



SYMPOSIUM

On the Origins of Parasite-Extended Phenotypes

David P. Hughes^{1,*†‡}

*Department of Entomology, Pennsylvania State University, University Park, PA 16802, USA; †Department of Biology, Pennsylvania State University, University Park, PA 16802, USA; ‡Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, PA 16802, USA

From the symposium “Parasitic Manipulation of Host Phenotype, or How to Make a Zombie” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

¹E-mail: dhughes@psu.edu

Synopsis Parasites that adaptively manipulate the behavior of their host are among the most exciting adaptations that we can find in nature. The behavior of the host can become an extended phenotype of the parasites within animals such that the success and failure of the parasite’s genome rely on precise change of the host’s behavior. Evolutionary biology was borne from the close attention of naturalists such as Wallace and Darwin to phenotypic variation in seeking to understand the origins of new species. In this essay, I argue that we also need to think about the origins of parasite-extended phenotypes. This is a more difficult task than understanding the evolution of textbook examples of novelty such as the eyes of vertebrates or the hooves of horses. However, new tools such as phylogenomics provide an important opportunity to make significant progress in understanding the extended phenotypes of parasites. Knowing the origins of parasite-extended phenotypes is important as a goal all by itself. But the knowledge gained will also help us understand why complex manipulation is so rare and to identify the evolutionary tipping points driving its appearance.

Introduction

Charles Darwin and Alfred Russell Wallace were excellent naturalists with a precise understanding of phenotypes and how they varied from individual to individual. Both were schooled on beetle collecting on British heaths (Berry and Browne 2008) and both took their entomologizing to far-flung locations. For Darwin, it was the well-known *Voyage of the Beagle* (1831–1836) (Darwin 1845) that acted as the catalyst for his theory of origins. For Wallace, the groundwork was laid on his near fatal trip to the Amazon and Rio Negro (1848–1852) where his boat (and thousands of samples) sunk on the return home (he was rescued after 10 days drifting in a leaky lifeboat [Berry 2002]). His discovery of what he called the *Organic Law of Change*, however, came during his extensive trip across the Malay Archipelago, memorialized in the eponymously titled book (Wallace 1869). Both men arrived at the theory of evolution by natural selection because of their exposure to many and varied phenotypes, experienced in diverse locations. Their thinking was also clearly influenced by geological processes and, in

the case of Darwin, by artificial selection of domesticated animals by humans (Darwin 1859). But by far the greatest impression for both men was made from their close attention to the phenotypes of animals. What then I wonder would they have thought of the extended phenotypes of parasites within the animals they so attentively studied?

The behavior of animals is sometimes due to the activities of the parasites within their bodies (Moore 2002). Some parasites have evolved the ability to adaptively control animal behavior in precise ways that enable the transmission of the parasite from one host to another. Now textbook examples of such behavioral manipulation are *Dicrocoelium dendriticum* brainworms inducing ants to bite into leaves to travel to the guts of ruminants (Moore 2002); hairworms causing crickets to jump into water to achieve mating (Thomas et al. 2002); or *Toxoplasma* changing the behavior of rats inducing a fatal feline attraction for the parasite to reach its definitive host where it reproduces (Berdoy et al. 2000; Webster 2001). Such examples of parasites affecting the behavior and morphology of parasites in



Fig. 1 The extended phenotype of *Ophiocordyceps unilateralis* in the SE Asian ant *Polyrhachis armata*. The mandibles of the ant can be seen embedded into the leaf vein which the fungus controlled it to bite before killing it and growing the large stalk from its head. This would have been very similar to that first sample collected by Wallace in 1859.

ways that increase transmission have come to be known as parasite-extended phenotypes (Dawkins 1982, 1990, 2004, 2012). In these examples, natural selection has shaped the genomes of parasites to control the phenotypes of the host they occupy. Multiple lines of evidence are emerging to illustrate the mechanisms by which parasites achieve this feat (Adamo 2012; Adamo and Webster 2013; Hughes 2013).

