Density-dependent prophylactic immunity reconsidered in the light of host group living and social behavior

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Abstract. According to the density-dependent hypothesis (DDP), hosts living at high densities suffer greater risk of disease and so invest more in immunity. Although there is much empirical support for this, especially from invertebrate systems, there are many exceptions, notably in social insects. We propose that (A) density is not always the most appropriate population parameter to use when considering the risks associated with disease and (B) behavioral defenses should be given a greater emphasis in considerations of a host’s repertoire of immune defenses. We propose a complementary framework stressing the connectivity between and within populations as a starting point and emphasizing the costs represented by disease above the risk of disease per se. We consider the components of immune defense and propose that behaviors may represent lower-cost defenses than their physiological counterparts. As group-living and particularly social animals will have a greater behavioral repertoire, we conclude that with group living comes a greater capacity for behavioral immune defense, most particularly for social insects. This may escape our notice if we consider physiological parameters alone.

Key words: disease resistance; host–pathogen interactions; immunocompetence; insects; social behavior.

INTRODUCTION

A key theme in the evolutionary ecology of disease is the degree to which hosts invest in defense against parasites and pathogens. Profitable paradigms in this area have been that (1) disease risk is a function, in the simplest instance, of host density (Anderson and May 1979, McCallum et al. 2001) and (2) this risk of disease leads to selection on the host to minimize the potential costs of disease (Hochberg 1991, Reeson et al. 1998, Wilson and Reeson 1998). The traits employed to minimize the costs of disease are often known as “immunocompetence,” although this term and its usage are not without their critics (Ryder 2003, Siva-Jothy et al. 2005, Viney et al. 2005).

A clear prediction that arises from these paradigms is that hosts living at high densities should invest more in defense than those living at low densities (Hochberg 1991, Reeson et al. 1998, Wilson and Reeson 1998, Altizer et al. 2003, Wilson and Cotter 2008). This hypothesis of “density-dependent prophylaxis” (DDP) has been supported in a range of studies, particularly with invertebrate hosts (e.g., Wilson and Reeson 1998, Wilson et al. 2003, Cotter et al. 2004, Wilson and Cotter 2008). Some of these have adopted a comparative, phylogenetic approach wherein physiological parameters such as hemocyte counts, cuticular melanization, or measures of hemolymph phenoloxidase titres are used as correlates of investment in defense against disease, and comparisons are made across species. Key papers adopting this comparative approach have characterized lepidopteran species according to a solitary or gregarious lifestyle with the hypothesis that the latter can be shown to invest more in defense against disease (Hochberg 1991, Wilson et al. 2003). However, these studies do not universally support the hypothesis, casting some doubt on the assumption that gregarious species will be universally exposed to a higher risk of disease. Alternative explanations can be found, such as the possibility that, for more aggregated populations, between-group transmission may be more difficult (Wilson et al. 2003) so disease risk may actually be lower.

An alternative yet complementary approach exploits a phenomenon displayed by several insect species, namely
density-dependent phase polyphenism, wherein the phenotype (or “phase state”) of some lepidopteran and orthopteran species varies with population density experienced by the mother and/or during development of immatures, and investment in immune defense is expected to vary accordingly. Thus, locusts reared at low densities display a suite of morphological and behavioral traits (e.g., cryptic coloration and a tendency to avoid conspecifics) and display the solitaria phase state. In contrast, individuals reared at higher densities (or simply exposed to conspecifics or associated stimuli: phase state is highly labile) tend to have a warning coloration and aggregate with conspecifics, part of the suite of traits that is known as the gregaria phase state (see Simpson et al. [1999] for a review). The expectation is that the gregaria-phenotype individuals invest more in immunity than do solitaria individuals. Indeed, one study of the desert locust (Schistocerca gregaria) has shown gregaria-phase individuals to have relatively greater hemocyte counts and resistance to a pathogenic fungus (Meta- rhizium anisopliae var. acridum) than their solitaria counterparts (Wilson et al. 2002). The plasticity of this phenomenon has allowed the comparison of genetically highly related lepidopteran or orthopteran individuals in high- and low-density treatments and has broadly shown a positive correlation between gregarious behavior and immune defense (Reeson et al. 1998, Wilson and Reeson 1998, Wilson et al. 2002, 2003, Cotter et al. 2004, Bailey et al. 2008, Reilly and Hajek 2008, Wilson and Cotter 2008). Similarly, organisms with variable densities, but which are not well known a priori for density-dependent phenotypic variability (such as the coleopteran mealworm Tenebrio molitor), may be revealed to have elevated investment in resistance when reared at higher densities (Barnes and Siva-Jothy 2000). Inevitably, however, this pattern is not always observed and particularly not in all of the parameters measured (Wilson and Reeson 1998, Wilson et al. 2002, Cotter et al. 2004, Wilson and Cotter 2008).

