Why should parasite resistance be costly?

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Parasite resistance is sometimes associated with fitness costs. Costs of resistance are fundamentally important in epidemiology, and in the ecology and evolution of host–parasite interactions. The cost of resistance is often envisioned as the cost of re-allocating limiting resources to resistance machinery from other traits. This popular paradigm has resulted in a spate of research that assumes a fitness cost to resistance. We comment on this trend and propose a working framework of various resistance means and mechanisms. Within these means and mechanisms, we suggest that many are not likely to incur significant fitness costs.

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RESISTANCE (see Glossary) to parasites has important implications for the agricultural, veterinary, medical, conservation, ecological and evolutionary sciences. With rapid advances in the understanding of the molecular basis of resistance, there has been increasing interest in the use of genetic engineering to introduce parasite resistance alleles into food crops and livestock. In addition, an understanding of resistance is necessary when considering the efficacy and safety of using parasites in the bio-control of pest species. A central issue in the study of resistance is understanding the factors that determine the appearance, spread and distribution of resistance alleles within populations and across geographical landscapes. One factor of paramount importance to all of the above is the potential COST OF RESISTANCE.

Resistance is frequently conceptualized as a resource-driven, mystical 'black box' (i.e. resistance is considered as a single trait that becomes more effective with increased resource input). Furthermore, resistance is often considered to reside solely in the domain of the immune system. Some recent empirical work has shown that parasite resistance sometimes comes with an associated FITNESS cost. This cost is often envisioned as the cost of re-allocating limited resources to resistance machinery from other fitness-related traits [1], and recent work often implicitly assumes that resistance is costly [1]. Here, we revisit the assumption of a cost of resistance. Further, we expand on previous work and propose a working conceptual framework detailing how parasite resistance could be accomplished and conclude that there are many important forms of parasite resistance which are unlikely to have fitness costs, relative to more susceptible phenotypes.

Means and mechanisms of resistance

Coustau *et al*. [2] advanced our understanding by looking inside the 'black box' of molecular resistance and reviewing the logic leading to the assumption that fitness costs are associated with parasite resistance. First, they listed the categories of the molecular basis of resistance to xenobiotics (e.g. pesticides and antibiotics). Second, they applied these categories to parasite resistance, emphasizing immunological forms of resistance. Coustau *et al*. [2] pointed out that fitness costs of resistance are often, simply, extensions of the molecular mechanisms used to accomplish resistance (e.g. through NEGATIVE PLEIOTROPY). They recognized four specific mechanisms by which molecular resistance could be achieved:
(1) increasing trait expression;
(2) decreasing trait expression;
(3) qualitative trait modification; and
(4) inducible trait expression.

Clearly, increasing the expression of DEFENSE-related molecular traits could increase resistance. However, decreasing the expression of something that is necessary for parasites (e.g. molecules that parasites use as cues to find their targets, such as the Duffy receptors used by the malaria parasite *Plasmodium vivax* to enter red blood cells [3]) can also lead to resistance. Qualitative modifications leading to immunological resistance are possible, for example, through the alteration of defense products used to recognize (Box 1) or fight parasites. Indeed, resistance to some parasites is associated with specific variants of recognition molecules in plants and animals [4–6]. Resistance could also be achieved by employing resistance traits only when necessary, that is using inducible defenses. As Coustau *et al*. [2] suggest, fitness costs of resistance are most likely to be associated with the first mechanism, and fitness costs are merely possible, but not necessary, with the other three mechanisms.

Glossary

Cost of resistance: Although the host could benefit from resistance by decreasing the effect of parasites or avoiding parasites, the defenses used to achieve resistance could have an associated fitness cost. When the cost of the defenses outweighs their benefit (e.g. defense in the absence of parasites), there could be a net fitness cost of resistance.
Defense: Any trait an organism uses to prevent or reduce the effects of infection (i.e. a trait used for resistance).
Fitness: The genetic contribution of an individual to the next generation, relative to other members of the same population. Parasites could reduce the fitness of an individual by reducing survival, in addition to reproduction (i.e. number and quality of offspring).
Knockout: To prevent the expression of a gene by blocking transcription, translation or the action of the gene product(s).
Over-expression: Increased gene transcription or translation, resulting in overproduction of the gene product(s) relative to the non-modified form.
Negative pleiotropy: When a gene has both positive and negative effects on different phenotypic traits.
Resistance: The ability of the host to avoid or diminish the effects of a particular parasite through the use or possession of defense traits, relative to other members of the same population.

