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Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid

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Parasitoids are among the most important natural enemies of insects in many environments. *Acyrthosiphon pisum*, the pea aphid, is a common pest of the leguminous crops in temperate regions. Pea aphids are frequently attacked by a range of endoparasitic wasps, including the common aphidiine, *Aphidius ervi*. Immunity to parasitoid attack is thought to involve secondary symbiotic bacteria, the presence of which is associated with the death of the parasitoid egg. It has been suggested that there is a fecundity cost of resistance, as individuals carrying the secondary symbionts associated with parasitoid resistance have fewer offspring. Supporting this hypothesis, we find a positive relationship between fecundity and susceptibility to parasitoid attack. There is also a negative relationship between fecundity and off-plant survival time (which positively correlates with resistance to parasitoid attack). Taken together, these results suggest that the aphids can either invest in defence (parasitoid resistance, increased off-plant survival time) or reproduction, and speculate that this may be mediated by changes in the aphids' endosymbiont fauna. Furthermore, there is a positive relationship between aphid size and resistance, suggesting that successful resistance to parasitoid attack may involve physical, as well as physiological, defences.

Keywords: *Acyrthosiphon pisum*; *Aphidius ervi*; costs of resistance; ecological immunity; life-history trade-off; parasitoid

1. INTRODUCTION

Parasitoids are perhaps the most important natural enemies of insects, potentially presenting a more important mortality factor than either predators or pathogens (Hawkins *et al.* 1997). Given the ubiquity of parasitoids in terrestrial ecosystems, it is perhaps unsurprising that their insect hosts have evolved a range of physiological and behavioural mechanisms to resist attack (reviewed in Godfray 1994). If there are life-history costs associated with resistance, then the adaptive benefit of investing in defence will be determined by the risk of exposure to attack. As the risk of attack is likely to vary in space and time, it is to be expected that resistance ability will vary between- and within-host populations. Understanding why such heritable variation in resistance is maintained is a fundamental component of the emerging discipline of ecological immunology (Sheldon & Verhulst 1996; Rolff & Siva-Jothy 2003).

Many parasitoids (endoparasitoids) oviposit their eggs inside the host's haemocoel. Here, the host may mount an immune response which, if successful, results in the death of the parasitoid and survival of the host. Within species, there is evidence from a limited range of taxa of variation in resistance to endoparasitoid attack both across (e.g. Kraaijeveld *et al.* 1998; Hufbauer 2002) and within (e.g. Henter & Via 1995; Kraaijeveld & Godfray 1997; Ferrari *et al.* 2001; Stacey & Fellowes 2002) populations.

To date, for host–parasitoid systems, the only successful studies to demonstrate costs of the ability to resist parasitoid attack have used *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and its parasitoids as a model system. *Drosophila melanogaster* utilises an innate immunological reaction known as cellular encapsulation to counter attack by hymenopteran endoparasitoids (reviewed in Fellowes & Godfray 2000; see also Kraaijeveld *et al.* 2002). Using an artificial selection approach, it was established that *D. melanogaster* resistant to attack by the braconid parasitoid, *Asobara tabida* Nees (Hymenoptera: Braconidae; Kraaijeveld & Godfray 1997), or the eucoilid parasitoid, *Leptopilina boulardi* Barbotin *et al.* (Hymenoptera: Eucoilidae; Fellowes *et al.* 1998, 1999) were poorer at competing for food during their larval stage. Trade-offs such as these will constrain the evolution of resistance, and may also influence the population and evolutionary dynamics of host–parasitoid interactions (Sasaki & Godfray 1999; Fellowes & Travis 2000).

Not all potential hosts utilise cellular encapsulation as a means of surviving parasitoid attack. The pea aphid (*Acyrthosiphon pisum* Harris; Homoptera: Aphididae) is attacked by a number of hymenopteran endoparasitoids. As with the system described above, the female wasp oviposits an egg into the host's haemocoel, which if successful, eventually kills the host. In contrast to the *D. melanogaster* system, there is no evidence of a cellular encapsulation response; instead in resistant pea aphids the parasitoid egg fails to mature, and quickly breaks down (Henter & Via 1995). Recent work suggests that symbiotic bacteria may play a role in this process.