These studies have been seminal in identifying the relationship between host density and investment in defense. However, they have clearly demonstrated that there is no simple and direct positive dependence of one upon the other. In particular, studies on eusocial insects have provided very little support for the DDP hypothesis (e.g., Pie et al. 2005). Thus, the initial assumptions (paradigms 1 and 2, above) may be too simplistic. We can also question the degree to which physiological parameters such as hemocyte counts, phenoloxidase titres, or even encapsulation responses, while being readily quantifiable, are appropriate measures of (or proxies for) total investment in defense (over and above the question of whether one is measuring an induced vs. constitutive component of defense, an issue we will not address here). An illustration of this complexity can be seen in a study in which a range of physiological responses of larvae of the lepidopteran Spodoptera littoralis are considered together (Cotter et al. 2004). In this example, it is found that some defenses increase with density while others do not and still others actually decrease, synthesized by Wilson and Cotter (2008). This highlights the importance of considering as many relevant parameters as possible (Siva-Jothy et al. 2005) and we emphasize that behavioral defenses are a key aspect to include.

It has been argued (Little et al. 2005) that invertebrate immunology has to some degree departed from a holistic, phenomenological approach (which characterized the initiation of the area of vertebrate immunology) in favor of a more reductionist approach that seeks similarities between vertebrate and invertebrate hosts as a starting point and/or focuses upon specific readily quantifiable physiological traits as correlates of investment in defense. As Little et al. (2005) point out, without considering the whole organism, and its progeny, responses to infection that could be expressed through life-history traits (Minchella 1985) or transgenerational changes in genotype (Elliot et al. 2003) will be overlooked. The same can easily be said of behavioral responses to infection, such as behavioral fever (Starks et al. 2000, Elliot et al. 2002a). It stands to reason that the whole suite of possible responses, whether physiological or behavioral, should be considered together.

An Earlier, and Broader, Definition of “Immunocompetence”

Immune defense (or “immunocompetence”) has been the subject of many reviews and much debate in the past few years. As empirical investigations have dug deeper into the mechanics of invertebrate immune systems, one early definition seems to have been left to one side. This definition is of particular relevance here: Owens and Wilson (1999:171) defined immunocompetence as “a measure of the ability of an organism to minimize the fitness costs of an infection via any means, after controlling for exposure to appropriate antigens” [our italics].

Whether or not the term “immunocompetence” meets with favor, we feel that this definition is of particular relevance when considering the selective forces that are expected to be operating and the possible evolutionary responses to these forces. It distills the host-parasite interaction into an effect on the host’s fitness and does not predefine mechanisms an organism might use to mitigate these effects. It is, indeed, the costs of infection which should drive host adaptation. This has been explored theoretically by van Baalen (1998), leading to the thought-provoking conclusion that, at high densities, resistance may no longer be beneficial as the force of infection is such that a host will eventually be reinfected and succumb to disease anyway. In such a situation hosts should invest instead in, say, rapid reproduction to increase fitness. (Although van Baalen’s model incorporated pathogen clearance by the hosts, the general result makes intuitive sense for other cases.) This type of life-history “escape attempt” should be considered part of
the organism’s immune defense as it could ameliorate the costs of infection (Minchella 1985). Thus, the definition given above can be interpreted to incorporate an almost unlimited suite of traits that may allow an organism to reduce the fitness costs of infection. This was highlighted by Little et al. (2005) when they emphasized that the full range of host responses to infection should be considered. In fact, in the original papers on density-dependent prophylaxis, behavioral responses were explicitly cited but were generally left for later consideration (Wilson and Reeson 1998). A notable exception to this was an empirical study by Reeson et al. (2000) in which host behavior was indeed shown to be an important component of the host’s density-dependent susceptibility. As the studies cited above have generated a body of empirical data on group-living (but nonsocial) insects, a number of papers have, particularly recently, explored the defenses employed by social insects to combat disease, concluding that individuals have increased resistance when in groups (Rosengaas et al. 1998, Tranilie et al. 2002) and employ diverse behavioral resistance mechanisms such as hygienic behaviors, increased brood care, waste management, behavioral fever, exclusion of infected individuals, and avoidance behaviors performed by infected individuals (Starks et al. 2000, Cremer et al. 2007, Uglevig and Cremer 2007, Cremer and Sixt 2009, Jackson and Hart 2009, Wilson-Rich et al. 2009). In the face of so many possible host responses contributing to overall host defense, we are presented with a problem in identifying the most biologically significant responses of a given system, and in untangling the individual and summative contributions of responses that at first sight appear to have less bearing on defense. In an effort to do this, we here consider how group living (which we use to redefine our x-axis) can lead to responses in terms of immune defense sensu lato (the y-axis). In particular, we compare predictions for nonsocial and eusocial insects.

