Inside JEB highlights the key developments in *The Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

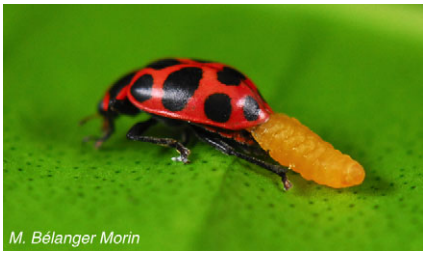
HOW PERNICIOUS PARASITES TURN VICTIMS INTO ZOMBIES

Parasites come in all shapes and forms. From skinny tapeworms that infest intestines to the microscopic infectious agent of malaria (*Plasmodium*), parasites are usually inconvenient and sometimes lethal. But there is one group of parasites that is particularly pernicious – they are the parasites that hijack their host's nervous system, turning their victims into zombies. 'The fact that parasites can so efficiently alter host behaviour is fascinating', says *JEB* Editor Michael Dickinson, from the University of Washington, USA, adding, 'There is something horrifying and wondrous about a tiny "implant" being able to control such a large animal machine'. What is more, it appears that these minute manipulators can have a significant, and often under-appreciated, impact on ecology, physiology and evolution, orchestrating the behaviour of vertebrates and invertebrates alike. 'Neuroparasitology is a science where science meets science fiction', Dickinson observes. However, the community tackling the thorny question of how parasites take possession of their hosts by manipulating their nervous systems, and the large-scale implications of these behavioural changes, is tiny. Shelley Adamo – an insect behavioural physiologist from Dalhousie University, Canada – adds that working with parasitic systems is particularly challenging because of the necessity of raising two different organisms in the lab.

Given the challenges faced by this small but dedicated community, Michael Dickinson and *JEB* colleague Janis Weeks invited Adamo and *Toxoplasma gondii* expert Joanne Webster from Imperial College, UK, to co-edit a special edition of *The Journal of Experimental Biology* dedicated to the burgeoning field of neuroparasitology, to learn more about how parasites turn their victims into zombies. Explaining that publication of the issue was preceded by a conference focusing on neuroparasitology in March 2012, Adamo says, 'One of the exciting things about the meeting was that it was the first time all of us had been together. There had never been a meeting entirely dedicated to this topic and usually there is a huge divide between people that work on vertebrate hosts and people that work on invertebrate hosts. We go to different meetings and publish in different journals, we read each other's work but never meet, so this was something that was really exciting'.

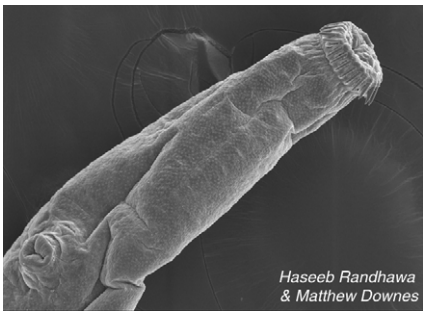
Launching the collection with her review, 'Parasites: evolution's neurobiologists', Adamo outlines the three strategies that parasites use to alter host behaviour. Explaining that the immune system is the host's first line of attack against an infection and that cytokines (factors released by the immune system) are known to modify behaviour to produce 'sickness behaviour', Adamo suggests, 'It may be a small evolutionary step from manipulating the host's immune system to prevent destruction, to manipulating it to secrete modulators that lead to a change in host behaviour' (p. 3). However, Adamo points out that in addition, many parasites directly influence their hosts by manipulating the release of neuromodulators, such as dopamine, octopamine and serotonin in the brain. Parasites also specificly target the expression of neurofunctional genes by secreting second messengers that directly impact gene expression, such as the selective activation of a neuronal transcription factor by *T. gondii* or the alteration of moulting hormones in caterpillars by baculovirus. Adamo adds that in contrast to neuroscientists, who target specific brain regions and systems to alter behaviour, parasites use a broader approach, targeting multiple brain structures *via* a selection of mechanisms, and she suggests that this is a strategy that the neuropharmaceutical industry would do well to emulate.

