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Genetic covariation between effectiveness and cost of defence in aphids

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Ecological immunology distinguishes between the long-term evolutionary costs of possessing defences against parasites and the short-term costs of using them. Evolutionary biologists have typically focused on the former in the search for constraints on the evolution of resistance. Here, we show in the peach-potato aphid, *Myzus persicae*, that short-term costs may be of equal evolutionary importance. Survivors of more resistant aphid clones suffered a higher reduction of fecundity upon parasitoid attack than survivors of more susceptible clones. This genetically based trade-off between benefits and costs of defence may limit the evolution of increased resistance and explain the maintenance of genetic variation for resistance under environmental variation in parasitism risk.

1. INTRODUCTION

Parasitoids are important natural enemies of aphids and exert strong selection on their hosts. Even so, aphid populations harbour ample genetic variation for susceptibility to parasitoids (Henter & Via 1995; von Burg *et al.* 2008). This variation may be maintained by costs of resistance. Two types of cost are usefully distinguished: the long-term evolutionary or constitutive costs of possessing the ability to resist (e.g. having an immune system), and the short-term actual or induced costs of using this ability when attacked (e.g. mounting an immune response). Attempts to demonstrate constitutive costs in aphids have yielded ambiguous results. Gwynn *et al.* (2005) reported that in the pea aphid, *Acyrthosiphon pisum*, clones with a high fecundity are more susceptible to parasitoids, but no such pattern was found in a study by Ferrari *et al.* (2001) in the same species and by von Burg *et al.* (2008) in the peach-potato aphid, *Myzus persicae*. In *Drosophila melanogaster*, on the other hand, lines selected for increased resistance to parasitoids show reduced larval competitive ability, consistent with constitutive costs of resistance (Kraaijeveld & Godfray 1997). Induced costs of resistance are generally un-disputed and readily measured (Schmid-Hempel 2005), but have so far received little attention in aphids.

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We have shown previously that aphids that successfully resisted parasitoids suffer from impaired fecundity (Vorburger *et al.* 2008). This is consistent with induced costs of resistance, albeit indistinguishable from negative effects of the parasitoid attack itself, e.g. envenomation by the parasitoid's sting. Here, we address two questions. (i) Does impaired fecundity upon parasitoid attack have a heritable basis? (ii) Is this fitness loss related to the observed level of resistance? These questions are important because if more resistant genotypes suffered a higher fecundity loss upon successfully resisting parasitoids, these induced costs would also be evolutionary in the sense that they slash the benefits of evolving increased resistance. This could constrain evolution and maintain genetic variation for resistance under an environmental variation of parasitism risk. Here, we demonstrate such a genetically based trade-off between benefits and actual costs of resistance in *M. persicae*.

2. MATERIAL AND METHODS

(a) *Study system*

Myzus persicae is a very polyphagous and economically important pest with a worldwide distribution (Blackman & Eastop 2000). The 16 clones used in this study were collected in 2003 at Bacchus Marsh, Australia, where *M. persicae* reproduces predominantly by obligate parthenogenesis (Vorburger *et al.* 2003). All clones possess different microsatellite genotypes. They are a subset of the clones used in a published study of life-history variation (Vorburger 2005), where their genotypes and collection details are available. The clones used here include nos. 5.1, 5.3, 5.6, 5.7, 5.8, 5.13, 5.15, 5.17, 5.19, 5.23, 6.9, 6.16, 6.18, 6.21, 7.9 and 7.10. Because facultative vertically transmitted endosymbiotic bacteria can affect a clone's phenotype, including the susceptibility to parasitoids (Oliver *et al.* 2003), all clones were tested for infection with known aphid endosymbionts as described in von Burg *et al.* (2008). Only clone 5.15 was found to harbour the bacterium *Regiella insecticola*.

Aphidius colemani is a solitary endoparasitoid of aphids that is commonly used in biocontrol. Female wasps oviposit a single egg into aphid nymphs. The larva develops inside the growing aphid until it kills the host to pupate inside its dried remains. This forms the characteristic mummy from which the adult wasp emerges. We originally obtained *A. colemani* from a commercial supplier (Andermat Biocontrol AG, Grossdietwil, Switzerland) and maintain it in the laboratory as large cage populations on Swiss clones of *M. persicae*.