**The Group-Living y-Axis: Population Density or Connectivity?**

Which independent variable best represents group living when we are concerned with host investment in defense? To take density as the independent variable (as has commonly been done) is to assume this to be the most important determinant of the likely costs of disease. It may not, however, even be the key determinant of the risk of infection, let alone the ensuing costs. For example, it is probably more realistic to take disease transmission as a mixture of frequency and density dependency rather than density dependency alone (Antonovics et al. 1995, Begon et al. 1999). For organisms that live at more or less fixed densities, an example being herbivorous spider mites (Oduor et al. 1997), we would expect frequency dependence to be predominant. Similarly, we may well expect highly social species such as eusocial insects to maintain a characteristic density, with a constant, possibly optimal (DeSouza et al. 2001, Altizer et al. 2003, DeSouza and Miramontes 2004), contact rate, while the colony expands to occupy more space. Frequency-dependent transmission has been considered to be essential in considering vertebrate disease dynamics and immune responses (Altizer et al. 2003). Despite all of this, studies of density-dependent prophylaxis in insects have often taken insect host density as the key independent variable to consider in laboratory assays. A notable example is a study by Pie et al. (2005) in which termite density is varied experimentally and over a short time period, and no effect on resistance to a fungal pathogen is found. This result should not come as a surprise: firstly, termites are unlikely to experience fluctuations in density, as explained above, so are unlikely to have evolved responses to this; secondly, if there were an increase in density, we would expect a colony-level response such as the production of more resistant workers, not individual responses over 20 days in nymphs that are already more than a year old; thirdly, we expect behavioral defense to be of key importance, as discussed below (see The y-axis: ...). Further to the above arguments, van Baalen and Beekman (2006) point out that, given a patchy distribution of potential hosts, contact rates (a measure of connectivity) within and between patches can differ, affecting epidemiological progress and therefore the likely evolutionary outcomes, an argument similar to that proposed by Wilson et al. (2003).

Since density-dependent transmission may not be expected in many systems, it may seem surprising that density-dependent prophylaxis (DDP) has found support in a number of systems. In baculovirus–caterpillar systems, for example, pathogen transmission may increase with host density in a mass action fashion (Dwyer 1991), but we also expect a saturating effect seen as a decline in per capita transmission at higher densities (D’Amico et al. 1996, Reeson et al. 2000, Wilson and Cotter 2008). Despite this decline in per capita transmission, disease risk still increases with density so we do see an adaptive increase in resistance of insects at higher densities (i.e., DDP) (Wilson et al. 2003).

In the face of these complexities, we suggest that a more suitable independent variable to consider (vs. host density) is some measure of connectivity between individuals and/or between populations, as this will determine in large part the chances for transmission to occur. In principle, this is much harder to quantify than is density. Connectivity can, however, be quantified when one considers animal behavior, in particular the behavior of animals displaying some degree of sociality, however limited this sociality may be. If we take behavioral connectivity to be a key parameter, therefore, we may be considering a better correlate of the risks of exposure to disease (where connectivity affects transmission) even though we remain a step away from the costs of disease. This cost, again, can influence the expected evolutionary outcomes (van Baalen and Beek-
There is, in fact, recent support for the paramount importance of connectivity: in Otterstatter and Thomson’s (2007) study of disease transmission in bumble bees, contact rates emerged as the only significant measured predictor of infection risk.