Alteration of host behaviour


M. Bélanger Morin

'When it comes to the larger field of change in host behaviour, Janice Moore literally wrote the book', says Adamo, pointing out that Moore, from Colorado State University, USA, discussed the ecological consequences and evolutionary mechanisms behind the behavioural changes exhibited by infected hosts in her 2002 book, *Parasites and the Behavior of Animals* (Moore, 2002). Building on this seminal publication in her current review, Moore describes how infected and potential hosts can exhibit a continuum of behavioural changes, ranging from novel actions that place the host at risk – such as directing intermediate hosts to seek predators – through to behaviours that benefit the host – such as swatting with the intention of avoiding infection (p. 11). In addition, she points out that as visually dominant animals, we may simply be missing a broad array of parasite-induced behavioural changes that may occur in the acoustic and olfactory domains,

concluding, 'How can we fail to wonder what manipulations we are missing in this wide world of information that lies just beyond our own senses?'

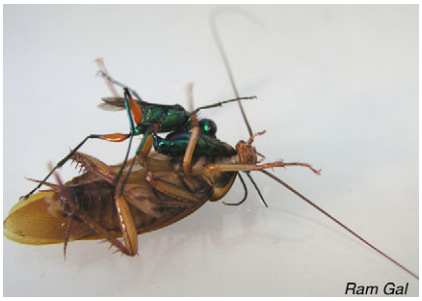


However, quantifying behaviour – natural or manipulated – is not a simple matter. Animals are not automata that uniformly reproduce identical textbook behaviours without variation. Each individual personalises their own suite of behaviours, each locating their own position on a range of behavioural continua – from shy to bold, from social to solitary – which define that individual's personality. Robert Poulin, an ecologist from the University of Otago, New Zealand, explains that instead of modifying individual behavioural traits, parasites target suites of interrelated traits in their host's behaviour: they alter the host's personality (p. 18). For example, it would be pointless for a parasite to increase the risk of transmission to its definitive host by altering behaviour without simultaneously making the intermediate host more conspicuous. Poulin lays out two potential strategies that parasites may use to alter personality. He says, 'They include increasing the variability in how an animal responds to a stimulus and altering the link between different behaviours (for example, decoupling two behaviours that would normally be expressed together, such that they become independent)'. However, having described how trematode infections impact a selection of behaviours in their intermediate hosts (amphipods), Poulin adds, 'The power of animal personality and behavioural syndromes to inform research on host manipulation by parasites will only be fully realised when underlying mechanisms are elucidated and linked to their phenotypic impacts'.

Continuing the theme that parasites impact multiple behaviours (phenotypic traits), Frank Cézilly, Adrien Favrat and Marie-Jeanne Perrot-Minnot from the Université de Bourgogne, France, define the phenomenon of multidimensionality in parasite-induced phenotypic alterations (where multiple behaviours are altered in concert) and sequential multidimensionality (where several behaviours are altered in succession) (p. 27). Having raised concerns that the currently reported extent of multidimensionality may simply reflect the degree to which a system has been studied, the trio outlines how targeting multiple behaviours could benefit parasites by increasing predation on intermediate hosts by the definitive host, but adds that as well as increasing the success of parasite transmission, some behavioural changes are likely to have occurred in response to the pressure to make infected hosts less attractive to non-host species. Explaining that the mechanisms that underlie parasite-induced phenotypic alterations are poorly understood, Cézilly and his co-authors suggest that the phenomenon could result from the infected host's immune response or differential activation of key neurochemical signalling pathways.

While behavioural manipulation clearly has cost implications for the parasite, Frédéric Thomas from the MIVEGEC centre in Montpellier, France, and an international team of collaborators propose that behavioural changes in infected hosts also incur costs for the victim, due to either increased activity or lost foraging opportunities when activity is reduced (p. 43). They suggest that parasites may have to balance the amount of energy that they invest in altering host behaviour against the cost to the host of the behavioural alteration, which could ultimately limit the host's life expectancy and impair the parasite's chances of being passed on. Suggesting that the energetic cost incurred by the host is a key constraint on parasite behavioural manipulation (defined as the host energetic resource constraint – HERC – hypothesis), Thomas and his colleagues go on to outline the sole demonstrated example of this trade off, where the spotted lady beetle *Coleomegilla maculata* is parasitised by the parasitic wasp *Dinocampus coccinellae.* In addition, they discuss other situations where parasites may need to regulate the amount of energy invested in altered behaviours by a host, and say, 'The relevance of the HERC hypothesis possibly lies in the fact that it may help us to understand different aspects related to the evolution of host manipulation by parasites'.