In addition to *Buchnera aphidicola*, an obligate mutualistic endosymbiont thought to provide nutritional benefits to the aphid (reviewed in Douglas 2003), pea aphids carry

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a number of additional microbial taxa which are facultatively associated with their hosts. A recent study found that experimental infection with secondary symbiont taxa known as PASS(R) and PABS(T) produced a significant increase in parasitoid resistance (20 and 40% increase, respectively; Oliver *et al.* 2003). A number of studies have found clonal variation in parasitoid resistance in the pea aphid (Henter & Via 1995; Hufbauer & Via 1999; Ferrari *et al.* 2001; Stacey & Fellowes 2002), and this variation in resistance is likely in large part to be explained by the presence or absence of such secondary endosymbionts. Given the advantages of carrying a symbiont that affects resistance to parasitoids, it is perhaps surprising that these symbionts have not spread to fixation throughout pea aphid populations. Infection with PASS(R) caused reduced fecundity and longevity (Chen *et al.* 2000), and it has been suggested that a trade-off between resistance to parasitism and fecundity may exist in the pea aphid (Oliver *et al.* 2003). An earlier study, however, failed to find any relationship between lifetime fecundity and parasitoid resistance in this system (Ferrari *et al.* 2001).

In this paper, we report the results of a study which tests the hypothesis that there is a relationship between susceptibility to attack by the endoparasitoid, *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) and fecundity (Oliver *et al.* 2003). Furthermore, we ask if there is a relationship between parasitoid resistance and aphid body size or off-plant survival time. These latter factors may reflect differential allocation of resources to growth and defence among the aphid clones.

2. MATERIAL AND METHODS

(a) *Study organisms*

Ten clones of the pea aphid *A. pisum* were established from single apterous individuals collected on red clover *Trifolium pratense* L. (Fabaceae) in Berkshire, UK during summer 2003. The clonal lineages established were reared separately on pre-flowering broad bean plants *Vicia faba* (Fabaceae; var. 'the Sutton dwarf'). Random amplification of polymorphic DNA analysis was carried out using commercial kits (Ready-To-Go RAPD Analysis Beads, Amersham Pharmacia Biotech) to confirm the unique identity of the clonal lines (results not shown). One clone was lost due to experimental error during the late fecundity trial, reducing the number of clones used to nine for part of the analysis.

Aphidius ervi is a solitary koinobiont (the host continues developing after attack) endoparasitoid, laying a single egg within the body cavity of its aphid nymph host. An infected host develops until the parasitoid wasp larva alters the developmental fate of the host, and causes the aphid to undergo a process known as mummification. The wasp larvae within this mummy consumes the remaining aphid tissue, pupates and emerges as an adult. *Aphidius ervi* is a generalist parasitoid of a large number of aphid species and is an abundant natural enemy of the pea aphid in the UK (Müller *et al.* 1999). The *A. ervi* used in this study were purchased from a commercial supplier (Koppert, UK).

Unless noted otherwise, all experiments were carried out in a 20 ± 1 °C temperature controlled room under constant light at ambient humidity (*ca* 80–85% RH). Each replicate was reared separately on *V. faba* plants for two generations prior to the study to avoid problems associated with pseudoreplication.

(b) *Parasitoid resistance*

The parasitism assay used was a modification of that used by Henter & Via (1995). Three adult apterous aphids were introduced into a single, 14 day old broad bean plant contained within a transparent cylindrical Perspex and gauze cage, approximately 30 cm high and 10 cm in diameter. This was replicated up to five times per clone. The aphids were allowed to reproduce for 24 h, then the adults and any excess nymphs were removed to leave 15 nymphs per plant. After the nymphs were 4 days old, and had reached the second larval instar, two naive, mated female *A. ervi* were added to the container and allowed to forage for 6 h before being removed. The aphids were allowed to mature upon the experimental plant for a further 10 days, by which time they had either mummified, or become adult. Each plant was inspected and numbers of remaining adult aphids and mummies recorded.

