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Review Article

Immunity, resistance and tolerance in bird–parasite interactions

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SUMMARY

*Interacting pathogens and hosts have evolved reciprocal adaptations whose function is to allow host exploitation (from the pathogen stand point) or minimize the cost of infection (from the host stand point). Once infected, two strategies are offered to the host: parasite clearing (resistance) and withstanding the infection while paying a low fitness cost (tolerance). In both cases, the immune system plays a central role. Interestingly, whatever the defence strategy adopted by the host, this is likely to have an effect on parasite evolution. Given their short generation time and large population size, parasites are expected to rapidly adapt to the environmental conditions provided by their hosts. The immune system can therefore represent a powerful engine of parasite evolution, with the direction of such evolutionary trajectory depending on, among other factors, (i) the type of mechanism involved (resistance or tolerance) and (ii) the damage induced by overreacting immune defences. In this article, I will discuss these different issues focusing on selected examples of recent work conducted on two bird pathogens, the protozoa responsible for avian malaria (*Plasmodium *sp.) and the bacterium* Mycoplasma gallisepticum.

Keywords *immunopathology, infection,* Mycoplasma gallisepticum*,* Plasmodium relictum*, virulence*

INTRODUCTION

In spite of the complexity of the vertebrate immune system, pathogens remain a pervasive threat for their hosts. The reason for this is that pathogens also respond to the threat imposed by the immune system by adopting a series of strategies that aim at escaping/reducing the effectiveness of the immune response (1). This can lead to a co-evolutionary arms race, where the two partners are continuously selected to avoid the cost of infection and the cost of immune clearance.

An additional layer of intricacy is brought by the observation that hosts can adopt different strategies to cope with an infectious menace. Hosts can resist the infection when immune defences keep parasite multiplication at bay and eventually clear the infection. However, hosts can also tolerate the infection. Tolerance refers to the capacity of hosts to bear the infection paying little or no fitness cost (2). The concept of tolerance was first discussed in the plant-herbivore literature and referred to the capacity of plants to remain productive in the face of herbivores and other pests (3). Only in recent years, the concept has been applied to animal host–pathogen interactions (2, 4, 5). Råberg and co-workers (2) described tolerance as the reaction norm of fitness (or health) over a range of parasite intensities (Figure 1). A flat slope relating fitness (health) to parasite burden would thus indicate a good tolerance to the infection. As such, tolerance is defined as a trait that can only be measured on groups of individuals (genotypes, clones, experimental groups, populations, species, etc.). Mechanisms of tolerance are diverse, and a few recent review papers have extensively discussed the different pathways leading to tolerance (6, 7). Broadly speaking, tolerance can arise because hosts can minimize the direct damage induced by pathogens or the damage induced by an overreacting immune response. In addition to this, capacity to tissue repair and intrinsic tissue susceptibility are other essential components of tolerance.

Making the distinction between tolerance and resistance has important consequences for our understanding of host strategies to face infectious diseases and parasite evolution (8). As mentioned above, however, animal ecologists have only recently fully appreciated the need to tease apart the different strategies that hosts can adopt to reduce the cost of infection. The number of studies that have addressed tolerance in animal–pathogen systems is rapidly increasing, even though most of the published work deals with

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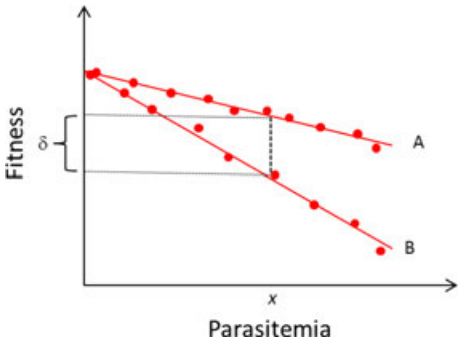

Figure 1 Tolerance as a reaction norm. The figure reports the change in host fitness (or health) as a function of increasing parasite burden. The two lines (A and B) represent different groups of individual hosts (they could be clones, experimental groups, populations, etc.). For both groups of individuals, fitness (or health) decreases with increasing parasitaemia. However, the slope of the regression line is steeper for group B. Group B is therefore less tolerant to infection than group A. This can be visualized at a given parasite burden (x) where individuals in group B have a δ fitness penalty compared with individuals in group A. Adapted from reference (2).

laboratory systems. Here, I will take advantage of very recent work conducted on bird–parasite associations to show that tolerance and resistance can rapidly evolve in natural populations exposed to epidemic waves.

Evolutionary biologists define parasite virulence as the fitness cost paid by infected hosts (9). It is striking to note that parasites do not exert similar costs to their hosts. Some parasites can persist for years in a latent form with little or no cost for the host; others produce extensive damage that can result in a rapid host death. Why is there this variability? What are the selection pressures that drive the evolution of virulence towards lethal or benign variants? How much of parasite evolution is due to differences in host defences? How does parasite virulence, in turn, drive the evolution of host defence strategies? Even though early work has seen virulence has an intrinsic parasite trait, it is now well established that virulence is a combination trait that depends on the parasite, the host and the environment where the interaction takes place (10).

During the last decades, theory on the evolution of parasite virulence has been erected on the assumption that there is a trade-off for the parasite between the benefits induced by within-host multiplication (higher number of propagules enhances the probability of transmission to new hosts) and the cost induced by host death (host death usually stops parasite transmission) (10). A parasite that reproduces rapidly has a higher chance to be successfully transmitted per unit time than a parasite that multiplies slowly. However, rapidly multiplying parasites are those that also risk killing the host. Parasites have therefore to cope with these conflicting selection pressures, on the one hand maximizing the number of propagules produced and on the other hand avoiding killing the host before any transmission has occurred. This general model of virulence evolution has been called the trade-off model and has received considerable attention from theoreticians and empiricists (see 10 for a recent review).