One of the most complex examples of parasites extending their phenotypes is the precise and complex manipulation of worker ant behavior by *Ophiocordyceps unilateralis* s.l. (Fig. 1) (Andersen et al. 2009; Hughes et al. 2011a; Andersen and Hughes 2012; Andersen et al. 2012). In this system, a fungal parasite controls animal behavior, causing worker ants to lock onto the underside of leaves by their mandibles before dying. Once dead the fungus grows a large stalk from the ant's cadaver. The function of such manipulation is the dispersal of spores from the stalk and the leaf provides a platform for spore release. Rather interestingly, Alfred Russell Wallace himself was the first scientist to collect samples of ants attached to the underside of leaves by their mandibles (*Polyrhachis merops* and *Echinopla melanarctos* at Tondano, a village in Celebes, Sulawesi). Unfortunately, we don't know what he made of ants attached to the underside of leaves and the samples have been lost in time. Darwin also observed textbook examples of parasites capable of controlling behavior; in his case, they were barnacles that he studied for 8 years (Høeg 1995). In that time he surely encountered one of the weirdest of all barnacles, *Sacculina*, which has evolved to control crab behavior. However, the morphological

reductions that typically accompany a transition to parasitism meant he did not recognize them for the barnacles they are. Within their extensive writings, neither of these great biologists discussed parasites living inside animals or their role in behavior.

Indeed, as Richard Dawkins who originally conceived of extended phenotypes (Dawkins 1982) recently pointed out, the extended phenotypes of parasites, although they are complex adaptations on par with any other adaptations in nature, have largely gone unnoticed by most evolutionary biologists (Dawkins 2012). Part of this blindness is due to the rarity of seeing nature through the eyes of parasites. Since parasites are cryptic it has been easy to overlook their effects. Recently, Moore (2012), a major developer of the field of parasites and behavior, recounted wonderfully the history of the field and how major advances have happened due to certain scientists taking sidelong views of animal behavior.

In 2014, the extended phenotypes of parasites are generally much better known. In many cases, we now are able to understand the mechanisms by which parasites can control behavior (Adamo 2012). We are also constantly adding new and intriguing examples such as nematodes and how they affect the behavior, color, and morphology of their hosts (Poinar and Yanoviak 2008; Yanoviak et al. 2008). In a number of cases, we have been able to see that manipulated behaviors also have significant knock-on effects (Lafferty and Kuris 2012). With this strong foundation there is every indication that the field will grow. It is therefore a good time to ask about the origins of these impressive parasite-extended phenotypes.

Part 1: Evidence from the past

Fossil record

One of the greatest lines of evidence for evolution by natural selection comes from the fossil record. A prime example of this, and one that fills many textbooks, is how the modern horse came to be such a large beast running across plains of grass on one toe, when its dog-sized ancestors scurried on four-toed hooves around Eocene forests over 50 Ma. Indeed, such transitions lead to Darwin's (1859) famous closing line of the origin where he stated "from so simple beginnings endless forms most beautiful and most wonderful have been, and are being evolved". Within this framework, we have come to understand the multiple steps in the evolution of the horse from *Eohippus* to *Equus* and by charting the correlated changes in traits from the feet to elongated legs to robust teeth and greatly enlarged zygomatic arches

capable of coping with a diet of grass. Darwin himself made a seminal contribution when he discovered the tooth of a long extinct horse from Patagonia (*Equus curvidens*) that established that the horse was once present in South America before going extinct and then being re-introduced by Europeans. What fossils offer, therefore, is a way to understand how complex phenotypes can come to be and how past populations were geographically distributed. We see this working well for understanding the lineages of horses and many other animals, such as the evolution of whales from terrestrial carnivores or birds from feathered dinosaurs. Can fossils illuminate our understanding of how parasites evolved the ability to control the behavior of their hosts?

Animal behavior does fossilize and we can use fossil impressions and amber fossils to better understand what animals did in the past (Boucot and Poinar 2011). An example of this is the discovery of nesting behavior in dinosaurs or avian-like sleeping positions in dinosaurs (Xu and Norell 2004). We can also discover past behaviors by examining impressions where animals either excavated the ground for burrows or constructed nests. Nest-building is of course also an extended phenotype (Dawkins 1982). But since nest-extended phenotypes are already solid constructions either carved out of soil and wood or built structures from dead material such as mud, sticks, feathers, and hair it is easier for them to fossilize. But can the behavioral changes of an infected animal fossilize? To ask whether the altered behavior of infected animals can be recorded from the fossil record we should first ask how frequently do we find fossil evidence of parasitism. It turns out that examples of infection can be directly deduced from observing fossils (Boucot and Poinar 2011, Ch4). For example, corals infected by copepods (deduced from copepod-generated cysts that form distinctive “Halloween pumpkin masks” that are evident in the fossil record).

