Hairworm anti-predator strategy: a study of causes and consequences

F. PONTON[1]*, C. LEBARBENCHON[1,2], T. LEFÈVRE[1], F. THOMAS[1], D. DUNEAU[1], L. MARCHÉ[3], L. RENAULT[3], D. P. HUGHES[4] *and* D. G. BIRON[1]

[1] *Génétique et Evolution des Maladies Infectieuses, UMR CNRS-IRD 2724, Equipe: 'Evolution des Systèmes Symbiotiques', IRD, 911 Avenue Agropolis, B.P. 64501, 34394 Montpellier Cedex 5, France*
[2] *Station Biologique de la Tour du Valat, Le Sambuc, 13200 Arles, France*
[3] *INRA, UMR BiO3P, Domaine de la Motte, BP 35327, 35653 Le Rheu Cedex, France*
[4] *Centre for Social Evolution, Institute of Biology, Universitetsparken 15, DK-21000 Copenhagen*

(*Received 4 May 2006; revised 7 June 2006; accepted 7 June 2006*)

SUMMARY

One of the most fascinating anti-predator responses displayed by parasites is that of hairworms (Nematomorpha). Following the ingestion of the insect host by fish or frogs, the parasitic worm is able to actively exit both its host and the gut of the predator. Using as a model the hairworm, *Paragordius tricuspidatus*, (parasitizing the cricket *Nemobius sylvestris*) and the fish predator *Micropterus salmoïdes*, we explored, with proteomics tools, the physiological basis of this anti-predator response. By examining the proteome of the parasitic worm, we detected a differential expression of 27 protein spots in those worms able to escape the predator. Peptide Mass Fingerprints of candidate protein spots suggest the existence of an intense muscular activity in escaping worms, which functions in parallel with their distinctive biology. In a second step, we attempted to determine whether the energy expended by worms to escape the predator is traded off against its reproductive potential. Remarkably, the number of offspring produced by worms having escaped a predator was not reduced compared with controls.

Key words: escape behaviour, gordian worm, parasite, predator, proteomics.

INTRODUCTION

Many animal species have evolved sophisticated morphological, physiological and behavioural adaptations to avoid succumbing to predation (Edmunds, 1974; Bertram, 1978; Elgar, 1989; Kavaliers and Choleris, 2001; Curio, 1993; Caro *et al*. 2004; Scott, 2005). These adaptations either reduce the probability of an attack (e.g. mimicry, crypsis and aposematic coloration), or lessen its chance of success (e.g. chemical defences, morphological weapons) (Magurran, 1999; Morin, 2003). The selective landscape in which parasites of animals evolve in response to predation pressures displays noticeable particularities. While virtually all free-living organisms (except mature top consumers) have predators, very few parasite species are directly concerned by predation, at least once inside their host (Combes, 2001). Rather, they inherit the predators of their host (Thomas *et al*. 2002*a*). This particular ecological context has in return favoured the emergence of original adaptive responses.

* Corresponding author: GEMI, UMR CNRS/IRD 2724, 911 av. Agropolis, BP 64501, 34394 Montpellier Cedex 5, France. Tel: +33 4 67 41 63 73. Fax: +33 4 67 41 62 99. E-mail: fleur.ponton@mpl.ird.fr

Hairworms, Dufour (Nematomorpha: Gordiida), typically develop in terrestrial arthropods, growing from a microscopic larva to a large worm that occupies most of the host cavity (Schmidt-Rhaesa, 1997, 2001). Once they reach this stage, hairworms emerge from their hosts and because adult males and females are free-living in aquatic environments, mature hairworms alter the behaviour of the insect host making them seek out and jump into water (Thomas *et al*. 2002*b*, 2003). That is, hairworms induce the suicide of their hosts. Once the host is in the water the adult worms then actively emerge, this takes from several seconds to several minutes (Thomas *et al*. 2002*b*). Once emerged the individuals begin searching for sexual partners (Thomas *et al*. 2002*b*). This emergence step is a critical period for the parasite with regard to predation risks, a time during which the writhing parasitized insect at the water surface is both attractive and highly vulnerable to a predator like a fish or a frog. Nevertheless, hairworms display a remarkable ability to escape from the digestive tract of predators following the predation of their host; they emerge alive from the mouth, gills or nose of the predators of their cricket hosts (Ponton *et al*. 2006).

The aim of this paper was to explore the physiological mechanisms underlying this anti-predator

doi:10.1017/S0031182006000904 Printed in the United Kingdom

response of hairworms. For this, we studied the proteomics response of worms able to escape from the gills or from the mouth of a fish predator. The study of the proteome with two key technologies of proteomics, two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MALDI-TOF), can provide a rapid and comprehensive view of the expression of entire genomes (Biron *et al*. 2005*a*). By permitting the study of the parasite proteome in action during the escaping behaviour, proteomics therefore offers, *a priori*, an excellent tool to understand the physiological basis of this anti-predator response. In a second step, to investigate whether the energy spent by the hairworm when exiting the predator is traded against reproductive effort, we studied the offspring number produced by pairs made with individuals having, or not, performed this behaviour.

