1 *Animal Behaviour review*

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3 **Better with your parasites? Lessons from evolved dependence and**
4 **conditionally helpful parasites for behavioural ecology**

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ABSTRACT

Interest in how parasites shape host behaviour has increased dramatically in recent years. The main focus of behavioural ecologists has been on the negative effects of parasites on host behaviour. However, there are instances in which infected hosts express more adaptive behavioural phenotypes and have higher fitness relative to uninfected hosts, suggesting that it is sometimes beneficial to be parasitized. For example, hosts can exhibit evolved dependence, wherein the host co-evolves with and comes to depend on parasites for the expression of adaptive host behaviours. Additionally, “conditionally helpful parasites” modify host phenotype in ways that benefit the host under particular conditions. These scenarios have been explored in the context of bacterial or fungal symbionts, but have been relatively unstudied with regard to metazoan parasites (e.g. trematodes, acanthocephalans, nematodes, and cestodes). We explore how these scenarios apply to hosts infected by metazoan parasites, and consider implications for research in behavioural ecology. We examine conditions under which infection should result in more adaptive host behavioural phenotypes, and implications for host fitness and evolution. We then discuss the implications of conditionally helpful parasites and parasites for which hosts have evolved dependence for laboratory studies of host behaviour and for conservation and reintroduction programmes.

Keywords: Evolved dependence, Hygiene Hypothesis, conditionally helpful parasites, personality, behavioural syndromes, plasticity

INTRODUCTION

Recent reviews reveal a growing awareness of parasites as critical to obtaining an ecologically and evolutionarily relevant understanding of host behaviour (Barber & Dingemanse, 2010; Kortet, Hedrick, & Vainikka, 2010; Poulin, 2013). Parasites influence host behaviour before and after infection. Parasites, particularly virulent parasites, exert direct and indirect costs on their hosts (e.g. Duncan & Little, 2007; Raffel, Martin, & Rohr, 2008; Rohr, Swan, Raffel, & Hudson, 2009), which may shape host behaviours over evolutionary time to decrease parasite encounter rates (Barber & Dingemanse, 2010; Kortet et al., 2010). Upon infection, the host phenotype may change because of host-driven adaptive responses that reduce the cost of infection, host-driven pathology that benefits neither host nor parasite, or parasite-driven manipulations of host phenotype that increase parasite transmission rates (Poulin, 2010). Phenotypic changes following infection are frequently multidimensional (Benesh, Valtonen, & Seppälä, 2008; Cézilly, Favrat, & Perrot-Minnot, 2013; Thomas, Poulin, & Brodeur, 2010), revealing that parasitized hosts may look and act very differently than unparasitized conspecifics.

Parasites are largely defined by the negative impacts they have on their hosts. However, the outcome of host-parasite interactions depends on particular features of the host-parasite system in question (Anderson & May, 1978; May & Anderson, 1978), and there exist conditions under which hosts may have *higher* fitness when infected. Evolved dependence occurs when two species co-occur

over long evolutionary time periods, and when selection favours phenotypic changes in one or both of the species in ways that increase fitness when the two species coexist (de Mazancourt, Loreau, & Dieckmann, 2005). If a host and parasite share a long evolutionary history, then selection may favour alterations in host phenotype to accommodate for and reduce the cost of inevitable infection. If this happens, the host may become “dependent” on the parasite; selection has driven changes in host phenotype that increase its fitness in the presence of a parasite and lead to fitness costs if a parasite is never encountered (Fellous & Salvaudon, 2009). Evolved dependence may have important implications for host behaviour, including implications for host personality, behavioural integration (i.e. behavioural syndromes), and behavioural plasticity. We discuss how evolved dependence may result in changes to host phenotype that are either fixed, where physiology and behaviour have shifted to a new baseline to accommodate infection, or variable, where physiology and behaviour exhibit flexibility or plasticity in response to infection occurring over a host’s lifetime.

Parasites may also modify host phenotype in ways that benefit the host under particular conditions (Fellous & Salvaudon, 2009; Michalakis, Olivieri, Renaud, & Raymond, 1992; Thomas, Poulin, Guégan, Michalakis, & Renaud, 2000). Parasites that modify host phenotype in ways that benefit the host under particular environment conditions while still exacting fitness costs under other environmental conditions are known as “conditionally helpful parasites” in the parasitology literature (Fellous & Salvaudon, 2009). These parasites can either induce novel phenotypes, or tinker with pre-existing phenotypes in ways that

benefit host survival or reproduction (Fellous & Salvaudon, 2009). For example, parasites can enhance host antipredator behaviour (Parker, Ball, Chubb, Hammerschmidt, & Milinski, 2009), and “love potion parasites” may enhance mating behaviours in ways that make individuals more attractive to the opposite sex (Lafferty, 1999). In many cases, these behavioural changes temporarily benefit both host and parasite. In the previous examples, increased host antipredator behaviour keeps the host alive until the parasite has matured to the point where it can survive predation by a definitive host predator, while enhanced mating behaviours are beneficial either because the parasite is sexually transmitted or because the mating behaviours attract predators that are the next host for the parasite. Whether the average fitness of infected hosts is greater than that of uninfected hosts depends on external conditions. For example, when predator density is high, hosts may not experience an average fitness increase from “love potion parasites”, as they may be consumed before they have an opportunity to reap reproductive benefits.