So, can manipulated behavior fossilize? In my own work on fungi controlling ant behavior I was motivated to ask whether the extended phenotypes of fungal parasites in ants could also fossilize (Hughes et al. 2011b). Following infection by *O. unilateralis*, worker ants ascend leaves and bite into the main veins before being killed (death-grip behavior). Since the ant dies upside down the fungus has evolved the ability to manipulate the mandibular muscles causing an effective lock-jaw and biting behavior (Hughes et al. 2011b). This results in a pair of mandible marks on the veins. Since leaves fossilize it was possible to ask whether we could discover in the fossil record an example of such death-grip behavior.

From the Messel deposits of Germany, Torsten Wappler, Conrad Labandeira, and I identified a fossil leaf 47 Myr old, which contained the tell-tale scars of ant manipulation by *Ophiocordyceps* (Hughes et al. 2011b). Indeed, we could be so precise as to infer that it was *O. unilateralis* as only this complex is so far known to induce biting into the veins.

Such direct evidence is possible in this host–parasite relationship because the manipulated ant leaves a signature on the leaf. In other systems, however, we can infer the earliest date when manipulation occurred if we find examples in the record of parasites that are today known to manipulate behaviors. For example, the group of obligate parasitic barnacles that infect crabs are the Rhizocephala. The species *Sacculina carcini* described in 1836 by J. Vaughan Thompson is typical and consists of an external sac outside the crab’s body with the majority of the body stretching as “roots” around the body and central nervous system of the host (Høeg 1995; Deutsch and Mouchel-Vielh 2003). This root forms after parasite’s entry into the host (as the kentrogon stage) and the female extrudes her “externa” through the ventral side of the host. This eventually becomes the eggmass of the parasite and the rhizocephalan parasite controls the host’s behavior in such a way as to oxygenate the parasite’s offspring. If the larval rhizocephalans land on male crabs, they can cause reversal of the host’s sex-role through the destruction of the adrenal gland, thereby turning male crabs into females capable of brooding the parasitic offspring (Høeg 1995). This manipulation is a clear example of a parasite-extended phenotype. Therefore, the discovery by Feldmann of a Miocene rhizocephalan from New Zealand that infected a male crab, which it feminized, is clear evidence that this form of manipulation has occurred at least since the Miocene (23 Ma) and possibly since the Cretaceous as Feldmann (1998) stated.

Another source of evidence is amber, which fossilizes small animals, notably insects and also small lizards on occasion (Rieppel 1980). Because amber can capture whole insects we can see details not possible in stone where insects only form compression or impression fossils (Grimaldi and Engel 2005). When amber is studied by researchers with a great awareness of parasitism, such as George Poinar who is an expert both on parasitic nematodes (Poinar 1979, 2003) and amber (Poinar 1992; Poinar and Poinar 1999), we get to see exciting fossil evidence of parasite-extended phenotypes. Prime examples are the mermithid nematodes and nematomorph hairworms. Both groups infect insects but require entry into water in order to reproduce and lay eggs. To

achieve this, they have convergently evolved the ability to manipulate insect behavior, causing infected insects to commit suicide in water (Poulin 1998). Both mermithids and hairworms manipulate the behavior of terrestrial insects to enter water. We do not have fossil evidence of insects actually entering water but we do have fossils of insects infected by both mermithids and hairworms (Boucot and Poinar 2011). As such the fossil record provides us with reasonable estimates for when such manipulation arose in these two groups.

Another group for which a fossil record might allow similar inference into the extended phenotypes of parasites are fungi that infect insects. George Poinar and others discovered *Paleoophiocordyceps coccophagus*, a fungal parasite of a scale insect from the Early Cretaceous (Upper Albian) (Sung et al. 2008). In this case, we don't suspect that the host insect was behaviorally manipulated but the ability to accurately identify the fungal group from amber offers the possibility of dating the emergence of certain groups of fungi which we know do manipulate modern insect behavior. This approach is complementary to the use of leaf scars of ants' death-grips induced by *O. unilateralis* (discussed above). Parallel to the fungal-insect examples are tetradonematid nematodes infecting ants. The nematode *Myrmeconema neotropicum* controls the behavior and color of turtle ants *Cephalotes atratus* so that infected workers whose gasters (posterior portion of abdomen) turn red ascend to the high canopy where they resemble fruit which birds are assumed to feed upon (Yanoviak et al. 2008). The nematode eggs reside in the gaster and in this way are distributed around the forest where they infect other ants (as worker ants eat nitrogen-rich bird droppings). Amazingly a fossil of this complex behavioral and morphological change exists. Poinar (2011, 2012) describes a worker ant of *Cephalotes serratus* infected by *Myrmeconema antiqua* in 20–30 Ma amber. Intriguingly the ant's gaster is punctured suggesting bird attack. Again, not conclusive evidence but given the similarity between the extant *M. neotropicum* and extinct *M. antiqua*, we can assume this genus of parasite has been manipulating ants for over 20 Myr.