(b) *Experimental procedures and analyses*

We split all test clones into eight sublines placed at random positions in eight different trays (randomized complete blocks). We maintained sublines on caged seedlings of radish (*Raphanus sativus*) at 20°C and a 16 hours photoperiod for two generations before the actual experiment took place in the third subline generation. This preparation serves to eliminate environmental maternal or grand-maternal effects that could be carried over from the stock culture. The test generation was started by transferring five adult aphids from each subline to two new plants (one for exposure to *A. colemani*, the 'parasitoid treatment' and the other for the 'control treatment'), where they reproduced for 24 hours before being discarded. After 2 days, when the offspring were 48–72 hours old, we counted the aphid nymphs on the plants assigned to the parasitoid treatment (mean colony size$=32.7 \pm 13.6$ s.d.), and exposed each of these colonies for 24 hours to two female *A. colemani* that were haphazardly selected from the stock population. This procedure ensured that mean and variation of host age at exposure were the same for all clones, which is important because aphid susceptibility to parasitoids varies with age. The chosen age of 48–72 hours corresponds to the period in which *M. persicae* is most susceptible to *A. colemani* (Martinou & Wright 2007). Two wasps on a small aphid colony for 24 hours is a very high level of parasitoid exposure and typically results in multiple attacks (parasitoid ovipositions) per aphid. Eight days after exposure to wasps, successfully parasitized individuals were recognizable as mummies and counted to calculate the proportion of aphids mummified as a measure of susceptibility to *A. colemani*. Proportions were arcsin-square root transformed (Sokal & Rohlf 1995) and analysed with ANOVA to test for the effect of clone, accounting for block, which was treated as a random effect. The

number of nymphs exposed to parasitoids was included as a covariate to account for unequal colony sizes among replicates. The design was not fully balanced because we lost three sublines in the preparation phase of the experiment. Therefore, we used Satterthwaite's approximation to produce error estimates for *F*-tests. Analyses were performed using the MIXED command in SPSS v. 11.5 (SPSS 2002).

Five surviving adults per replicate from the parasitoid treatment (or all remaining when fewer than five were not mummified) were transferred to new plants and allowed to reproduce for 24 hours. The same was done with five adults per replicate from the control treatment. From offspring counts, we calculated the daily fecundity per individual. In *M. persicae*, daily fecundity of young adults exhibits tight positive correlations with lifetime reproduction as well as other components of fitness, including offspring size as an estimate of offspring quality (Vorburger 2005). Fecundity was analysed by ANOVA testing for the effects of treatment, clone, and their interaction, accounting for block effects. Again, the design was not fully balanced because in several replicates of the parasitoid treatment, all aphids were mummified, leaving no survivors for fecundity measurements.

3. RESULTS

As expected, based on a previous study (von Burg *et al.* 2008), the proportion of individuals mummified differed significantly among clones ($F_{15,101.2}=6.919$, $p<0.001$). Colony size had a slight but significant positive effect on the proportion mummified ($B=0.006\pm0.002$ s.e., $F_{1,105.8}=7.252$, $p=0.008$). Variation among clones remained significant, even when the most resistant *R. insecticola*-bearing clone 5.15 was excluded ($F_{14,95.3}=2.41$, $p=0.006$). We then compared the fecundity of the surviving aphids from these trials with that of the aphids from the control colonies that had not been exposed to parasitoid attack. With 3.59 ± 0.28 (s.e.) offspring per day, survivors from the parasitoid treatment were less fecund on average than aphids from the control treatment (5.13 ± 0.24 offspring per day; $F_{1,174.5}=27.43$, $p<0.001$). This fecundity reduction differed significantly among clones (clone×treatment, $F_{15,173.4}=2.15$, $p=0.010$), and it was more severe in survivors of more resistant clones than in survivors of more susceptible clones (figure 1). Particularly, the survivors of the most resistant *R. insecticola*-harbouring clone 5.15 were almost completely sterilized by the parasitoid attack. Yet, even if this clone is excluded from the dataset, the correlation between costs and benefits of defence remains significant (figure 1).

