Slugs & Snails: Agricultural, Veterinary & Environmental Perspectives

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Slugs & Snails

Agricultural, Veterinary & Environmental Perspectives

Chaired by G B J Dussart

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in association with The Malacological Society of London
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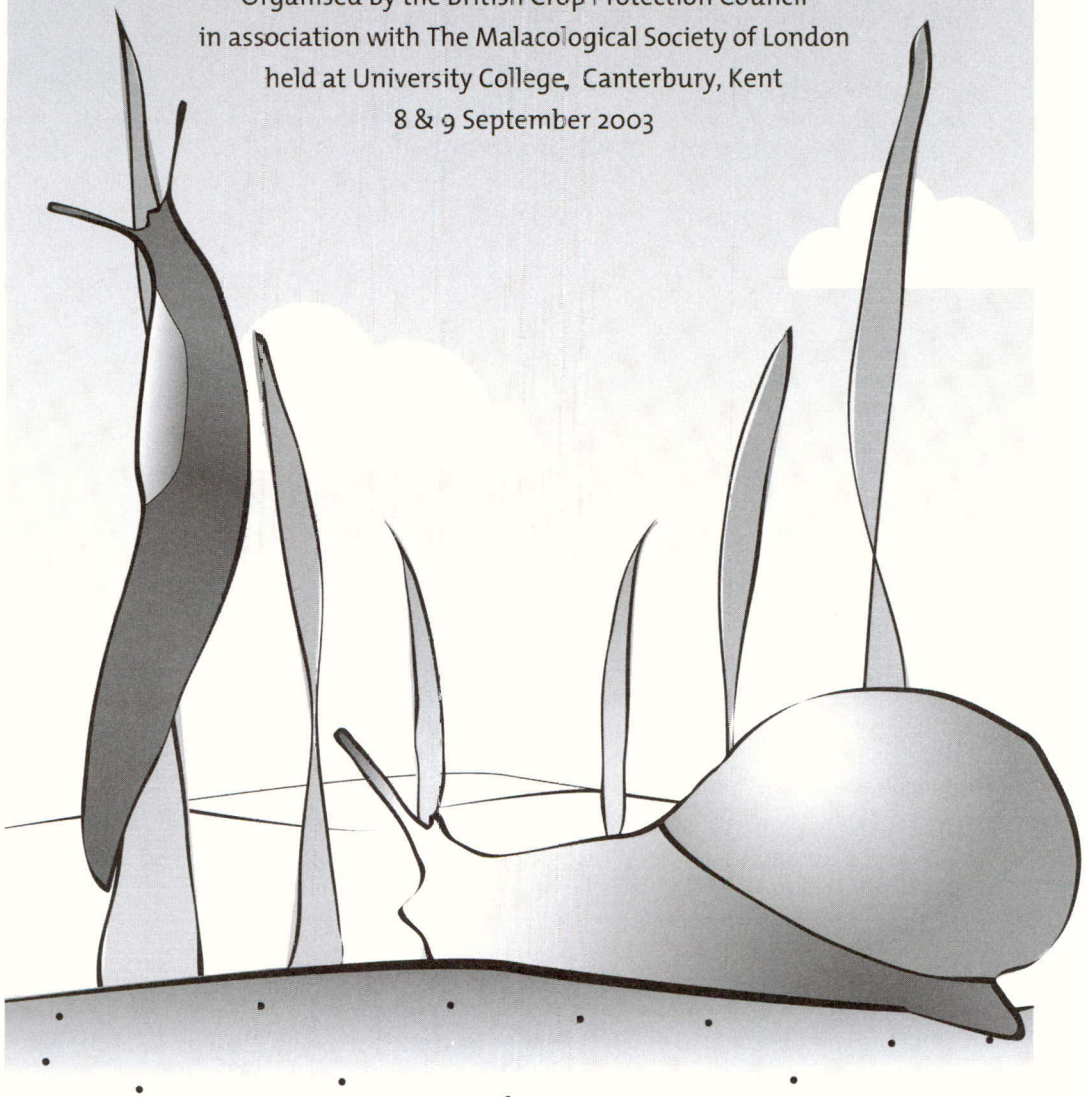