Continuing by focusing on one particular group of parasites, the insect parasitoids, Thomas and colleagues describe how these animals exploit their hosts during a single life stage – for the development of their young – in a specialised form of behavioural alteration known as 'bodyguard manipulation' (p. 36). The team describes how hosts are either manipulated to provide defence for developing pupae or exploited as incubators to feed and protect larvae. After listing the species that have selected this particularly gruesome form of parasitism, Thomas and his co-authors show that some more conventional parasites also manipulate the behaviour of their host to become more bodyguard-like. By reducing risky behaviour in the host, parasites can prevent unproductive predation by alternative hosts during non-infective life stages. Suggesting that manipulative parasitism, such as the bodyguard manipulation, evolved to provide protection for vulnerable insect life stages, the team concludes that the relatively simple life cycles of parasitic wasps makes them ideally suited to addressing the question of the trade-offs between the benefits of the manipulation and the costs – such as altered fecundity and longevity – incurred by the parasite.



Following on from Thomas's proposal, Frederic Libersat and Ram Gal from the Ben Gurion University of the Negev, Israel, discuss their ground-breaking work teasing apart the neuronal mechanisms that allow parasitic jewel wasps to rob cockroaches of their capacity for independent movement (p. 47). Inflicting non-lethal injections to initially paralyse and then incapacitate the cockroach's ability to move of its own free will, the wasp then leads the docile victim to a burrow where it is entombed with a single egg, and subsequently consumed by the developing larva and pupa. Describing how the wasp injects venom directly into the supra- and sub-oesophageal ganglion in the cockroach brain – which modulate locomotion – with extraordinary precision, Libersat explains that the venom targets the victim's ability to walk spontaneously by reducing its drive in addition to raising the level of neuronal activity required to stimulate and maintain walking. The duo concludes by recounting a series of neuropharmacological experiments, which showed that the supra- and sub-oesophageal ganglion control the insect's drive to walk.

Closing the section dedicated to the manipulation of host behaviour by parasites, Kevin Lafferty from the US Geological Survey and Jenny Shaw from the University of California Santa Barbara, USA, asked whether the type of host, type of parasite or the site of infection affected the host's change in behaviour (p. 56).

Explaining that intermediate hosts either become less active – reducing their chances of evading a predator – or more active and alter their choice of environment – increasing the probability of an encounter with a predator – Lafferty and Shaw report that more parasites manipulated their host's activity than their microhabitat selection. Considering the locations that parasites occupy in the bodies of their hosts, the duo explains that many target the body cavity, muscles and central nervous system. Finally, Lafferty and Shaw outline what is currently known about how parasites manipulate the host's nervous and endocrine systems to alter its behaviour.

Neuroimmunological mechanisms of behaviour manipulation

Having considered the impact of parasitic infections on host behaviour, the collection now switches to review what is known about the neuroimmunological mechanisms that mediate these changes. Simone Helluy from Wellesley College, USA, explains that Adamo initially proposed that the host's natural neuroinflammatory response to infection could bring about some of the behavioural changes (Adamo, 2002). After surveying the influence that various parasites located either in the body cavity or in the brain have on the behaviour of several gammarid species (p. 67), Helluy points out that the different infections, 'induce similar behavioural pathologies characterised by the alteration of sensorimotor pathways'. Having recognised that infected gammarids adopted the same posture as other crustaceans (such as lobsters) that had been treated with the neurotransmitter serotonin, Helluy showed that both motor and sensory aspects of the infected gammarid's behaviour are likely to be modulated by the neurotransmitter. She goes on to speculate that serotonin corrupts signals from the olfactory and visual system, leading the infected crustacean to swim toward, instead of away from, light. Drawing insights from vertebrate and invertebrate innate immune responses to parasites, Helluy suggests that the inflammatory processes in the nervous system, triggered by infection, are responsible for disruption to the crustacean's serotonergic system, leading to altered behaviour.

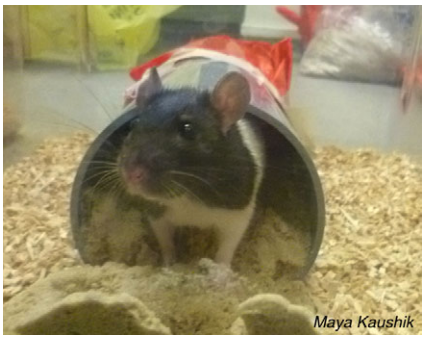
Moving on to the impact of parasites on vertebrates, Celia Holland from Trinity College, Ireland, and Clare Hamilton from University College Dublin, Ireland, describe the effect of the dog parasite *Toxocara canis* on mice and humans, the dead-end hosts (i.e. they do not pass on the infection) where it can damage vision and cause psychological effects in humans (p. 78).