Genes and genomes

One of the most famous drawings in the history of biology is Charles Darwin's phylogenetic tree, drawn in his "B" notebook on *Transmutation of Species*. This simple sketch was revolutionary because it posited that species alive today, which may hardly appear related because of very different phenotypes,

do in fact trace their lineages to a common ancestor. The forks in the tree leading to two branches represent some past, unseen event in evolutionary history, where one species gave rise to two. These species are sufficiently similar that we recognize they are closely related. The sketch is made that bit more powerful because Darwin wrote "*I think*" above it. Ever since then, phylogenetic trees have been hypothetical reconstructions of relationships among living groups (Dayrat 2003). Initially such trees were based on morphological characters, which is how Darwin himself presumably imagined the data although he did not put species names on the tree. It was the German biologist, Ernst Haeckel (1834–1919), who began putting names on the tips of the branches. He, in 1866, coined the word *phylogeny* in his *Generelle Morphologie der Organismen* (Dayrat 2003). There is an interesting history within Haeckel's work (Dayrat 2003) but for the current purposes his focus on morphology and establishing a language for comparisons helped subsequent researchers understand how species are related. In 1988, the use of ribosomal genes as characters ushered in an era of far more data where gene sequences were used to infer relationships among distantly related animals (Field et al. 1988). Ever since then molecular characters have been the primary tool for inference. We have moved from one or a few genes to multiple genes and whole genomes.

The abundance of phylogenetic and phylogenomic data is an exciting opportunity for researchers interested in the extended phenotype of parasites. Having species-level phylogenies of animals offers the possibility of examining which animals are infected by a group of parasites and which species among all those that are infected, are also manipulated. Some excellent notable examples are recent phylogenies of flies (Wiegmann et al. 2011), ants (Moreau and Bell 2013), and bees (Danforth et al. 2006). It is now possible to use those data to make phylogenies of the extended phenotypes of parasites. The concept of the extended phenotype posits that natural selection acts on the genomes of parasites to express behaviors in phenotypes of the hosts they infect. To better understand how extended phenotypes arise, a species-level phylogeny of a group of animals, some manipulated and some not, is valuable. Onto this tree the manipulated traits can be mapped. It is ideal to work with a group of parasites that display a range of manipulated traits, ideally varying in complexity. This, then, would be a phylogeny of extended phenotypes: *parasite behaviors mapped onto phylogenies of hosts genes*. Then, the task would be to analyze the character-traits to ask whether certain taxa of

hosts were more likely to be manipulated than others. This approach would view each animal species as an ecological niche for which we ask two questions: have parasites colonized that niche and have such parasites evolved the ability to manipulate the host? Such exciting questions are possible because of the abundance of data on the phylogenetics and phylogenomics of animals.

The phylogenies of animal groups provide opportunities to map the occurrence of extended phenotypes. But we can also gain insights into the evolution of extended phenotypes of parasites by resolving relationships among parasites that have evolved the ability to control behavior. This is more difficult because fewer researchers work on parasites compared with the number working on non-parasitic organisms. There are many people interested in resolving the relationships of the insect family of the ants (Formicidae) compared with the family of parasitic nematodes, Mermithidae, that manipulate the behavior of ants. As such, there are fewer phylogenies for taxa of parasites and even fewer for those taxa that contain manipulators and non-manipulators. With that said, there are still some interesting opportunities and the affordability of sequencing means researchers interested in generating such data are in a good position. With data on parasite relationships in hand it would be possible to examine whether some evolutionary patterns are discernable that facilitated the evolution of manipulation. For example, why are the acanthocephalans so dominated by behavioral manipulators? Or if mermithids and hairworms convergently evolved the manipulation of insect behavior, has that led to similar, or different, patterns of radiation? These are all good questions relevant to the evolutionary origins of parasite-extended phenotypes.

