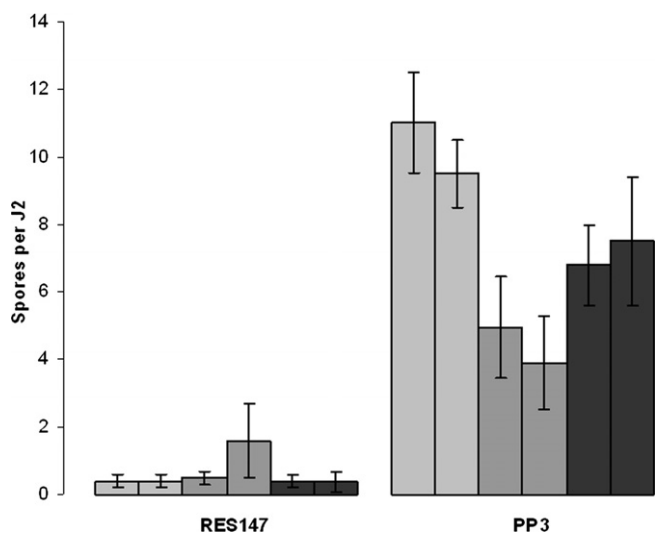


Fig. 6. Comparison between mean endospore attachment to second-stage juveniles from single juvenile descent lines of *Meloidogyne hapla* Line VW9K1 (light grey), VW9K2 (dark grey) and VW9K3 (black) which were forced to reproduce through meiotic parthenogenesis and exposed to *Pasteuria* populations RES147 and PP3. SEM are shown. Using ANOVA,  $P < 0.001$ .

A comparison of the standard error expressed as a proportion of the mean across the groups of nematodes reproducing by obligate parthenogenesis and those reproducing by facultative meiotic parthenogenesis showed a large overlap and there was no statistically significant difference between those (Fig. 7). Subsequent testing of lines following 9 months of continuous culture showed the levels of attachment remained similar (K.G. Davies, unpublished data).

#### 4. Discussion

The initial stage in the infection of root-knot nematodes by *P. penetrans* involves the attachment of endospores to the J2 cuticle. Numerous studies have shown that isolates of the bacterium differ in their ability to encumber specific nematode populations (Davies et al., 1988, 2001; Espanol et al., 1997). However, to date there have been no specific comparisons made between or within root-knot nematode species that maintain different reproductive strategies. We show here that there is considerable within-strain variation in the attachment of endospores both to root-knot nematodes that reproduce by obligate parthenogenesis and to those that reproduce by facultative meiotic parthenogenesis. We also produce evidence that the level of variation in attachment to both groups of nematodes does not differ significantly.

Comparing the attachment of endospores to J2s of single juvenile descent lines derived from Mi020804 encumbered with either three or 26 endospores, revealed considerable overlap in the numbers of adhering endospores. The same overall result is obtained independently with each of the *Pasteuria* populations tested, although in general *Pasteuria* isolate PP3 shows greater ability to encumber the J2s than does isolate RES147. There were statistically significant differences in levels of mean attachment between the individual egg masses originating from the 3S line ( $P < 0.05$ ) and the 26S line ( $P < 0.001$ ). This suggests that there may be more involved than an inherited genetic component that affects the surface of the J2 cuticle and renders it more or less susceptible to endospore encumbrance.

The numbers of endospores adhering to J2s of the *M. hapla* strains VW8 and VW9 were similar. Within each *Pasteuria* population there was no statistically significant difference between these nematode strains and there was an overlap with respect to their standard errors. Similarly, the attachment of endospores to J2s of single J2 descent lines of *M. hapla* showed a high degree of consistency both within and between lines. However, the differences between endospore attachment of RES147 and PP3 to lines VW8K1 and VW9K1 and VW9K2 suggests that these nematode lines had functional differences in their cuticle surfaces. Nematode line VW9K3 appears to enable intermediate numbers of endospores to attach. These results suggest that endospore attachment is under some form of genetic (or epigenetic) control as the number of endospores that attach is related to the parental type.