The Malacological Society of London

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CONTENTS

Page

SESSION 3
POSTER SESSION

predation on slugs and the use of DNA-based slug monitoring techniques to improve controls. Conversely, the fact that many human activities threaten gastropod biodiversity is also examined. A presentation on the role of gastropods as pollution indicators leads into *Session 2 – Physiology of Slugs and Snails* which provides insights into the control of pests, species conservation and pollution monitoring. The use of aquatic snails to monitor environmental oestrogen, the response of the gastropod immune system to pathogens, the impact of pollution on gastropod stress-proteins and the metabolic redeployment of body-wall materials during reproductive maturation are described. A description of the biochemistry of a combination of molluscicide with a fusion protein leads into the *Session 4 – Prospects for Control*. Here perspectives are offered on the risks and benefits of molluscicide treatments, and the suitability of caffeine as a potential new molluscicide. A system to assess the risk of slug damage by means of a climate model, tested in France since 1999, is also described. Snails have recently becomes pests of sewage works, and a successful technique for control is discussed.

To achieve improved methods of control of pest species, it is essential to understand factors that influence the abundance of terrestrial gastropods. *Session 5 – Ecology and Behaviour* examines responses of slugs and snails to chemicals and predators. The interactions between pest slugs, particularly *Deroceras* and *Arion*, and predatory beetles are discussed and the behavioural responses of slugs to molluscicidal pellets are examined. The control of an introduced snail pest in Australian citrus orchards is described. Although copper is often used to control molluscs, in extreme habitats, high concentrations can sometimes be tolerated and examples of *Helix* from North Wales are examined. Slugs and snails are good invaders, but can, themselves be invaded by parasites, a situation exemplifed by the invasive aquatic snail *Potamopyrgus*. Parasitism features again in *Session 6 – Integrated Pest Management (IMP)* which examines novel approaches to management of mollusc pests and how these approaches can be integrated with other measures. The release and establishment of *Sarcophaga*, an exotic biological control agent for the snail pest *Cochlicella* in Australia is described and a comparative study of slug control by *Phasmarhabditis* in lettuce and sprouts in Belgium is discussed. Both integrated management of slug and snail pests in ornamental nurseries and the integrated control of slug damage in horticultural field crops are considered. Novel approaches are a fundamental aspect of IPM and it is necessary to be alert to new possibilities. In this context, results from screening African plants for mollusc repellence are described.

The oral presentation sessions are supported by *Session 3 – Poster Papers* which comprises an eclectic mix of papers, ranging from aspects of heliciculture in Greece to systems for countering parasitologically important snails in China.

The joint British Crop Protection Council and Malacological Society Symposia of 1989 and 1996 were 'state of the art' meetings and the current Symposium follows the tradition in both offering a synopsis of the present state of affairs, as well as offering prospects for the future.

G B J Dussart
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August 2003

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The Malacological Society of London

