

Genotype and temperature influence pea aphid resistance to a fungal entomopathogen

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Abstract. The influence of temperature on life history traits of four *Acyrtosiphon pisum* clones was investigated, together with their resistance to one genotype of the fungal entomopathogen *Erynia neoaphidis*. There was no difference among aphid clones in development rate, but they did differ in fecundity. Both development rate and fecundity were influenced by temperature, but all clones showed similar responses to the changes in temperature (i.e. the interaction term was nonsignificant). However, there were significant differences among clones in susceptibility to the pathogen, and this was influenced by temperature. Furthermore, the clones differed in how temperature influenced susceptibility, with susceptibility rankings changing with temperature. Two clones showed changes in susceptibility which mirrored changes in the *in vitro* vegetative growth rate of *E. neoaphidis* at different temperatures, whereas two other clones differed considerably from this expected response. Such interactions between genotype and temperature may help maintain heritable variation in aphid susceptibility to fungal pathogen attack and have implications for our understanding of disease dynamics in natural populations. This study also highlights the difficulties of drawing conclusions about the efficacy of a biological control agent when only a restricted range of pest genotypes or environmental conditions are considered.

Key words. *Acyrtosiphon pisum*, biological control, *Erynia neoaphidis*, genotype by environment interaction, resistance, temperature.

Introduction

All organisms face attack by natural enemies. Given the enormous fitness costs of susceptibility to attack, it is not surprising that an incredibly diverse range of defences has evolved. However, when considering populations within species, there is generally heritable variation in defence traits present, whereas the *a priori* expectation may be that natural selection would lead to the fixation of beneficial

traits. There are a number of reasons why heritable variation may be maintained in such circumstances, including negative genetic correlations and genotype-by-environment interactions (Stearns, 1992).

Work on pea aphids [*Acyrtosiphon pisum* (Harris); Hemiptera: Aphididae] has shown high levels of heritable variation in resistance to parasitoid wasps, coccinellid predators and fungal pathogens (Milner, 1982; Henter & Via, 1995; Losey & Denno, 1998; Hufbauer & Via, 1999; Ferrari *et al.*, 2001; Hufbauer, 2002; Stacey & Fellowes, 2002). Much effort has focused on life-history trade-offs as a mechanism constraining the evolution of resistance in *Drosophila*-parasitoid (Fellowes & Godfray, 2000) and some other systems (Sayyed & Wright, 2001). However, similar trade-offs have not been found in pea aphid–parasitoid and

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pea aphid–entomopathogen systems (Ferrari *et al.*, 2001). This paper reports the results of a study investigating whether genotype-by-environment interactions may help explain the presence of heritable variation in a pea aphid–entomopathogen system. In common with the majority of insect herbivores, the two most important environmental factors influencing aphid life histories are plant quality and ambient temperature. Although previous work has shown that plant quality does not influence pea aphid resistance to entomopathogen attack (Fellowes *et al.*, unpublished data), there is some evidence that temperature can affect levels of susceptibility (Stacey & Fellowes, 2002). In addition, the growth rate of the entomopathogen itself may also be influenced by temperature (Hsiao *et al.*, 1992; Papierok *et al.*, 1993; Morgan *et al.*, 1995; Dara & Semtner, 1998; Feng *et al.*, 1999).

The results of a study investigating how temperature influences the resistance of four *A. pisum* genotypes against one genotype of the fungal entomopathogen *Erynia neoaphidis* Remaudière and Hennebert (Zygomycetes: Entomopathorales) are reported here. *Erynia neoaphidis* is a widespread entomopathogen that frequently attacks pea aphids and can cause high mortality (Milner, 1982, 1985; Pickering *et al.*, 1989; Vilcinskas & Götz, 1999). The potential for natural epizootics has encouraged the study of this fungal pathogen as a potential biological control agent against several aphid species (Wilding *et al.*, 1986, 1990; Schmitz *et al.*, 1993). Previous studies have suggested that temperature is one of the most important factors determining the likely occurrence of an epizootic (Hall & Bell, 1960, 1961; Morgan *et al.*, 1995). At the same time, other studies have indicated significant variability between pea aphid clones in their resistance/susceptibility to *E. neoaphidis* (Milner, 1982, 1985; Ferrari *et al.*, 2001). The current study is the first to investigate how genotype and temperature interact to determine the outcome of the aphid–pathogen interaction, and to examine whether an interaction between temperature and genotype may result in the maintenance of heritable variation in resistance.