Even though a few experimental models have provided supportive evidence for the trade-off model of virulence evolution (11–13), in many host–parasite interactions there is no simple relationship between parasite density (the number of parasites per infected host) and the cost of the infection (14). It should also be noted that this theoretical framework works poorly for macroparasites that do not multiply within their final host.

There are several reasons why parasite multiplication and host damage can be decoupled, one being that the cost of infection might be more due to an overreacting host defence rather than a direct damage due to parasite multiplication (14, 15). In several host–parasite systems, host damage arises by a misdirected or an overexpressed immune response, a phenomenon called immunopathology. Recent theoretical work has suggested that immunopathology-induced disruption of the covariation between parasite density and host damage does not necessarily invalidate the trade-off model of parasite virulence, but it can substantially alter the evolutionary outcome (16–19). Indeed, if immunopathology damage is an increasing function of parasite multiplication (the more the antigenic stimulus, the stronger the immune response), then parasites are predicted to evolve towards lower virulence because highly multiplicative strains will pay the cost of direct host damage plus the immunopathology-induced cost. On the contrary, if immunopathology arises independently of parasite multiplication (a starting signal is enough to trigger immunopathology), then we expect parasites to become nastier because any prudent (slowly reproducing) parasite would nevertheless pay the immunopathology cost. Subsequent theoretical work has refined these predictions, showing that an additional important factor affecting the evolutionary outcome is how disease severity is measured (19).

The task of the immune system is not necessarily to clear the infection. In many cases, it might be more rewarding to coexist with the parasite instead of declaring the war. Even though the two terms refer to different processes, infection tolerance and immunological tolerance do overlap to a certain extent (20). As mentioned above, infection tolerance involves a wide array of mechanisms, including the down-regulation of many effectors that confer immunological tolerance (a nonresponsive immune system even when an antigenic stimulus is present). As for most immunological pathways, immunological tolerance involves different redundant mechanisms. Central

tolerance operates during the negative selection of T cells with a very high affinity to self-MHC molecules occurring in the thymus; peripheral tolerance arises when self-reactive cells that have escaped the negative selection are anergized or suppressed by regulatory T cells (21). Anti-inflammatory cytokines produced by macrophages and regulatory T cells also play a prominent role during the resolution of an inflammatory response and are essential components of organismal homoeostasis during an infectious insult (22).

Immunological tolerance is a mechanism that controls and prevents immunopathology. Tolerant hosts, thus, may pay a minimal cost of infection because they are protected by the immunopathology cost. Again this is likely to have substantial fitness consequences for the parasites and drive their evolution. For instance, when tolerance is due to a down-regulated immune response, parasites are freed from the selection induced by the host immune system that breaks down the antagonistic co-evolutionary interactions between the hosts and the parasites. However, an alternative view suggests that tolerance reduces the cost of virulence traits for highly exploitative parasite strains (infected hosts tolerate the infection and the parasite achieves its transmission). Therefore, tolerant hosts might actually select for more virulent parasites (8, 20, 23).

The interplay between resistance, tolerance, immunopathology and parasite virulence is a fast-moving area of research. However, for obvious reasons, most of the studies that have tackled these questions have used laboratory model systems (2, 4, 23). This is understandable given the need to perform controlled infections, assess parasite density, measure immune traits involved in resistance, tolerance and immunopathology, and assess parasite and host fitness, which is rarely doable in the wild. However, one potential drawback of laboratory studies is that they neglect the fact that the interaction between the host immune response and the parasitic strategy of host exploitation takes place in an environment that is variable in both space and time (24). Ecological complexity is therefore an additional important source of variation affecting the relationship between immunity, resistance, tolerance and virulence.

Birds offer the opportunity to complement laboratory studies under controlled conditions with a more realistic work conducted under natural situations. The study of bird–pathogen interactions in nature combined with laboratory studies have proved a powerful combination, particularly for the two infectious diseases discussed below. In this article, I will review some recent results illustrating the evolution of resistance/tolerance in birds and the potential consequences for parasite evolution using avian malaria parasites and the bacterium *Mycoplasma gallisepticum* as model systems.

AVIAN MALARIA

Haemosporidia (*Plasmodium, Haemoproteus, Leucocytozoon*) parasites have been reported to infect a wide range of bird species, worldwide (25). As for mammalian *Plasmodia*, the agent of avian malaria is transmitted from bird to bird by a dipteran vector. The life cycle of avian *Plasmodia* involves the multiplication by asexual reproduction (merozoites) in the bird host. Merozoites can also mature into gametic forms (gametocytes) that are infectious for the mosquito where a sexual reproduction occurs. Merozoites multiplication induces the burst of infected red blood cells and this usually produces the anaemic crisis observed in avian and mammalian hosts.

Traditionally, the study of avian malaria parasites has been carried out using natural populations of hosts (26–29). The advent of modern molecular techniques has promoted the discovery of an unsuspected diversity of parasite lineages and confirmed that, as for mammalian *Plasmodia*, individual hosts harbour mixed infections (30–32). Unravelling the cost of infection and the resistance/tolerance towards avian malaria has been a more challenging task, because as mentioned above this usually requires the use of experimental infections.