MATERIALS AND METHODS

Data collection

We used as a model one of the most common insect-hairworm systems of Southern France, that is the cricket *Nemobius sylvestris*, Bosc (Orthoptera: Gryllidae), parasitized by the hairworm, *Paragordius tricuspidatus*. *Nemobius sylvestris* infected by *P. tricuspidatus* were captured at night in July 2004 as described by Ponton *et al*. (2006). Infected crickets were captured just before they jumped into water. To avoid the possible confounding effects on the proteomics expression of multiple infections and/or parasite-sex specific factors, only male hairworms singly infecting a male insect host were used for 2-DE.

The predator used for predation experiments was the fish *Micropterus salmoïdes*, a common predator species in the river and lakes from southern France, easy to maintain at the laboratory. Previous tests revealed that about 25% of worms are able to exit this predator (Ponton *et al*. 2006). Four *M. salmoïdes* (body length 15–25 cm) were caught with a lure from the wild 2 weeks before the experiment. They were maintained in the Station Méditerranéenne de l'Environnement Littoral (Sète) in a large tank containing 6 m^3 of constantly aerated freshwater and fed with uninfected *N. sylvestris*.

Experimental protocol

Predation experiments were performed as described by Ponton *et al*. (2006). In all cases hairworms that were able to escape from the predator did so within 10 min following the ingestion of the infected cricket. These worms were immediately retrieved after their exit from the fish. The first category comprised worms able to escape the digestive tract of fish by the mouth or the gill (Ingested – Escaped, I-E). A second category was worms having emerged normally from their insect host (Not Ingested – Non-Stressed, NI-NS). For this, infected crickets were placed in a tank of fresh water until the emergence of the worm. These NI-NS were maintained in freshwater and allowed to swim for 5 min before being preserved. Considering that being the victim of predation constitutes a stressful event for worms, we might expect the expression of several protein spots typically linked to general stress responses in the proteome of predated worms. In an attempt to control for this effect, we placed worms that had just emerged in another kind of stressful environment, a draining habitat since in natural conditions this represents a hostile and potentially lethal environment (Non Ingested – Stressed, NI-S). To simulate the draining of the habitat, hairworms were laid into a thin water layer on a table for 5 min. Finally, the fourth category comprised worms that did not succeed in exiting the fish predator. These worms (Ingested – Non-Escaped, I-NE) were retrieved by gastric tubing 20 min after the ingestion of the parasitized cricket. For each category, hairworms were immediately frozen in liquid nitrogen during the retrieval process and then conserved at $-80\,^{\circ}C$.

Two-dimensional gel electrophoresis (2-DE)

For the 4 categories, 5 hairworms were cut into fine equal pieces on an ice bath and under sterile conditions. Following Biron *et al*. (2005*b*), each sample was rinsed 4 times in a Tris-HCl (10 mM, pH 7·4) solution. Then water soluble and especially constitutive proteins were extracted in a homogenizing solution (urea 15M, Tris-HCl 10 mM, pH 7·4, 5% (v/v) β-mercaptoethanol, ampholytes 2%, pH 3–10) as described by Biron *et al*. (2005*b*). Protein concentration was estimated (Bradford, 1976) then standardized at 2 μg/μl (Biron *et al*. 2005*b*). The protein samples were stored at $-80\,^{\circ}C$ prior to electrophoresis separation on 2-DE. The two dimensional gels were done following the protocol of Biron *et al*. (2005*a*). At least 4 IPG strips (ImmobilineTM, DryStrip gels; Bio-Rad, USA) of pH 3–10 were run per treatment. Gels were stained using tetrathionate-silver nitrate (Oakley *et al*. 1980; Rabilloud *et al*. 1994).

Computer analyses

At least 3 well-replicated 2-DE gels were preserved and used for computer analyses of the various hairworm categories described above. Replicated gels for the same treatment were compared using ImageMaster^{TM} 2D Platinum Software Version 5.0 (Amersham Biosciences, UK; GENEBIO, Switzerland), common protein spots observed at least on 2 replica gels were retained. The best gel obtained for

each category was then used to build a 2-D master gel showing the differential expression of the hairworm proteome between the 4 categories. Spots differentially expressed between the 4 categories should reflect more the variability inter-category than the variability intra-category (Tastet *et al*. 1999; Francis *et al*. 2006). The isoelectric-point (pI) and molecular weight (Mw) scales of 2-DE gels were determined using a protein standard kit from Bio-Rad (USA). Crowded protein spot areas and areas containing high molecular weight protein spots were not well defined and thus discarded from the analysis.