In this *Review*, we investigate how evolved dependence and conditionally helpful parasites can result in infected hosts that express more adaptive behaviours than uninfected hosts. We focus mainly on typical parasites – those that infect one host that they do not kill, and produce offspring that do not infect the current host - and trophically transmitted parasites, which infect one host that must be consumed by the next host in the life cycle. Conditionally helpful parasites and parasites that drive evolved dependence would not apply to most pathogens, which reproduce inside the host and must be eradicated by the

immune system, or to parasites that reduce host fitness to zero or near zero soon after infection (e.g. most parasitoids and parasitic castrators). While our main interest is in metazoan parasites, we will draw many of our examples from the literature on invertebrate hosts and their associated symbionts (mainly bacterial) as more research has been done in this area. We will often use the term symbiont (i.e. one of two species that engage in long-term interactions), as this term reflects that the outcome for interacting partners can grade from mutualism to parasitism depending on various factors (e.g. Leung & Poulin, 2008).

For both evolved dependence and conditionally helpful parasites we first provide background on these fields of research, discuss how these phenomena may specifically impact host behaviour, and discuss conditions under which we would expect to observe evolved dependence or conditionally helpful parasites impacting host behaviour. We then explore the importance of evolved dependence and conditionally helpful parasites for host fitness and evolution, and discuss why it is crucial to consider these phenomena in laboratory studies quantifying host behavioural phenotypes. Finally, we emphasize that the loss of parasites in an ecosystem can have a cascading effect when hosts that depend on these parasites find themselves suddenly uninfected. Thus, parasites should be considered an important conservation priority.

EVOLVED DEPENDENCE

Background

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129 Evolved dependence of hosts on their parasites can have profound
130 implications for the host. For example, female parasitoid wasps (*Asobara tabida*)
131 that co-evolved with *Wolbachia* (a bacterial symbiont) lost the ability to produce
132 mature oocytes following antibiotic treatment that removed *Wolbachia* (Dedeine
133 et al., 2001). Oocytes in uninfected *A. tabida* females exhibit increased
134 apoptosis, and this rampant apoptosis seems to be inhibited in the presence of
135 *Wolbachia* (Pannebakker, Loppin, Elmans, Humblot, & Vavre, 2007). Another
136 classic example of evolved dependence is the amoeba *Amoeba discoides*, which
137 became infected by a fitness-reducing bacterium in the lab (Jeon, 1972). The
138 fitness of infected amoebae returned to normal after five years of laboratory
139 culturing in the presence of the bacteria, but during that time some amoebae lost
140 the ability to form clones when the bacteria were removed (Jeon, 1972). One
141 possibility is that, over the course of the infection period, selection favoured *A.*
142 *discoides* individuals that offset the cost of infection by, for example co-opting
143 bacterial mechanisms for the completion of asexual reproduction. Because some
144 amoebae became dependent upon the bacteria to complete asexual
145 reproduction, the bacteria have effectively become mutualists where the bacteria
146 benefit from having a habitat in which to live, while the amoeba benefits from
147 being able to reproduce asexually. However, the benefits to the host arise not
148 because the parasite provides an immediate benefit, but because the host has
149 changed in some way that reduces the cost of parasitism.

Evolved dependence is explored in the literatures of parasitology (Fellous & Salvaudon, 2009), plant disease (Salvaudon, Héraudet, & Shykoff, 2008) and herbivory (de Mazancourt et al., 2005), and is known in the medical literature as the “Old Friends” or “Hygiene Hypothesis” (Rook, 2009). The Hygiene Hypothesis posits that the vertebrate immune system evolved in the face of chronic infections by microbes (e.g. bacteria transmitted through faeces) and helminths (monogeneans, trematodes, cestodes, and nematodes), and the presence of some of these organisms became important for the development and fine-tuning of the immune system (Rook, 2008; Rook, 2009; Strachan, 1989; Zacccone, Burton, & Cooke, 2008). These old friends needed to be tolerated by vertebrate immune systems because they were either ubiquitous and benign in small numbers, or because mounting an immune response to eradicate the symbiont would cause more damage to the host than would allowing it to persist (Rook, 2012). Old friends are thus organisms that are 1) encountered regularly such that an individual is likely to deal with them throughout their lives, 2) encountered over many generations, and 3) are not highly virulent. Organisms such as hookworms (*Necator americanus*) meet these criteria for our human ancestors and were likely old friends, but highly virulent diseases that only became common more recently when humans began to live in dense settlements (e.g. measles or smallpox) do not meet these criteria and so are likely not old friends (Armstrong, 2012; Rook, 2012). Selection favoured hosts that adjusted their physiology to reduce the costs associated with chronic infection, or

integrated parasites into the immune system by using the presence of parasites as a cue to stimulate proper immune function.

Details on the mechanisms underlying the Hygiene Hypothesis are still emerging. However, one hypothesis is that helminths stimulate the production of dendritic cells and regulatory T-cells that control the inflammatory response and that sample the body to determine what belongs (e.g. what cells are “self”) and what does not (Rook, 2009). Our modern hygienic environment reduces exposure to old friends, which may reduce the production of dendritic and regulatory T-cells that function to suppress the inflammatory response, consequently causing a rise in the frequency of diseases associated with excessive inflammation. These diseases include autoimmune diseases (e.g. irritable bowel syndrome, Crohn’s disease) and allergies, and recent work suggests that the loss of old friends may increase the risk of anxiety, depression, and some cancers (Elliott & Weinstock, 2009; Jackson, Friberg, Little, & Bradley, 2008; Maizels & Wiedermann, 2009; Matricardi & Hamelmann, 2009; Rook & Lowry, 2009). Administering eggs from relatively benign helminths has reduced symptoms of autoimmune diseases and allergies in clinical trials (Elliott & Weinstock, 2009; Jouvin & Kinet, 2012).