Contact rates alone will not cover many systems as transmission often does not occur through direct contact between live individuals. For example, most insect-pathogenic fungi kill their host and then sporulate from the cadaver; meanwhile, insect viruses may kill the host and liquefy it, infective particles then being ingested by new hosts, or may be transmitted via feces. Nevertheless, inter-individual behavior will affect transmission. If we consider a caterpillar and a virus, then it is virions deposited on a substrate such as leaves that will affect transmission. Caterpillars may avoid conspecifics or may aggregate with them. These behaviors are components of connectivity and may have greater relevance than density. Once adult, moths can affect transmission through dispersal and mating behavior; again, these behaviors make up connectivity between individuals or populations and cannot be described by density alone. The specific measure of connectivity that is most applicable will vary according to the system and in particular the modes of transmission of the important parasites and pathogens. Thus, it may be sufficient to consider contact rates for direct transmission, a product of host feeding rates and local density for oral transmission, number of mating partners for sexually transmitted diseases, and so on.

As our focus is on the behavior of potential hosts, we now explore a few, hopefully illustrative examples where behavior may mitigate against density-dependent transmission (before our explicit consideration of relative roles of behavioral and physiological defenses). In these situations, we propose that a measure of connectivity is of far greater predictive power than density.

**Example Systems in which Density Dependence May Best Be Replaced by a Measure of Connectivity**

The interaction of ants from different colonies can be characterized by very low physical contact rates (with notable exceptions such as slave-making ants). Ant territories often have buffer zones, and the use of pheromone trails within territories greatly reduces the opportunity for inter-colony interaction. The adaptive explanations offered for territoriality are often centered around the consequences of competition (e.g., Adler and Gordon 2003). At the same time, juvenile stages of herbivorous microarthropods such as spider mites or hemipterans (aphids, whitefly etc.) are frequently sessile. This fact can easily be explained by their feeding ecology.

In both instances, groups of individuals represent a genetically homogeneous, high-density patch that can serve as an ideal resource for an invading pathogen (but see van Baalen and Beekman 2006, Wilson-Rich et al. 2009). The danger to the potential hosts in each instance is that neighboring patches of conspecifics may serve as sources of inoculum, so we should expect selection against connectivity between patches. We should expect, if disease risk acts as a selective force, that individuals should minimize their contact rates with pathogens by avoiding interactions with neighboring colonies, as is the case with territorial ants such as attine leafcutter ants (Jutsum 1979), or by adopting a sessile lifestyle where possible, as appears to be the case with herbivorous microarthropods (Elliot et al. 2000b). Thus, selection to minimize disease risk is as valid an explanatory hypothesis for these behavioral adaptations as any of the more apparent and conventional explanations.

Indeed, the selective pressure to avoid infecting kin may well be a driving force in structuring social-insect colonies. Social-insect workers may take on increasingly more dangerous tasks outside the colony as they age, and avoid returning to the colony so as to avoid infecting kin (Schmid-Hempel 1998). In these social insects, we therefore expect behavioral interactions to be of far greater importance in determining disease risk than density per se.

Meanwhile, if we consider the migratory behavior of locusts (marching as nymphs or swarming as adults), we can find distal explanations for this behavior based upon massive local resource depletion (Despland and Simpson 2000, Despland et al. 2000) or reduction of predators’ capacity to track groups (Reynolds et al. 2009), and proximal explanations based upon avoidance of cannibalistic conspecifics (Bazazi et al. 2008). In this situation, it would seem at first sight that the risks of disease would be tremendous. However, any infective propagules produced from infective individuals will be left behind as the band or swarm moves on. Meanwhile, infected locusts will allocate time to increased thermoregulation (behavioral fever) so as to combat infection (Elliot et al. 2000a) so will also be left behind. In this example, it is entirely possible that the complexities of group living lead to a lower risk of exposure to disease rather than the reverse. In fact, in this system, we have little idea how pathogens such as the fungus *Metarhizium anisopliae* var. *acridum* might actually be transmitted in the field. We do know, however, that not keeping up can be lethal (Simpson et al. 2006, Bazazi et al. 2008). Thus, an alternative explanation for Wilson and colleagues’ (2002) observation of elevated immune responses in gregaria-phase locusts, may be that this allows infected animals to invest less time in behavioral fever and so being left behind or eaten. The difference is subtle but important: in this alternative explanation for the published results, disease risk per se is not greater for the high-density group livers; rather, the costs of disease are greater to them.