4. DISCUSSION

Our results indicate that in a collection of clonal genotypes of *M. persicae*, the loss of fecundity in individuals surviving an attack by *A. colemani* increases with the effectiveness of the clones' defences against this parasitoid. The fecundity reduction upon parasitoid attack might have been somewhat underestimated in our experiment. Competition among adults was similar in both treatments (even parasitized aphids are actively feeding until shortly before mummification), but the higher fecundity of aphids that were not attacked meant that they experienced stronger competition with their own offspring before their fecundity was measured on a new plant. Thus, the adults from the control treatment might have been less well nourished than the surviving adults from the parasitoid treatment. This might explain
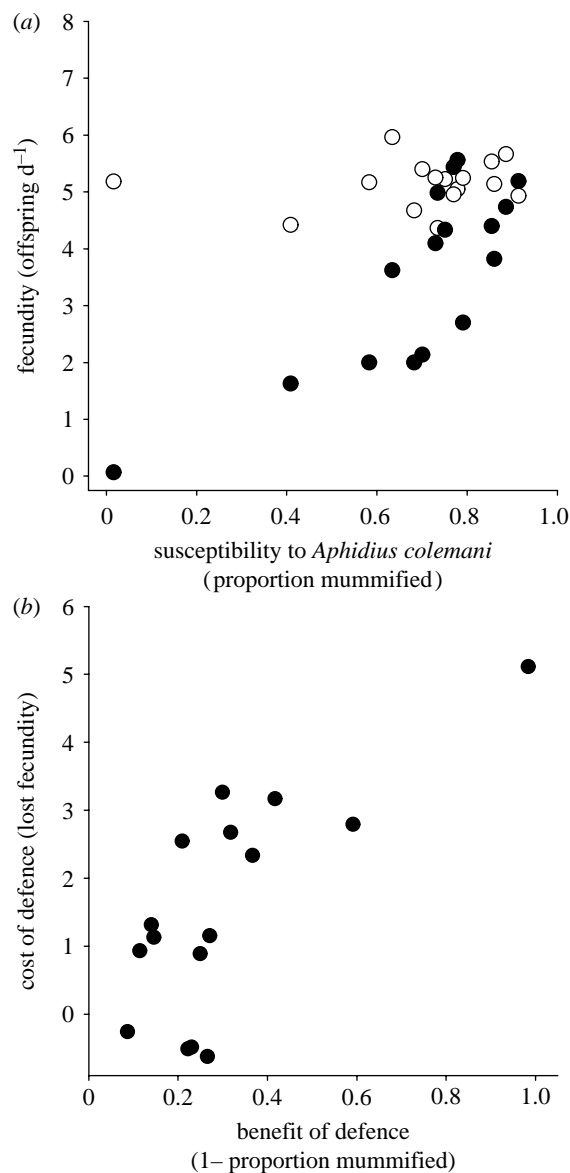
Figure 1. The fitness loss of individuals exposed to but not mummified by parasitoids (*A. colemani*) varies among clones of the aphid *M. persicae* and is higher in clones with a low susceptibility to the parasitoid. (*a*) Susceptibility is unrelated to mean clonal fecundity in the control treatment (open circles; $r=0.156$, $p=0.564$, $n=16$), but correlates significantly with the mean fecundity of survivors in the parasitoid treatment (filled circles; $r=0.794$, $p<0.001$, $n=16$). (*b*) Plotting the same data differently shows that the above relationships result in a significant correlation between the benefits and induced costs of defence ($r=0.740$, $p=0.001$, $n=16$). This correlation remains significant after exclusion of the most resistant clone (top right), which differs qualitatively from the other clones by harbouring the facultative endosymbiotic bacterium *R. insecticola* ($r=0.575$, $p=0.025$, $n=15$).

why some clones appeared not to lose any fecundity when attacked (figure 1*b*), but it cannot explain the observed correlation between lost fecundity and susceptibility, because the clones with the strongest fecundity loss had experienced the least competition with their offspring. We do not know whether this correlation is caused by higher energetic demands of induced defence in more resistant clones or simply by a higher sensitivity to the negative effects of the

parasitoid attack itself. For example, more resistant clones could be more susceptible to the venom that parasitoids inject at oviposition. In either case, this can be interpreted as a cost of resistance, yet one that is expressed only when an aphid has indeed been attacked. Whatever the mechanistic basis of the observed genetic correlation, it reduces the selective advantage to resistant genotypes and may thus be important for the maintenance of genetic variation for susceptibility to parasitoids in aphid populations (Henter & Via 1995; von Burg *et al.* 2008). The role of *R. insecticola* in this variation is presently unclear. Unlike other facultative endosymbionts, such as *Hamiltonella defensa* or *Serratia symbiotica*, *R. insecticola* does not appear to affect resistance to parasitoids in pea aphids, where endosymbionts are best studied (Oliver *et al.* 2003). In the example of *M. persicae* here, the much higher resistance of the only clone harbouring *R. insecticola* suggests a protective effect of this bacterium, too. However, the critical tests of this hypothesis are yet to be carried out.

Because the size of our experiment precluded directly observing and quantifying all parasitoid attacks, it could be criticized that the clonal variation in fecundity loss upon resisting parasitoids might just reflect variation in the frequency at which different clones were attacked by *A. colemani*. Given our results, however, this would mean that the most mummified were the least attacked clones, and vice versa, which is unlikely. Also, we obtained the same pattern of higher fecundity loss in more resistant clones in a pilot experiment, in which we used only three clones but ensured that all aphids were stung exactly once (electronic supplementary material). Thus, we would argue that our results are consistent with *M. persicae* exhibiting genotypic variation along an axis from fighting parasitoids strongly, but at high costs to survivors, to fighting parasitoids weakly, yet at little fitness cost to the few survivors in which this proves sufficient. We want to emphasize two aspects of this finding. First, genetic variation for a cost of resistance has rarely been demonstrated. We are only aware of one similar result: Hoang (2002) found significant variation among isofemale lines of *D. melanogaster* in the reduction of adult fitness upon surviving a parasitoid attack at the larval stage. Second, here it is an induced cost of actual defence that constrains the evolution of increased resistance, rather than the 'usual suspect', a frequently assumed (but rarely demonstrated) constitutive cost of defence (Kraaijeveld *et al.* 2002). In fact, an attempt to demonstrate the latter in the very same clones has previously failed (von Burg *et al.* 2008). This suggests that the study of genetic variation for the costs of actually mounting a defence deserves increased attention also in other systems and may possibly elucidate some of the many examples where evolutionary costs of resistance have so far proven elusive.

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