During the last few years, a number of laboratory-conducted studies have stressed that passerine species can have strikingly different infection dynamics when infected with naturally occurring *Plasmodium* strains. (33–36). Palinauskas *et al.* (33) infected 5 passerine species with the same generalist *Plasmodium relictum* (lineage SGS1) and investigated the parasitaemia and the associated costs for the hosts. While starlings (*Sturnus vulgaris*) were fully resistant to the infection, the other four species showed a variable pattern of resistance/tolerance. House sparrows (*Passer domesticus*) were partially resistant because 50% of inoculated birds established a successful infection, whereas 100% of chaffinches (*Fringilla coelebs*), crossbills (*Loxia curvirostra*) and siskins (*Carduelis spinus*) were susceptible to the infection. Within the susceptible species, infection intensity showed huge variation with siskins and crossbills having the highest peak of parasitaemia. However, when looking at the reduction in haematocrit (the proportion of red blood cells, a good proxy of infection-induced fitness cost), only the two species with experimental highest parasitaemia seemed to suffer from the infection. This study therefore strongly suggests that avian hosts exhibit interspecific variation in their propensity to be resistant/tolerant to *Plasmodium* parasites.

The co-infection with two *Plasmodium* species (*Plasmodium relictum* and *Plasmodium ashfordi*) led to a very different outcome depending on the host species (34). Whereas starlings were again fully resistant to the

infection by the two parasites, siskins and crossbills were highly susceptible, with parasitaemia in double-infected birds being higher than in single infected hosts. The two susceptible species appear to differ in terms of tolerance to the infection. Indeed, even though siskins and crossbills have similar peak parasitaemia, siskins paid a much smaller cost of infection (a smaller reduction in haematocrit values and no infection-induced mortality).

This experimental work therefore shows that generalist malaria parasites infecting a large number of host species nevertheless achieve quite different infection dynamics and incur quite different costs for their hosts possibly due to a combination of resistance and tolerance processes. A pending question is what accounts for this interspecific pattern of resistance/tolerance even for closely related host species. Variation in life history traits among species has been suggested to explain specific propensity to invest in costly inflammatory response (20). However, the species used by Palinauskas *et al.* (33, 34) have similar paces of life.

Immunologically naïve hosts, in particular those that have not coevolved with avian malaria, are predicted to suffer more from infection. The accidental introduction of avian malaria in the Hawaiian archipelago provides a textbook illustration of a rapid evolutionary change in a novel host–parasite association. Avian malaria became a serious threat for Hawaiian honeycreepers when the mosquito vector *Culex quinquefasciatus* was introduced in the early 20th century (37, 38). Prevalence of infection and parasitaemia were high in honeycreepers, and the infection induced a substantial drop in body mass, haematocrit and finally high mortality (39–42). As a consequence, lowland areas that provided a favourable environment to the mosquito and therefore to *Plasmodium* transmission became unfavourable for the bird hosts, and the populations of several honeycreepers went eventually extinct in lowland areas and established refuges at high altitudes, where temperature is too low to allow mosquito survival (37, 38). In 2002, a survey of Hawaiian honeycreepers in lowland areas found that the populations of the amakihi (*Hemignathus virens*) recovered in number, comprising from 24.5% to 51.9% of the avian community, in spite of very high prevalence (24–40% if estimated by microscopy, 55–83% if estimated by serology) (43). Genetic structure of high- and low-altitude populations further suggested that individuals that recolonized low-altitude sites did not come from high-altitude refuges, but likely originated from residual lowland populations that were continuously exposed to malaria imposed selection (44, 45). Finally, the finding that prevalence was still high in this expanding population possibly suggests that tolerance rather than resistance rapidly evolved in amakihi (even though data on parasitaemia are needed to confirm this). The rapid spread of resistance/tolerance to malaria also suggests that standing genetic variation was possibly present in the amakihi, before the spread of malaria. It should be noted that amakihi was the only honeycreeper to show such evolved pattern of resistance, further stressing the among-host variability shown by experimental infections of European passerines (33–36).

Additional evidence for resistance to malaria parasites comes from population genetics studies focusing on immune genes involved in the antigen presentation process. Screening of genes of major histocompatibility complex (*Mhc*) class I and II in different European passerines has reported a protective role of *Mhc* diversity and specific alleles towards the infection with different *Plasmodium* lineages in terms of both prevalence and parasitaemia (46–48). Moreover, when multiple populations were surveyed, alleles conferring a protective effect were found to be population-specific, suggesting a co-evolutionary interaction between the host and the parasite, potentially promoting local adaptation (49). More recent work using next-generation sequencing has shown that distinct *Mhc* supertypes confer qualitative (prevalence) and quantitative (parasitaemia) protection against two *Plasmodium* species (*P. relictum* and *P. circumflexum*) in one wild population of great tits (*Parus major*) (50).

The involvement of specific immune effectors in the process of resistance/tolerance to malaria parasites has been recently shown by a series of experimental studies conducted in the domestic canary (*Serinus canaria*)–*Plasmodium relictum* association.

