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INFECTIVITY, MULTIPLE INFECTIONS, AND THE GENETIC CORRELATION BETWEEN WITHIN-HOST GROWTH AND PARASITE VIRULENCE: A REPLY TO HOCHBERG

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Abstract.—Two experimental studies, Ebert (1994) and Ebert and Mangin (1997), described a genetic correlation between parasite virulence and the number of transmission stages found in the hosts. It was concluded that this correlation is evidence that within-host growth rate of the parasite is positively correlated with virulence. Hochberg (1998) has criticized this interpretation, arguing that differential infectivity and density-dependent parasite growth could confound the results. Here I point out that density dependence is unlikely to have confounded the results, but, at least for Ebert (1994), differential infectivity is likely to have been a confounding factor. However, Ebert and Mangin (1997) measured infectivity and showed that strains with higher infectivity had lower virulence, which is contrary to Hochberg's hypothesis. In summary, I conclude that differential infectivity played a role in the assessment of the correlation between parasite reproduction and virulence, but that differential within-host growth remains the most likely explanation for the correlation observed in Ebert and Mangin (1997).

Key words.—*Daphnia magna*, dose effects, genetic correlation, *Glugoides intestinalis*, parasite, trade-off, within-host growth.

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The study of the evolution of virulence centers on understanding the proximate and ultimate causes of the damage done by a parasite to its host. A major breakthrough in this field was the hypothesis that, for many diseases, virulence is a necessary by-product of the interaction between the parasite and the host (Ewald 1980; Anderson and May 1982; May and Anderson 1982; Ewald 1983). According to this hypothesis the evolution of virulence is constrained by genetic correlations between virulence and other parasite fitness components, such as within-host growth, reproduction, or survival. For example, within-host survival of the myxoma virus is negatively correlated with virulence (Fenner and Ratcliffe 1965; Anderson and May 1982; May and Anderson 1982), and bacteria-phage reproduction is positively correlated with virulence (Bull et al. 1991; Bull and Molineux 1992). In recent studies, Ebert (1994) and Ebert and Mangin (1997) presented data from a microsporidian gut parasite (*Glugoides intestinalis*, formerly *Pleistophora intestinalis* [Larsson et al. 1996]) in the planktonic crustacean *Daphnia magna*, which are consistent with the presence of a positive genetic correlation between virulence and within-host growth. These studies were the subject of a correspondence by Hochberg (1998), in which he pointed out a potential problem in the interpretation of the data. He argues that the experiments were not suitable to demonstrate a trade-off between virulence and within-host growth of the parasite, because they did not control for multiple infections. Here, I reply to his comment in two parts. First, I use evidence from the *D. magna*-microparasite system to show that the relationships proposed by Hochberg (1998) between the number of successful infections, within-host competition, and virulence do indeed exist and that these are an important conceptual addition to the current view of the role of trade-offs in virulence research. Second, I review the two studies in question and point out that in Ebert (1994) differential infectivity might have confounded the results, but this is not the case in the study by Ebert and Mangin (1997). Unless specified, I refer to virulence in this reply as parasite-mediated morbidity and mortality.

Hochberg (1998) points out that for many parasites there are dose effects, that is, the greater the number of infective particles that enter the host, the more intense the infection. High doses usually go hand in hand with more severe disease, leading to reduced host fecundity and survival. Hochberg argues that variation in parasite infectivity (a measure for the likelihood that a single parasite transmission stage will enter the host) produces variation in the infective dose and therefore variation in virulence (but see Schmid-Hempel and Schmid-Hempel [1993] for exception from this pattern). Since higher within-host growth rates may also lead to higher parasite sporeloads (equals number of parasite transmission stages resulting from an infection) and possibly higher virulence, the effects of differential infectivity and differential within-host growth rate cannot easily be disentangled. In *D. magna*, several microparasites are known to cause more severe disease when the dose is higher, for example, the microsporidium *G. intestinalis* (Ebert 1995), the bacterium *Pasteuria ramosa* (Ebert et al. 1998), and the fungus *Metschnikowiella biscuspidata* (Ebert, unpubl. data). For *P. ramosa* it was also shown that higher host susceptibility (i.e., when few spores are needed to produce an infection) leads to stronger fecundity reduction (Ebert et al. 1998). Thus, the data are in full agreement with Hochberg: dose effects and differential susceptibility can confound the assessment of virulence and other correlated traits.