However, it is important to note that not all individuals that live in modern, hygienic environments are plagued with autoimmune and allergic diseases, and the relationship between parasites and the host immune response is not always straightforward. For example, some studies find that helminth parasites can also increase inflammation (e.g. Graepel et. al., 2013). Additionally, administration of

helminth eggs does not always alleviate the symptoms of autoimmune diseases (e.g. Voldsgaard et al., 2015), and scientists still debate whether the increase in autoimmune and allergic diseases is caused by loss of old friends versus other changes to our modern environment (Honeyman & Harrison, 2009). Whether or not an individual experiences autoimmune or allergic diseases in a hygienic environment likely depends on factors that include host genotype, the severity of infection (e.g. how many parasites an individual is infected with), interactions between the host microbiome and helminth parasites, interactions between multiple helminth species infecting the same host, and whether or not infection happens early or late in life (Maizels & Nussey, 2013; Maizels, McSorley, & Smyth, 2014; McKay, 2009; Zaiss et al. 2015). These complexities make studying the importance of evolved dependence of host behaviour on parasites a potentially daunting task, but unravelling this complexity will yield a more ecologically relevant understanding of host behaviour.

Salient observations from the Hygiene Hypothesis include: 1) infection by some helminth parasites is nearly inevitable (or was so before the advent of modern hygiene), 2) persistent presence of parasites has selected for vertebrate hosts that accommodate for and reduce the cost of infection, 3) these accommodations increase fitness in the face of inevitable infection, and 4) mechanisms that accommodate for infection may become fixed over many generations (e.g. through genetic assimilation, Pigliucci & Murren, 2003), and so the loss of parasites reduces the fitness of hosts that now accommodate for infection with no benefit.

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219 *Lessons for Behavioural Ecology*

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221 Evolved dependence could occur when a parasite manipulates the
222 production of compounds that control the expression of behavioural traits. For
223 example, helminth parasites secrete compounds that manipulate the host
224 immune system to create more favourable conditions for the parasite (Maizels et
225 al., 2004; Maizels & Wiedermann, 2009). Interactions between the immune
226 system and the nervous system are important for the expression of behaviour
227 (Adamo, 2013; Demas, Adamo, & French, 2011; McCusker & Kelley, 2013), and
228 adjustments in host physiology to account for the secretion of immunoreactive
229 compounds by parasites likely have implications for host behaviour.

230 One major way in which parasite-secreted compounds impact the immune
231 system is by reducing the host's inflammatory response (Maizels et al., 2004).
232 The degree of inflammatory response is linked to pace-of-life characteristics at
233 both the species and individual level (Sears, Rohr, Allen, & Martin, 2011), and
234 behavioural traits associated with pace-of-life characteristics include dispersal,
235 activity levels, sociability, exploratory behaviour, boldness, aggressiveness, and
236 parental care (Réale et al., 2010). Other behaviours that change following
237 immune system activation, and thus may be important for evolved dependence
238 studies, include activity, depression-like behaviour, memory, and feeding
239 behaviour (McCusker & Kelley, 2013; Williamson et al., 2016). For example, a
240 recent study found that adult rats whose mothers were infected by tapeworms

(*Hymenolepsis diminuta*) and who were themselves infected by tapeworms and *Escherichia coli* as pups performed better in a memory task than uninfected rats following an immune system challenge (Williamson et al., 2016). In rats that were not infected by tapeworms, bacterial infection early in life hypersensitized the immune cells in the brain and led to cognitive dysfunctions (i.e. reduced memory, Williamson et al., 2016). This study highlights that when hosts are infected with helminths during early stages of life, the infection can tune host immune responses and have lifelong implications for host behaviour.

Another group of parasites that may be important for evolved dependence are parasites that manipulate host phenotype to increase the likelihood that the parasite is transmitted to its next host. These parasites exert multidimensional changes to host phenotype, including changes in neurotransmitter activity, coloration, phototaxis, activity, habitat preference, and success at finding a mate (Benesh et al., 2008; Cézilly et al., 2013; Cézilly & Perrot-Minnot, 2010; Hughes, Brodeur, & Thomas, 2012; Lafferty, 1999; Moore, 2002; Thomas et al., 2010). The mechanisms through which these parasites induce behavioural changes are currently unclear, but may include secretion of neuroactive compounds and interactions between the immune and nervous systems (Adamo, 2012, 2013). As has been noted elsewhere, when these parasites are highly prevalent over many generations, selection might favour hosts that change their physiology to account for the phenotype-manipulating compounds secreted by behaviourally manipulative parasites, and such hosts would exhibit inappropriate behavioural phenotypes if parasites were lost (Read & Braithwaite, 2012).

Changes in physiological mediators that result from a long evolutionary association with parasites could modify average host behavioural traits, the integration of behavioural traits (i.e., behavioural syndromes), and behavioural plasticity. The multidimensional changes in host phenotype that result from infection by phenotype-manipulating parasites may result in different behavioural correlation structures between infected and uninfected individuals (Barber & Dingemanse, 2010; Kortet et al., 2010; Poulin, 2013), amplification or masking of existing behaviour-physiology correlations (Killen, Marras, Metcalfe, McKenzie, & Domenici, 2013), or wholesale changes in the physiological regulatory networks that underlie behavioural expression (e.g. Cohen, Martin, Wingfield, McWilliams, & Dunne, 2012). Parasites are known to alter key nodes in physiological regulatory networks – ‘integrator’ molecules like serotonin, testosterone, and interleukins that exert pleiotropic effects on many aspects of the phenotype, including behaviour (Cohen et al., 2012; Donaldson, Nautiyal, Ahmari, & Hen, 2013; Ketterson, Atwell, & McGlothlin, 2009; McGlothlin & Ketterson, 2008). Examples of integrators whose production is altered by infection with behaviour-modifying parasites include cortisol (Weinersmith, Hanninen, Sih, McElreath, & Earley, 2016), testosterone (Lim, Kumar, Dass, & Vyas, 2013), and serotonergic and dopaminergic activity (Lafferty & Shaw, 2013; Perrot-Minnot & Cézilly, 2013).