During the course of a malaria infection, a wide array of immune effectors are activated. The first acute phase stimulates an inflammatory response with the release of cytotoxic compounds followed by acquired response and antibody production. Previous exposure to the pathogen confers a partial protection to a subsequent infection, a phenomenon coined premunition by very early work on avian malaria (51). Cellier Holzem *et al.* (52) infected immunologically naive domestic canaries with *Plasmodium relictum*. Thirty-four days after this primary infection, when the birds had recovered their initial haematocrit and body mass values, surviving canaries were re-infected with the homologous strain. In agreement with the idea of premunition, re-exposed birds were better able to cope with the infection, keeping parasitaemia at lower levels and managing to maintain constant haematocrit and body mass. Primary infected canaries produced more haptoglobin, a protein of the acute-phase response, compared with noninfected birds. However, haptoglobin did not differ between primary and secondary infected birds, suggesting

that while inflammatory effectors are involved in the control of the initial acute phase of the infection, long-lasting partial immunity relies on memory effectors.

Pioneering work conducted on rodent malaria has stressed the importance of host immunity as a component of malaria virulence. Pro-inflammatory cytokines are important immune effectors involved in malaria resistance. Up-regulation of pro-inflammatory cytokines is often associated with a resistance phenotype prone to immunopathology damage. On the contrary, up-regulation of anti-inflammatory cytokines confers a susceptible phenotype to microparasites and a protection towards immunopathology. Long *et al.* (53, 54) used phenotypic manipulations of both pro- and anti-inflammatory cytokines in mice infected with *Plasmodium chabaudi*. They found that blockade of IL-10 (an anti-inflammatory cytokine) reduced parasitaemia but, nevertheless, exacerbated malaria virulence (i.e. mouse mortality) (53). Similarly, blocking the TNF-α receptors induced an increase in parasite density while reducing disease severity (54). Overall, there is strong evidence based on human and rodent studies that malaria virulence has an immune-based component (55, 56).

Building on this previous work, Bichet *et al.* (57) experimentally infected domestic canaries whose inducible nitric oxide synthase (iNOS) activity was inhibited by a drug (aminoguanidine). Inducible nitric oxide synthase catalyses the production of nitric oxide (NO), a nitrogen reactive species with cytostatic and cytotoxic effect on different *Plasmodium* species both *in vitro* and *in vivo* (58). In agreement with the expected effect of iNOS inhibition, birds treated with the drug were less able to keep *Plasmodium* multiplication under control, and the parasitaemia of iNOS-inhibited animals tended to increase with time, whereas parasitaemia of control birds dropped after day 14 post-infection. Interestingly, however, in spite of higher parasitaemia, iNOS-inhibited birds did not pay a higher cost of infection because haematocrit values were similar for iNOS-inhibited and control birds. This result parallels those reported for *Plasmodium chabaudi*-infected mice and suggests that the cost of higher parasitaemia in iNOS-inhibited birds might be compensated by a reduced cost of immunopathology. Overall, these results also point towards a possible trade-off between resistance and tolerance. As mentioned above, the control of the acute proliferation of asexual malaria parasites relies on several inflammatory effectors. Up-regulating the inflammatory response however adds a potential immunopathology toll to the overall cost of infection. Breaking down immunological tolerance therefore constitutes a possible mechanism underpinning a physiological trade-off between resistance and tolerance.

A pending important question is now how parasites do adapt to hosts depending on the defence strategy (resistance vs. tolerance) and the possible trade-off between strategies. Again insight into the possible evolutionary trajectory followed by parasites experiencing particular immune environments comes from studies on rodent malaria, where *Plasmodium chabaudi* serially passaged in vaccinated mice evolved to become a more serious threat to their host (59). The reason for increased virulence of parasites evolving in vaccinated host lies on the relaxed cost of virulence. Vaccinated hosts are protected from infection-induced mortality but they still contribute to parasite transmission (60). Therefore, rapidly growing parasites are favoured in vaccinated hosts and can be highly pathogenic in nonvaccinated hosts.

Evidence in support to parasite evolution as a function of host immunity (61) also comes from a recent study involving *Plasmodium relictum*-infected canaries. Cornet *et al.* (62) assessed the infection dynamics and the cost of infection in canaries facing two diets. Birds enjoying a protein- and vitamin-enriched food were better able to control parasite growth (they had lower parasitaemia, and peak parasitaemia was reached earlier than for control, nonsupplemented hosts). Protein and vitamins are important environmental determinants of immune competence as shown in several organisms, including humans (63,64). Therefore, reduced parasitaemia in food-supplemented birds is consistent with an improved resistance. Nevertheless, food-supplemented birds also paid the highest per-parasite cost of infection (Figure 2a). In a follow-up experiment, parasites grown in food-supplemented and control hosts were inoculated in another group of hosts following a fully factorial design (parasites grown in food-supplemented hosts passaged in food-supplemented and in control hosts; parasites grown in control hosts passaged in food-supplemented and in control hosts) (62). This allowed disentangling parasite origin from the current host environment. After a single passage, parasites issued from the control, tolerant hosts induced the highest parasitaemia, suggesting that they had been selected for higher multiplication rate. The effect of parasite origin largely predominated compared with the effect of the current host environment, which further suggests that increased multiplication rate in passaged parasites resulted from genetic selection instead of phenotypic plasticity. Parasites issued from hosts kept on a nonsupplemented diet (the tolerant ones) also induced the highest damage in the subsequent hosts, in terms of both haematocrit reduction and body mass loss (Figure 2b,c) (62). These results are therefore in agreement with the idea that tolerance might favour the evolution of more virulent parasite strains. It is noteworthy that a single passage was enough to elicit a measurable