Materials and methods

Aphid and pathogen clones

Four pea aphid clones were used in the study, coded 9, 18, 21 and 25 as previously described (Ferrari *et al.*, 2001). The clones were originally collected from *Vicia* spp. in Berkshire and were maintained as monoclonal cultures on broad bean *Vicia faba* L. (var. ‘the Sutton’) in a controlled temperature room at 20 ± 1 °C, under a 16:8 h light: dark regime and at ambient humidity. The fungal pathogen used was the isolate X4 strain of *Erynia neoaphidis*, obtained from the Rothamsted Research reference collection. This isolate has been maintained in culture by passage through apterous adult *A. pisum* (Roy *et al.*, 1999).

Aphid life-history traits

The development rate of the four aphid clones was studied at five constant temperatures (12, 15, 20, 25 and 27 °C). A fluctuating regime was not chosen as previous work revealed that clones reared at fluctuating temperatures showed the same level of susceptibility as clones reared at a constant intermediate temperature (Stacey & Fellowes, 2002). Temperatures above 30 °C were avoided as pea aphids show high mortality under such conditions. For each replicate, five newly moulted apterous adult pea aphids were placed onto a bean plant 24 h prior to the experiment, and allowed to produce nymphs. This was repeated four times for each clone/temperature treatment. After 24 h, the adult aphids were removed, leaving equally aged nymphs on the plants. The plants were covered using a clear plastic and nylon mesh cage and placed in one of five controlled temperature rooms, each set at the appropriate temperature. The number of days from larviposition to adult moult was recorded, until all aphids had reached adulthood. The first nine apterous pea aphids to reach adulthood from each clone and temperature treatment were transferred individually onto new plants, covered, and kept in the same controlled temperature rooms. After 4 days, the number of offspring produced was counted as a measure of fecundity.

Growth of E. neoaphidis in vitro

A temperature growth curve for vegetative growth of *E. neoaphidis in vitro* was determined by measuring the rate of increase in diameter of fungal colonies kept at constant temperatures (5, 10, 15, 18, 20, 23, 25, 28, 30 and 32 °C, with 16:8 h photoperiod). Plugs of fungus from an established culture were removed with a 6-mm-diameter cork borer and transferred individually to the centre of 9-cm diameter Petri dishes containing a medium of Sabouraud dextrose agar supplemented with milk and egg yolk (SEMA) (Wilding & Brobyn, 1980). Four replicate plates were set up for each temperature treatment. The Petri dishes were triple ventilated and were individually placed in sealed sterile plastic boxes with a vial of distilled water to provide moisture. Vegetative growth of the colony was measured every three days for 26 days. Two diametrically opposite lines were drawn across the lid of each Petri dish crossing over the inoculum plug. The colony diameter was measured along these lines, with the mean of the two lines giving an estimate of colony size. Colony diameter was plotted against incubation period to calculate the rate of growth (mm growth per day) at each temperature. Plotting growth rate against temperature provided the temperature dependent rate of vegetative growth of *E. neoaphidis*.

Susceptibility of aphids to E. neoaphidis

The susceptibility of the four pea aphid clones to *E. neoaphidis* was tested at four temperatures (12, 15, 20

and 25 °C). Twenty, 1-day-old apterous adult aphids per treatment (replicated eight times) were placed into glass vials and exposed to a shower of conidia from one sporulating aphid cadaver for 90 min at 20 °C (Ferrari *et al.*, 2001), after which time the cadavers were removed and placed over glass slides for 45 min to assess the variation in dose of conidia. These cadavers had been produced from *E. neoaphidis*-infected adult pea aphids prior to the experiment. The treated aphids were left in the vials for a further 2 h to allow the conidia to germinate at a standard temperature and humidity across treatments. *Erynia neoaphidis* conidia generally take 2–4 h to germinate at 20 °C (Vilcinskas & Götz, 1999).

After inoculation aphids were placed on to 5-cm tall bean plants (two plants per pot, grown in multipurpose compost). Each 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. Thirty-two chambers were used (four temperatures × eight replicates), and were randomly positioned within a 12 ± 1 °C controlled temperature room, 16:8 h light regime and at ambient humidity. Eight of the chambers were not turned on (the 12 °C treatment). The remaining 24 chambers were switched on to heat to one of the three other temperature treatments. Integral fans continually circulated air throughout each chamber. After 6 days, the percentage of infected cadavers per total number of aphids left in each pot at the end of the experiment was calculated. Only originally treated adults were considered when calculating infection rates; any infected offspring could be readily identified as they had yet to reach the adult stage.