The example of convergent evolution points the way to another productive area of research, which is comparative genomics. Take for example the fungus, *O. unilateralis*, that causes its ant host to bite vegetation. These fungi belong in the Phylum Ascomycota and Family Ophiocordycipitaceae. Another group of fungi called entomophthoralean fungi are in the newly erected Phylum Entomophthoromycota. These two taxa of fungi both cause insects to bite onto leaves before dying (Roy et al. 2006, 2010). They even both cause ants to bite leaves (Hughes et al. 2011a). Nevertheless, their most recent common ancestor lived more than 500 Ma (Hibbett et al. 2007). Since they both induce the same extended phenotypes but have taken different evolutionary pathways, we can attempt to detect the signatures of manipulation within their genomes. An

exciting question is whether, given their different starting points, do we find similarities? For example, within the family Ophiocordycipitaceae, the ability to manipulate ants to bite vegetation has arisen independently (D. Hughes et al., unpublished data). This evidence is based on multi-gene phylogenies and in the future it will be exciting to examine the genomes of these more closely related fungi and ask how convergent evolution of parasite-extended phenotypes leads to changes at the level of the genome. Searches for the compounds that behaviorally manipulating fungi have evolved can take lessons from the excellent work on plant pathogenic fungi where genomes have provided extensive evidence of the ways that fungi control the metabolism of plants (Cornell et al. 2007; Soanes et al. 2008).

Part 2: Natural history

The ability to sequence genes and infer relationships will not be valuable if we do not know what effects parasites have on the behavior of their hosts. The sub-field of behavioral ecology focused on parasite manipulation is very interesting and was excellently reviewed by Moore (2012) recently. Essentially, the message from this historical overview is that discovering behaviors in animals that are due to parasites manipulating them only happens when a solid understanding of the behavior of non-infected animals is understood. The manipulated animals stand out because they are so different. To a person new to this area, it might seem that nothing can be less obvious than a hairworm-infected cricket launching itself into a pool of water where the worm to emerges and the cricket dies (Biron et al. 2005; Ponton et al. 2006), or a rat approaching a cat because of the *Toxoplasma* infection within its brain (Berdoy et al. 2000). However, these insights only come from continued and close attention to animal behavior. This point is well illustrated by the 100-year old saga of the “red-berry turtle-ant species.”

I already mentioned the infection of turtle ants, *C. atratus*, by a trophically transmitted tetradonematid nematode parasite (*M. neotropicum*) that controls the color, behavior and morphology of infected workers. The function of this multi-dimensional control is assumed to be the inducement of birds, the parasite's paranteic host, to consume infected ants, thereby enhancing transmission. These ants typically are all black and have long spines on the central part of their body (thorax) that serves to dissuade predators from eating them. The challenge for the nematode is to enter the bird. The nematode sits in the ant's gaster (terminal part of the abdomen). It

changes the color of the gaster from black to red. It also makes the gaster 14 times easier to pull off so the bird consumes what appears to be a red-berry without ingesting the spines. The change in color is so vivid that the infected ants originally were considered a different variety of the species, *C. atratus*, described in 1894 (Hughes et al. 2008). It was only through the familiarity of Stephen Yanoviak, with these ants' behavior and ecology (Yanoviak et al. 2003, 2005) that the relationships of ants, parasites, and birds, became known. Working with the previously mentioned expert on nematodes, George Poinar, they could piece together the story and discover a dramatic extended-phenotype that might appear obvious in hindsight, but remained unrecognized for more than a century.

An important message, then, is that fieldwork and observation of behavior under natural conditions are crucial. This is necessary both to discover new examples of parasite-extended phenotypes and to better understand systems that appear to be examples of adaptive manipulation of hosts' behavior by parasites but where the full details remain unclear. For example, a poster child of manipulation is *Leucochloridium paradoxum* a flatworm parasite that infects snails (*Succinea putris*), leading to multi-colored pulsating tentacles that bring to mind caterpillars on vegetation. As dramatic as such changes are and as parsimonious as the manipulation hypothesis was for such a long time (Moore 2012) it was only recently recognized that infected snails do behave differently (Wesołowska and Wesołowski 2013). Yet, we still don't know whether increased predation by birds occurs. What is needed is more fieldwork and controlled experiments. One nice model of the type of experiments possible is the excellent work on killifish infected by trematode parasites (Lafferty and Morris 1996). Infected and manipulated fish were kept in pools with uninfected fish. Predators of the fish (birds) have access to the pens with both infected and uninfected fish. Impressively, the authors discovered that infected fish were 31 times more likely to be consumed (Lafferty and Morris 1996). This then serves as an example of how a precise understanding of animal behavior based on extensive observations and consideration of the animal's natural history can inform decisive experiments.