The second part of Hochberg's argument is that density dependence limits within-host growth and thus parasite reproduction. As the parasite biomass inside the host increases,

density-dependent processes become stronger; and once the parasite biomass reaches a certain density (approaching a carrying capacity of the host resources), parasite growth might decline and so might parasite fecundity and survival (the details of this depend on the form of density dependence). Higher dose, higher infectivity, and higher within-host growth rate are all factors that lead to a more rapid approach to the carrying capacity, and thus intensify density-dependent effects. In the absence of density dependence, all three factors could lead to an increase in sporeload. However, if sporeload is measured after density-dependent processes have acted, sporeload might not be a reliable estimator of these factors and the correlation between sporeload and virulence might even become negative. There is good experimental evidence for density-dependent within-host regulation for helminth parasites (Anderson and May 1991; Poulin 1998). Experiments with two microparasites of *D. magna* have also given evidence for density-dependent within-host growth. For the yeast *M. biscuspidata*, density dependence was so strong that high spore doses killed the host before any parasite transmission stages were produced (Ebert, unpubl. data). A similar relationship was found for the bacterial parasite *P. ramosa*, although some transmission stages were produced even at the highest doses (Ebert et al. 1998, unpubl. data). In summary, I agree with Hochberg that the assessment of parasite reproduction reflects not only within-host growth, but is also influenced by parasite infectivity (or dose) and the strength of within-host density dependence. I also agree that by bringing the effects of dose-dependent virulence and density-dependent sporeload together, it is difficult to draw conclusions on a trade-off between within-host growth and virulence when only sporeload and virulence are measured.

Hochberg (1998) specifically criticized Ebert (1994) and Ebert and Mangin (1997). In both studies it was found that sporeload was positively correlated with virulence. For Ebert (1994), only the first part of Hochberg's argument applies, as density dependent parasite growth is unlikely to be important 15-days postinfection, when sporeload was measured. I have two arguments for this. First, infecting *D. magna* with three different doses of *G. intestinalis* resulted in an increase in sporeload with increasing dose (Ebert 1995), but within-host growth rate (in this case assessed by counting sporeload at day 6 and 11) was not lower in those cases where sporeload was higher, as would be predicted if density dependence were acting. Second, sporeloads in hosts more than 30-days post-infection were several times higher (see fig. 1 in Ebert and Mangin 1997) than the highest counts made at day 15 in Ebert (1994), indicating that sporeloads were far below the carrying capacity when assessed at day 15 postinfection. (Note that Ebert [1994] counted sporophorous vesicles, whereas Ebert and Mangin [1997] counted spores. One sporophorous vesicle contains between eight and 32 spores [Larsson et al. 1996].) Therefore, I believe that the highest sporeload at day 15 did not result from intermediate levels of successful infections, as speculated by Hochberg (compare his fig. 1c). I agree, however, that it is probable that the number of successful infections were higher in those clones that also suffered the highest virulence. Therefore, the correct conclusion from Ebert (1994) should be that the number of parasite spores in the host might be a function of host susceptibility, and thus the number of successful infections, and within-host growth rate. I want to emphasize, however, that this does not make any difference to the central point of Ebert (1994). The data clearly indicated that the parasite causes less harm in association with novel hosts than when infecting hosts from the same population, and that this effect has a genetic basis. Whether differential within-host growth rate or infectivity is the key trait producing high variance in virulence is a different question. The data indicated that differential infectivity explained at least some proportion of the overall variance in virulence. However, in a following study Ebert and Mangin (1997) provided evidence that higher infectivity was not the cause of higher virulence, and that differential within-host growth was the likely reason for differences in virulence.