ABBREVIATIONS

Where abbreviations are necessary the following are permitted without definition

acceptable daily intake	ADI
acetolactate synthase	ALS
acetyl CoA carboxylase	ACCase
acid dissociation constant	pKa
acid equivalent	a.e.
active ingredient	a.i.
approximately	c.
base pair	bp
becquerel	Bq
body weight	b.w.
boiling point	b.p.
British Standards Institution	BSI
by the author last mentioned	*idem.*
centimetre(s)	cm
Chemical Abstracts Services Registry Number	CAS RN
coefficient of variance	CV
colony-forming unit(s)	cfu
compare	cf.
concentration x time product	ct
concentration required to kill 50% of test organisms	LC_{50}
correlation coefficient	*r*
counts per minute	cpm
cultivar	cv.
cultivars	cvs.
dalton	D
day(s)	d
days after treatment	DAT
degrees Celsius (centigrade)	°C
degrees of freedom	df
Department of Environment, Food & Rural Affairs	Defra
disintegrations per minute	dpm
dose required to kill 50% of test organisums	LD_{50}
dry matter	d.m.
Edition	Edn
editor	ed.
editors	eds
emulsifiable concentrate	EC
enzyme-linked immuno-sorbant assay	ELISA
fast-protein liquid chromatography	FPLC
Food and Drugs Administration	FDA
for example	e.g.
freezing point	f.p.
gas chromatography-mass spectrometry	gc-ms
gas-liquid chromatography	glc
genetically modified	GM
genetically modified organism	GMO
gram(s)	g
gravity	*g*
growth stage	GS
hectare(s)	ha
high performance (or pressure) liquid chromatography	hplc
high volume	HV
hour	h
infrared	i.r.
inner diameter	id
integrated crop management	ICM
integrated pest management	IPM
International Organization for Standardization	ISO
in the journal last mentioned	*ibid.*
Joules	J
Kelvin	K
kilobase pair	kb
kilodalton	kD
kilogram(s)	kg
kilogram(s) per hectare	kg/ha
kilometres per hour	km/h
least significant difference	LSD
litre(s)	litre(s)
litres per hectare	litres/ha
logarithm, common, base 10	log
logarithm, natural	ln
low volume	LV
mass	*m*
mass per mass	*m/m*
mass per volume	*m/V*
mass spectroscopy	ms
maximum	max.
maximum residue level	MRL
melting point	m.p.
metre(s)	m
metres per second	m/s
milligram(s)	mg
milligrams per litre	mg/litre
milligrams per kg	mg/kg
millilitre(s)	ml
millimetre(s)	mm
minimum	min.
minimum harvest interval	MHI
Ministry of Agriculture, Fisheries and Food (England & Wales) (now Defra)	MAFF
minute (time unit)	min
moisture content	M.C.
molar concentration	M
mole	mol
molecular weight (relative)	*Mr*
no observed adverse effect level	NOAEL

ABBREVIATIONS continued

no observed effect concentration	NOEC
no observed effect level	NOEL
no significant difference	NSD
nuclear magnetic resonance	nmr
number average diameter	n.a.d.
number median diameter	n.m.d.
octanol/water partition coefficient	K_{ow}
organic matter	o.m.
page	p.
pages	pp.
parts per billion	ppb
parts per million	ppm
parts per trillion	ppt
pascal	Pa
percentage	%
polyacrylamide gel electrophoresis	PAGE
polymerase chain reaction	PCR
post-emergence	post-em.
power take off	p.t.o.
pre-emergence	pre-em.
pre-plant incorporated	ppi
probability (statistical)	*P*
relative humidity	r.h.
revolutions per minute	rev/min
second (time unit)	s
standard error	SE
standard error of the difference	SED
standard error of the mean	SEM
soluble powder	SP
species (singular)	sp.
species (plural)	spp.
square metre	m^2
subspecies	ssp.
surface mean diameter	s.m.d.
suspension concentrate	SC
systemic acquired resistance	SAR
tandem mass spectrometry	MS-MS
technical grade	tech.
temperature	temp.
that is	*i.e.*
thin-layer chromatography	tlc
time for 50% loss; half life	DT_{50}
tonne(s)	t
tonne(s) per hectare	t/ha
ultra low volume	ULV
ultraviolet	u.v.
United Kingdom	UK
United States	US
United States Department of Agriculture	USDA
vapour pressure	v.p.
variety (wild plant use)	var.
volume	*V*
volume median diameter	v.m.d.
water dispersible granule	WG
weight	*wt*
weight by volume	*wt/v*
(mass by volume is more correct)	(m/V)
weight by weight	*wt/wt*
(mass by mass is more correct)	*(m/m)*
wettable powder	WP

less than	<
more than	>
not less than	≮
not more than	≯

Multiplying symbols-		Prefixes
mega	$(x\ 10^{6})$	M
kilo	$(x\ 10^{3})$	k
milli	$(x\ 10^{-3})$	m
micro	$(x\ 10^{-6})$	μ
nano	$(x\ 10^{-9})$	n
pico	$(x\ 10^{-12})$	p

SESSION 1

INTRODUCTION AND SCENE SETTING

Chairman: Dr G R Port
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Molluscs, molecules and man: towards new perspectives in host-parasite interactions and future control strategies