Conclusion

If I were asked to nominate my personal epitome of Darwinian adaptation, the ne plus ultra of natural selection in all its merciless glory, I might

hesitate between the spectacle of a cheetah out-sprinting a jinking Tommie in a flurry of African dust, or the effortless streamlining of a dolphin, or the sculptured invisibility of a stick caterpillar, or a pitcher plant silently and insensibly drowning flies. But I think I'd finally come down on the side of a parasite manipulating the behavior of its host-subverting it to the benefit of the parasite in ways that arouse admiration for its subtlety, and horror at the ruthlessness in equal measure. Dawkins (2012)

Parasites that control the behavior of their hosts are fascinating. Knowing the mechanisms by which one organism controls the behavior of another is a wholly relevant area of inquiry for modern biology. This is especially true where the parasite is a microbe because the organism without a brain has evolved control over one with a brain. Already we have begun to see important advances (Poulin 2011; Adamo 2012; Hughes 2013). Alongside this we are seeing how parasites that control behavior are ecologically important and this area, no doubt, will also increase in importance (Lafferty and Kuris 2012). Given the still nascent state of this field, what is the value of advocating the importance of also considering the origins of parasite-extended phenotypes?

One important reason is contained within the quote by Dawkins (above) that considers parasite-extended phenotypes as adaptations on par with anything else in nature, even the elegant hunting behavior of a cheetah. This is certainly true, but unlike the capture of prey, the compound eye, or even the horse-example that opened this essay, many of the parasite-extended phenotypes we now observe cannot be viewed within a lineage of incremental changes from so "simple a beginning" (Darwin 1859). There is not a species of nematode hairworm that causes crickets to do half a jump into a body of water. Ancestrally, some worms requiring reproduction in water must have managed to manipulate their terrestrial insect hosts to select slightly more humid environments, thereby conferring a selective advantage to the parasite as the emerging worm would be able to travel the remaining distance to a body of water without desiccating. For the evolution of the compound eye, we can see in extant forms many eyes at different stages of complexity and so we can imagine the transitions. Not so for behaviorally manipulating parasites. Thus, thinking about origins makes us aware that this complexity came from somewhere and must be explained.

Another reason origins are important is because most parasites do not, in fact, control the behavior

of their hosts. The diversity of parasites is very high, with some suggestions that parasites might account for half of life on earth (Poulin and Morand 2000, 2005). We have no estimate of what percent of all parasites have evolved complex manipulation of their hosts' behavior, but it is likely to be very low, perhaps less than 0.5%. It has been assumed that manipulation is energetically expensive (Poulin 2011) which is likely why it is rare; most parasites are able to transmit without it. Knowing this, it becomes even more curious as to why it ever evolved. Parasite-extended phenotypes are one of the most impressive adaptations of parasites and clearly they can affect transmission. However, its rarity suggests special conditions are required. Thinking about origins for extended phenotypes, then, can help us understand the importance of variation in nature, just as it did for Wallace and Darwin.

Acknowledgments

The author is very grateful to Kelly Weinersmith and Zen Faulkes for organizing this symposium and inviting the participation. The author dedicates this essay to Richard Dawkins for his seminal contribution to parasites and animal behavior and to George Poinar Jr for seminal contributions understanding both parasites and their past.

Funding

This work was supported by the Society for Integrative and Comparative Biology (Division of Invertebrate Biology, Division of Animal Behavior, and Division of Neurobiology); The American Microscopical Society; the National Science Foundation (IOS 1338574), and funds from Penn State.

