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IDEAS AND PERSPECTIVES

Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment

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Abstract

Numerous studies have revealed genetic variation in resistance and susceptibility in host–parasite interactions and therefore the potential for frequency-dependent selection (Red Queen dynamics). Few studies, if any, have considered the abiotic environment as a mediating factor in these interactions. Using the pea aphid, *Acyrthosiphon pisum*, and its fungal pathogen, *Erynia neoaphidis*, as a model host–parasite system, we demonstrate how temperature can mediate the expression of genotypic variation for susceptibility and virulence. Whilst previous studies have revealed among-clone variation in aphid resistance to this pathogen, we show that resistance rankings derived from assessments at one temperature, are not conserved across differing temperature regimes. We suggest that variation in environmental temperature, through its nonlinear impact on parasite virulence and host defence, may contribute to the general lack of evidence for frequency-dependent selection in field systems.

Keywords

Coevolution, entomopathogen, evolution of resistance, genetic variation, pea aphid, temperature, virulence.

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INTRODUCTION

According to the Red Queen hypothesis of host-parasite coevolution, a host or a parasite must keep 'running' in an evolutionary landscape in order to stay in the contest. A general assumption in many studies that have considered this phenomenon is that the landscape is a flat surface, and that what determines who has to keep up with whom is how fast host and parasite run. Laboratory studies in particular tend to deliberately flatten the surface by limiting environmental variability, with conditions then manipulated to encourage host or parasite to run to alter the relative distance between them (see References below).

The starting point for many studies of host-parasite coevolution has been to examine genetic variation for defence and virulence, as the basis for frequency-dependent selection. To this end, genetic variation for defence and virulence in ectotherm animal–parasite associations has been demonstrated for *Drosophila* and certain parasitoids (Kraaijeveld *et al.* 1998), *Daphnia* and bacteria (Ebert *et al.* 1998), a freshwater snail and trematode worm (Lively 1989), and in field-collected pea aphids and their parasitoids and pathogens (e.g. Ferrari *et al.* 2001). Detailed laboratory studies on *Drosophila*-parasitoid systems have further demonstrated that resistant host genotypes can be selected for under high rates of parasitism, and that this increase in resistance is costly to the host (Kraaijeveld & Godfray 1997). In addition, common snail genotypes have been shown to be disproportionately infected by their trematode parasite (Dybdahl & Lively 1998; Lively & Dybdahl 2000). All these findings are consistent with the Red Queen hypothesis.

In the field, however, where environmental variability is not controlled and the speed and relative distance of the racers is not artificially determined, there has been some difficulty in demonstrating that laboratory-observed genetic variation can lead to frequency-dependent selection. In pea aphids, for example, among-clone variation for resistance to two parasitoids and a fungal entomopathogen has been demonstrated (Henter & Via 1995; Henter 1995; Ferrari *et al.* 2001), yet there is no evidence that high parasite pressure leads to an increase in frequency of resistant clones over the season (Henter & Via 1995). Significantly, no measurable trade-offs between resistance and other fitness components have been identified in this system (Ferrari *et al.* 2001). Indeed, though widely invoked, evidence for trade-offs in animals is rare and then, generally only demonstrated under constant laboratory conditions such as in Kraaijeveld

& Godfray (1997). Where environmental variation is included, costs of resistance may vary (Bohannan & Lenski 2000).

A number of studies have now demonstrated that host susceptibility and disease virulence are condition dependent (e.g. Ferguson & Read 2002; Wilson *et al.* 2002; Yourth *et al.* 2002) and that one key condition appears to be temperature (e.g. Blanford & Thomas 1999). However, variation in environmental temperature (and therefore ectotherm body temperature) has not generally been considered in experimental tests for genetic variation in host–parasite interactions. In cases where the prevailing environmental conditions are very stable, or where both host and parasite share the same thermal optima and are adapted to perform similarly across a temperature range, then this may be justified. Interestingly, frequency-dependent selection has been demonstrated in endotherm–parasite interactions (Eady *et al.* 1996) and in other systems where the nature of the interacting organisms makes thermal effects unlikely (Soler *et al.* 2001) (though this is not to say that conditions for the Red Queen hypothesis are exclusively met in such systems; see Dybdahl & Lively 1998; Lively & Dybdahl 2000). On the other hand, where host and parasite have more discrete thermal performance profiles and where temperatures regularly fluctuate across the range of these reaction norms, the simplification and associated assumptions are much more questionable; even subtle nonlinearities in the respective responses of host and parasite to temperature could lead to large 'genotype × genotype–environment' interactions that could dramatically alter the pattern of frequency-dependent selection.