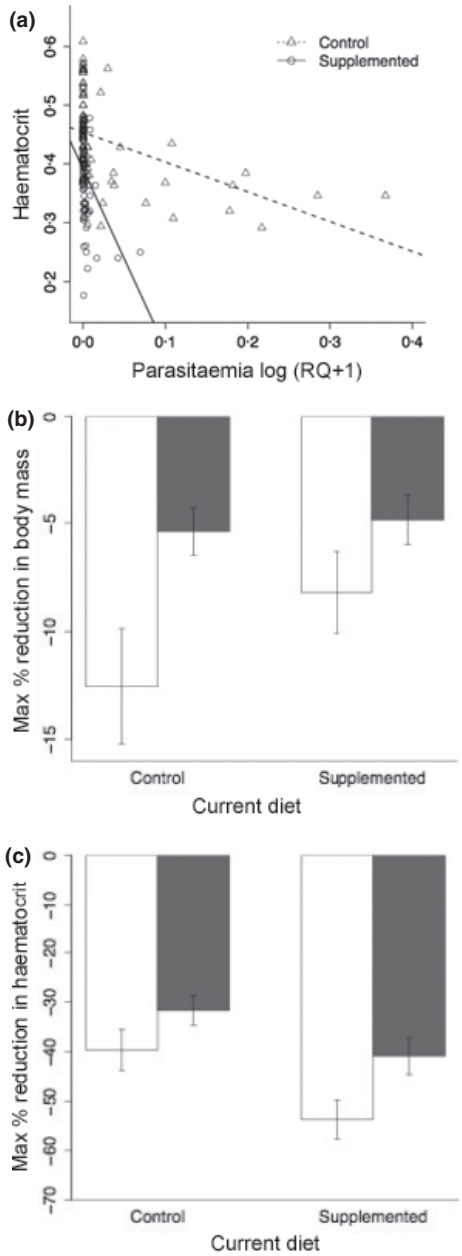

Figure 2 (a) Cost of infection with *Plasmodium relictum* in domestic canaries maintained on a control (triangle) or protein- and vitamin-supplemented (dots) diet. The slope relating haematocrit and parasitaemia is steeper for supplemented birds, showing that they paid a higher per-parasite cost. (b) and (c) Parasites grown in control nonsupplemented hosts (white bars) induce a higher damage (body mass and haematocrit loss) when passaged to new hosts compared with parasites grown in supplemented hosts (grey bars), whatever the diet of the new hosts (current diet) [From reference (62)].

effect on parasite multiplication and virulence. Inoculated parasites were isolated from naturally infected house sparrows and certainly contained multiple clones. High genetic variation among inoculated parasites speeds up the response to selection exerted by the immune system and this most likely reproduces the natural situation where parasites have high degree of genetic variation and large population size.

Assessing the relationship between resistance, tolerance and fitness is for obvious reasons much more difficult in natural populations. Nevertheless, Stjernman *et al.* (65) reported a nonlinear relationship between survival and intensity of infection with the malaria parasite *Haemoproteus majoris* in naturally infected blue tits (*Cyanistes caeruleus*) (Figure 3). Whereas poor survival prospect of heavily parasitized birds might indicate the direct cost of the infection, reduced survival of individuals with low parasitaemia might reflect the cost of hyper immunity. Maximal survival is therefore achieved when birds balance the costs of an over-reactive immune response and the benefits of parasite clearance.

MYCOPLASMA GALLISEPTICUM

Mycoplasma gallisepticum is a pathogenic bacterium of poultry causing respiratory diseases and conjunctivitis. Among others, swollen eyes are a typical symptom of the disease (Figure 4a). In the 1993–1994, house finches (*Carpodacus mexicanus*) with swollen eyes were observed in the area around Washington DC (66). Even though *Mycoplasma* can infect other passerine species, house finches were shown to be particularly susceptible to the disease (67). The infection reduced both the survival prospect and the reproductive success of house finches (68, 69). The number of infected birds rapidly increased with a substantial impact on the population dynamics (68, 69). As for

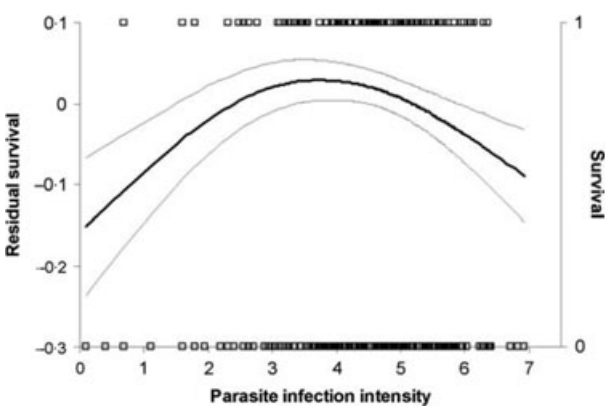

Figure 3 Nonlinear relationship between host survival (after controlling for the effect of sex and year) and intensity of infection with the haemosporidian *Haemoproteus majoris* in the bleu tit. The fit corresponds to a cubic spline function ± SE. Squares indicate the raw data [From reference (65)].