These are just a few examples of the many behavioral traits that can affect the costs incurred by disease. Our suggestion is that, in many situations, these behaviors are likely to be far more important than host density per se. Accordingly, the best way to approach these systems...
is to look at connectivity, that is, behavioral interactions between individuals that might result in transmission or otherwise affect the costs of disease. This is unlikely to be simple, but Otterstatter and Thomson (2007) have shown that it is feasible. Our prediction, then, goes hand-in-hand with DDP (density-dependent prophylaxis): greater connectivity between infective and susceptible hosts will lead to greater costs of disease and so investment in immune defenses. For the sake of argument, we can call this “connectivity-dependent prophylaxis” (CDP). In the gregarious locust example above, it might be interesting to consider a fungal pathogen (e.g., *Metarhizium anisopliae* var. *acridum*), which must kill the host in order to be transmissible; in such a system, connectivity between infectives and susceptibles will be far lower than straight density might indicate as the locusts will move past the dying locust or else consume it.

As with DDP, we can expect feedback in this system. In the case of DDP, individuals that live at higher densities may be expected to invest more in immune defenses but may also be expected to respond by reducing their tendency to aggregate. In the case of CDP, we may expect individuals to reduce connectivity so as to avoid infection, as has been proposed as a response to predation (Reynolds et al. 2009). It may then be that some of the variation we see in behaviors (ant territoriality, microherbivore sessility, locust marching) have arisen in part to reduce connectivity and therefore vulnerability to disease. This would be a pleiotropic benefit of the behavior and we could consider the minimization of the costs of disease to have been a selective force of a comparable magnitude, potentially even the dominant force, leading to these behavioral traits. Unfortunately, unpicking the evolutionary history of highly stable behavioral traits is unlikely to be a very fruitful exercise. An interesting product of this argument, though, is that it emphasizes the value in considering behavior as a component, just as any more apparent physiological mechanism, of an organism’s suite of disease defenses. A recent example of this sort of argument is one by Pontoppidan et al. (2009), where ants restricting their foraging to the canopy is described as a behavioral defense against contamination by fungal pathogens in the understory.

**The y-axis: How to Defend?**

We have argued that the total investment in immune defense may incorporate conventional physiological parameters such as the production of hemocytes or a plastic phenoloxidase response to invasion by a pathogen, but may equally incorporate behavioral prophylaxes or (low cost) behavioral responses (Fig. 1). Whether behavioral or physiological, resistance may be constitutive or induced, perhaps reflecting the costs of mounting the defenses (but see Hamilton et al. 2008), but we do not consider this explicitly in this paper. We may, though, expect physiological responses to be costly (Barnes and Siva-Jothy 2000, Siva-Jothy et al. 2005, Wilson and Cotter 2008), perhaps more so than behavioral responses (Elliot et al. 2005, Schulenburg and Ewbank 2007). Indeed, our prediction is that the relative costs of behavioral vs. physiological defenses will decrease with connectivity in the case of group livers in the first instance (Fig. 1B) and more so with sociality (Fig. 1C). For group-living, but nonsocial, hosts, we expect certain behaviors (e.g., avoidance of conspecifics) to be cheaper than physiological defenses, so we may see behavioral
defenses assuming a proportionately greater role as connectivity increases. It is even feasible that behaviors such as cannibalism, while driven by protein deficit (Simpson et al. 2006), have a pleiotropic benefit of improving immune function and so reducing susceptibility to pathogens (Lee et al. 2006, Povey et al. 2009). In the case of social insects, our case for the paramountcy of behavioral defenses is far greater and we have four reasons to propose this. One reason is that there may be greater pleiotropic benefits of behavioral than physiological immune defense. Grooming can increase resistance to pathogens (Yanagawa and Shimizu 2007), but may have rewards beyond reductions in disease risk, such as contributing to social cohesion, thereby rendering this form of investment in defense relatively cheap. Another is that kin selection is likely to be a strong force in social grouping, so making it more likely that cooperative behaviors to reduce disease will evolve (e.g., an individual employing behavioral fever in a honey bee colony will benefit its sisters as well as itself; Starks et al. 2000). The third is that, although physiological responses can be inductive, we expect behavioral responses to be highly labile (Hart et al. 2002, Hughes and Cremer 2007), so the costs of an induced (or otherwise tuned) response to disease risk can be held off until this response is required, and even avoided entirely once the risk is perceived to have passed. Finally, larger social groupings will have the potential for more sophisticated (thus potentially more effective and lower cost) responses to disease through cooperative behaviors (e.g., Hughes et al. 2002, Traniello et al. 2002, Cremer et al. 2007, Cremer and Sist 2009, Jackson and Hart 2009).