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Box 1. The cost of recognition variation

The first step in many successful inducible defenses is recognizing the attack. Defense activation requires recognizing a cue from the parasite which reliably signals that the host is, or will be, attacked. In the immune system of animals, receptors bind to parasites and activate host defenses [a]. Similarly, plants also use receptors (many are called R genes) to recognize parasites (or pathogens) and activate their defenses [b].

Qualitative variation in the molecular receptors that hosts use to recognize parasites could be some of the best examples of cost-free means of achieving parasite resistance. There are so many different parasite taxa, combined with diversifying selection within taxa to avoid recognition by the host, that most hosts are unable to recognize all of the parasite species or strains that attack them. In other words, owing to a limited number of genes coding for recognition alleles, there are constraints on the particular recognition alleles an individual can have. The constraint entails fitness costs, but the cost is based on differential susceptibility to parasites and is not present in the absence of parasites. Although variation in recognition alleles is likely to be cost-free and all the examples of recognition in Table 1 have no known costs, there could be exceptions if the recognition allele is negatively pleiotropic or if the allele is accompanied by increased expression of the receptor. The outcome of this scenario is that hosts could exert selection on parasites to avoid recognition. Parasites, in turn, exert selection on hosts to recognize them despite their adaptations to avoid detection. This is expected to cause frequency-dependent cyclical antagonistic co-evolution. This form is cyclical in that the frequency of recognition alleles in hosts and evasion alleles in parasites cycle over time, with neither side ever winning. This scenario of cycling recognition alleles has been called trench warfare [c].

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Given the recent trend in implicitly assuming that there are fitness costs associated with parasite resistance, we would like to expand on the important point by Coustau *et al.* that there could be cost-free forms of resistance [2]. We propose five categories for the general means of defense by which resistance could occur or evolve. These are alterations in the:
(i) host immune system;
(ii) host biochemistry;
(iii) host behavior;
(iv) host life-history; and
(v) host structure (see examples in Table 1).

The immune system (i) achieves resistance through chemical and cellular mechanisms, which recognize and respond to non-self entities. The immune system also operates outside of the host by secreting immune products to attack or deter parasites that have not yet entered the host (e.g. humans secrete immune defenses onto the skin and into the gastrointestinal tract [7,8]). Biochemical resistance (ii) relies on non-immunological molecules that could have antiparasitic properties, be used by parasites to find their hosts or be used as nourishment within the host. For example, humans achieve a degree of apparently cost-free resistance to malaria through various alterations to molecules in and on the surface of red blood cells (apart from the sickle cell hemoglobin S trait) [3,9]. Resistance might also be achieved by using behavioral traits (iii), by avoiding parasites, removing parasites, self-medicating or by mutualistic interactions with other species. Behavioral traits, which might contribute to resistance, are well documented in animals [10]. The reader is likely to use behavioral defense traits as well! (See Fig. 1.) Life history (iv) changes could lead to resistance, for example, by minimizing the duration of susceptible life-history stages, changing the timing of reproduction or producing offspring better suited to resist parasites. Physical aspects of host morphology (or structure) (v) could also be used to deter, repel or remove parasites in what is termed structural resistance. Altogether, there are several widely differing means of defense, which could be used as part of a defense network to achieve parasite resistance (Table 1).