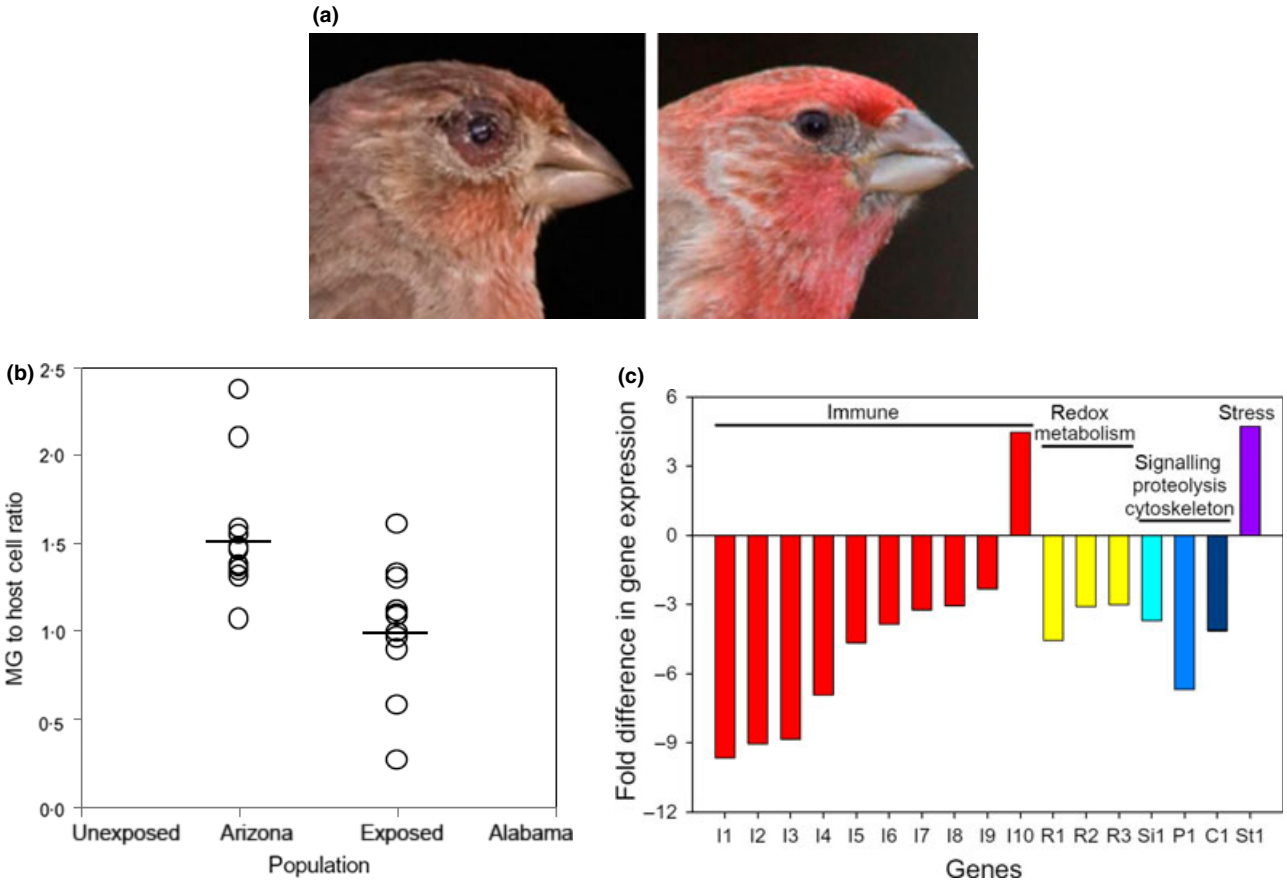

Figure 4 (a) Symptoms of *Mycoplasma gallisepticum* infection in the house finch (copyright GE Hill). (b) Bacterial load in the conjunctivae of house finches from a coevolved (Alabama) and a naïve (Arizona) population. (c) Differential gene expression between the two populations. Negative values indicate lower expression in birds from the naïve (Arizona) population relative to the coevolved one. I1 to I10 refer to the following immune genes: T-cell Ig and mucin domain containing-4; MHC class II-associated invariant chain I1; lectin, galactoside-binding soluble-2-protein; programmed death ligand 1; TCR β-chain; Ig J; neutrophil cytosolic factor-4; Ig superfamily member 4A isoform a; parathymosin; and complement factor H. R1 to R3 refer to the following redox metabolism genes: thioredoxin; spermidine/spermine N1-acetyltransferase variant 1; and squalene epoxidase. Si1, P1 and C1 refer to RhoA GTPase, ubiquitin C and lymphocyte cytosolic protein genes, respectively. St1 refers to heat shock protein 90a [From reference (71)].

the avian malaria in the Hawaiian archipelago, the arrival of the epidemic wave has been rapidly followed by a decrease in the percentage of birds showing the symptoms of the disease (70). This has led to the hypothesis of selection for resistance in exposed hosts.

In 2007, Bonneaud *et al.* (71) investigated the evolution of *Mycoplasma* resistance in one house finch population in Alabama that was exposed to the pathogen early on during the epidemic wave (a 12-year exposure period). They experimentally infected birds from Alabama with a local *Mycoplasma* strain. As a comparison, they also infected house finches from Arizona, a region where house finches have never experienced the disease. As expected, Alabama birds harboured a lower bacterial load in the conjunctivae compared with Arizona finches (Figure 4b). Between-population differences in bacterial load were mirrored by a differential pattern of gene expression in response to the experimental infection. Among the 52 identified genes with known function, 38% and 21% showed a post-infection expression change in Arizona and Alabama, respectively. This post-infection expression change was due to genes in Arizona birds being more down-regulated (80% of 20 genes) compared with Alabama individuals (27% of 11 genes). When focusing on experimentally infected birds only and looking at the post-infection gene expression changes, all 52 genes were differentially expressed in birds from the two populations and again this was due to Arizona individuals having 90% of these genes down-regulated post-infection (10% in Alabama birds).

