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Strong parasitoid-mediated selection in experimental populations of aphids

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Clonal diversity in asexual populations may be maintained if different clones are favoured under different environmental conditions. For aphids, parasitoids are an important variable of the biotic environment. To test whether parasitoids can mediate selection among host clones, we used experimental populations consisting of 10 clones of the peach–potato aphid, *Myzus persicae*, and allowed them to evolve for several generations either without parasitoids or in the presence of two species of parasitoid wasps. In the absence of parasitoids, strong shifts in clonal frequencies occurred, mostly in favour of clones with high rates of increase. The parasitoid *Diaeretiella rapae* hardly affected aphid densities but changed the outcome of competition by favouring one entirely resistant clone and disfavouring a highly susceptible clone. *Aphidius colemani*, the more infective parasitoid, strongly reduced aphid densities and dramatically changed host clonal frequencies. The most resistant clone, not a successful clone without parasitoids, became totally dominant. These results highlight the potential of temporal or spatial variation in parasitoid densities to maintain clonal diversity in their aphid hosts.

Keywords: clonal selection; competition; *Myzus persicae*; parasitoids; resistance

1. INTRODUCTION

Clonal selection in an asexual population is a powerful process and, at least in stable and homogeneous environments, should lead to a rapid erosion of clonal diversity. However, natural populations of clonal organisms typically harbour substantial genotypic diversity. This suggests that the temporal and/or spatial heterogeneity of natural environments is able to maintain variation because clones differ in their relative fitness across environments.

In aphids, hymenopteran parasitoids are a major source of mortality and should thus exert strong directional selection on increased resistance. Nevertheless, studies on pea aphids, *Acyrthosiphon pisum*, show that natural populations exhibit ample genetic variation for resistance (Henter & Via 1995; Ferrari *et al.* 2001). These seemingly discrepant results are interpreted as evidence for trade-offs between resistance and other components of fitness, e.g. fecundity, which was supported by a study by Gwynn *et al.* (2005). In the peach–potato aphid, *Myzus persicae*, resistance against two of its parasitoids, *Aphidius colemani* and *Diaeretiella rapae*, also exhibits strong among-clone variation (von Burg *et al.* submitted). However, in that species no trade-offs were detected. Resistance against the two parasitoids was unrelated to life table estimates of the clones' finite rate of increase and fecundity (von Burg *et al.* submitted). Yet, even in the absence of a trade-off, the lack of a positive correlation between resistance and reproduction suggests some potential for genotype×environment interactions. Under conditions primarily selecting for increased resistance (high parasitoid density), different clones should be favoured from those favoured under conditions primarily selecting for high reproduction (low parasitoid density). Parasitoids might thus mediate the outcome of competition among aphid clones. Here, we tested this hypothesis in experimental populations of *M. persicae*.

2. MATERIAL AND METHODS

(a) *Study system*

We used 10 Australian clones of *M. persicae*, representing a subset of those analysed for life-history variation in Vorburger (2005), where the collection information and microsatellite genotypes of these clones are reported. Their numbers are 5.1, 5.3, 5.7, 5.13, 5.15, 6.9, 6.18, 6.28, 7.9 and 7.10. These clones were chosen to encompass the whole range of existing estimates of their finite rate of increase (mean F_i'; Service & Lenski 1982), measured by Vorburger & Ramsauer (submitted) on radish, *Raphanus sativus*, the host plant also used in this experiment. Note that one clone, 5.15, was found to be entirely resistant to parasitoids, possibly due to its harbouring the facultative endosymbiotic bacterium *Regiella insecticola* (von Burg *et al.* submitted).

The aphidiine wasps *D. rapae* and *A. colemani* are solitary endoparasitoids of aphids with a relatively wide host spectrum. Both are important natural enemies of *M. persicae*. Owing to their use as biocontrol agents, they occur sympatrically with *M. persicae* almost globally, including Australia, where our aphid clones were collected. Female parasitoids lay a single egg into aphid nymphs. The larva develops inside the living aphid before the last larval instar kills its host, pupates within its exoskeleton and finally hatches as an adult from the characteristic 'mummy'. We originally obtained *A. colemani* from a commercial supplier (Andermatt Biocontrol AG, Grossdietwil, Switzerland) and *D. rapae* from a laboratory colony at IACR, Rothamstead, UK, and maintained them in our laboratory on Swiss clones of *M. persicae*.