References

- Adamo S. 2012. The strings of the puppet master: how parasites change host behavior. In: Hughes DP, Brodeur J, Thomas F, (eds.). Host manipulation by parasites. Oxford: Oxford University Press. p. 36–51.
- Adamo SA, Webster JP. 2013. Neural parasitology: how parasites manipulate host behaviour. *J Exp Biol* 216:1–2.
- Andersen S, Ferrari M, Evans HC, Elliot SL, Boomsma JJ, Hughes DP. 2012. Disease dynamics in a specialized parasite of ant societies. *PLoS One* 7:e36352.
- Andersen SB, Gerritsma S, Yusah KM, Mayntz D, Hywel-Jones NL, Billen J, Boomsma JJ, Hughes DP. 2009. The life of a dead ant: the expression of an adaptive extended phenotype. *Am Nat* 174:424–33.
- Andersen SB, Hughes DP. 2012. Host specificity of parasite manipulation: zombie ant death location in Thailand vs. Brazil. *Commun Integr Biol* 5:163–5.
- Berdoy M, Webster JP, Macdonald DW. 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc R Soc Lond B Biol Sci* 267:1591–4.
- Berry A, (ed.). 2002. Infinite tropics: an Alfred Russel Wallace anthology. London/New York: Verso.
- Berry A, Browne J. 2008. The other beetle-hunter. *Nature* 453:1188–90.
- Biron DG, Marche L, Ponton F, Loxdale HD, Galeotti N, Renault L, Joly C, Thomas F. 2005. Behavioural manipulation in a grasshopper harbouring hairworm: a proteomics approach. *Proc R Soc B Biol Sci* 272:2117–26.
- Boucot AJ, Poinar GO. 2011. Fossil behavior compendium. Boca Raton: CRC Press, Inc.
- Cornell MJ, Alam I, Soanes DM, Wong HM, Hedeler C, Paton NW, Rattray M, Hubbard SJ, Talbot NJ, Oliver SG. 2007. Comparative genome analysis across a kingdom of eukaryotic organisms: specialization and diversification in the fungi. *Genome Res* 17:1809–22.
- Danforth BN, Sipes S, Fang J, Brady SG. 2006. The history of early bee diversification based on five genes plus morphology. *Proc Natl Acad Sci USA* 103:15118–23.
- Darwin C. 1845. Journal of researches into the natural history and geology of the countries visited during the voyage of H.M.S. Beagle round the world, under the Command of Capt. In: Fitz Roy R.N, 2nd ed. London: John Murray (The Voyage of the Beagle), John Murray London.
- Darwin C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. London: John Murray.
- Dawkins R. 1982. The extended phenotype. Oxford: W.H. Freeman.
- Dawkins R. 1990. Parasites, desiderata lists and the paradox of the organism. *Parasitology* 100:563–73.
- Dawkins R. 2004. Extended phenotype—but not too extended. A reply to Laland, Turner and Jablonka. *Biol Philos* 19:377–96.
- Dawkins R. 2012. Foreword to host manipulation by parasites. In: Hughes DP, Brodeur J, Thomas F, (eds.). Host manipulation by parasites. Oxford: Oxford University Press. p. xi–xiii.
- Dayrat B. 2003. The roots of phylogeny: how did Haeckel build his trees? *Syst Biol* 52:515–27.
- Deutsch JS, Mouchel-Vielh E. 2003. Hox genes and the crustacean body plan. *Bioessays* 25:878–87.
- Feldmann RM. 1998. Parasitic castration of the crab, *Tumidocarcinus giganteus* Glaessner, from the Miocene of New Zealand: coevolution within the Crustacea. *J Paleontol* 72:493–8.
- Field KG, Olsen GJ, Lane DJ, Giovannoni SJ, Ghiselin MT, Raff EC, Pace NR, Raff RA. 1988. Molecular phylogeny of the animal kingdom. *Science* 239:748–53.
- Grimaldi D, Engel MS. 2005. Evolution of insects. Cambridge: Cambridge University Press.
- Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, et al. 2007. A higher-level phylogenetic classification of the fungi. *Mycol Res* 111:509–47.
- Høeg JT. 1995. The biology and life cycle of the Rhizocephala (Cirripedia). *J Mar Biol Assoc UK* 75:517–50.
- Hughes D. 2013. Pathways to understanding the extended phenotype of parasites in their hosts. *J Exp Biol* 216:142–7.