Among the different genes with differential expression, 10 were directly linked with immunity (Figure 4c). Nine of these 10 immune genes were down-regulated in birds from

Arizona. The tenth gene (complement factor H) was up-regulated in Arizona birds. However, this gene restricts the activation of the complement cascade and is therefore functionally consistent with the expression pattern of the other immune genes. Overall, birds from Arizona showed a pattern of down-regulation of their immune response. This pattern nicely fits with the known immunosuppressive action of *Mycoplasma* on their chicken hosts. After 12 years of exposure to the pathogen, house finches were thus able to overcome the infection-induced immunosuppression and restore an effective immune protection. To further confirm this view, Bonneaud *et al.* (71) also compared the pattern of gene expression between birds from Alabama sampled in 2000, after only 5 years of exposure to the bacterium. The gene expression of these birds resembled the 2007 Arizona birds more than the 2007 Alabama individuals, strongly suggesting that the observed pattern was due to a microevolutionary change that occurred with time rather than a geographical (environmental-based) variation. A further study comparing the pattern of gene expression in birds from Alabama and Arizona at 3 and 14 days post-infection (72) concluded a possible role of innate immunity in *Mycoplasma* resistance.

The *Mycoplasma*–house finch system also provides a rather unique opportunity to investigate the cost of pathogenesis (the cost directly induced by the parasite) and the cost of immunity (the cost of host defences) in a more natural context than the experiments involving avian malaria described above. The emergence of the epidemics in the East United States, the rapid evolution of host resistance and the persistence of immunologically naïve populations in the West can almost be considered as a natural experiment that might allow to test the following predictions: if the cost of infection is mostly due to the direct damage of the pathogen, then hosts from Arizona (the nonexposed population) should suffer the most; if immunological resistance incurs costs and these constitutes the bulk of the fitness reduction in infected birds, then exposed (Alabama) hosts should suffer the most. Bonneaud *et al.* (73) used the same populations of house finches to measure changes in body mass intervening during the first 14 days post-infection as a proxy of infection cost. Overall, birds from the coevolved population lost more body mass than birds from the naïve population, and interestingly, the relationship between bacterial load and loss in body mass was reversed in the two populations (Figure 5a). Whereas bacterial load was negatively correlated with body mass loss in Arizona birds, indicating that most heavily infected birds lost more mass, the sign of the correlation was reversed in Alabama birds. Birds with the lowest bacterial load suffered the most intense mass reduction in Alabama. One possible interpretation of these results is that body

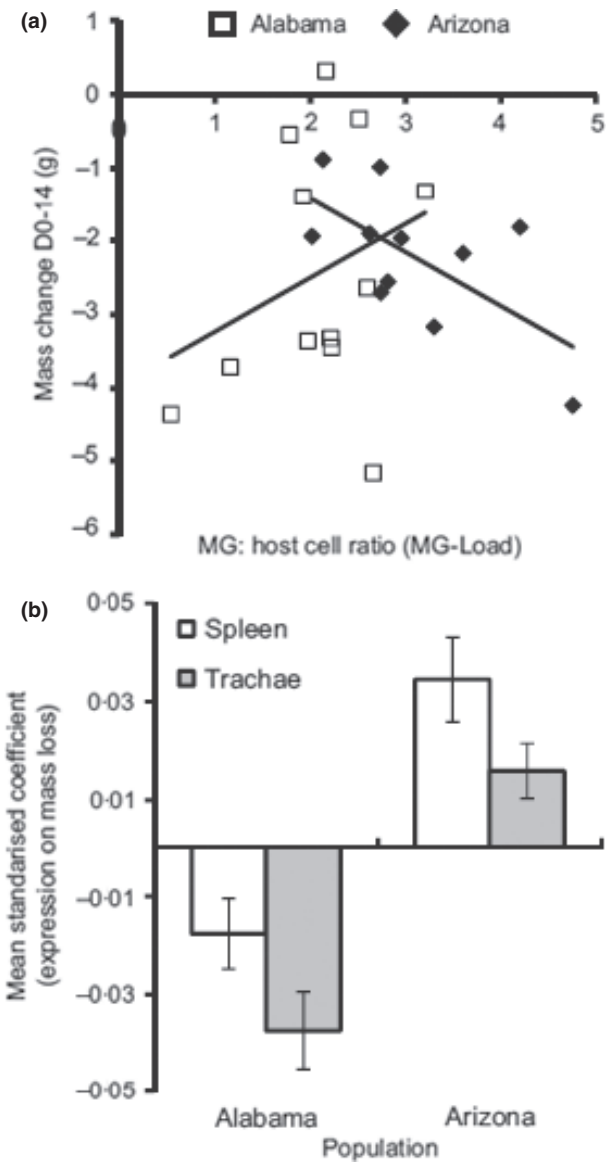

Figure 5 (a) Relationship between changes in body mass during the course of the infection (mass at day 0 minus mass at day 14 post-infection) and bacterial load in *Mycoplasma*-infected house finches. Squares indicate birds from the coevolved population, diamonds birds from the naïve population. (b) Changes in body mass during the course of the infection as a function of protective immunity (mean of the slopes of the regression between mass load and gene expression). Expression of genes conferring protective immunity is associated with body mass loss in birds from the coevolved population (Alabama) [From reference (73)].

mass loss represents two different components of the cost of the infection in the two populations: cost of immunological resistance in Alabama and cost of parasite damage in Arizona. In agreement with this view, the pattern of immune gene expression (indicating a protective immunity) was associated with a higher body mass loss in Alabama

than in Arizona (Figure 5b). These results therefore nicely confirm in a more natural setting the findings reported for malaria parasites. Immunological costs, whatever their nature (energetic or self-reactivity) and whatever the conferred protection (resistance or tolerance), substantially contribute to determine parasite virulence.