(b) *Experiment*

Our experiment consisted of 21 caged populations of *M. persicae* that were subject to three treatments with seven replicates each: (i) without parasitoids, (ii) with *A. colemani*, and (iii) with *D. rapae*. The cages consisted of modified 20 l plastic aquaria with ventilation windows and a lateral access for exchanging plants. Cages were placed in random order under metal halide lamps (16 hour photoperiod) in a temperature-controlled room at 20°C. They were fitted with four 10 cm plastic flower pots, each containing six radish plants. Aphid populations were founded by placing five young adults of each clone on the plants in every cage. Five days later, when aphid populations had grown to approximately 1500–2000 individuals, we added 10 female and 10 male wasps to those cages subject to parasitoid treatments. Twice a week, plants were watered and one pot with new two-week-old plants was added to each cage to replace the pot with the oldest plants. At weekly intervals, we determined a relative index of host and parasitoid densities. For the aphids, we clipped the largest leaf of the pot with the youngest plants and counted all individuals within three randomly placed squares of 0.64 cm^2 (total area 1.92 cm^2). For the parasitoids, we placed a lamp directly above each cage (parasitoids are attracted to light) and counted all wasps on the perspex cage lid within 30 randomly placed squares of the same size (total area 19.2 cm^2). Eight weeks after the addition of parasitoids, corresponding to approximately 6–8 aphid generations, the experiment was terminated by collecting 100 adult aphids from each cage for genotyping. To obtain a random sample of adults, we shook all aphids off the plants into a bowl, mixed them thoroughly and repeatedly scooped out small portions from which all adults were collected until the required number was attained. Two cages from the *A. colemani* treatment, where aphid densities were low, yielded less than 100 adults (83 and 34). Aphids were

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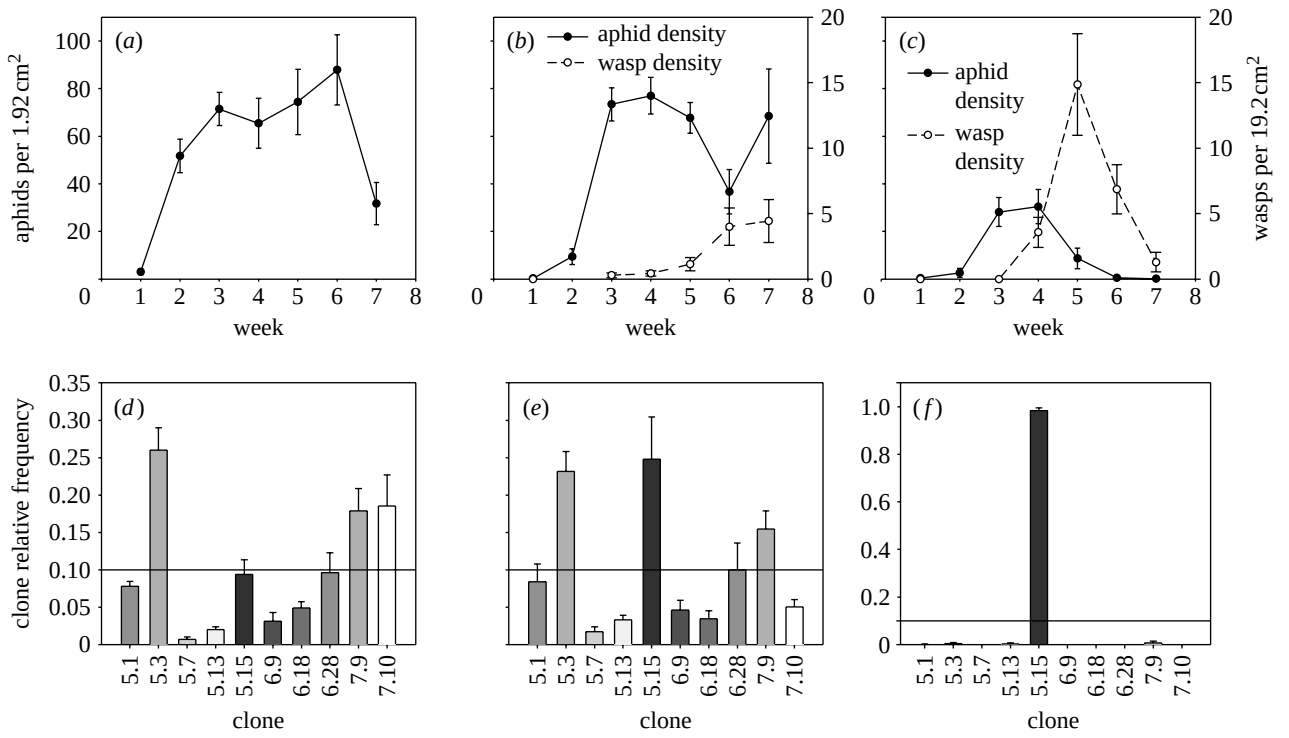