To illustrate this argument, we report here on a study in which we investigate the effect of temperature on variation in resistance of pea aphids to the fungal pathogen, *E. neoaphidis*. As indicated above, variation in resistance between clones has been demonstrated in a simple laboratory setting (Ferrari *et al.* 2001). Our aim was to examine how resistance varies under a wider range of more realistic fluctuating temperatures and in particular, whether the ranking of resistance between clones is independent of environment.

MATERIALS AND METHODS

We selected four clones (clones 9, 18, 21 and 25) spanning a range of susceptibility to *E. neoaphidis* as defined in Ferrari *et al.* (2001). Procedures for inoculating aphids and other basic experimental and statistical techniques followed those of Ferrari *et al.*

Prior to experimentation, all aphids were cultured on broad bean seedlings at 15 °C, 60% RH and a 16 : 8 L : D cycle, according to Ferrari *et al.* (2001). Twenty 1-day-old apterous adult aphids per treatment (replicated eight times) were taken from these cultures and placed on to 5-cm-tall bean plants. Each plant pot (6 cm diameter) was covered with a 10-cm-tall cage made from acetate and nylon gauze. The pots were placed in independently heated, thermostatically controlled chambers (34 × 24 × 20 cm), each holding four pots, one for each clone. Twenty-four chambers were used (three temperatures × eight replicates), and were randomly positioned within a 18 ± 1 °C CT-room, with a 16L : 8D light regime and ambient humidity. We investigated three fluctuating temperature regimes of 18–25 °C, 18–28 °C and 18–31 °C, with the upper temperatures maintained for just 4 h during the middle of each day, before returning to 18 °C. The upper temperature limits for the 4-hour exposure periods were chosen based on field-collected temperature data recorded during the summer of 2001 in the aphid habitat where the clones originated (Ferrari *et al.* 2001). After 10 days, the percentage of mummies (i.e. aphids that had died from the pathogen and were showing characteristic signs of mycosis) per total number of aphids left in each pot was calculated (as in Ferrari *et al.* 2001). The proportion of mummies formed was angular transformed before analysis using ANOVA, with clone as a random factor and temperature as a fixed effect, blocked by incubation chamber.

RESULTS

In line with Ferrari *et al.* (2001), we observed a significant difference between clones in susceptibility to the fungal pathogen ($F_{3,56} = 4.1$, $P < 0.01$, see Fig. 1b–d), with no significant block effects of the temperature chambers ($F_{21,56} = 0.51$, $P > 0.05$). More interestingly, there was a significant effect of temperature regime on aphid susceptibility ($F_{2,56} = 20.4$, $P < 0.001$) and a significant interaction between temperature fluctuation and aphid clone ($F_{6,56} = 2.3$, $P < 0.05$). Such an interaction would not be expected if between-clone variation in resistance was independent of temperature.

DISCUSSION

The results above reveal two important patterns. First, overall levels of aphid susceptibility vary with temperature, with exposure to the higher temperatures reducing susceptibility to very low levels (zero for all but one clone). Thus, all clones may be effectively resistant under certain conditions, indicating the possibility of 'environmentally acquired immunity', irrespective of any intrinsic resistance. Secondly, within the range of temperatures examined here (which are representative of the field environment), ranking of clonal resistance does not remain constant. Thus, a clone identified as immune in the study by Ferrari *et al.* (2001) (i.e. clone 21) is revealed to be moderately susceptible under the