More recently, Adelman *et al.* (74) explored explicitly the role played by inflammatory effectors in the resistance/tolerance of house finches experimentally infected with *Mycoplasma gallisepticum*. They used the same house finch populations (Alabama and Arizona) studied by Bonneaud *et al.* (71–73), but birds were infected with a strain of *Mycoplasma* isolated soon after the emergence of the epidemics. They also focused on pro- (IL-1β) and anti-inflammatory (IL-10) effectors as mediators of tolerance to infection. Interestingly, they showed that birds originating from Alabama were more tolerant to the infection (they had a better health for a given pathogen load), even though this depended on the method used to assess tolerance, than birds from Arizona. Birds from Alabama also had a lower expression of the pro-inflammatory cytokine IL-1β. This result suggests that (i) the evolution of tolerance might imply the down-regulation of pro-inflammatory effectors and (ii) such microevolutionary shift can occur during very short periods of time.

CONCLUSION AND FUTURE DIRECTIONS

At least two documented cases of bird–pathogen interactions show that epidemic waves emerging in immunologically naïve hosts do initially have devastating effect on the populations of their hosts, but this early stage is rapidly followed by the emergence of resistance/tolerance. The rapidity of host recovery, in particular when considering the *Mycoplasma* epidemics, strongly suggests that standing genetic variation exists in host population for traits that confer protection towards infectious diseases, be they resistance or tolerance traits. These findings mirror the textbook example of the myxoma virus that, following its deliberate release in Australia to keep control of the rabbit population, rapidly selected for resistant hosts (75). They also highlight the value of studying natural parasite invasions/epidemics, as we can watch evolution of resistance or tolerance in action.

Even though we are still far away from having a full picture of the genetic changes intervening on hosts exposed to these major epidemic waves, innate immune genes (72) and *Mhc* genes (76) have been shown to rapidly respond to parasite-exerted selection pressures, pending the existence of standing genetic variation in the population. Nevertheless, while the classical view has been to consider that epidemic waves select for resistant hosts, accumulating evidence indicates that tolerance can be an effective alternative mechanism that hosts can use to cope with pathogens. However, we still have a partial understanding of the sources of variation in resistance/tolerance among species, populations or individuals. A simple food manipulation experiment (62) showed how environmental traits can have profound effects on tolerance to infection. It would certainly be worth conducting similar experiments in the wild. The immunological mechanisms involved in resistance/tolerance also deserve to be better studied, as illustrated by the excellent work done on the association between house finches and *Mycoplasma gallisepticum* (71–74). For instance, it would be extremely interesting to explore the immunological traits underlying the interspecific variation in resistance/tolerance to avian malaria observed in some passerine hosts (33–36).

Adopting a resistance vs. a tolerance strategy can also have profound effects on parasite evolution. However, several pieces of information are still missing if we want to have a better understanding of the antagonistic selection pressures between host immune system and invading pathogens and predict the co-evolutionary trajectories. For instance, down-regulation of anti-inflammatory effectors does exacerbate the cost of the infection by adding an immunopathology component to the direct parasite damage. The evolutionary consequences for the parasites are likely to depend on the transmission consequences of a down-regulated inflammatory response. While some parasites do suffer from exacerbated costs of immunopathology paid by their hosts, other can enjoy an improved reproductive success and transmission by over-stimulating the inflammatory response, by outcompeting other parasite species/strains (77) or enhancing the accessibility to host tissues (78). Therefore, the co-evolutionary trajectories between hosts and pathogens are likely to be species-specific and difficult to forecast in the absence of detailed information on the interactions between the host immune response and parasite growth and transmission. Similarly, parasites that produce both transmissible and nontransmissible stages might elicit different immune protection, with specific effectors targeting the transmissible stages, with a major impact on parasite fitness. In some instances, self-harm might even represent a host defence that reduces the amount of resources that are available to the parasite, as recently suggested for the destruction of noninfected red blood cells in mice infected with *Plasmodium chabaudi* (79).

A fascinating but still poorly studied phenomenon deals with the evolutionary consequences of the parasite manipulation of the host immune response (1, 80). As mentioned above, pathogens might adaptively exacerbate the

inflammatory response for their own spread and persistence; however, more commonly, parasites aim at down-regulating and evading the host immune response (81). Interestingly, some pathogens can do both. *Mycoplasma* initially up-regulates the inflammatory response, and the associated break down of the epithelial cell layer facilitates the spread of the bacterium (82). Later on, the infection induces a down-regulation of T-cell activity (83). Similarly, a rodent malaria species (*Plasmodium yoelii*) has been shown to up-regulate regulatory T cells (84). The evolutionary consequences of immune evasion can be far reaching for both parasite virulence and host defences. Immune evasion mechanisms are often responsible for the pathogenesis of the infection (85), and life history theory tells us that parasite fitness is more sensitive to mechanisms that avoid early clearance even if they induce a later cost to the host (86). The study of the intertwined connections between parasite manipulation of the immune system, virulence and host defences is still in its infancy. At the moment, we ignore for instance if immune evasion strategies are genetically variable (but see 87) and how hosts can neutralize subverted immune functions. Interestingly, the evolution of house finches in response to the *Mycoplasma* epidemics suggests that resistance has arisen by escaping the bacterium-induced sabotage of the immune system.

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