Protein identification by MALDI-TOF mass spectrometry

Once initial analyses suggested protein spots of interest, new gels were run and silver stained following the method described by Schevchenko *et al*. (1996) in order to excise candidate protein spots. Identification of proteins, peptide digestion and MALDI-TOF analysis were done following the protocol of Biron *et al*. (2005*c*). Protein identification was obtained by conducting a database search of the peptide mass generated from MALDI analysis. Identification of proteins was performed using ALDENTE (http://www.expasy.org/tools/aldente) and PROTEIN PROSPECTOR MS-FIT (http://prospector.ucsf.edu) software. Monoisotopic peak lists were imported into ALDENTE and PROTEIN PROSPECTOR MS-FIT software with the following search parameters: OTHER METAZOA in the species field, pI $\pm$ 2·0, Mw $\pm$ 30%, one missing cleavage, tryptic digestion, carbamidomethylation as a cysteine modification and oxidation of methionine (Wilkins and Williams, 1997; Barrett *et al*. 2005). Based on cross-species concepts for the protein identification, we did a parsimony search by taking into consideration closest Nematomorpha taxa (Wilkins and Williams, 1997; Lester and Hubbard, 2002; Barrett *et al*. 2005; Gasteiger *et al*. 2005). Actually, given the poor number of protein sequences in Nematomorpha, we consider valid results given proteins belonging to Nematoda taxa as it is considered as the Nematomorpha closest phylogenetic group (Hanelt *et al*. 2005). Search tolerance was set at 100 ppm with a MH+ charge state. Proteins that were retained had: the highest score, the higher significant '*P*-value' ($P<0{\cdot}05$, i.e. the probability that observed match is a random event), a minimum of missed cleavages, a minimum of Δppm between the molecular mass of the experimental peptides and the corresponding theoretical peptides, a theoretical pI/Mw close to the experimental pI/Mw (Wilkins and Williams, 1997; Lester and Hubbard, 2002; Barrett *et al*. 2005; Gasteiger *et al*. 2005). Matching peptides with missed cleavages were considered as relevant only when there were 2 consecutive basic residues or when arginine and lysine residues were followed by a proline or acidic residues inside the peptide amino acid sequence (Bécamel *et al*. 2002; Gasteiger *et al*. 2005).

Hairworm crossing

In order to investigate the reproductive potential of hairworms following predation, we estimated the offspring number produced by pairs made with individuals having accomplished the escape behaviour (I-E) and hairworms that were not predated (NI-NS). Again only hairworms, singly infecting a male insect host, were considered. By randomly choosing male and female hairworms, 4 kinds of pairs were made: ♂NI-NS × ♀NI-NS ($n=11$), ♂NI-NS × ♀I-E ($n=15$), ♂I-E × ♀NI-NS ($n=22$), and ♂I-E × ♀I-E ($n=15$). Length measurements revealed that there was no significant size difference between individuals from the different kind of pairs (mean $\pm$ S.E.: ♂NI-NS: 11·12 $\pm$ 0·34 ($n=11$) × ♀NI-NS: 11·60 $\pm$ 0·36 ($n=11$), ♂NI-NS: 10·30 $\pm$ 0·29 ($n=15$) × ♀I-E: 11·75 $\pm$ 0·31 ($n=15$), ♂I-E: 10·98 $\pm$ 0·25 ($n=21$) × ♀NI-NS: 11·55 $\pm$ 0·26 ($n=22$), and ♂I-E: 10·73 $\pm$ 0·29 ($n=15$) × ♀I-E: 11·58 $\pm$ 0·31 ($n=15$); males: Kruskal-Wallis $H_3=5{\cdot}31$, $P=0{\cdot}15$; females: Kruskal-Wallis $H_3=0{\cdot}47$, $P=0{\cdot}93$). Pairs were maintained individually in small cups (diameter, 2 cm; height, 5 cm) filled with constantly oxygenated freshwater and under 22 $\pm$ 1 °C and a LD 12:12 cycle. Males were removed after mating (4 days after pair formation). Females were kept in the cups and these were examined daily to determine the delay required for a complete larval hatching. We added ethanol 70% in all cups 52 days after mating to kill the worms and larvae, since by then the large majority of the larvae had successfully hatched. Larval counting was done under a microscope (Leica DM LB) with a Thoma chamber. For each reproductive pair, larval concentration was determined in 30 ml of ethanol 70%. Prior testing ensured that 12 sample cups were sufficient to obtain a reliable estimation of the larval quantity. Concentration was determined by randomly choosing tubes from the different categories.

RESULTS

Analysis of 2D gels

Our results demonstrated a differential expression of specific proteins in the proteome of worms able to escape. Fig. 1 shows the differential expression of the hairworm proteome in the different categories. A total of 553 protein spots was detected, 358 of them (i.e. 64·74%, see Figs 1 and 2) being common between the 4 categories. We considered that a protein spot was likely to be linked to the predator escape behaviour when it was either present or absent specifically in the Ingested-Escaped category.