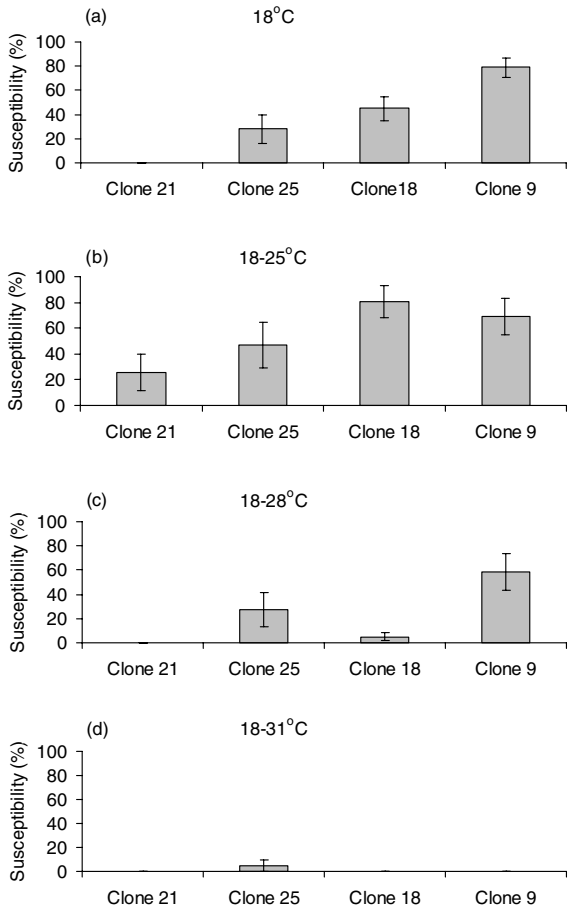

Figure 1 Susceptibility (mean percentage fungus mummies formed ± SE) of four pea aphid clones to the fungal pathogen *E. neoaphidis* at four different temperature regimes: (a) original susceptibility at constant 18 °C reproduced from Ferrari *et al.* (2001); (b) susceptibility under a fluctuating regime of 18 °C (20 h) and 25 °C (4 h); (c) as for (b) but with a fluctuation of 18/28 °C; (d) as for (b) but with a fluctuation of 18/31 °C.

18–25 °C regime. Similarly, clone 18 is recorded as moderately susceptible at 18 °C but becomes the most susceptible clone at 18–25 °C. The susceptibility of clone 9, on the other hand, tends to remain fairly constant, until, that is, the upper temperature reaches 31 °C, whereupon it becomes effectively immune.

Mechanisms for these two patterns are unclear. Antifungal compounds and melanization may be important but detailed analysis of the immune response of aphids is lacking. Resistance mechanisms such as cellular encapsulation have been shown in only a few aphid–parasite interactions (Carver & Sullivan 1988). It may be hypothesized, however, that some of the effect derives from high temperatures affecting *E. neoaphidis* protoplast survival, in a similar manner to that described in Carruthers *et al.* (1992). Whatever the mechanisms, it is clear that the ranking of clones based on their susceptibility at one temperature may have little relevance to other temperatures, and that even subtle differences in temperature (i.e. 18–25 vs. 18–28) may have marked effects on susceptibility. Whilst we have explored a range of realistic fluctuating regimes, similar effects also result under different constant temperatures (D. Stacey, unpublished PhD thesis, University of London, London). This has clear implications for interpreting simple measures of genetic variation in resistance and for any subsequent assessments such as correlated resistance to other parasites and trade-offs with resistance.

Support for the generality of these results is available in another important evolutionary study-system. Fellowes *et al.* (1999), examining the potential for cross-resistance in *Drosophila* against its parasitoids, also highlighted a role of temperature. Against the parasitoid *Asobara tabida*, a change in the thermal regime from 20 °C to 25 °C conferred higher resistance to both selected and unselected *Drosophila* lines by some 40%. Interestingly, this temperature-induced increase in resistance in unselected lines was comparable with the level of resistance exhibited by selected lines at 20 °C. Moreover, while the authors of the study emphasize a simple effect of temperature acting to increase resistance, examination of the results presented indicate a much more variable effect, with temperature increasing resistance in certain host-parasite combinations, whilst having little effect or even reducing resistance in others (Fellowes *et al.* 1999). Beyond host-parasite studies that specifically consider frequency-dependent selection, examples of relevant thermal effects have also been shown in other insect–fungal pathogen associations (Blanford & Thomas 1999), in amphibian–monogenean interactions (Jackson & Tinsley 2002), in fish–parasite interactions (Le Morvan *et al.* 1998) and in reptile–bacteria interactions (Bernheim *et al.* 1978), among others. Yet further support for an influence of environment comes from the study by Ackermann *et al.* (2001), which revealed that conclusions about responses to selection in life history experiments with *Drosophila*, depended on the environment in which the assay was performed.

Returning to pea aphids, as indicated previously, in spite of there being no correlations between resistance and fecundity, host plant quality or susceptibility to other parasites (Ferrari *et al.* 2001), evidence for increase in resistant clones in the field is lacking (Henter & Via 1995). We suggest that temperature, though not necessarily the only environmental determinant (cf. Ackermann *et al.* 2001; Ferguson & Read 2002), may be an important mediator of resistance and virulence and hence, frequency-dependent selection. Thus, under one set of conditions selection may act in one direction, whilst under a different set, it may act in another. Given the variability in temperature that can exist across even very small spatial or temporal scales, the net

effect on frequency-dependent selection at the population level may be zero, or at the very least, different from that predicted from one set of conditions in the laboratory. As such, temperature may quite literally create coevolutionary hot and cold spots in a geographical mosaic (Gomulkiewicz *et al*. 2000).

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