(c) *Fecundity*

Three adult apterous aphids were placed on a 14 day old *V. faba* plant within the sealed experimental cages. This was replicated 10 times per clone. Aphids were first counted after 4 days (early fecundity; nymphs that generally develop prior to the females final moult to adulthood). On the 6th day of the experiment, the adults were moved to fresh plants to maintain high plant quality, and then the number of offspring counted after a further 8 days (late fecundity).

(d) *Body size*

Body size measurements were taken from a subset (five adults per replicate) of aphids from the first week of the fecundity experiment. A calibrated dissection microscope and graticule were used to measure hind tibia length, as this appendage is desiccation resistant and gives a reliable indication of body mass in the pea aphid (Nicol & Mackauer 1999).

(e) *Off-plant survival time*

The progeny of adults used in the fecundity assay were allowed to mature and 15 individuals per replicate (up to 10 replicates per clone) were transferred to 10 cm diameter Petri dishes and placed in a 19 °C, 16 : 8 L/D incubator at ambient humidity (*ca* 80–85% RH). The mean time to death was calculated per Petri dish, and this was used as the value for each of the replicates. A census was taken every 8 h until all aphids had died.

(f) *Body fat and water content*

Aphid fat and water content were examined following a slightly modified version of a simple petroleum ether extraction (Cockbain 1961). Ten freshly emerged adult aphids per replicate were killed by brief exposure to -20 °C and placed into 2.5 ml glass vials, with their weights recorded to the nearest milligram. The aphids were then dried for 24 h at 70 °C prior to fat extraction, and the water content of the samples recorded. To extract fat from the desiccated aphid material, the samples were kept in 2.5 ml of petroleum ether (b.p. 40–60 °C) for 48 h, with the ether changed every 12 h using a micro-pipette. Immediately following this, samples were air dried for 24 h and then re-weighed. The amount of ether soluble fat extracted was thus not directly measured, but instead inferred by subtraction.

(g) *Statistical analyses*

Life history data were compared using ANOVA, following tests to confirm that the data met normality assumptions. Proportion data were angular transformed prior to analysis (Zar 1999), and regressions (or multiple regressions, where appropriate) were performed on the transformed data. A conservative approach of taking mean clonal values for traits of interest was used for all regressions. All analyses were performed using S-PLUS 6.1 (Insightful Corp. 2002).

3. RESULTS

(a) *Clonal variation*

There was significant variation among clones in resistance to attack by *A. ervi* ($F_{9,29}=9.85$, $p<0.001$), in early and late fecundity ($F_{9,76}=15.19$, $p<0.0001$; $F_{8,61}=8.58$, $p<0.0001$), hind tibia length ($F_{1,14}=7.54$, $p<0.001$) and off-plant survival time ($F_{9,61}=8.58$, $p<0.0001$). There was no difference among clones in proportion water ($F_{8,53}=1.51$, $p=0.18$), but there was significant difference in proportion body fat ($F_{8,53}=6.43$, $p<0.0001$).

(b) *Resistance and life-history traits*

There was no relationship between body size (hind tibia length) and fecundity or off-plant survival time across clones (early fecundity: $F_{1,8}=1.26$, $R^2=0.14$, $p=0.3$; late fecundity: $F_{1,7}=0.35$, $R^2=0.05$, $p=0.57$; off-plant survival time: $F_{1,8}=0.4$, $R^2=0.05$, $p=0.55$). However, there was a (near) significant relationship between fecundity and off-plant survival (early fecundity: $F_{1,8}=5.77$, $R^2=0.42$, $p<0.05$, figure 1; late fecundity: $F_{1,7}=4.31$, $R^2=0.38$, $p=0.077$). Therefore three multiple regression analyses were conducted. First, with hind tibia length and either late or early fecundity, and then with hind tibia length and off-plant survival time as the explanatory variables, and susceptibility to *A. ervi* as the response variable.