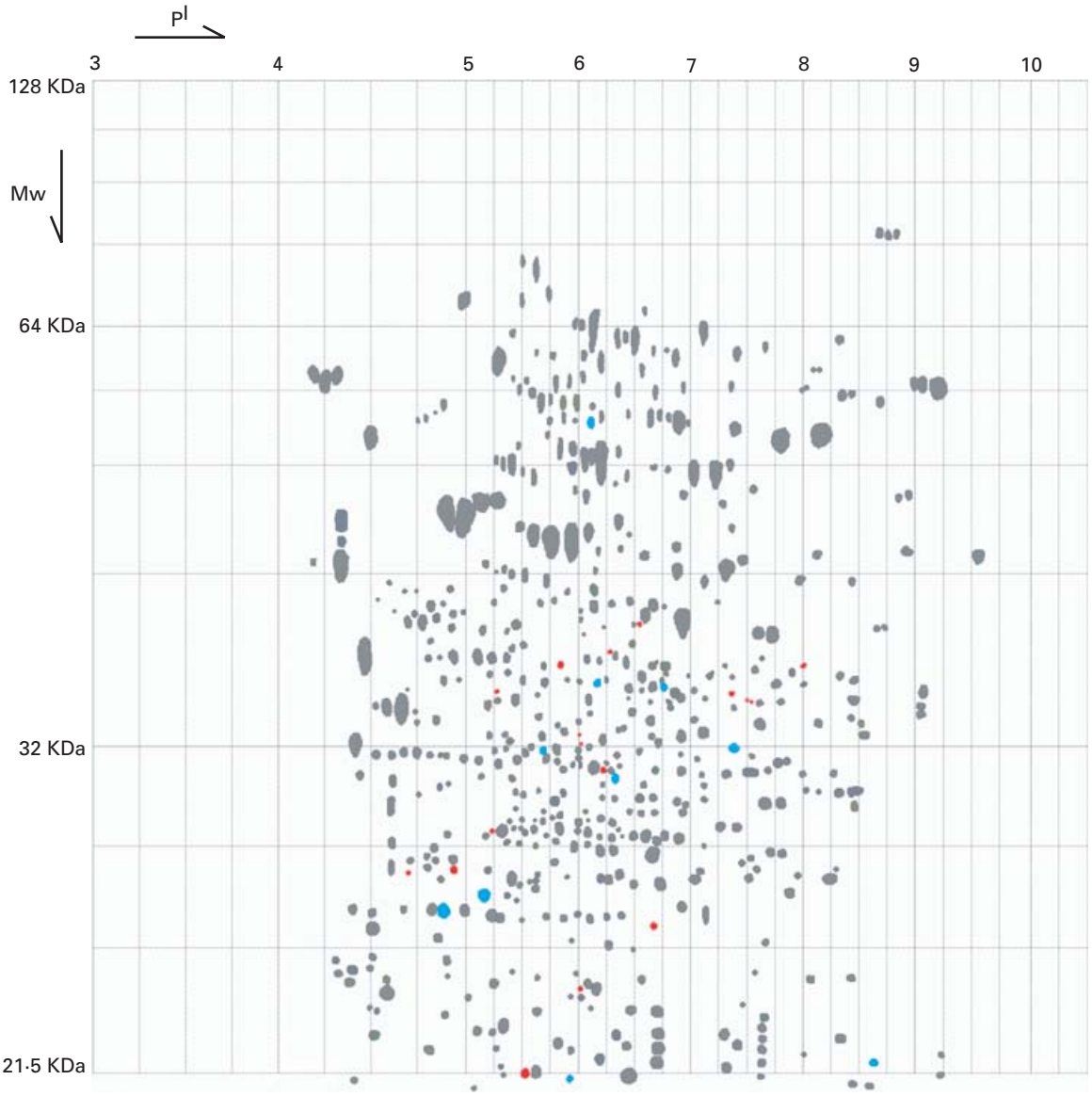

Fig. 1. Two-dimensional synthetic gel of *Paragordius tricuspidatus* showing proteome response to the 'escape behaviour' (worms Ingested – Escaped).

- Common and non-specific protein spots,
- Proteins specifically induced on the proteome of Ingested-Escaped worms,
- Proteins specifically suppressed on the proteome of Ingested-Escaped worms.

Applying this criterion, 4·88% of the total of protein spots (i.e. 17 present and 10 absent) were considered as linked to the escaping behaviour (see Figs 1 and 2).

Identification of candidate proteins

PMF analyses were performed on the 27 protein spots probably linked to the escaping behaviour. Good PMF were obtained for the 27 candidate protein spots (see Electronic Supplementary Material S1). Since actin is highly conserved (Sheterline *et al.* 1996) it is thus a nice positive control to evaluate the MALDI-TOF protocol used in our experiment. Searches in SwissProt and TrEMBL protein databases confirmed that the control protein spot belongs to the Actin family (Table 1 and Electronic Supplementary Material S1). For the candidate protein spots, we identified 7 with PMF (see Table 1). Three protein spots, specifically absent from the proteome of Ingested-Escaped hairworm category, were identified as (i) a torsin-like protein precursor, (ii) an ATP-dependent protease and (iii) an enzyme belonging to the phosphoglycerate kinase (see Table 1). In addition, 3 proteins specifically present in the Ingested-Escaped category were identified as (i) a protease involved in the ATP-dependent degradation of

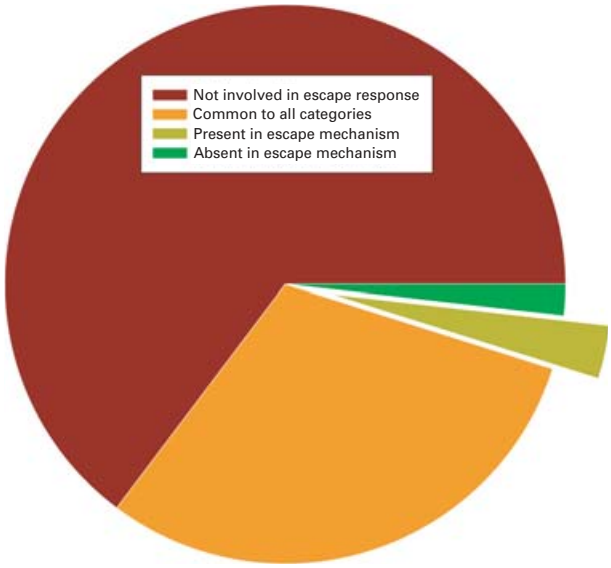

Fig. 2. Percentages of protein spots from the synthetic gel classified in 3 categories: (i) common proteins (ii) proteins not involved in escape mechanisms (iii) proteins involved in escape mechanisms (detected during the phenomenon (present), not detected during the phenomenon (absent)).

ubiquitinated proteins, (ii) a protein intervening in intracellular signalling and cytoskeletal regulation and (iii) a protein constitutive of the large ribosomal subunit (see Table 1). A final induced protein was identified as belonging to the DUF672;1 family but its function is unknown.