The five means of defense used to achieve parasite resistance could be used both externally and internally to the host (Fig. 1). These means of defense could be used to prevent parasites from finding or entering hosts and to combat parasites outside of the host body (i.e. operate externally), or to decrease the negative fitness effects of the parasites on hosts after the parasites have found or entered the host (i.e. operate internally). A particular means of defense, as a result of constraints and/or enabling mechanisms, could operate predominantly either internally or externally. Within each of the five means of defense (i–v), the mechanisms (1–4) outlined by Coustau *et al.* [2] for molecular resistance will also operate. Parasites then could only achieve successful infections by contending with an external and internal defense network (Fig. 1). In this scenario, hosts could use numerous combinations of means of defense (i–v) and their respective subcomponent mechanisms (1–4) to achieve parasite resistance. The combination of defenses used or evolved should

Table 1. Examples of the means and mechanisms of defense used by hosts to achieve or evolve resistance to parasites[a]

Defense trait expression	Resistance trait	Host–parasite system	Fitness costs[b]		Refs
			Documented	Likely	
Immunology					
Increase	Increased hemocyte numbers (defensive blood cells)	Fruitfly–parasitoid	Yes	NA	[16,18]
Qualitative	HLA alleles (recognition)	Human–malaria	No	No	[30]
Qualitative	HLA alleles (recognition)	Human–HIV virus	No	No	[31]
Qualitative	MHC II alleles (recognition)	Human–hepatitis C virus	No	No	[32]
Qualitative	MHC IIB alleles (recognition)	Salmon–bacteria	No	No	[6]
Inducible	Antimicrobial peptides (kill parasites)	Fruitfly–bacteria, fungi	No	No	[33]
Biochemistry					
Increase	Increased melanization of cuticle (kills parasites)	Insect–parasitoid and insect–fungi	No	Yes	[34]
Decrease	Duffy antigen – cytokine receptor (impedes parasites)	Human–malaria	No	No	[3,9]
Decrease	Enzyme G6PD (lowers pathology)	Human–malaria	Yes	NA	[35]
Qualitative	α^{+}-globin (lowers pathology)	Human–malaria	No	No	[9]
Qualitative	Hemoglobin C (prevents/reduces infection)	Human–malaria	Yes	NA	[36]
Qualitative	Hemoglobin E (lowers pathology)	Human–malaria	Yes	NA	[37]
Qualitative	Hemoglobin S (prevents/reduces infection)	Human–malaria	Yes	NA	[3]
Qualitative	PRNP gene (prevents infection)	Human–prions	No	No	[38,39]
Qualitative	R genes (recognition)[c]	Plant–virus, plant–bacteria, plant–fungi, plant–nematode	No	No	[40]
Inducible	Chitinase (kills parasites)	Plant–fungus	No	No	[41]
Behavior					
Increase	Programmed grooming rate (removes parasites)	Antelope–ectoparasite	No	Yes	[10]
Decrease	Activity levels (infection avoidance)	Lizard–haemogregarine	No	Yes	[42]
Qualitative	Selective foraging (avoiding infected feces)	Cow–nematode	No	No	[10]
Qualitative	Nest fumigation (use plants that kill parasites)	Bird–lice and bird–mite	No	No	[43]
Inducible	Visiting cleaner fishes (removes parasites)	Fish–gnathiid isopod	No	No	[44]
Inducible	Behaviorally regulated fever (kills parasites)	Grasshopper–microsporidea	No	No	[45]
Life history					
Decrease	Pupation time (decreases parasite virulence)	Mosquito–microsporidea[d]	Conditional	NA	[46]
Decrease	Maturation size (reproduce before castration)	Snail–trematode	Yes	NA	[47]
Inducible	Alter offspring growth and reproductive strategy	Lizard–mite	No	No	[48]
Structure					
Increase	Cuticle thickness, melanization, and spines (impedes parasites)	Insect–parasitoid wasp	No	Yes	[34,49]
Increase	Peritrophic membrane thickness (impede parasites)	Arthropod–endoparasite	No	Yes	[50]
Qualitative	Leaf stomatal topography (impede infection)	Plant–fungi	No	No	[41]
Qualitative	Gill cleaner structure (remove parasites)	Crab–rhizocephalan	No	No	[51,52]
Inducible	Spleen size (remove parasites)	Rats–*Trypanosoma* spp.	No	No	[53]