This research set out to test the hypothesis that cuticle variation is greater in a facultatively parthenogenetic root-knot nematode (*M. hapla* Race A) line derived from a single J2 than in a line derived from a species reproducing by obligate parthenogenesis (*M. incognita*). These results show that this is clearly not the case and that a high degree of cuticle variability, as revealed by *Pasteuria* endospore attachment, is maintained in root-knot nematodes reproducing by obligate parthenogenesis. There is an ongoing debate about the adaptive potential of asexually reproducing organisms and clonal lineages (Lushai and Loxdale, 2002; Lushai et al., 2003). It has long been recognised that root-knot nematodes reproducing by obligate parthenogenesis are polyploid (aneuploid) (Triantaphyllou, 1985; Trudgill and Blok, 2001) and that there may be a high degree of heterozygosity between homologous chromosomes within each line. In addition, there are many examples of these asexual root-knot nematode species gaining ability to circumvent host resistance genes, most notably the tomato gene Mi-1 (reviewed in Williamson and Kumar, 2006; Castagnone-Sereno, 2006). However, these changes occur at a low frequency compared with those that we have observed in spore attachment. This begs the question as to the circumstances and mechanism(s) by which asexually reproducing nematodes derived from a single individual J2 differ significantly in attachment levels of endospores. One could argue that the differences that we see are due to environmental factors. In fact, nematodes are known to change their surface composition in response to environmental signals. For example, a stage-specific surface antigen switch is induced in *Caenorhabditis elegans* by a chemical signal present in spent nematode culture medium (Grenache et al., 1996; Olsen et al., 2006). In animal parasitic nematodes, rapid changes in the surface occur during transition from pre- to post-parasitic forms (Proudfoot et al., 1993), and phytohormones have been shown to alter the surface of plant parasitic nematodes (Akhkha et al., 2002, 2004). However, in our experiments nematodes were cultured on the same host plant cultivar and under the same conditions. Egg masses were all hatched in water without exposure to plant hormones. Therefore, it is unli-

kely that the differences that we see are due to environmental effects.

Another explanation is that a specialised, epigenetic mechanism has evolved to allow nematodes to survive (co-evolve) with this specific obligate parasite.

It is recognised that parasites and their hosts are locked in a co-evolutionary arms race and both animal parasites, such as trypanosomes (Taylor and Rudenko, 2006) and pathogenic bacteria (van der Woude and Bäuml, 2004) have special mechanisms to switch their surface coats to evade immune systems (phase variation). Presumably root-knot nematodes and *Pasteuria* have co-evolved over a long time and therefore special genetic and epigenetic mechanisms have come into existence for the survival of each species. Sexually reproducing organisms show an ability through epigenetic phenomena, such as incomplete penetrance, to generate phenotypic variability and it is likely that similar mechanisms will also undoubtedly operate in parthenogenetically reproducing organisms as, theoretically, they are likely to use every means available to produce phenotype variation in the face of homozygosity.

The nematode cuticle is multilayered, comprising of cross-linked collagen overlaid by an electron dense epicuticle, the surface of which, the glycocalyx, contains carbohydrates. In animal parasites there is evidence that carbohydrate epitopes have a role in host immunity (Haslam et al., 2000; Appleton and Romaris, 2001; Skelly and Wilson, 2006), and recent work with gastrointestinal nematodes of sheep suggests that only a proportion of the worms within a population express a particular surface antigen (Maass et al., 2007). This demonstrates the ability of the animal-parasitic nematodes to modulate the surface coat of their cuticle to generate heterogeneity that is of functional importance. *C. elegans* has recently become a model for studying innate immunity and host pathogen interactions (Millet and Ewbank, 2004; Gravato-Nobre and Hodgkin, 2005) and there is evidence that carbohydrates on the surface coat are necessary for microbial adhesion. For example, studies of changes to the cuticle surface of a *srf-3* *C. elegans* mutant, involving a gene that encoded a nucleotide sugar transporter, prevented the bacterium *Mycobacterium nematophilum* from adhering to the rectum (Höflich et al., 2004). Other *srf* mutants have shown altered susceptibilities to being trapped by fungi (Mendoza de Gives et al., 1999a). However, to date no endospores of *P. penetrans* have been able to adhere to wild type *C. elegans* or any of the surface mutants so far tested (Mendoza de Gives et al., 1999b). More recent research on *C. elegans* has demonstrated that multiple gene loci may be involved in interactions in which bacterial surface colonisation is an initial stage of infection (Gravato-Nobre et al., 2005; O'Rourke et al., 2007; Yook and Hodgkin, 2007). The fact that a nematode is able to switch its cuticle surface through signalling genes that are under environmental control (Grenache et al., 1996; Olsen et al., 2006) suggests several mechanisms may be operating. If this is the case in *C. elegans*, similar interactions between genes may also govern the sur-

face properties of root-knot nematodes and their environment could also affect the ability of *Pasteuria* endospores to attach to the cuticle. The application of findings from the recent studies of innate immunity in *C. elegans* and animal parasitic nematodes, combined with the information presented here, that the attachment of endospores differs in progeny of a single individual, will increase our understanding of nematode cuticle variation and aid in the development of environmentally benign parasitic nematode control strategies.

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