Reproductive output

There was no significant difference between the mean number of larvae produced by the different kinds of pairs (mean number of larvae ± S.E.: ♂NI-NS × ♀NI-NS ($n=11$), 41 590·9 ± 17595; ♂NI-NS × ♀I-E ($n=15$), 35 916·7 ± 15068; ♂I-E × ♀NI-NS ($n=22$), 61 215·2 ± 12 442 and ♂I-E × ♀I-E ($n=15$), 49 661·1 ± 15 068; Kruskal-Wallis ANOVA, $\chi^2=1{\cdot}70$, D.F. = 3, $P=0{\cdot}64$). Given the high P-value and moderately large sample sizes, we conclude that no difference in fecundity exists between the categories. Because the data were non-normally distributed and we used non-parametric tests then a Power analysis was inappropriate (David Nash, personal communication).

DISCUSSION

Gordian worms have evolved a novel and simple solution to predation of their host: they crawl out from the digestive tract of the predator. Achieving this remarkable feat relies upon both their fusiform morphology and evolved adaptations to the harsh environment of the digestive tract. Not only can these worms survive a predation event but they apparently reproduce afterwards without any reduction of reproductive potential.

Our data has shown that this behaviour coincides with particular physiological mechanisms detectable with proteomics tools. We identified 27 proteins whose expression was specifically altered in the proteome of worms able to exit the predator. Identification of candidate proteins indicates a modification of the muscular activity since some of the identified torsin-like proteins are involved in repetitive muscle contractions and twisted postures (Breakefield *et al*. 2001). Additionally, proteins probably involved in intracellular signalling and cytoskeletal regulation were observed. A modified ATP synthesis also appears to occur and may facilitate escape. Using our approach we did not detect biological signatures of toxic component synthesis that might serve to elicit a vomit response by the predator. This could argue that worms use a simpler anti-predator strategy that involves active self-propelled exit from the hostile environment of the vertebrate gut.

Although this study only focused on physiological aspects detectable with proteomics tools, it seems likely that the efficiency of the escaping behaviour results from the synergistic action between the intense muscular activity and the filiform morphology of hairworms coupled with the rigidity of their cuticle (Protasoni *et al*. 2003; Schmidt-Rhaesa, 2003). The filiform morphology probably enhances mobility under conditions such as those within the vertebrate gut. The rigidity, and unique arrangement of the cuticle, probably also protect hairworms from mechanical and chemical attacks during escape. Further studies would be necessary to determine the stimuli that induce the escape behaviour in worms once inside the predator. Acidic pH levels, digestive enzymes and/or mechanical constraints could be included in the factors which signal to the worm the urgency of displaying such an anti-predator response.

Life-history theory predicts that anti-predator behaviour should have a cost that is traded off against other components of fitness (Stearns, 1992; West-Eberhard, 2003). The general problem of searching for such trade-offs involving anti-predation behaviour has recently be raised and centred on the potential compensation by prey following predator avoidance (Lind and Cresswell, 2005). Clearly, no study has demonstrated an ability to escape the gut following ingestion so determining, *a priori*, what possible costs are involved is difficult. For this reason no study has measured the fecundity of an organism after it survived ingestion by a predator. Remarkably, we found no significant difference between the mean numbers of larvae produced by the different kinds of pairs. At least in our experimental approach, predation did not have a negative effect on reproductive output. Of course this absence of evidence is not evidence for absence taking into account

Table 1. Identification of hairworm-specific proteins to Ingested-Escaped (I-E) category

	Identity of protein spots	Protein name	Accession number (SwissProt/ TrEMBL)	pI_Mw Exp. *pI_Mw Theo.*	No. of peptides matched (sequence coverage (%))	*P* value	Family of the protein according to Pfam database of Sanger institute	Known function according to SWISS-PROT, TrEMBL and Pfam database
Absent in I-E proteome	*Actin*	Actin-2	P10984*	5,30_43 000 *5,30_41 543*	6 (22%)	$4{,}8{\cdot}10^{-4}$	Actin; 1	Actin
	PA01	Torsin-like protein (precursor)	Q95NU5*	7,40_31 973 *6,48_38 648*	4 (13%)	$4{\cdot}10^{-4}$	Torsin; 1	Chaperon proteins implied in biochemical pathways leading to repetition of muscular contractions.
	PA03	YME1 protein homolog	P54813*[a]	5,16_25 991 *9,10_74 454*	4 (7%)	$1{,}4{\cdot}10^{-1}$	AAA; 1 Peptidase_M41; 1	Putative ATP-dependent protease.
	PA07	Hypothetical protein T05H10.8 in chromosome II	Q10004*	6,17_34 386 *4,90_42 202*	4 (11%)	$1{,}9{\cdot}10^{-12}$	PGK	Phosphoglycerate kinase (PGK) is an enzyme that catalyses the formation of ATP to ADP and vice versa. PGK is found in all living organisms and its sequence has been highly conserved throughout evolution.
Present in I-E proteome	*PC12*	Hypothetical protein Y40H7A.3	Q9XWA3*	7,37_33 708 *7,49_29 390*	4 (22%)	$9{,}4{\cdot}10^{-4}$	DUF672; 1	This family includes several proteins of unknown function.
	PC13	Hypothetical protein CBG11069	Q61GT9**	5,30_33 741 *9,36_48 586*	7 (18%)	$1{,}8{\cdot}10^{-5}$	AAA; 1	The 26S protease is involved in the ATP-dependent degradation of ubiquitinated proteins.
	PC15	Hypothetical protein CBG12407	Q61DP0**	6,70_24 795 *6,51_49 864*	4 (14%)	$4{,}5{\cdot}10^{-5}$	Arm; 1	The «Armadillo/beta-catenin-like repeat» proteins function in various processes, including intracellular signalling and cytoskeletal regulation.
	PC20	Hypothetical protein T04A8.11	Q22140*	6,21_31 093 *9,41_25 360*	3 (16%)	$6{,}5{\cdot}10^{-4}$	Ribosomal_L16; 1	Ribosomal protein L16 is one of the proteins from the large ribosomal subunit.