- Hughes DP, Andersen S, Hywel-Jones NL, Himaman W, Bilen J, Boomsma JJ. 2011a. Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal infection. *BMC Ecol* 11:13.
- Hughes DP, Kronauer DJC, Boomsma JJ. 2008. Extended phenotype: nematodes turn ants into bird-dispersed fruits. *Curr Biol* 18:R294–5.
- Hughes DP, Wappler T, Labandeira CC. 2011b. Ancient death-grip leaf scars reveal ant–fungal parasitism. *Biol Lett* 7:67–70.
- Lafferty KD, Kuris AM. 2012. Ecological consequences of manipulative parasites. In: Hughes DP, Brodeur J, Thomas F, (eds.). *Host manipulation by parasites*. Oxford: Oxford University Press. p. 158–69.
- Lafferty KD, Morris AK. 1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology* 77:1390–7.
- Moore J. 2002. *Parasites and the behavior of animals*. Oxford: Oxford University Press.
- Moore J. 2012. A history of parasites and hosts, science and fashion. In: Hughes DP, Brodeur J, Thomas F, (eds.). *Host manipulation by parasites*. Oxford: Oxford University Press. p. 1–14.
- Moreau CS, Bell CD. 2013. Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution* 67:2240–57.
- Poinar G. 2003. Trends in the evolution of insect parasitism by nematodes as inferred from fossil evidence. *J Nematol* 35:129–32.
- Poinar G. 2012. Nematode parasites and associates of ants: past and present. *Psyche* 2012: 13 p.
- Poinar G, Yanoviak SP. 2008. *Myrmeconema neotropicum* nsp, a new tetradonematid nematode parasitising South American populations of *Cephalotes atratus* (Hymenoptera: Formicidae), with the discovery of an apparent parasite-induced host morph. *Syst Parasitol* 69:145–53.
- Poinar GO. 1992. *Life in amber*. Stanford: Stanford University Press.
- Poinar GO. 2011. *The evolutionary history of nematodes: as revealed in stone, amber and mummies*. The Netherlands: Brill.
- Poinar GO, Poinar R. 1999. *The amber forest: a reconstruction of a vanished world*. Princeton: Princeton University Press.
- Poinar GO Jr. 1979. *Nematodes for biological control of insects*. CRC Press, Inc.
- Ponton F, Lebarbenchon C, Lefevre T, Thomas F, Duneau D, Marche L, Renault L, Hughes DP, Biron DG. 2006. Hairworm anti-predator strategy: a study of causes and consequences. *Parasitology* 133:631–8.
- Poulin R. 1998. Evolution and phylogeny of behavioural manipulation of insect hosts by parasites. *Parasitology* 116:S3–11.
- Poulin R. 2011. Parasite manipulation of host behavior: an update and frequently asked questions. In: Brockmann HJ, (ed.). *Advances in the study of behavior*. Burlington (NJ): Elsevier. p. 151–86.
- Poulin R, Morand S. 2000. The diversity of parasites. *Quart Rev Biol* 75:277–93.
- Poulin R, Morand S. 2005. *Parasite biodiversity*. Washington (DC): Smithsonian Books.
- Rieppel O. 1980. Green anole in Dominican amber. *Nature* 286:486–7.
- Roy HE, Vega FE, Chandler D, Goettel M, Pell J, Wajnberg E, (eds.). 2010. *The ecology of fungal entomopathogens*. Dordrecht: Springer.
- Roy HED, Steinkraus C, Eilenberg J, Hajek AE, Pell JK. 2006. Bizarre interactions and endgames: Entomopathogenic fungi and their arthropod hosts. *Annu Rev Entomol* 51:331–57.
- Soanes DM, Alam I, Cornell M, Wong HM, Hedeler C, Paton NW, Rattray M, Hubbard SJ, Oliver SG, Talbot NJ. 2008. Comparative genome analysis of filamentous fungi reveals gene family expansions associated with fungal pathogenesis. *PLoS One* 3:e2300.
- Sung GH, Poinar GO, Spatafora JW. 2008. The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal–arthropod symbioses. *Mol Phylogenet Evol* 49:495–502.
- Thomas F, Schmidt-Rhaesa A, Martin G, Manu C, Durand P, Renaud F. 2002. Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? *J Evol Biol* 15:356–61.
- Wallace AR. 1869. *The Malay Archipelago: the land of the orang-utan and the bird of paradise. A narrative of travel, with studies of man and nature*. London: MacMillan and Company.
- Webster JP. 2001. Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. *Microbes Infect* 3:1037–45.
- Wesołowska W, Wesołowski T. 2013. Do Leucochloridium sporocysts manipulate the behaviour of their snail hosts? *J Zool* 292:151–5.
- Wiegmann BM, Trautwein MD, Winkler IS, Barr NB, Kim J-W, Lambkin C, Bertone MA, Cassel BK, Bayless KM, Heimberg AM. 2011. Episodic radiations in the fly tree of life. *Proc Natl Acad Sci USA* 108:5690–5.
- Xu X, Norell MA. 2004. A new troodontid dinosaur from China with avian-like sleeping posture. *Nature* 431:838–41.
- Yanoviak SP, Dudley R, Kaspari M. 2005. Directed aerial descent in canopy ants. *Nature* 433:624–6.
- Yanoviak SP, Kaspari M, Dudley R, Poinar G. 2008. Parasite-induced fruit mimicry in a tropical canopy ant. *Am Nat* 171:536–44.
- Yanoviak SP, Nadkarni NM, Gering JC. 2003. Arthropods in epiphytes: a diversity component that is not effectively sampled by canopy fogging. *Biodivers Conserv* 12:731–41.