Thus, we expect that, while insects that live in groups may well have higher global densities than their more solitary counterparts, their living in social groups will come hand-in-hand with greater behavioral complexity. With social behavior, we expect to see less investment in the physiological responses normally sought in experimental studies and more investment in the behavioral component of immune defense (see Fig. 1C).

**Conclusion**

To conclude, we have offered an adjustment to the framework in which we consider how host density (broadly speaking) may affect investment in defense against disease. We think our connectivity-based framework is broader than density-dependent prophylaxis and may well explain some of the unusual empirical results. In particular, we emphasize that overlooking behavioral defenses may imply missing half of the story (or even more than half). We also propose that for social insects in particular, the suite of behavioral defenses may be of paramount importance. We propose that pleiotropy may have acted upon some aspects of group living so that selection for behaviors (or lack of behaviors) could have occurred to reduce disease risk in addition to other, more apparent benefits. This is not to exclude pleiotropic benefits of physiological defenses, of course—grasshoppers and locusts living at higher densities may have a more melanized cuticle, which represents elevated physiological defenses against pathogens and this melanization may also have pleiotropic benefits as aposomatic coloration (Sword et al. 2000).

We have emphasized that studies intended to elucidate such mechanisms must integrate physiological and behavioral mechanisms where possible. This promises to be challenging as it is far from likely that such combined defenses will have additive effects. That said, if it is possible to relate these combined defenses to consequences in terms of disease progress and virulence, then it should be quite possible. Wilson et al.’s (2002) study of locust immune defense considers physiological defense (hemocyte levels and antibacterial activity) and, although it does not quite incorporate behavioral fever as an experimental variable, the study does allow the hosts to display this defense behavior. So, studies that combine defenses as independent variables are quite possible.

Finally, we have considered van Baalen’s (1998) idea that, at high densities, defense may be a lost cause, as a further potential explanatory force for lower investment in defense at high densities. This argument can also be used to predict low connectivities (territoriality, low movement rates, avoidance behaviors) in animals living at high densities (Fig. 1C).

There are several key areas around which we have deliberately skirted in this paper. One is the distinction between constitutive and induced defenses. This will strongly affect both physiological and behavioral defenses but it is too important an area to be given a brief treatment here. It is, though, worth pointing out that we may initially expect behavioral defenses to tend to be induced rather than constitutive, due to their inherent lability. However, if physiological defenses are, as we have suggested, more costly, then it is these defenses that we expect to be more inducible. Behavioral defenses may then tend to be more constitutive, as seen with grooming and division of labor in social insects. Another consideration, to which we have only alluded, is host or host-offspring life-history change as a type of defense. This can apply equally to solitary animals (Minchella 1985), to group-living nonsocial animals (Elliot et al. 2003, 2005), and to social insects (Schmid-Hempel 1998). While other areas are likely to be important, such as the effects of a variable external environment on defenses (Thomas and Blanford 2003, Lee et al. 2008, Lazzaro and Little 2009), it seems that the interplay between diverse defense mechanisms over both evolutionary time scales and the lifetime of an individual or colony may initially provide the most interesting insights (Siva-Jothy et al. 2005, Fefferman et al. 2007, Cotter et al. 2008).

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