Short Communication

Characterisation of a nucleopolyhedrovirus and Spiroplasma sp. bacterium associated with outbreaking populations of the Antler moth Cerapteryx graminis

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A B S T R A C T
A broad survey was undertaken to characterise microbes associated with larval outbreaks of the Antler moth Cerapteryx graminis in Cumbria, United Kingdom. A nucleopolyhedrovirus present in all sampled populations at ≤5% prevalence, was characterised via restriction fragment length polymorphism and partial sequencing the Polyhedrin, Lef-8 and Lef-9 genes; indicating a previously uncharacterised species most closely related to Agrotis ipsilon NPV. A survey of the host-associated bacterial community detected a species phylogenetically related to Spiroplasma sp., a male-killing phenotype previously isolated from Lepidoptera and Coleoptera, present at <63% prevalence in larvae. The implications of these associated microbes for host population dynamics are discussed.

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1. Introduction

The Antler moth Cerapteryx graminis (Lepidoptera: Noctuidae) is an agricultural outbreak pest that can cause the devastation of upland grassland (Heath et al., 1979). It is widely distributed throughout Europe and North America, being abundant throughout much of the United Kingdom with a southern range limit of 45°N (Entwistle and Rivers, 1972; Morris, 1979; Möttus et al., 1998). It inhabits meadows, open ground, hills and acidic moorland above 200 m with its larvae feeding on gramineous species (Entwistle and Rivers, 1972; Danell and Ericson, 1990). Previous studies have shown that it has the potential to change the composition of meadow pasture directly, through grazing and fertilisation by their faeces, and indirectly increasing herb growth due to the reduction in dense grass swards (i.e. through competitive release; Danell and Ericson, 1990).

Long-term datasets on Antler moth abundance in the United Kingdom (the Rothamsted Insect Survey; Woiwood and Harrington, 1994) indicate that UK populations exhibit 7-year cycles. In this study, as part of a broader investigation into the factors contributing to the population dynamics of Antler moths, we conducted an investigation into the microorganisms associated with the larval stages. The majority of previous research has been undertaken on cyclic populations of forest dwelling Lepidoptera and associated microorganisms (Dwyer and Elkinton, 1993; Dwyer et al., 2000; Myers, 1988; Tanada and Fuxa, 1987). Baculovirus epidemics have often been reported following host outbreaks (Entwistle and Evans, 1985; Tanada and Fuxa, 1987), and are thought to have potential in regulating cyclic host populations (Anderson and May, 1980, 1981; Briggs and Godfray, 1996; Liebold et al., 2000; Myers, 1988). We detected a novel nucleopolyhedrovirus (NPV) and a Spiroplasma sp. endosymbiont, believed to be a male-killing phenotype. Here, we characterise each microbe via molecular methods and explore the possible biological associations between the host, bacteria and virus.

2. Methods and materials

During June 2008, there were large outbreaks of Antler moth larvae in the English Lake District National Park (northern England). Larvae were sampled at three outbreak sites in June 2008 (Helvellyn, Matterdale and Kentmere). Quadrat counts were used to determine larval densities along altitudinal transects, using three randomly positioned 0.5 m × 0.5 m quadrats at each 10 m point along the transect, commencing where larvae were first observed. Transects were over 100 m long, with a minimum of 30 quadrat counts per population. Random collections of live larvae and NPV cadavers were made over a wide spatial area at each site, to ensure a representative sample for each population. Live larvae were reared individually under laboratory conditions until adult emergence.

Field-collected NPV was purified from individual cadavers, a method modified from Hunter-Fujita et al. (1998). Briefly, each infected cadaver was homogenised in sterile 1.5 ml microtubes with 400 µl of 0.1% sodium dodecyl sulphate (SDS). The virus was pelleted at 8000 g for 3 min and resuspended in 750 µl sterile
Milli-Q water. Viral polyhedra were dissolved by adding 3 μl Proteinase K (20 mg/ml), 10 μl 1 M Na₂CO₃ and 15 μl 10 % SDS. The nucleic acids from this mixture were phenol/chloroform extracted twice, dialysed and stored at 4°C until use. Purified viral DNA was digested with the endonucleases EcoRI, XhoI, HindIII, EcoRV and BamHI (New England Biolabs), fractionated in 0.6 % agarose gels, and band sizing undertaken using Quantity One software (BioRad Inc., USA).

Polymerase Chain Reaction (PCR) was used to amplify a portion of the NPV Polyhedrin, Lef-8 and Lef-9 genes, following the standard protocol of Jelhe et al. (2006a). Concatenated sequences of baculovirus Lef-8, Lef-9 and Polyhedrin/Granulin genes are suitable markers to mirror viral speciation (Jehle et al., 2006a). Reaction mixtures (50 μl) contained PCR buffers (10 mM Tris–HCl pH 8.3 at 25°C, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin), 5 μM each of dATP, dTTP, dCTP and dGTP, 0.1 μM of each primer, 1 unit Taq polymerase (Qiagen), and approximately 10 ng of insect genomic DNA template. PCRs were done with the settings (i) 94°C for 5 min, 1 cycle; (ii) 94°C for 20 s, 52°C for 20 s, 72°C for 30 s, 40 cycles; and (iii) 72°C for 5 min, 1 cycle. PCR products were separated by Denaturing Gradient Gel Electrophoresis (DGGE) using the TV400-DGGE system (Progen Scientific Ltd., London, UK). Gels consisted of 1 mm thick 6 % polyacrylamide with a denaturing gradient of 30–70% (100% denaturant corresponds to 7 mol/L urea and 40% v/v deionized formamide) and 1 × TAE buffer (40 mM/L Tris–acetate (pH 8.0) and 2 mM/L EDTA). Electrophoresis was performed at 60°C and 80 V in 1 × TAE running buffer.
buffer for 16 h, and gels were stained with SYBR Gold nucleic acid stain (Invitrogen). The DNA bands were excised with a sterile razor-blade and placed in 100 µl of sterile distilled water at 94 °C for 5 min to elute DNA for direct sequencing.

3. Results and discussion

Surveys indicated that larvae were confined within a fairly narrow altitudinal band, between 630–810 m, 610–760 m and 700–770 m at Helvellyn, Matterdale and Kentmere, respectively. Mean abundances reached 172 larvae per m² at Helvellyn and Matterdale (range = 0–340 larvae per m², quadrant number = 54), and 60 larvae per m² at Kentmere (range = 0–76 larvae per m², quadrant number = 30).

NPV mortality was recorded in all three Antler moth populations, with virus prevalence in the field being 0.74% at Matterdale and 5.08% at Kentmere (accurate NPV mortality was not recorded at Helvellyn). NPV mortality was spatially widespread throughout the populations, found in 4 of 54 quadrats at Matterdale and 7 of 30 quadrats at Kentmere (accurate NPV surveys were not recorded at Helvellyn). NPV infection was confirmed using standard Giemsa staining and standard microscopy methods (Hunter-Fujita et al., 1998), with the virus designated as C. graminis NPV, CegrNPV. Viral DNA was isolated individually from 11, 17 and 10 cadavers from Helvellyn, Matterdale and Kentmere, respectively. Investigation of the NPV populations indicated low genotypic variation, with only two variants being found in all populations, assigned CegrNPV-var1 and CegrNPV-var2.

Diversity and phylotype of partial 16S rRNA bacterial sequences isolated from Antler moth samples.

<table>
<thead>
<tr>
<th>No.</th>
<th>Accession No.</th>
<th>Isolate ID</th>
<th>Genus</th>
<th>Clade</th>
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References