If parasites directly secrete “integrators” or modify their endogenous titers, selection might act to alter the physiological (and genetic) landscape of the host in ways that make the parasite essential for the expression of adaptive host

behaviours. For example, if infection is inevitable, this might lead to relaxed selection on genetic variances-covariances among integrator-mediated traits in the host because the parasite essentially takes on the role of 'integrator'. While the ramifications of such selection might not be evident when parasites are present (host phenotype appears 'normal'), the sudden loss of parasites will result in dramatically different, perhaps maladaptive, patterns of correlation among phenotypic traits, aberrant behaviours, and strong selection favouring hosts that retained pre-parasite genetic variance-covariance structures. The magnitude of the fitness loss to the host will depend on the extent to which the host has ceded control to the parasite. If host populations harbour enough standing genetic variation for the production of integrator molecules, or if such variation is present but simply was not expressed in the presence of parasites, then selection should act to return host production of these molecules to an appropriate level (e.g. re-appearance of diversionary displays in stickleback with reversion to ancestral environmental conditions: Foster et al. 2015). However, loss of parasites and their associated impact on integrator production could result in fitness-reducing behavioural phenotypes for many hosts in the population, and could dramatically reduce host population size.

It is possible that insufficient population variation in the production of integrator molecules exists, which could lead to extinction of the host population upon removal of the parasite. This could occur with strong selection against the maintenance of genes related to integrator production (e.g. if there were significant associated costs) but this seems unlikely given the importance of such

integrators for maintenance of homeostatic states and given that there are not obvious associated costs. Variation in host integrator production might be lost if genes associated with the integrator molecule accumulate mutations during an extended period of relaxed selection when the parasite was mainly controlling the production or reception of these compounds (e.g. Lahti et al. 2009). For parasites to completely take over the production of integrator molecules, these parasites would need to be vertically transmitted so they can produce integrators during host development, the parasite would need to produce large enough quantities of the integrator to completely replace host production of these compounds, and the parasite would likely need to be present throughout the host's body as integrator molecules are necessary in multiple body compartments. Systems that meet all of these criteria, and meet them over long enough time periods for evolved dependence to arise, are likely to be vanishingly rare. Thus, it is more likely that hosts will modify their production of integrator molecules in response to infection rather than ceding complete control of their production to the parasite.

We know that genetic variances-covariances and, to a lesser extent phenotypic correlation structure (integration), can drive or constrain evolutionary change (Dochtermann & Roff, 2010; Lande & Arnold, 1983), and influence the degree to which organisms are flexible/plastic in response to environmental change (Earley, Hanninen, Fuller, Garcia, & Lee, 2012; Ketterson et al., 2009; Schlichting, 1989). We also know that concentrations of some of the integrator molecules that are manipulated or otherwise affected by parasites exhibit heritable variation and, because these integrators often have pleiotropic effects,

selection on integrator concentrations can drive wholesale changes to the phenotype and thus the variance upon which selection can act (Cox, McGlothlin, & Bonier, 2016). There also is growing evidence that such integrators can alter patterns of genetic variance-covariance among traits, which can have profound evolutionary consequences (Cox et al. 2016). Dependence on parasites thus has enormous implications for understanding the evolutionary trajectory of host behaviour in the presence and absence of parasites, as well as the ability of hosts to express adaptive phenotypes in the face of rapid environmental change.

Will Evolved Dependence be Fixed or Variable?

Understanding the details of a host-parasite study system will be important for predicting whether hosts have evolved to become dependent on parasites, and for understanding whether parasites will drive to fixation the mechanisms that hosts use to accommodate for infection or select for plasticity/flexibility in response to infection. Here, we forward a framework for understanding the evolutionary mechanisms underlying and the responses associated with host dependence on parasites.

Under fixed evolved dependence (Table 1), the present day form of a behavioural trait in uninfected hosts is not the same as the ancestral form because of parasite-driven selection on host phenotype. That is, selection has favoured hosts that alter endogenous levels of physiological mediators and behaviour to accomodate for changes in host physiology driven by infection.

These changes become fixed (e.g. through genetic assimilation, Pigliucci & Murren, 2003) when: 1) nearly all individuals in a population are infected by a parasite over many generations, 2) the number of parasites or their physiological impacts on their hosts is constant across generations, and 3) the costs of maintaining flexibility or plasticity outweigh the benefits in environments where parasites are always encountered.

Variable evolved dependence occurs when the presence of parasites is important for the development and/or maintenance of behaviour (Table 1). Under variable evolved dependence, selection acts on host plasticity (i.e. irreversible changes in host phenotype that result from responses to early life environment) and flexibility (i.e. reversible changes to the phenotype throughout life) (Piersma & Drent 2003; Beaman, White, & Seebacher, 2016). Hosts capable of adjusting their physiology and behaviour in response to infection have higher fitness than those that do not. Evolved dependence should be flexible if all adults in a population are infected, but parasite infection intensity varies within a generation. Plasticity in response to infection should be favoured when the acquisition of parasites early in life is variable, but environmental conditions that might mediate parasite-associated fitness costs and benefits remain stable throughout adulthood. Plasticity could also be favoured if there are particular environmental characteristics that forecast parasite prevalence during adulthood (i.e. animals do not necessarily need to be infected during early life for plasticity to be favoured).

There are many typical parasites and trophically transmitted parasites that reach 100% prevalence in the host population, and these host-parasite systems

may be good starting places for laboratory studies. We suggest lab rearing the hosts, and conducting controlled infections on a subset of these hosts while taking care to mimic ecologically relevant infection patterns. When fixed evolved dependence has arisen, hosts that do not receive controlled infections should show deficits relative to infected conspecifics in the concentrations of physiological mediators and/or in patterns of behaviour including activity, feeding, and memory/learning. Ideally, such experiments would be conducted on multiple host populations that differ in their history with the parasite. In this case, controlled infections in a population that lacks recent evolutionary experience with the parasite should result in detrimental host behaviour in infected individuals, while controlled infections in the population that co-occurred with the parasite and that may exhibit evolved dependence should result in more adaptive behaviours in infected individuals.