Statistical analysis showed a significant effect of fecundity and hind tibia length on resistance to parasitoid attack (with early fecundity in model: $F_{2,7}=17.68$, $R^2=0.83$, $p=0.002$; with late fecundity in model: $F_{2,6}=8.62$, $R^2=0.74$, $p<0.02$). Further examination of the data showed a positive relationship between fecundity and susceptibility (early fecundity: $\beta=0.62$, $t_7=3.73$, $p=0.007$; late fecundity: $\beta=0.5$, $t_7=2.4$, $p=0.056$; figure 2), and a negative relationship with hind tibia length (with early fecundity in model: $\beta=-0.48$, $t_7=-2.93$, $p=0.02$; with late fecundity in model: $\beta=-0.59$, $t_6=-2.82$, $p=0.031$, figure 3).

Repeating the analysis with off-plant survival time and hind tibia length as explanatory variables again explained a significant amount of the variation in susceptibility ($F_{2,7}=19.51$, $R^2=0.85$, $p<0.0014$). As previously, there was a negative relationship between hind tibia length and susceptibility ($\beta=-0.58$, $t_7=-3.85$, $p=0.006$). Here, a significant negative relationship between off-plant survival time and resistance was found ($\beta=-0.6$, $t_7=-3.97$, $p=0.005$; figure 4).

(c) *Relationships between other traits*

Variation in aphid water or lipid content did not explain a significant proportion of the variance in aphid fecundity, off-plant survival time or susceptibility to parasitoid attack.

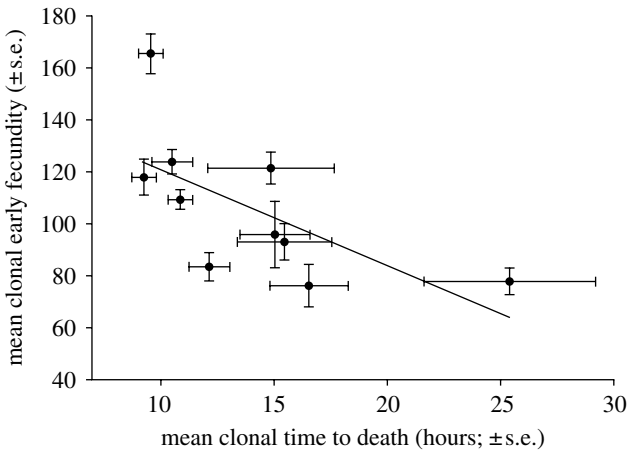

Figure 1. Negative relationship between mean clonal early fecundity (measured as number of offspring larviposited within 4 days of reaching adulthood) and mean clonal off-plant survival time in hours. Analysis was performed on the mean values for each clone ($n=10$) and standard errors are shown for each value.

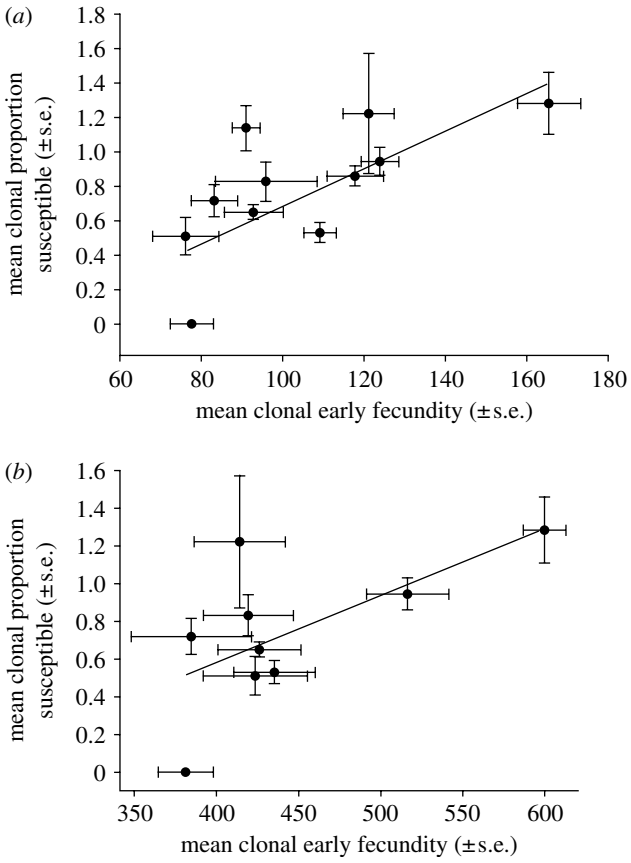

Figure 2. Positive relationship between mean clonal susceptibility to parasitoid attack (angular transformed) and (*a*) mean clonal early fecundity ($n=10$) or (*b*) mean clonal late fecundity (measured at 12 days; $n=9$). See figure 1 for further details.