Note: Experimental (Exp.) pI and Mw were obtained according to location of protein spot on gel, Theoritical (Theo.) pI and Mw were obtained according to protein banks.
* *Caenorhabditis elegans*; ** *Caenorhabditis briggsae*; [a] fragment.

our sample size but seems to indicate that worms do not suffer a negative effect on their reproductive output when they escape from a predator. Further research is necessary to clarify the cost of the escaping behaviour for worms, since we did not measure the performances of the different kinds of worms in the other steps of the reproductive process. For instance, it could be possible that worms having experienced a predation event subsequently have a reduced survival and/or swimming activity making them less able to find a sexual partner. In addition, further studies would be necessary to determine the frequency of this anti-predator response in natural populations in relationship with the local frequency/identity of predators.

In conclusion, it is safe to say that the unique behaviour of hairworms following a predation event upon their host predator is an impressive phenomenon. An intense muscular activity seems to be the main physiological process underlying this anti-predator response.

We thank Nathalie Galéotti and Emmanuelle Demey-Thomas from the proteomics platform of Montpellier Génopôle® for their help concerning the mass spectrometry analysis. We thank Mr F. Bonhomme (Station Méditerranéenne de l'Environnement Littoral, Sète), Mr J. L. Lafaurie and the thermal station of Avènes-Les-Bains for their cooperation during the field study. We thank David Nash for statistical advice. This work was supported by la région Languedoc-Roussillon and by an ACI 'jeunes chercheurs' grant to F. Thomas. The experiments comply with the current laws of the country in which they were performed.

REFERENCES

Barrett, J., Brophy, P. M. and Hamilton, J. V. (2005). Analysing proteomic data. *International Journal for Parasitology* **35**, 543–554.

Becamel, C., Galeotti N., Poncet, J., Jouin, P., Dumuis, A., Bockaert, J. and Marin, P. (2002). A proteomic approach based on peptide affinity chromatography, 2-dimensional electrophoresis and mass spectrometry to identify multiprotein complexes interacting with membrane-bound receptors. *Biological Procedures Online* **4**, 94–106.

Bertram, B. C. R. (1978). Living in groups: predators and prey. In *Behavioural Ecology : an Evolutionary Approach* (ed. Krebs, J. R. and Davies, N. B.), pp. 64–96. Blackwell Scientific Publications, Oxford.

Biron, D. G., Moura, H., Marché, L., Hughes, A. and Thomas, F. (2005*a*). Towards a new conceptual approach to 'parasitoproteomics'. *Trends in Parasitology* **21**, 162–168.

Biron, D. G., Joly, C., Marché, L., Galéotti, N., Calcagno, V., Schmidt-Rhaesa, A., Renault, L. and Thomas, F. (2005*b*). First analysis of the proteome in two nematomorph species, *Paragordius tricuspidatus* (Chordodidae) and *Spinochordodes tellinii* (Spinochordodidae). *Infection, Genetics and Evolution* **5**, 167–175.

Biron D. G., Marché, L., Ponton, F., Loxdale, H., Galeotti, N., Renault, L., Joly, C. and Thomas, F. (2005*c*). Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach. *Proceeding of the Royal Society of London, B* **272**, 2117–2126.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram-quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.

Breakefield, X. O., Kamm, C. and Hanson, P. I. (2001). TorsinA: movement at many levels. *Neuron* **31**, 9–12.

Caro, T. M., Graham, C. M., Stoner, C. J. and Vargas, J. K. (2004). Adaptive significance of antipredator behaviour in artiodactyls. *Animal Behaviour* **67**, 205–228.

Combes, C. (2001). *Parasitism, The Ecology and Evolution of Intimate Interactions*. The University of Chicago Press, London.

Curio, E. (1993). Proximate and developmental aspects of antipredator behavior. *Advances in the Study of Behavior* **22**, 135–238.

Edmunds, M. (1974). *Defence in Animals*. Essex: Longman.

Elgar, M. A. (1989). Predator vigilance and group size in mammals and birds: a critical review of the empirical evidence. *Biological Review* **64**, 13–33.