Studying variable evolved dependence would require creating multiple infection treatments (e.g. low, medium, and high infection intensities), and quantifying how host behavioural phenotype and fitness change across the lifetime of the host. Studies assessing plasticity in response to infection should expose animals to parasites early in life, and should quantify behaviour and fitness during adulthood. Quantifying flexible responses to infection would entail exposing individuals to parasites at various stages of adulthood, and subsequently assessing behaviour and fitness. Animals that are exposed to ecologically relevant infection intensities should adopt a more appropriate behavioural phenotype for the particular environment that they are experiencing

during adulthood and would have higher fitness than animals that were unexposed.

CONDITIONALLY HELPFUL PARASITES

Background

Conditionally helpful parasites are those that change host phenotype in ways that benefit the host under some conditions, but remain detrimental under other conditions (Fellous & Salvaudon, 2009; Michalakis et al., 1992; Thomas et al., 2000) (Table 1). For example, *Paramecium* are infected by bacteria of the genus *Caedibacter*, and the bacteria produce toxins that are released from their host paramecium and kill paramecium competitors (Kusch & Görtz, 2006). The bacteria are costly to harbour and reduce *Paramecium* fitness, but infected hosts experience higher relative fitness than uninfected conspecifics that die due to toxin exposure. In this example, the parasite provides the host with a completely novel trait (a toxin), but parasites can also modify pre-existing traits in ways that are adaptive for the host (Fellous & Salvaudon, 2009). With respect to behaviour, hosts may experience genetic or developmental constraints that prevent the expression of adaptive behavioural phenotypes. Parasites may be able to help the host overcome these constraints, and sculpt the host phenotype in mutually beneficial ways. Fellous and Salvaudon (2009) noted that selection on hosts may either remove host constraints to allow the host to express a beneficial

behavioural phenotype in the absence of infection (and thus eliminate the benefit of infection), or selection may favour hosts that harbour parasites if the average fitness of infected hosts is higher than that of uninfected hosts (e.g. because the parasite moves the host to a new adaptive peak).

Examples of conditionally helpful parasites include instances where parasites limit the ability of new and potentially more harmful parasites to establish in the host (Haine, Boucansaud, & Rigaud, 2005; Mideo, 2009), provide hosts with a competitive advantage (Kusch & Görtz, 2006), protect against abiotic stressors (Duncan et al., 2010; Sánchez et al., 2016; Sures, 2003, 2008), improve host antipredator behaviour towards predators in instances where parasites could not survive transmission to these predators (Maure, Daoust, Brodeur, Mitta, & Thomas, 2013; Médoc, Rigaud, Bollache, & Beisel, 2009; Parker et al., 2009; Weinreich, Benesh, & Milinski, 2013), or increase reproductive success (Dass et al., 2011; Haine et al., 2004). These benefits can come in the form of novel functions to the host (e.g. the production of a novel toxin that harms host competitors) (Kusch & Görtz, 2006), or modifications to existing traits such as antipredator behaviour (Fellous & Salvaudon, 2009; Médoc et al., 2009).

The outcomes of these interactions depend on many factors (Bronstein, 1994; Bronstein, 2001; Egger & Hibbett, 2004; Fellous & Salvaudon, 2009), including symbiont and host genotype (Duncan et al., 2010; Koch & Schmid-Hempel, 2012; Oliver, Degnan, Hunter, & Moran, 2009; Oliver, Moran, & Hunter, 2005), abiotic conditions (Dusi et al., 2014; Kersch & Fonseca, 2005), or the

presence or absence of natural enemies (Oliver, Campos, Moran, & Hunter, 2008). For example, a bacterial symbiont (*Holospora undulata*) increases the fitness of only one of two examined *Paramecium caudatum* (a ciliate) clones, and only did so in the presence of osmotic stress (Duncan et al., 2010). The symbiont was costly to harbour and is thus only conditionally helpful, suggesting that no clones should harbour the symbiont under less adverse conditions.

Lessons for Behavioural Ecology

The effects of conditionally helpful parasites may be most identifiable during times when the parasite has a low probability of being transmitted to its next host because selection may favour parasites that modify host behaviour to increase host survival during this sensitive time period. For vertically or horizontally transmitted parasites, a time lag may occur when appropriate subsequent hosts are abundant only during particular times of year, or when appropriate hosts are rarely encountered. One particularly important time lag is the period between initial infection and the time at which the parasite is capable of infecting its next host (Chubb, Ball, & Parker, 2010; Médoc & Beisel, 2011; Parker et al., 2009). For example, immature trophically transmitted parasites die if a predator consumes the host, even if the predator was the appropriate next host for the parasite. Selection may favour parasites that alter host behaviour to decrease the host's probability of predation until the time when the parasite is competent to infect its next host, and may continue to change host phenotype in

ways that reduce predation by non-host predators to increase the probability that the current host is consumed by an appropriate host predator (Chubb et al., 2010; Médoc & Beisel, 2011; Parker et al., 2009). *Pomphorhynchus laevis*, an acanthocephalan parasite, manipulates refuge use of their amphipod host, *Gammarus pulex*, during the parasite maturation period, which decreases the probability that the amphipods will be consumed by trout (*P. laevis*' definitive host) (Dianne et al., 2011). This same parasite, however, is associated with reduced refuge use and increased risk of trout predation after the parasite matures (Dianne et al., 2011). In this example, increased host survival during parasite maturation may not increase overall host fitness because infected *G. pulex* consume less food (Dianne, Perrot-Minnot, Bauer, Guvenatam, & Rigaud, 2014). However, this example reveals that parasites are associated with changes in behavioural traits important for survival.