[a]Abbreviations: HLA, human leukocyte antigen; MHC, major histocompatibility complex; NA, not applicable; PRNP, prion protein.
[b]When fitness costs have not been documented, the likelihood of costs have been based on whether individual with the trait is likely to have a lower fitness in the absence of parasites than other members of the same population without the same trait. For inducible traits, this is limited solely to possessing the ability to induce and not inducing the trait in the absence of parasites. Furthermore, low magnitude costs could be ameliorated or even overcome as a result of secondary compensatory mutations (see text). The examples chosen here should be regarded as possibilities and are not meant to be either definitive or exhaustive.
[c]Plant R genes are listed under biochemistry because plants are not considered traditionally as having immune systems.
[d]Conditional fitness costs were observed in the mosquito-microsporidea system depending on food availability.

depend on several host-specific factors, including the available genetic variation within the host population, the capacity for learning or innovating new behaviors, and the cost to benefit ratio of each defense mechanism. Combes has also proposed a set of filters – the encounter and compatibility filters – for parasite virulence and abundance (and by implication, host resistance) which generally operate in a similar conceptual manner [11,12].

When a new trait, resistance or otherwise, first appears, it is often associated with costs (i.e. through negative pleiotropy). Although the initial resistance mutation might be costly, it should be recognized that if it conveys a fitness advantage under some circumstances, subsequent secondary mutations could occur which decrease the fitness costs of the trait. These secondary compensatory mutations are partly responsible for the widespread evolution of antibiotic resistance in medicine and agriculture. Furthermore, the compensated resistant genotypes sometimes have a greater fitness than susceptible genotypes, even in the absence of antibiotics [13]. This suggests that the fitness costs of resistance traits could depend not only on what means and mechanisms of defense are used, but also on the evolutionary stage of the host during which these costs are measured.

Methods for the investigation of costs

There are two complementary ways of studying resistance: one involves detailed mechanistic

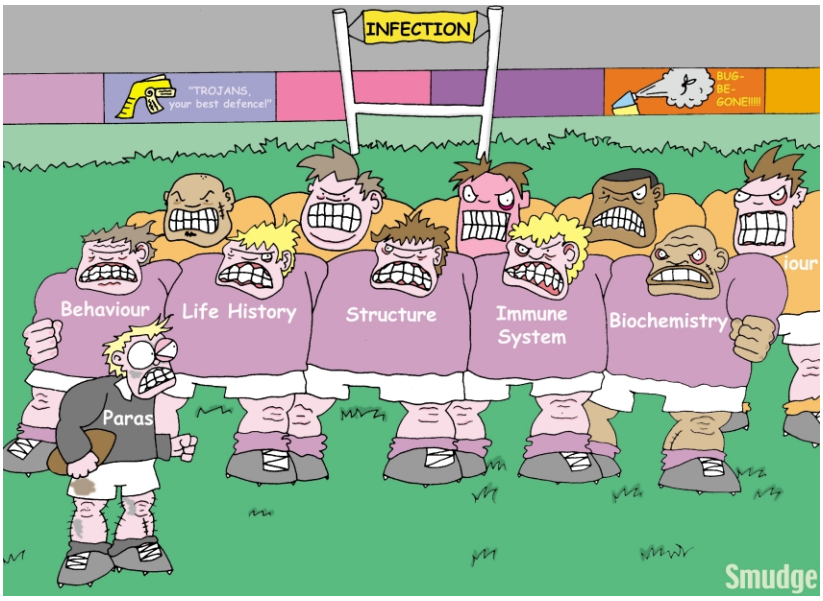

Fig. 1. On the playing field of host–parasite interactions, parasites must infect a host by penetrating the external defenses of the host (represented by the front line in the rugby game). Once past the external defenses of the host, the internal defenses must be defeated (represented by the back line in the rugby game) before the parasites can reach their goal of a successful infection. Within the external and internal defenses, there are five means of defense (indicated on the rugby shirts of the front line players), each of which have four sub-component defense mechanisms (see text). The advertisements also show behavioral traits used by humans to defend against parasites. Cartoon by Neil Smith.