In Ebert and Mangin (1997), 12 lines of *G. intestinalis* were tested on the same host clone. These lines were isolated from experimental *D. magna* cultures which had been maintained for 14 months under different demographic conditions. For these 12 lines we measured not only virulence (host mortality) and sporeload (spores per host), but also transmissibility (infectivity, standardized for dose). These three traits do not relate to each other in the way proposed by Hochberg (1998). As for Ebert (1994), sporeload was highest in lines with the highest virulence (Pearson correlation $r = 0.84$, $n = 12$, $P = 0.0005$), but this correlation is not explained by differential infectivity, as the more transmissible parasite lines were less virulent and produced lower sporeloads (figs. 1 and 2 in Ebert and Mangin 1997). The effect of density dependence, which I believe was nevertheless present, was apparently not strong enough to influence these relationships in the way suggested by Hochberg (1998). I conclude therefore that the genetic correlation between sporeload and virulence found by Ebert and Mangin (1997) was largely a reflection of differential within-host growth and was not strongly influenced by differential infectivity.

Consequences of a Correlation between Infectivity and Virulence

The central point of the trade-off argument for the evolution of virulence is that between-host transmission is constraint by a genetic correlation between virulence and other parasite fitness components. Hochberg points to yet another form of this argument, which had not yet been discussed within the framework of the evolution of virulence: a positive correlation between infectivity and virulence. Experimental evidence supports the presence of such a trade-off in the *P. ramosa-D. magna* system (Ebert et al. 1998). Host clones with a lower ID25 (infective dose required to infect 25% of test animals) suffered stronger fecundity reduction per infected female. Hochberg speculates that, under these conditions, virulence could put an upper constraint on the evolution of parasite transmission. This might be the case under certain, but not all, conditions. A crucial factor will be the shape of the functional constraint between virulence and infectivity, as well as the frequency of multiple infections experienced by a host under natural conditions. The frequency of multiple infections has been described to vary strongly with ecological conditions (Herre 1995; Frank 1996; Ebert

and Mangin 1997). A detailed epidemiological model might help to shed light on this complex issue.

Defining Virulence

Hochberg (1998) proposed a strict definition of virulence. I have avoided defining virulence in such a strict way, because it creates the type of confusion Hochberg wishes to avoid (for discussion see Levin [1996], Ebert [1999], and Read et al. [1999]). For most purposes, it is sufficient and meaningful to describe virulence as parasite-mediated morbidity and mortality. This description encapsulates the entire range of disease symptoms that reduce host fitness, regardless of whether virulence is an evolved character or an accidental coincidence. For specific cases, precise definitions of virulence are clearly required and these should precede any discussion on specific aspects of its evolution. I am not aware of cases where this approach has proven problematic and I do not see a need for a consensus on one strict definition as proposed by Hochberg (1998). In evolutionary terms, virulence has many different facets and meanings, and its restriction to a specific subset of cases would not only produce confusion and hinder its discussion, but also would require new terms for those cases not included in the definition. Hochberg attempts to broaden and generalize the definition by including ecological aspects of the host-parasite interaction. However, other aspects are still missing, such as the effect of parasites on host fecundity, which is an essential feature of the evolution of vertically transmitted parasites or the expression of virulence in accidental hosts. Therefore, following Levin (1996), I suggest the term virulence to be used as a general description for parasite-mediated host morbidity and mortality and to use context-specific definitions wherever necessary.

Conclusion

I agree with Hochberg (1998) that differential infectivity can be a confounding factor in assessing genetic correlations between parasite fitness components. In the earlier of the two studies discussed here, differential infectivity certainly played a role in shaping the correlation between sporeload and virulence (Ebert 1994). In the second study, infectivity was shown to vary in the opposite direction of what was proposed by Hochberg, indicating that its influence on the overall correlation between sporeload and virulence was negligible (Ebert and Mangin 1997). Differential within-host growth remains as the most likely explanation for the positive correlation between virulence and sporeload.

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