Compensatory responses are a class of behaviours that may be important candidates for behavioural targets of conditionally helpful parasites. When hosts become infected, they may compensate for infection in a number of ways, and parasites might also benefit from host compensatory responses (Lefèvre et al., 2008, 2009). For example, this might occur if hosts increase foraging activity to offset the costs of infection and experience elevated risk of predation by definitive host predators. Also, if a host becomes infected by a parasite that imposes survival costs, the host may increase investment in current reproduction to compensate for a reduced probability of surviving to future breeding seasons. If the parasite is transmitted during sexual encounters, the parasite may

manipulate host behaviour (e.g. mating displays) in ways that make the host more attractive to mates. This would benefit the parasite by increasing the number of hosts to which it is transmitted, and would benefit the host by providing it with more mating opportunities. This scenario may not be so implausible, and manipulation of reproductive behaviours by sexually transmitted parasites is becoming an active research area (Adamo, 2014). Indeed, male rats infected by *Toxoplasma gondii* are preferred over uninfected males, even though the parasite can be transmitted through sex to both the female and her pups (Dass et al., 2011). Theory predicts that parasites should induce compensatory behaviours mainly through virulence (e.g. extracting energy to induce the host to change its phenotype) rather than by paying a cost to manipulate host phenotype (Lefèvre et al., 2008, 2009). However, we may expect to see parasites pay a cost to manipulate compensatory behaviours if the fitness benefits to parasites that pay a cost to manipulate dramatically outweigh the benefits achieved by parasites that induce compensation through virulence.

Studies quantifying the costs and benefits of conditionally helpful metazoan parasites across a host's lifetime are needed. Particularly promising systems are those where the interests of hosts and parasites are clearly aligned. As noted earlier, both the host and a trophically transmitted parasite die if the host is consumed by a predator that is not an appropriate next host for the parasite. In these cases, the parasite may induce antipredator behaviour in the host that increases the survival of both (Parker et al., 2009). This increased survival could benefit the host if it accelerates reproduction or affords infected

hosts more time to find and copulate with mates. However, this benefit will likely be context-dependent, and could change depending on the availability of potential mates or the density of predators. Thus studies are needed that quantify the fitness of infected and uninfected hosts across host lifetimes, and across a variety of ecological conditions.

FITNESS AND EVOLUTIONARY IMPLICATIONS

Imagine a theoretical maximum value of a host behavioural trait that is positively linked to host fitness and is achieved by only one or a few host genotypes in a population in the absence of parasites (Figure 1), and assume that different genotypes express different behavioural phenotypes in the absence of parasites. We explore several ways in which the evolutionary trajectory of a population can be influenced by changes in behavioural variation arising from evolved dependence and conditionally helpful parasites.

Evolved Dependence

Multiple host genotypes might converge upon an adaptive behavioural phenotype in the presence of parasites through evolved dependence. If hosts have evolved to express new physiological and behavioural maxima to accommodate for infection, then most hosts will exhibit inappropriate phenotypes without parasites (Figure 1a). When individuals experience higher fitness in their

infected relative to their uninfected state, selection may favour parasite-seeking behaviours or eliminate parasite avoidance behaviours. Under evolved dependence, parasitism has the potential to reduce host behavioural variance while preserving genetic diversity (i.e. a possible mechanism maintaining cryptic genetic variation) (Ghalambor, McKay, Carroll, & Reznick, 2007). Parasites may thus shield some host genotypes from selection, preventing evolutionary change. The loss of parasites would subsequently increase host phenotypic variation and expose genetic variation, inducing a pulse of evolutionary change in the population (e.g. Pfennig et al., 2010). This reveals that the loss of parasites may be important from a conservation standpoint, because it could expose individuals in a host population to selection pressures from which they were previously protected by parasitism.

Despite living in a hygienic environment where parasite encounter rates are greatly reduced, only some humans develop autoimmune diseases and allergies in the absence of parasites. In both human and non-human animal populations, there exists substantial variation in immune responsiveness, including variation in markers suggestive of autoimmune disease (Graham et al., 2010; Limaye et al., 2007; Maizels & Nussey, 2013). This variation is likely maintained because of fitness trade-offs (Graham et al., 2010). We may expect similar host genotype-specific behavioural responses to infection, with some host genotypes experiencing higher fitness in their infected states while other experience higher fitness in an uninfected state. Host genotypes that do not exhibit evolved dependence on parasites may experience a steep fitness decline

when parasites are encountered (e.g. Figure 1b square genotype), whereas genotypes that exhibit evolved dependence would show higher fitness in the presence of parasites (Figure 1b, circle and triangle genotype). If there is variation among host genotypes in the phenotypic response to being infected, parasites might reveal or mask host phenotypic variation upon which selection can act and this can have dramatic effects on the evolutionary trajectory of host populations. Such effects of parasites on behavioural diversity parallels recent work on how developmental plasticity might facilitate or constrain morphological and behavioural evolution (Foster, 2013; Pfennig et al., 2010).

Conditionally Helpful Parasites

With conditionally helpful parasites, all or some of the genotypes in a host population might experience higher fitness in the presence of parasites (e.g. Duncan et al., 2010). If parasites experience increased fitness when the fitness-maximizing host behaviour is achieved, then parasites may be more beneficial to hosts expressing a suboptimal phenotype when uninfected (Figure 1a; variation in slopes). Imagine that bolder individuals have higher reproductive success, but are also more likely to be consumed by predators (as observed in Smith & Blumstein, 2008). If parasites induce boldness in their current host to increase transmission to the definitive host, then host fitness will increase if, on average, infected hosts are consumed by the parasite's definitive host *after* reproduction occurs (i.e. after they have the opportunity to benefit from increased boldness).