Statistical analysis

Aphid life-history traits. The mean number of days to adulthood for the aphids on each plant was calculated (four replicates for each treatment) and converted to development rate (1/days). The data were analysed using ANCOVA, with temperature as the covariate and clone as a factor. Fecundity data were analysed using ANOVA with Poisson errors in GLIM, with temperature and clone as factors because the count data were non-normally distributed (Crawley, 1993). The change in deviance resulting from the deletion of a factor with Poisson errors results in an asymptotic chi-square distribution, explaining the use of a chi squared statistic below (Crawley, 1993). All other statistics were calculated using the Statistica Software Package (Statsoft Inc., 1998).

Growth of Erynia neoaphidis in vitro. A nonlinear regression model was fitted to the growth rate data to describe the temperature-growth rate profile of the fungus and identify the optimum temperature for vegetative growth. The model used was:

$$\text{Growth rate} = \left[e^{(t/a)} / (b + (e^{(t/c)^2}) \right]$$

where t = temperature, and a , b and c are constants identified in the regression procedure (Thomas & Jenkins, 1997).

Susceptibility of aphids to E. neoaphidis. The proportion of infected cadavers formed was angular transformed before analysis using ANCOVA, with clone and temperature as factors and spore dose [\log_{10} (+1) transformed] as a covariate.

Results

Aphid life-history traits

Clones did not differ between each other in their rate of development ($F_{3,15} = 1.75$, $P = \text{NS}$), although there was a significant effect of temperature ($F_{1,15} = 910.8$, $P < 0.0001$) (Fig. 1). Temperature also affected aphid fecundity (chi squared = 90.3, d.f. = 1, $P < 0.0001$), and there was a significant difference between the clones in fecundity (chi squared = 11.36, d.f. = 3, $P < 0.01$). The interaction term was nonsignificant (chi squared = 1.68, d.f. = 3, $P = \text{NS}$) (Fig. 2). Mortality rates in this experiment were very low, with no significant differences between the clones or treatments. Only individuals that reached adulthood were included in the analyses.

Growth of E. neoaphidis in vitro

The best-fit model for vegetative growth rate of *E. neoaphidis* across temperatures gave parameter values for the constants of $a = 3.6$, $b = 50$ and $c = 8.9$, and explained a significant amount of the variance ($F_{3,32} = 248.9$, $P < 0.0001$, $r^2 = 0.91$) (Fig. 3). From this profile, the estimated optimum temperature for vegetative growth on SEMA was 18.4 °C.

Susceptibility of aphids to E. neoaphidis

The clones differed significantly from each other in their response to the fungus ($F_{3,93} = 12.1$, $P < 0.0001$), and susceptibility was also affected significantly by temperature ($F_{3,93} = 3.77$, $P = 0.013$) (Fig. 4). The interaction between clone and temperature was significant ($F_{9,93} = 7.57$, $P < 0.0001$). The density of conidia in the shower had a significant effect on susceptibility ($F_{1,93} = 6.97$, $P = 0.01$), but explained only a small amount of variation ($r^2 = 0.07$).

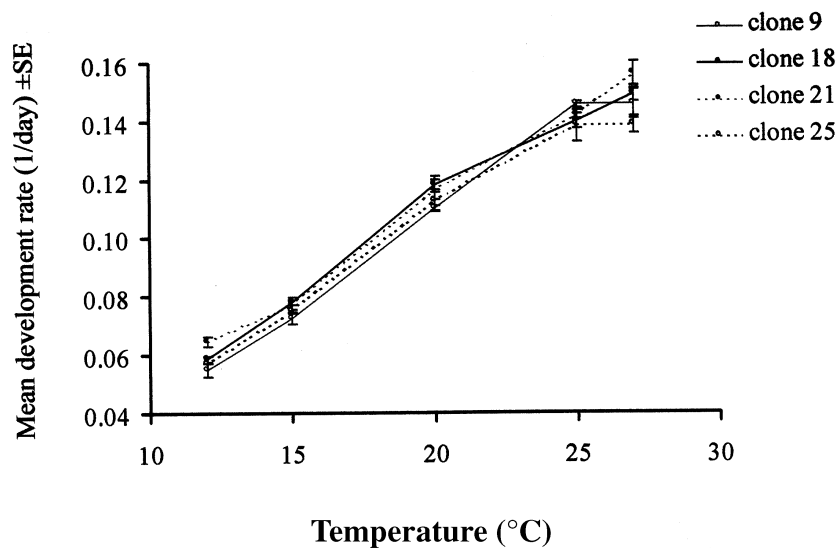


Fig. 1. Mean development rate \pm SE of four pea aphid clones reared at different temperatures.