4. DISCUSSION

We provide evidence for a trade-off between the ability to resist parasitoid attack and fecundity in the pea aphid. This is the second species in which a cost of resistance to parasitoids has been reported, and the first in which resistance is thought to be mediated by endosymbionts.

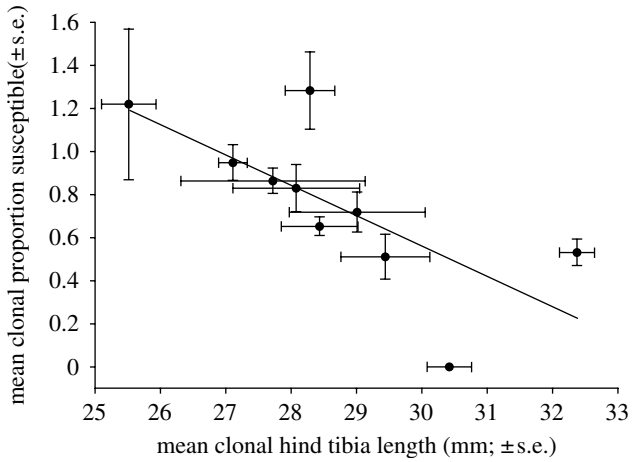

Figure 3. Negative relationship between mean clonal susceptibility to parasitoid attack (angular transformed) and mean clonal hind tibia length ($n=10$). See figure 1 for further details.

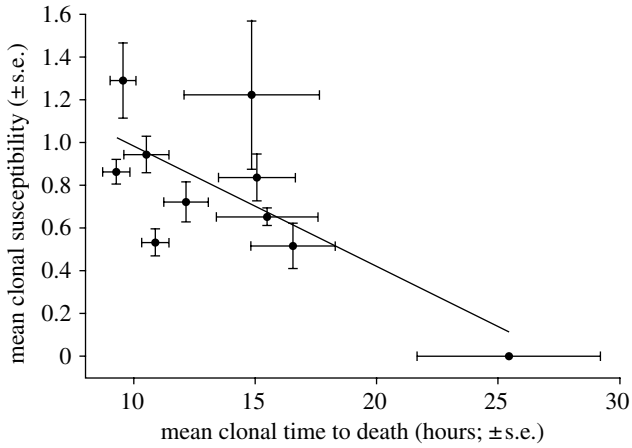

Figure 4. Negative relationship between mean clonal susceptibility to parasitoid attack (angular transformed) and mean clonal off-plant survival time ($n=10$). See figure 1 for further details.

Such trade-offs will constrain the spread of resistance in this system. This trade-off may be driven by changes in nutritional investment, as off-plant survival time positively correlated with resistance, but negatively correlated with fecundity, suggesting that resistant clones invest more in survival than reproduction. Furthermore, we found a positive relationship between adult aphid size and resistance. Given that the parasitoids are attacking second instar aphid nymphs, this correlation is perhaps surprising, but this is likely to reflect variation in size during the second instar stage. The lack of a relationship between adult size and fecundity was unexpected, as such relationships are considered almost ubiquitous in insect systems.

Fecundity is perhaps the most important component of fitness in organisms such as the pea aphid, which compensate for high mortality rates with rapid reproductive potential (Dixon 1998). Hazell *et al.* (2005) found that there is no relationship between lifetime fecundity and competitive ability in the pea aphid. This suggests, in contrast to previous work with *D. melanogaster* (reviewed in Fellowes & Godfray 2000), that resistance and competitive ability do not trade-off against each other. Indeed, Hazell *et al.* (2005) found that clones which have higher fecundity tend to produce more winged (dispersing) offspring, thus reducing their ability to compete on a single host plant. Furthermore, Hazell *et al.* (2005) found that there was no correlation across pea aphid clones in early and lifetime fecundity. These observations may explain the lack of a trade-off between pea aphid lifetime fecundity and resistance to parasitoids (Ferrari *et al.* 2001) or between resistance and competitive ability (Steaphan Hazell, unpublished data). We did find a significant correlation between early (first 4 days) and late (first 12 days) fecundity, but this was strongly influenced by the most fecund clone. Pea aphids can survive and reproduce for up to four weeks after their final moult into adulthood when reared under optimum conditions (Hazell *et al.* 2005), so our measures of clonal fecundity are unlikely to reflect the ranking of clones when life-time fecundity is considered.