Assuming that behavioural variation in uninfected hosts is linked to underlying genetic variation, parasite alteration of host behaviour would preserve genetic diversity but reduce behavioural diversity within a population. Parasite-induced shifts of behavioural traits towards a fitness-maximizing trait value would mimic directional selection.

With conditionally helpful parasites, some host individuals might benefit and others might suffer from parasitism. If a host genotype expresses a behavioural phenotype that already is well suited to its environment, infection would likely impose costs (e.g. energy loss to the parasite), but provide no added fitness benefits (Figure 1c, square genotype). For other individuals, the costs of infection may be offset (Figure 1c, circle genotype) or more than offset (Figure 1c, triangle genotype) if parasites alter host phenotype in ways that benefit both parasite and host. For example, parasites that manipulate a male sexual trait (e.g. mating behaviours) may increase the male host's susceptibility to definitive host predators while also increasing the male's attractiveness to females ("love potion parasites") (Lafferty, 1999). If a male is able to express a high value of this sexual trait independent of parasites and if parasites are unable to further increase attractiveness (e.g. because of constraints on elaboration), then parasitism may simply reduce male fitness. However, males incapable of achieving high levels of attractiveness might benefit from parasites that increase host attractiveness. Provided these altered hosts have an opportunity to breed before they are consumed by the parasite's definitive host, they may experience a net fitness benefit from infection. Under these dynamics (Figure 1c),

609 conditionally helpful parasites would protect some genotypes from selection while
610 exposing others. Host genotypes that express phenotypes associated with lower
611 fitness in the absence of parasites would experience selection to reduce
612 antiparasite behaviours, or would experience selection for parasite seeking
613 behaviours. Genotypes that express fitness-maximizing phenotypes in the
614 absence of parasitism would experience selection for parasite avoidance
615 behaviours or stronger immune responses post-infection. If parasites were
616 unavoidable, then this dynamic would result in a pattern mimicking stabilizing
617 selection.

618 619 **RESEARCH AND CONSERVATION IMPLICATIONS**

620
621 Evolved dependence and conditionally helpful parasites reveal that hosts might
622 match their behaviour to the environment more accurately and experience higher
623 fitness in the presence than in the absence of parasites. This has important
624 implications for studies on host behaviour and conservation biology.

625 626 *Laboratory Studies on Host Behaviour*

627
628 Nearly every wild animal is infected by one or more parasites (Poulin & Morand,
629 2000; Price, 1980; Shaw & Dobson, 1995), yet laboratory and field studies often
630 do not consider the contribution of parasites to behavioural variation. This is
631 either because parasites are not quantified in wild-caught individuals, antibiotics

or antihelminthics are given to hosts prior to making behavioural measurements, or because laboratory breeding removes parasites from the population. It is clearly important to study uninfected individuals to delineate factors contributing to host behaviour. However, an ecologically and evolutionarily relevant understanding of host behaviour requires teasing apart the relative contributions of the host and its parasites, including consideration of whether hosts have come to depend on parasites for the expression of adaptive behavioural phenotypes. In the Appendix we provide an empirical approach for determining whether the host or the parasite is driving changes in host behavioural phenotype. Additionally, animals “deprived” of the microbes and helminths with which they coevolved will likely have impaired immune systems (Rook 2008, 2009). Considering whether hosts have evolved dependence on parasites may provide insight in to why some organisms are more difficult to acclimate to parasite-free lab conditions than others.

Conservation

Parasites should rank as an important conservation priority because they comprise over 50% of the metazoan biodiversity on the planet (Poulin & Morand, 2000; Price, 1980; Toft, 1986), and play important roles in ecological and evolutionary processes (Gómez & Nichols, 2013; Gómez, Nichols, & Perkins, 2012; Hatcher, Dick, & Dunn, 2006, 2012; Lafferty et al., 2008; Mouritsen & Poulin, 2005). Symbionts, including parasites, constitute the majority of

biodiversity lost when host population sizes decline or when hosts go extinct (Dunn, Harris, Colwell, Koh, & Sodhi, 2009; Koh et al., 2004). We argue that parasites should also be considered a conservation priority because they may be instrumental in allowing their charismatic free-living hosts to match their behavioural phenotype to the environment. If hosts exhibit evolved dependence or benefit from conditionally helpful parasites, then the loss of parasites important for the expression of host phenotype would increase the host's susceptibility to extinction. We thus join others in advocating for parasite co-reintroductions, and incorporation of principles from the Hygiene Hypothesis into host captive breeding and reintroduction programmes (Jørgensen, 2015; Schulte-Hostedde & Mastromonaco, 2015; Spencer & Zuk, 2016). Coupling parasite and host reintroduction could increase the likelihood of successful host establishment in reintroduction programmes.

CONCLUDING REMARKS

Research on the holobiont (i.e. the host plus its microbial symbionts) is revealing that symbionts have profound impacts on host ecology and evolution (Archie & Theis, 2011; Bordenstein & Theis, 2015; Cryan & Dinan, 2012; Dheilly, 2014; Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012). Here we have argued that parasite symbionts may play an important role in the host's ability to produce adaptive behavioural phenotypes, and in behavioural evolution. Exploring the importance of evolved dependence on parasites and conditionally helpful

parasites for host behaviour is both exciting and a bit daunting. Predicting which aspects of host phenotype will change following infection for the benefit of both host and parasite and the implications, both immediate and evolutionary, of these phenotypic changes will require an understanding of host-parasite ecology and evolution. Quantifying the mechanisms through which parasites manipulate host phenotype will be informed by integrating neuroscience, endocrinology, genomics, proteomics, metabolomics, and immunology (Adamo, 2013; Biron & Loxdale, 2013; Hughes, 2013). Investigators interested in exploring evolved dependence and conditionally helpful parasites will thus have an exciting opportunity for cross-disciplinary collaboration, and we envision that such collaboration will result in a more ecologically and evolutionarily relevant understanding of host behavioural phenotype.