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ABSTRACT

Since the earliest settled communities, gastropod molluscs have been crop pests, as well as a source of disease afflicting agriculturalists and their livestock. Trends such as global warming, increasing population pressure and rapid transportation and admixture of alien species to areas devoid of natural competitors/predators, are exacerbating this situation in both developed and developing countries. Drugs provide effective, if often expensive, medical and veterinary treatments against gastropod vectored disease. However, mounting evidence suggests increasing drug resistance by parasites, prompting calls for detailed investigations of gastropod host-parasite interactions with the aim of producing novel interventions. Similarly, chemical treatment of crop pests is expensive, time consuming, and unpredictable, often affected by vagaries of weather/micro-climate. The majority of chemical and biological control programmes rely upon either empirical approaches or an imperfect knowledge of gastropod host-parasite interactions. Here we review the potential of new genomic approaches to elucidate the molecular basis of these interactions, and suggest how they might contribute towards more targeted control.

INTRODUCTION

Gastropod molluscs are important crop pests and intermediate hosts for a variety of medical and veterinary diseases. In many temperate areas 30-70% of main root crops, such as potatoes, may suffer slug damage, depending upon weather and local farming practices (Moens, 1980). Although cereal crops may be less affected, a population of one slug per $100m^2$ could consume 250kg of plant material per hectare each day (Mallet, 1973). As the intensity of slug damage varies with weather conditions, existing control measures are often difficult to deploy effectively, and crop damage may be locally devastating (Port & Port, 1986).

Similarly unpredictable and potentially devastating are the effects of gastropod pest introductions on subsistence crops. In South East Asia production of rice, the staple diet of 60% of the world's population (Coosemans & Mouchet, 1990), is badly affected by the snail *Pomacea canaliculata*, an alien from South America. The presence of egg toxins may have assisted in survival and spread of this species in an ecosystem lacking the predators to deal with such chemical defences (Snyder & Snyder, 1971). However, more importantly the freshwater environment acts as an effective medium for disease transmission. The most

widespread gastropod vectored infection, schistosomiasis, is estimated to afflict around 200 million people in 75 countries in the developing world (Chitsulo, *et al.*, 2000). The impact of this disease is exacerbated by the dependency of subsistence agricultural communities on irrigation projects, serving 70% of land suitable for crops in Africa. Additionally, some 165 million cattle may be infected with *Schistosoma* species (De Bont & Vercruysse, 1998).

The Gastropoda clearly contains some important pest species, the impact of which could be greatly ameliorated by strategies based on more detailed information than currently exists of interactions at the molecular level for their host-parasite systems, and for chemical control. However, most snails and slugs have substantial genome sizes, often in the order of 1Gbase, and many small, poorly differentiated chromosomes (20 homologous pairs is not an uncommon number), which makes their genomes difficult to investigate. Being easy to maintain in the laboratory *Biomphalaria glabrata* has been central to investigate and confirm the genetic basis of host-parasite compatibility (Richards, *et al.*, 1992). Here we describe our recent approach to elucidate the molecular basis of snail-schistosome interactions, and discuss how such recent advances in molecular biology may be used to investigate a variety of medical, veterinary and commercially important questions fundamental to gastropod control.

SNAILS AND SCHISTOSOMES: A CASE STUDY

Many species of freshwater planorbid snail are intermediate hosts of trematode parasites. The widespread genus *Biomphalaria* contains intermediate hosts for *S. mansoni*, responsible for intestinal schistosomiasis in Africa, Arabia, Madagascar, South America and the Caribbean. Schistosomiasis remains a debilitating disease in many agrarian communities, especially in sub-Saharan Africa, and causes severe morbidity in the absence of control interventions. Recent efforts at combating or managing human schistosomiasis are based largely on integrated approaches that focus on the prevention, detection, and treatment of infected individuals, the latter with chemotherapy using praziquantel, the only generally available drug. However, there is increasing concern that parasite tolerance to praziquantel may increase with long-term usage and alternative control approaches must be investigated. Additionally, the development of an effective vaccine remains elusive. Consequently, research efforts have been directed towards eliminating snail vectors by modification of snail habitat, and biological control, but with the focus on molluscides, many of which produce collateral damage to fisheries or important ecosystem components. The design and application of many molluscicides has been empirical, and their mode of action at the molecular level remains largely uncertain, precluding systematic improvement. Current efforts to control freshwater snails are therefore largely untargeted, demanding a more specific approach to interrupt transmission. Alternative effective control measures may involve limiting the parasite's access to compatible snails.