Explaining that the immune response of mice can limit the degree of cerebral infection, Holland and Hamilton describe how infected animals are less active, appear to be less timid and less afraid of open spaces, novelty and predators, and suffer memory impairment. The degree of behavioural alteration is affected by the extent of the cerebral infection. Adding that *Toxocara* produces inflammatory responses in the brain of infected animals, causing sickness behaviour, the duo point out that infection also alters neurotransmitter profiles in infected brains. However, they say that, 'As of now, these changes in immune parameters have not been correlated with observed behavioural changes or defects in the same experimental animals', and they suggest that simultaneously measuring behaviour, the level of immunity factors and the extent of the *Toxocara* infection in mice would help our understanding of cerebral *Toxocara* infection.

Drawing the section to a close, Adamo says, 'We invited Robert McCusker and Keith Kelley to contribute because they are neuroimmunologists and they can explain how immune systems influence brain function'. Outlining the symptoms of depression and sickness behaviour (including fever, nausea and achiness), McCusker and Kelley go on to discuss the pathways that transmit the signal that an infectious agent has been recognised by the immune system to the brain to initiate the behavioural changes (p. 84). Explaining that inflammatory cytokines, such as TNFα and the interleukin IL-1β, are produced when infectious agents bind to specialised receptors on cells of the innate immune system, McCusker and Kelley add that the inflammatory response triggers specific behaviours that are independent of other physiological effects of infection. In addition, they describe that certain cytokines and other inflammatory factors trigger sickness behaviour, while others primarily act to amplify this behavioural response, by interacting directly with peripheral neurones to send signals to the brain, or by acting directly within the brain by crossing the blood–brain barrier. They say, 'The immune system communicates with the brain to regulate behaviour in a way that is consistent with animal survival'.

Behavioural manipulation by *Toxoplasma*

If ever there was an emblematic example of a parasite hijacking a vertebrate and manipulating the unfortunate zombie, it must be *Toxoplasma gondii*. Picked up by rats and mice from cat faeces, the parasite dramatically alters the rodents' behaviour, so that the infected animals become fatally attracted to the smell of cat urine in a bid to pass *Toxoplasma* on to its definitive host (the cat). There, it reproduces sexually, releasing the infective life stage of the parasite into the cat's intestine ready to be passed on to the next generation of unsuspecting rodents. However, infective parasites have no control over which host they contact, so they are routinely passed on to creatures other than cats – such as humans and domestic livestock – where the parasite reproduces asexually, forming cysts in various tissues (including the brain) which may remain for the rest of the victim's life.


Maya Kaushik

Outlining the impact that a *Toxoplasma* infection has on the behaviour of hosts ranging from cats and rodents to otters and humans, Joanne Webster and colleagues from Imperial College, UK, and the University of Leeds, UK, make the case that *Toxoplasma* may be a contributory factor in some cases of human schizophrenia given its presence in the brains of infected individuals and our long lifespan (p. 99). The team also suggests that *Toxoplasma*-infected rats may provide a good model for human schizophrenia symptoms, because of similarities in the groups of behaviours manifested by the infected rodents and schizophrenic patients, in addition to providing insight into the mechanisms underlying behavioural changes in infected hosts.

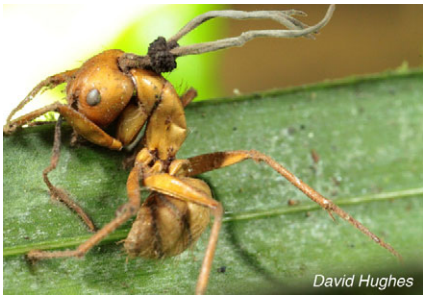
However, *Toxoplasma* transmission is not restricted to the trophic route though the food chain. Ajay Vyas from the Nanyang Technological University, Singapore, explains that the parasite can also be transmitted sexually in the semen of infected dogs and rodents to females and their pups (p. 120). According to Vyas, female rats find infected males that are carrying other parasites – including ectoparasites and protozoa – unattractive. In contrast, when presented with males infected with *Toxoplasma*, the females find them more attractive than parasite-free males. Remarkably, infected males produce more testicular testosterone than uninfected males. Vyas explains that this could account for their greater attractiveness to females and to their increased boldness, which raises the risk of predation.