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**APPENDIX. IS THE HOST OR THE PARASITE DRIVING CHANGES IN HOST
PHENOTYPE?**

Studies exploring links between parasitism and host behaviour are typically conducted in one of two ways: 1) correlations between host behaviour and parasite loads are examined in wild-caught, naturally infected individuals or, 2) host behaviour is quantified before and after controlled infections on laboratory-reared animals. While the former method may reveal interesting correlations between parasite loads and host behaviour, it is impossible to differentiate between host traits that increased the host's risk of parasitism from host traits that were changed following infection. Studies on laboratory bred animals in which host behaviour is quantified before and after controlled infections allows researchers to differentiate between traits that predispose an individual to infection from traits that change following infection. It will be important to understand host-parasite ecology so that controlled infections can be conducted at an ecologically relevant time in the host's life; infections that typically occur during development will likely have different effects on host behaviour than infections acquired during adulthood.

Because parasites may have multidimensional impacts on host phenotype (Benesh, Valtonen, & Seppala, 2008; Cézilly, Favrat, & Perrot-Minnot, 2013; Thomas, Poulin, & Brodeur, 2010), and because these phenotypic changes may have dramatic fitness implications, it is crucial to understand the links between

parasitism and host phenotype. Changes in host phenotype following infection may arise as a consequence of increased energetic demands placed on the host, host-initiated effects (e.g. arising from the host's immune response), parasite-initiated effects (e.g. secretion of neuroactive compounds), or some combination of these factors (Hart, 2011; McCusker & Kelley, 2013; Moore, 2013; Poulin, 1995). The best approach to understanding the mechanisms underlying shifts in host phenotype will vary depending on the particular host-parasite system in question, but we suggest a general approach here. For example, suppose the magnitude of the host's phenotypic response to infection varies as a positive, linear function of infection intensity (Figure A1, see below). Host phenotypic responses could be due to infection-related compromises to energy balance that result from a combination of the parasite's energy intake and the cost of mounting an immune response. If energy balance drives host phenotype shifts, then we should be able to reveal a similar phenotypic response by limiting energy intake in uninfected hosts; the energetic toll exacted on the host by increased parasite intensity could then be evaluated by progressively decreasing energy availability to uninfected individuals. To determine if host-initiated factors drive phenotypic changes in response to infection, one could apply the mechanical or antigenic stimuli that evoke host responses without actually infecting the host. For example, to mimic infections by trematode metacercariae, one could surgically place appropriately sized glass beads that have been seeded with an antigenic substance (e.g. sheep red blood cells) in the organ typically invaded by the parasite. Adjusting the number of beads placed in the host might induce

1173 parasite intensity-dependent effects. Once the indirect effects of energy
1174 restriction and host response to infection have been quantified, the magnitude of
1175 phenotypic change induced by these factors can be subtracted from the overall
1176 change in phenotypes observed in infected hosts. The resulting value will provide
1177 an estimate of the magnitude of parasite manipulation of host phenotype.

1178
1179 **Figure 1.** Fitness implications of parasitism. The square, circle, and triangle
1180 represent three host genotypes that produce different values of a behavioural
1181 trait linked to fitness. The square host genotype expresses a behavioural
1182 phenotype that achieves a theoretical fitness maximum in the absence of
1183 parasitism. The values to the left of the dashed line represent fitness outcomes in
1184 the absence of parasites (NP), while values to the right of the dashed line
1185 represent fitness outcomes following parasitism (P). See text for more details. a)
1186 Infection alters host behaviour and mimics directional selection by reducing
1187 phenotypic diversity while preserving genotypic diversity. b) Host genotypes with
1188 relatively low fitness prevail in the presence of parasites, and those that are fittest
1189 in the absence of parasitism suffer when parasitized. Genotypic diversity is
1190 maintained if host genotypes that benefit from parasitism are able to acquire
1191 parasites while genotypes that suffer from parasitism are able to avoid it. c)
1192 Infection reduces phenotypic diversity and potentially genotypic diversity,
1193 resulting in a pattern that mimics stabilizing selection when parasitism is
1194 unavoidable.

Figure A1: Relative influence of factors affecting host phenotype. The solid black line represents the observed change in phenotype as the number of parasites infecting the host increases (i.e. the sum of host-initiated effects, parasite-initiated effects, and the energetic costs of infection). The bottom dashed line represents changes in host phenotype resulting from the energetic costs associated with parasitism. The middle dashed line represents the effects of energy restriction combined with the host's response to the presence of an antigen (foreign, parasite-associated molecules). Parasite-initiated effects can then be estimated as the difference between the overall change in phenotype in response to infection (solid black line), and the host's phenotypic response to energy restriction and the presence of an antigen (everything below the middle dashed line). While the relationships between the various factors impacting host phenotype are depicted here in a simplified, linear manner, the function describing these relationships is likely much more complex and probably varies considerably across host-parasite systems. We also recognize that each of the three contributing factors might contribute in different ways to various aspects of host phenotype (e.g. one sigmoidal, one exponential, and one linear), and that different host traits (e.g. behavioral, physiological, morphological, or even various traits within each of these categories) might change in unique ways with parasite infection intensity.

Figure 1

Figure A1

Acknowledgements

KLW was funded by an American Dissertation Fellowship from the American Association of University Women (AAUW) and block grants from the Graduate Group in Ecology at the University of California Davis. This *Review* arose from work supported by a National Science Foundation Doctoral Dissertation Improvement Grant (IOS-1110639) awarded to KLW. We thank Andrew Bibian, Shannon Carter, Patrick Clay, Andy Sih, the Sih Lab, the Earley lab, Judy Stamps, Benjamin Toscano, and the anonymous reviewers for insightful comments that greatly improved the quality of this manuscript.