Underpinning this approach is a need to elucidate interactions between the developing parasite and the snail's internal defence system (IDS), identifying those host factors that disrupt, influence, or promote trematode survival in the snail. Little is known of the molluscan IDS compared to vertebrate immune systems. Molluscs lack an adaptive immune system, instead using various innate, non-adaptive mechanisms, involving cell-mediated and humoral reactions that interact to recognize and eliminate invading pathogens or parasites (Loker, 1994). Haemocytes play a primary role in snail IDS, aggregating in response to trauma, phagocytosing small particles (such as bacteria or yeast), and encapsulating larger ones (such

as trematodes). Activation of haemocytes may rely upon lectins, which have been implicated as mediators of non-self recognition in invertebrates and are considered to play a role in the humoral immunity of molluscs (Horak & van der Knaap, 1997). Haemocytes detect schistosome-derived molecules presumably through direct binding to cellular receptors, or indirectly through detection of molecules released from other host tissues in response to infection. Killing mechanisms use non-oxidative and oxidative pathways (Bayne, *et al.*, 2001a), with the production of toxic reactive oxygen intermediates (ROIs). Haemocyte immune responses to invading parasites are thought to be controlled by receptor-mediated signal transduction pathways (Yoshino, *et al.*, 2001), probably with several types involved in interactions between the invading parasites and snail haemocytes (reviewed in Lockyer, *et al.*, 2003). As yet, little is known about these pathways or the genes associated with mechanisms of resistance, although the existence of snails naturally resistant to the parasite provides an opportunity to use modern genomic approaches to investigate the genetic basis of resistance/ susceptibility.

There is considerable disparity in availability of genome sequence data for different components of the snail-schistosome-human system (intermediate host, parasite and definitive host). Much progress has been made with helminth gene discovery programmes (Foster & Johnston, 2002), but compared to the genomic studies of other invertebrate vectors, such as *Anopheles gambiae* (Aultman, *et al.*, 2002; Hoffman, *et al.*, 2002), molecular studies of *B. glabrata* are in their infancy. Availability of all three relevant genome sequences would allow rapid progress in the identification of novel strategies for controlling the development of the trematode in the intermediate host. With this motivation an international consortium has recently formed to pursue a genome initiative in *B. glabrata* (http://biology.unm.edu/biomphalaria-genome/index.html), a major objective of which is construction of a bacterial artificial chromosome (BAC) library, recently approved by the National Human Genome Research Institute. The genome size is estimated to be 931 Mbases, with a GC content of 46%, and the 18 chromosomes are small and undifferentiated.

Despite a marked increase in the application of molecular methods, information on the nature of genes involved in the host-parasite relationship remains rudimentary. However, the application of modern genomics to such 'non-model' organisms has become a reality with the advent of approaches permitting investigation of uncharacterized genes expressed in response to specific stimuli, such as parasite infection, or exposure to a toxic substance.

MOLECULAR STRATEGIES FOR TARGETING THE GENETIC BASIS OF MEDICAL, VETERINARY AND COMMERCIAL TRAITS IN MOLLUCS

Processes involved in host-parasite interactions, such as non-self recognition and signal transduction, may be associated with differential gene expression. In order to understand their regulation, relevant sets of differentially expressed genes must be identified, cloned and characterized. Differential gene expression can be analysed independently by several approaches including Differential Display (DD), Expressed Sequence Tag (EST) methodologies, and Suppression Subtractive Hybridization (SSH), as well as high throughput parallel methods such as DNA microarray technology. However, the power of these approaches is greatly increased when they are used serially, with SSH, DD and EST derived transcripts arrayed to identify gene constellations expressed in response to specific stimuli. Collectively, these approaches have begun to make it feasible to correlate mRNA patterns in