Continuing with the theme of the effect of *Toxoplasma* infection on behaviour, Glenn McConkey and colleagues from the University of Leeds, UK, and Joanne Webster explain that during chronic infection the parasite forms cysts in the host brain (p. 113). Some studies suggest that in rats the cysts target regions of the brain associated with fear, possibly explaining why the rodents lose their fear of cats. However, there is currently no evidence of selective dispersal in human brains. Yet, the team reports a novel mechanism that could still account for specific neurological disorders in humans. According to McConkey, *Toxoplasma* cysts produce an enzyme that catalyses the synthesis of a precursor of the dopamine neurotransmitter. Although this precursor could be synthesised in all infected cells, it would only lead to increased dopamine levels in circuits that synthesise the machinery necessary for the conversion of the precursor to dopamine. 'By this mechanism, although the parasite infects many brain regions, only catecholamine neurones would be affected and behaviours associated with these neurones', the team says.

Concluding the section dedicated to *Toxoplasma*, Jaroslav Flegr from Charles University, Czech Republic, reviews the physiological and neurological impact of *Toxoplasma* on humans (p. 127). Estimating the personality profiles of infected and uninfected individuals with standard psychological questionnaires, Flegr explains that the personality differences that he has identified become more pronounced with time. In addition to reducing reaction times in infected individuals and suppressing their immune systems, male hosts also appear more masculine and are, on average, 3 cm taller than uninfected males. Flegr also reviews the evidence suggesting that *Toxoplasma* is involved in the initiation of severe schizophrenia, although he adds that individuals with Rhesus positive blood groups appear to be protected from some effects of infection.

New approaches to understanding host behaviour manipulation

Having discussed a wide variety of behavioural changes, neural mechanisms and the effects of *Toxoplasma gondii*, the collection switches attention to novel approaches that will allow us to further understand how parasites manipulate their hosts. In a second review by Perrot-Minnot and Cézilly, they discuss the use of pharmaceutical drugs to alter host behaviour in order to identify the role that specific behavioural changes have on parasite transmission (p. 134). In addition, they explain how neuropharmacology can be used to identify the role of specific neural systems in multidimensional behavioural manipulations – where a range of behaviours is altered (either simultaneously or in succession) as a result of parasitic infection. The duo goes on to emphasise the need to develop an explicit neuroethological framework for the study of parasitic manipulation in order to learn about and better understand the neural mechanisms that underpin parasitic behavioural manipulation.


David Hughes

Also emphasising the power of modern approaches, David Hughes from Pennsylvania State University, USA, points out that these analyses can help us to understand the mechanisms that underpin animal behaviour. Explaining that the manipulation of behaviour in the host can be thought of as an extended phenotype of the parasite, Hughes says that as 'natural selection has acted on both the genome of the parasite and the host to control a single phenotype (behaviour in the host), understanding diverse pathways from genes to phenotypes will help us tackle the important question in evolutionary biology: what is the mechanistic basis of animal behaviour?' (p. 142). Presenting case histories – such as the effect of a parasitic fungus on the behaviour of ant hosts and how a single parasitic baculovirus gene triggers infected gypsy moth caterpillars to climbing to the top of vegetation to propagate the infection – Hughes says, 'We have a fabulous opportunity to not only advance our own understanding of the co-evolutionary dynamics between animals and the parasites that manipulate them but also contribute to evolutionary behaviour more generally'.

Concluding the collection, David Biron from CNRS, France, and Hugh Loxdale from the Royal Entomological Society, UK, discuss the use of 'parasito-proteomics' – the study of the reaction of the host and parasite genomes through the expression of their proteomes during complex interactions (cross-talk) between the two – to understand how parasites manipulate behaviour and to identify key genes involved in the alteration (p. 148). Describing the impact of several parasites on the proteome of their hosts – such as the hairworm, which mimics some of the host's neuromodulators in order to drive infected insects to commit suicide in water – the duo goes on to show that pathways involved in similar behavioural modifications across different hosts are evolutionarily convergent. However, Biron and Loxdale also warn of the many pitfalls that may confound these complex studies, and point out that elucidating the peptidome of manipulating parasites is a major future goal to understand the mechanism of host behavioural manipulation by parasites.

To the future

Reflecting on the papers that have been collected together in this special issue, Adamo says, 'Parasites don't work as simple bullets hitting one target. One of the things to come out of the symposium and this collection is that we have to think of parasitic manipulation in terms of multiple mechanisms working simultaneously. It means that many standard neuro-techniques are going to be tough to use. The new molecular techniques presented by Biron, Hughes and McConkey will be important for showing us how you get specific behavioural changes through what look like non-specific mechanisms'.

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Moore, J. (2002). *Parasites and the Behavior of Animals*. Oxford: Oxford University Press.

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