Discussion

There is significant variation among pea aphid clones in resistance to the fungal entomopathogen *E. neoaphidis*. The pattern of resistance between clones observed at 20 °C closely matched that reported in a similar assay conducted at a constant 18 °C by Ferrari *et al.* (2001). Importantly, however, the likelihood of a given genotype succumbing to *E. neoaphidis* infection varied with temperature. In part, this is likely to result from variation in fungal growth across temperature. Whilst we have no direct measure of the effect of temperature on pathogen growth *in vivo*, and certain studies such as those by Thomas & Jenkins (1997) and

Väninen (1999) show that temperature may have subtly different effects on pathogen growth *in vivo* versus *in vitro*, the *in vitro* vegetative growth profile provides guidance as to the temperature optimum and thermal sensitivity of *E. neoaphidis*. This *in vitro* profile reveals a temperature optimum of approximately 18 °C with a characteristic fall-off in growth as temperature moves above or below this optimum. This temperature optimum and growth profile is broadly consistent with studies on a range of entomophthoralean fungi (Hall & Bell, 1961; Hall & Papierok, 1982; Carruthers & Haynes, 1986; Morgan *et al.*, 1995). Consequently, it may be expected that the pathogen would do better (i.e. cause greater and/or more rapid mortality) in aphid populations

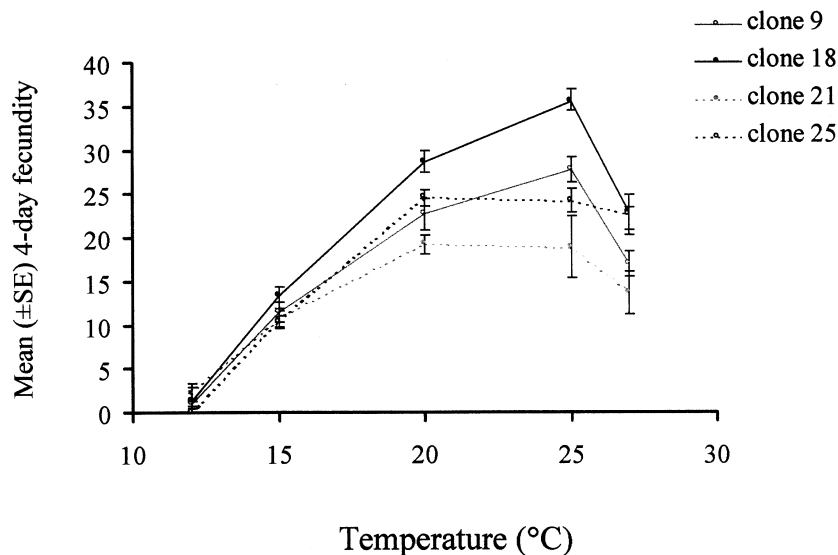


Fig. 2. Mean fecundity (number of offspring produced in four days) \pm SE of four pea aphid clones reared at different temperatures.

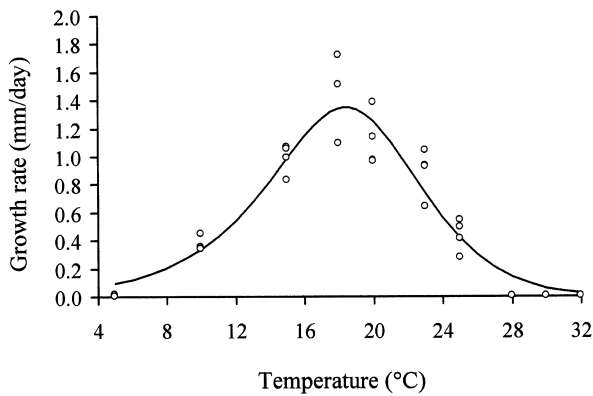


Fig. 3. Vegetative growth rate (mm/day) of the entomophthoralean fungus *Erynia neoaphidis* growing on SEM agar after 26 days incubation cultured at different temperatures.

maintained at 20 °C, than those maintained at 12 °C or 25 °C. This prediction appears to hold for clones 9 and 25, where susceptibility closely follows the pattern of *E. neoaphidis* growth rate, suggesting changes in mortality across temperature reflect differences in the development rate of the pathogen.

In contrast, clones 18 and 21 show very different responses. Clone 18 rapidly increases in susceptibility with temperature, before reaching a plateau, whereas clone 21 becomes increasingly susceptible as temperatures increase. In effect, there is a crossing of susceptibility reaction norms, indicating that, as temperature changes, the clone that is most likely to survive also changes. This suggests that in addition to the direct effects of temperature on pathogen growth, aphid physiological or immunological factors are also important in determining susceptibility. In summary, these data indicate both pathogen growth and host defence to be influenced by temperature, but not necessarily in identical ways. Surprisingly, although the mortality rates of the four pea aphid clones

following exposure to *E. neoaphidis* strongly differed with temperature (i.e. a strong interaction effect), the other traits measured either did not vary among clones, showed an interaction effect (for development rate) or varied among clones but showed no interaction effect (for early fecundity).