Francis, F., Gerkens, P., Harmel, N., Mazzucchelli, G., De Pauw, E. and Haubruge, E. (2006). Proteomics in *Myzus persicae*: Effect of aphid host plant switch. *Insect Biochemistry and Molecular Biology* **36**, 219–227.

Garin, J., Ferro, M., Rolland, N. and Joyard, J. (2001). Stratégies en protéomique: outils, limites et développement. *1ère Ecole thématique de biologie végétale*.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D. and Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. In *The Proteomics Protocols Handbook* (ed. Walker, J. M.), pp. 571–607. Totowa, Humana Press, NJ-USA.

Hanelt, B., Thomas, F. and Schmidt-Rhaesa, A. (2005). Biology of the Phylum Nematomorpha. *Advances in Parasitology* **59**, 243–305.

Kavaliers, M. and Choleris, E. (2001). Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neuroscience and Biobehavioral Reviews* **25**, 577–586.

Lester, P. J. and Hubbard, S. J. (2002). Comparative bioinformatic analysis of complete proteomes and protein parameters for cross species identification in proteomics. *Proteomics* **2**, 1392–1405.

Lind, J. and Cresswell, W. (2005). Determining the fitness consequences of antipredation behaviour. *Behavioural Ecology* **16**, 945–956.

Magurran, A. E. (1999). The causes and consequences of geographic variation in antipredator behaviour. In *Geographic Variation in Behavior. Perspectives on Evolutionary Mechanisms* (ed. Foster, S. A. and Endler, J. A.), pp. 139–163.

Morin, P. J. (2003). *Community Ecology*, pp. 104–135. Blackwell Science.

Oakley, B. R., Kirsch, B. R. and Moris, N. R. (1980). A simplified ultrasensitive silver stain for detecting

proteins in polyacrylamide gels. *Analytical Biochemistry* **105**, 361–363.

Ponton, F., Lebarbenchon, C., Lefèvre, T., Biron, D. G., Duneau, D., Hughes, D. P. and Thomas, F. (2006). Parasite survives predation on its host. *Nature, London* **440**, 756.

Protasomi, M., De Eguileor, M., Cngiu, T., Grimaldi, A. and Reguzzoni, M. (2003). The extracellular matrix of the cuticle of *Gordius panigettensis* (Gordioiidae, Nematomorpha): observations by TEM, SEM and AFM. *Tissue and Cell* **35**, 306–311.

Rabilloud, T., Vuillard, L., Gilly, C. and Lawrence, J. J. (1994). Silver-staining of proteins in polyacrylamide gels: a general overview. *Cellular and Molecular Biology* **40**, 57–75.

Schmidt-Rhaesa, A. (1997). Nematomorpha. In *Sübwasserfauna Mitteleuropas* 4/4 (ed. Schwoerbel, J. and Zwick, P.), pp. 1–17. Gustav Fischer-Verlag, Stuttgart.

Schmidt-Rhaesa, A. (2001). The life cycle of horsehair worms (Nematomorpha). *Acta Parasitologica* **46**, 151–158.

Schmidt-Rhaesa, A. (2003). Ultrastructure of an integumental organ with probable sensory function in *Paragordius varius* (nematomorpha). *Acta Zoologica* **85**, 15–19.

Scott, G. (2005). Avoiding predation: staying alive against the odds. In *Essential of Animal Behavior*, pp. 143–165. Blackwell Science, Oxford.

Sheterline, P., Clayton, J. and Sparrow, J. C. (1996). *Actins* 3rd Edn. Academic Press, London.

Shevchenko, A., Wilm, M., Vorm, O. and Mann, M. (1996). Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. *Analytical Chemistry* **68**, 850–858.

Stearns, S. C. (1992). *The Evolution of Life Histories*. Oxford University Press, Oxford.

Tastet, C., Bossis, M., Gauthier, J. P., Renault, L. and Mugniéry, D. (1999). *Meloidogyne chitwoodi* and *M. fallax* protein variation assessed by two-dimensional electrophoregram computed analysis. *Nematology* **1**, 301–314.

Thomas, F., Brown, S. P., Sukhdeo, M. and Renaud, F. (2002*a*). Understanding parasite strategies: a state-dependent approach? *Trends in Parasitology* **18**, 387–390.

Thomas, F., Schmidt-Rhaesa, A., Martin, G., Manu, C., Durand, P. and Renaud, F. (2002*b*). Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? *Journal of Evolutionary Biology* **15**, 356–361.

Thomas F., Ulitsky, P., Augier, R., Dusticier, N., Samuel, D., Strambi, C., Biron, D. G. and Cayre, M. (2003). Biochemical and histological changes in the brain of the cricket *Nemobius sylvestris* infected by the manipulative parasite *Paragordius tricuspidatus* (Nematomorpha). *International Journal for Parasitology* **33**, 435–443.

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press, Oxford.

Wilkins, M. R. and Williams, K. L. (1997). Cross-species protein identification using amino acid composition, peptide mass fingerprinting, isoelectric point and molecular mass: a theoretical evaluation. *Journal of Theoretical Biology* **186**, 7–15.