evaluation (i.e. determining the proximal manner by which resistance is achieved) and the other involves quantifying resistance in natural populations. The mechanistic approach is often used in medicine, agriculture and in evolutionary biology. Studies of resistance to xenobiotics [2,14,15] also lie within this area of study. However, it should be noted that xenobiotic resistance is fundamentally different from parasite resistance because chemicals lack the ability to co-evolve and are much more limited in their interactions with a host. The second approach is more often used in epidemiological, ecological and evolutionary studies. Quantifying resistance in natural populations includes experimental manipulations where organisms are randomly assigned to uninfected control and experimental infection treatments, in addition to studies where individuals of known genotype (i.e. resistant or susceptible) are exposed to pathogens and compared with uninfected individuals. Studies of resistance in natural populations are often performed without knowing the mechanism(s) and/or mean(s) used to achieve resistance.

When the genetics of resistance are known, or host populations differ in susceptibility, the costs of resistance related to specific genetic lineages can be estimated in terms of fitness parameters, including survival, reproduction and growth. Artificial selection experiments are frequently used for this purpose. Artificial selection for increased resistance could be achieved by selecting for the gross (phenotypic) manifestation of the entire defense network; for example, selecting for the absence of infection or low parasite numbers per host following exposure. Resistant selection lines with increased trait expression might re-allocate resources to achieve resistance. However, other potential responses to selection for increased resistance might not require increased resource input to achieve resistance, contrary to recent suggestions [16]. Instead of selecting for overall parasite resistance, a much clearer picture would be presented by having multiple selection lines on the specific means of defense used to achieve resistance (e.g. trait increase, decrease and control) followed by assays on the effects on fitness-related traits and overall parasite resistance. It might also be beneficial to maintain selection for a long enough time to allow compensatory mutations to occur. Alternatively, KNOCKOUT [17] or OVER-EXPRESSION of the means of defense used to achieve resistance should provide the clearest demonstration of fitness costs.

Competition experiments between resistant and susceptible genotypes are also a good means of revealing the costs of resistance. These types of studies are commonly used with antibiotic-resistant bacteria and costs of resistance as small as 0.5% can be detected [15]. However, costs could also be environmentally dependent, such that they are minimal in some (e.g. benign) environments and are greater when inter- and or intra-specific competition increase or in non-benign environments [18]. These methods could be used to determine if there is a cost of resistance or, conversely, to demonstrate that resistance is not costly!

Conclusions

We noted that much recent work has operated under the implicit assumption that parasite resistance is costly. Contrary to that view, we and Coustau *et al*. [2] suggest that the answer to the question posed in the title of this paper is that a cost of resistance is not always necessary. Furthermore, the likelihood and magnitude of such costs depend on how resistance is achieved.

There is a large body of theory dealing with host–parasite interactions, that does not assume a cost of resistance. Indeed, most theories of host–parasite interactions belong to one of three classes based on assumptions concerning the costs of resistance. First, there is the body of theory where resistance is explicitly assumed to be costly (although actual costs vary) and parasite evolution is not considered explicitly [19–21]. Second, there is the body of theory in which costs are not incorporated or not assumed at all. Many of the cyclical antagonistic co-evolution models [22–26] belong to this category. Resistance in these models could be envisioned as operating through the cost-free mechanisms within any of the means of defense outlined here (i–v). Third, there are the models where host evolution is ignored and thus resistance (and much less the costs of resistance) do

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not come into play. Examples of this include many of the models dealing with the evolution of virulence [27], and epidemiological or ecological host–parasite models [28,29].

These three bodies of theory, one set assuming fitness costs of resistance, one assuming no costs of resistance and another not considering costs of resistance at all, are not mutually exclusive. Resistance can be achieved through any of the mechanisms in any of the means of defense given here (Fig. 1). Recognizing that there are many types of cost-free and costly forms of parasite resistance is the first step. The next step forward is to integrate them into future theories, models and experiments. We must recognize these important and diverse aspects of host–parasite relationships in order to gain a better understanding of resistance and of host–parasite interactions in general.

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