Previous work has suggested that pea aphids belong to biotypes that are either susceptible or resistant to *E. neoaphidis* (Milner, 1982, 1985). The data presented here show that resistance is not so categorical but varies along a continuum (Ferrari *et al.*, 2001) and that where particular clones lie on this continuum is influenced by temperature. Such patterns could, in part, reflect the fact that mortality was measured at the same time postinfection for all 'clone × temperature' combinations. Thus, the experiment may not be providing a measure of absolute susceptibility or resistance, as all clones may have reached the same level of mortality if the course of the infection had been allowed to run. However, if this is the case, then the snapshot measure of pathogen-induced mortality in the experiment provides a measure more akin to pathogen virulence (number dead over a given time period) than absolute host susceptibility, which is still interesting from a disease dynamic perspective. Moreover, the colonies were observed until plant quality deteriorated (8+ days post infection) and, although there was a small increase in pathogen-induced mortality with time, this did not alter the outcome of the experiment (data not shown). Given this, and the fact that colonies of most aphid species are quickly discovered and attacked by natural enemies under field conditions (Müller & Godfray, 1999), it could be argued that the differences in disease levels observed between clones, whether due to differences in absolute susceptibility or virulence, are ecologically relevant and have both evolutionary and population-dynamic implications.

From an evolutionary perspective, the crossing of reaction norms is of considerable interest as it can help maintain heritable variation in a trait (Stearns, 1992; Schlichting &

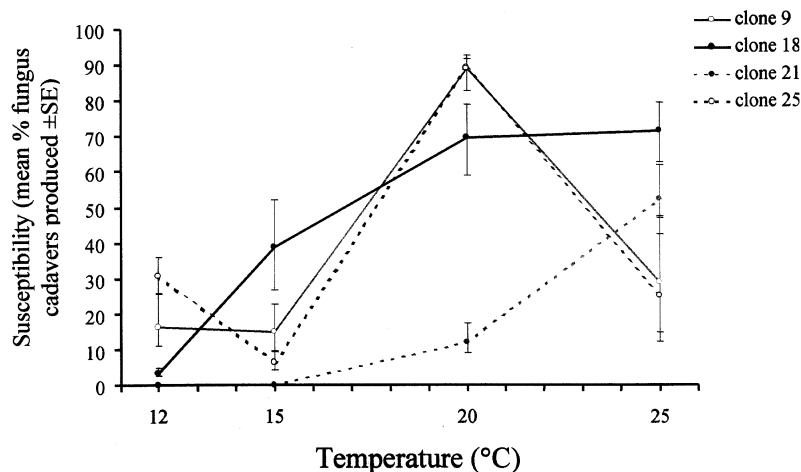


Fig. 4. Susceptibility (mean percentage fungus cadavers formed \pm SE) of four pea aphid clones to the entomophthoralean fungus *Erynia neoaphidis* when reared at four temperatures.

Pigliucci, 1998). However, it must be remembered that the susceptibility reaction norms in the current study differ from many other studies in that they reflect 'genotype \times genotype \times environment' interactions (i.e. host resistance \times pathogen virulence \times temperature), rather than more simple 'genotype \times environment' interactions (i.e. host resistance \times temperature). This applies even though only one pathogen genotype was utilized in the present study. Given that there are many isolates of *E. neoaphidis* which vary in pathogenicity to *A. pisum* (Milner, 1982), it is likely that the observed patterns of susceptibility would change if the aphid clones were exposed to a different isolate. However, although the 'genotype \times genotype \times environment' interactions make patterns of susceptibility more difficult to understand, the outcome is the same: the most resistant clone varies with temperature and as a consequence, fluctuating environmental temperatures will help maintain heritable variation in resistance to the pathogen in natural populations.

Most studies on host resistance or pathogen virulence are conducted under very restricted and regulated environmental conditions. The present results suggest that such work may not always reflect the underlying ecological [e.g. by influencing indirect (Pope *et al.*, 2002) or direct (Arthurs & Thomas, 2001; Welch *et al.*, 2001) interactions between species] patterns and interactions that exist in natural populations. Furthermore, such results may have implications for the development of entomopathogens as agents of biological control (Blanford & Thomas, 1999).

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