S: PMF OBTAINED FOR PROTEIN SPOTS SPECIFICALLY INDUCED OR SUPPRESSED FROM THE INGESTED-ESCAPED CATEGORY

	PMF																
PA01	1151,740	1184,594	1279,794	1334,910	1448,945	1562,956	1618,080	1732,122	2015,287	2150,097							
PA02	1334,972	1448,946	1617,953	1731,870	1813,592	2149,201	2367,663	2564,453	2582,348	2907,665	3107,169						
PA03	1005,527	1022,538	1279,801	1334,916	1448,952	1506,796	1562,973	1618,096	1732,131	1788,918	2042,048	2204,027	2313,176	2345,024	2419,236	2435,241	
PA04	1002,562	1005,533	1022,541	1049,553	1332,804	1448,967	1486,802	1506,824	1651,899	1764,947	1788,951	1910,877	1949,031	2042,054	2150,083	2313,181	
	2419,232	2435,231	2440,268														
PA05	992,598	1184,634	1332,804	1334,953	1448,981	1553,808	1651,890	1732,140	1764,962	1785,948	1788,951	1939,095	2061,101	2150,088	2419,233	2435,230	
	2440,255	2564,225															
PA06	992,602	1220,699	1332,791	1651,888	1788,456	1788,931	1840,893	1949,061	2420,232	2435,239	2808,254						
PA07	900,537	916,571	918,602	936,589	990,646	992,643	1008,642	1131,790	1203,822	1205,845	1223,850	1295,891	1316,836	1332,817	1418,980	1474,959	
	1491,000	1511,033	1567,058	1583,054	1799,202												
PA08	1184,628	1219,705	1316,811	1332,798	1334,938	1448,962	1651,901	1764,940	1788,938	2150,082	2419,231	2435,239	2440,272	2584,180			
PA09	1316,763	1332,743	1448,919	1785,902	1788,892	2419,244	2435,238	2808,364	3094,679								
PA10	1010,553	1334,937	1448,969	1606,900	1618,101	1688,026	1788,944	2246,209	2269,122	2313,184	2435,234	2831,161					
PC11	1332,771	1448,958	1784,942	1788,956	1813,926	2151,087	2198,945										
PC12	1151,804	1184,650	1279,841	1332,802	1334,948	1449,004	1618,140	1651,933	1732,160	1788,983	1998,993	2015,306	2061,101	2150,092	2226,118	2435,224	
	2440,243	2502,210	2536,256														
PC13	926,623	936,586	992,594	1008,638	1011,668	1129,674	1190,745	1203,777	1205,802	1279,814	1295,839	1332,811	1338,875	1422,946	1448,946	1510,994	
	1583,006	1622,924	1666,049	1708,760	1788,928	1950,082	2435,263										
PC14	1184,621	1279,766	1332,786	1334,907	1448,943	1784,928	1788,913	1824,887	2419,228	2435,242	2808,310	3094,626	3110,660				
PC15	1316,792	1332,794	1542,751	1652,922	1764,960	1824,949	1840,922	1939,098	2150,085	2261,111	2435,247	2566,261	2584,178				
PC16	1007,678	1151,742	1265,763	1279,804	1334,905	1448,946	1553,745	1562,953	1618,076	1732,093	1847,140	1901,235	2015,280	2150,097	2419,238	2435,272	
PC17	900,499	1007,642	1151,740	1279,777	1334,883	1448,921	1732,101	1788,921	2061,042	2150,070	2435,250						
PC18	1279,845	1788,968	2047,098	2057,025	2150,092	2368,984	2418,212	2566,244	2584,177	2808,239							
PC19	971,335	1151,754	1184,628	1279,802	1332,785	1334,937	1448,967	1618,101	1651,889	1732,124	1784,945	1788,489	1788,943	2150,094	2313,204	2435,263	
PC20	900,527	1184,625	1448,970	1652,947	1788,936	2061,089	2150,091	2435,243	2566,256	2584,188	2831,155						
PC21	1151,745	1279,784	1334,914	1410,733	1448,962	1784,937	1788,928	1899,923	2127,053	2566,321	2584,234	2831,258					
PC22	1448,957	1688,028	1813,933	1994,004	2091,901	2150,090	2198,954	2210,069	2349,217	2411,973	2418,239	2441,961	2566,268	2584,205	2831,195		
PC23	1279,821	1334,945	1448,979	1732,147	1813,932	1883,957	2150,090	2198,956	2435,256								
PC24	900,537	1184,611	1219,674	1279,771	1332,773	1334,903	1448,940	1651,869	1764,927	1949,122	1998,959	2058,977	2061,071	2400,043	2435,260	2440,286	
	2536,324	2566,270	2823,327														
PC25	1007,685	1151,741	1279,792	1334,918	1448,944	1618,083	1651,861	1788,932	1813,908	1998,955	2061,075	2297,139	2357,142	2398,022			
PC26	900,520	1151,750	1184,609	1279,799	1334,924	1448,964	1618,103	1732,125	1788,933	1918,957	2061,091	2313,186	2355,156	2435,257	2441,943	2972,500	
PC27	900,523	1151,754	1184,606	1279,796	1334,908	1448,953	1618,079	1652,907	1732,114	1784,938	1788,927	1998,960	2150,091	2435,264			
Actine	945,541	967,504	976,440	1086,601	1130,539	1141,723	1171,546	1248,628	1256,763	1291,775	1359,672	1499,650	1515,727	1516,758	1763,924	1772,871	
	1790,880	1954,000	1960,879	1991,943	2113,909	2215,060	2698,464										