The physiological basis of resistance in the pea aphid is currently poorly understood, but infection with a secondary symbiont (PASS(R)) has been shown to confer increased parasitoid resistance (Oliver *et al.* 2003). The presence of the symbiont is associated with fecundity costs in an experimentally infected pea aphid clone (Chen *et al.* 2000), and it has been suggested that a potential trade-off may exist between fecundity and parasitoid resistance (Oliver *et al.* 2003). Our evidence indicates that this proposed trade-off exists, but that it primarily results from a loss of early fecundity. Inspection of the figures shows that the trade-off is weak for later fecundity, with the significant result being driven by one clone, and the effect of fecundity is not significant at the traditional threshold value of $p=0.05$. Repeating this work with a new series of clones provide support for the trade-off between parasitoid immunity and early fecundity, but not for later fecundity (David Gwynn, unpublished data). One possible explanation for this result is that by measuring fecundity, we are measuring a proxy of development time, as all trials were started at the same time. If variation in early fecundity indirectly results from differences in timing of final moult into adulthood, rather than directly from investment in reproduction, then our understanding of the trade-off is altered, albeit while the trade-off itself remains. We are currently examining this possibility.

Off-plant survival times showed a negative relationship with fecundity, again suggesting a change in allocation of resources from reproduction to survival. We expected that clonal variation in off-plant survival would be explained by variation in the water or fat content of the aphids. This was not so, and it would be of considerable interest to understand the mechanistic basis of the trade-off between fecundity and off-plant survival time. These data suggest that our results are unlikely to be confounded by condition-dependency (Van Noordwijk & De Jong 1986; Cotton *et al.* 2004; Rolff *et al.* 2004).

The positive relationship between ability to resist parasitoid attack and pea aphid adult size was unexpected. Adult size can be an indication of overall quality in insects (e.g. Ryder & Siva-Jothy 2001). It is possible that the factors inferring increased parasitoid resistance in the pea aphid, whether mediated by secondary symbionts or other mechanisms, give other, as yet unexamined, benefits with relation to other aspects of aphid physiology and development. However, there was no relationship between

aphid size and fecundity across clones. This result is surprising, given the frequent assumption of size–fecundity relationships in insects, and may bring into question the suggestion that clonal size reflects physiological quality.

This lack of a relationship between size and fecundity may be due to competition between primary and secondary symbiont populations, but we treat this result with some caution. More recent work with a larger number of clones suggests that a positive correlation may exist (David Gwynn, unpublished data). Infection with the PASS(R) symbiont acts to suppress *Buchnera* populations, indeed in young adult aphids the titres of *Buchnera* in an infected clone were half that of its uninfected counterpart (Koga *et al.* 2003). Depending on the genetic and physiological basis of adult size, a reduction in energy intake during this critical, early reproductive period may explain the lack of a size/fecundity relationship. The reduction seen in *Buchnera* number also provides a potential mechanistic basis to the trade-off found between parasitoid resistance and fecundity.

All organisms face attack by natural enemies. Understanding how trade-offs constrain the evolution of resistance is a key constituent of ecological immunology; and although these trade-offs may not be obvious or ubiquitous (e.g. Little *et al.* 2002), they may provide critical insights into the community ecology of victim–enemy interactions (Fellowes & Kraaijeveld 1998). The substantial variation in susceptibility to parasitoids found in pea aphids is likely to be maintained by a combination of temporally and spatially fluctuating selection pressures and the fecundity costs of resistance. Just how these factors affect host–parasitoid interactions in the field is relatively unexplored terrain; we suggest that pea aphids and their natural enemies are likely to provide an excellent model system to explore these questions.

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