Figure 1. (*a*)–(*c*) Temporal trajectories of indices of aphid and parasitoid densities during the course of the experiment and (*d*)–(*f*) estimated proportions of the 10 clones of *M. persicae* at the end of the experiment: (*a*,*d*) no parasitoids, (*b*,*e*) *Diaeretiella rapae* and (*c*,*f*) *Aphidius colemani*. The reference line indicates the clones' relative frequencies at the start of the experiment (10% each). Error bars depict 1 s.e.

placed individually in Eppendorf tubes and frozen at −80°C until DNA extraction. Each individual was genotyped at two microsatellite loci (M86 and myz9; Sloane *et al.* 2001; Wilson *et al.* 2004), which was sufficient to distinguish the 10 clones.

Although the original plan was to not interfere with the populations, we found parasitism rates early in the experiment to be much higher than expected from preliminary trials and thus decided to cull parasitoids for fear of losing replicates due to host extinction. The first generation of mummies was reduced to 40 mummies per cage. After that, we removed adult wasps from the cages twice weekly during the first three weeks for *D. rapae* and during all eight weeks for *A. colemani*, because the latter species is the more infective of the two parasitoids. Despite more extended culling of *A. colemani*, wasp densities were higher and aphid densities were lower in this treatment than in the *D. rapae* treatment (figure 1).

(c) *Statistical analyses*

The weekly estimates of aphid and parasitoid densities were compared among treatments with repeated-measures ANOVAs, applying the Greenhouse–Geisser (G–G) adjustment for tests of within-subject effects when the sphericity assumption was not met. To test whether each clones' estimated proportions per cage at the end of the experiment differed among treatments, we used a generalized linear model with a logit link and, due to some overdispersion, quasibinomial errors as implemented in the R software package (R Development Core Team 2006). As recommended by Crawley (2005) for quasibinomial fits, *F*-tests rather than χ^2-tests were used to compare deviances of models with and without the effect of treatment. To account for multiple testing, a Bonferroni-adjusted level of significance was used (0.05/10=0.005). These analyses were followed by pairwise comparisons among treatments using the same test for clones with a significant overall difference.

3. RESULTS

Aphid densities differed significantly among treatments ($F_{2,18}=65.87$, $p<0.001$), and so did their temporal trajectories (treatment×time interaction: G–G; $F_{6.7,60}=5.46$, $p<0.001$). Without parasitoids and in the presence of *D. rapae*, aphid populations increased rapidly and conditions became very crowded (figure 1*a*,*b*). In the *A. colemani* treatment, aphid populations grew more slowly, decreased again after week 4 and nearly went extinct by the end of the experiment (figure 1*c*). Wasp population densities also differed between the two parasitoid treatments ($F_{1,12}=9.20$, $p=0.010$), with differences also in their temporal development (treatment×time interaction: G–G; $F_{1.9,23.2}=8.25$, $p=0.002$). The density of *D. rapae* increased slowly over time, whereas the density of *A. colemani*, despite extended culling of

Table 1. Summary of the analyses of treatment effects on the final proportions reached by the 10 clones of *Myzus persicae* in the experimental populations. (The overall difference among treatments was significant for all clones after Bonferroni correction. The pairwise comparisons show which treatments did (>, <) or did not (enclosed within parentheses) differ significantly at $\alpha=0.017$ (0.05/3 pairwise comparisons). Np, no parasitoids; Dr, *Diaeretiella rapae*; Ac, *Aphidius colemani*.)

clone	*F*	*p*	pairwise comparisons
5.1	17.71	<0.001	(Np, Dr)>Ac
5.3	45.43	<0.001	(Np, Dr)>Ac
5.7	9.24	0.002	(Np, Dr)>Ac
5.13	7.96	0.003	Dr>Ac[a]
5.15	107.49	<0.001	Np<Dr<Ac
6.9	11.29	<0.001	(Np, Dr)>Ac
6.18	20.75	<0.001	(Np, Dr)>Ac
6.28	9.86	0.001	(Np, Dr)>Ac
7.9	19.43	<0.001	(Np, Dr)>Ac
7.10	30.53	<0.001	Np>Dr>Ac

[a] Np did not differ significantly from any other treatment.

parasitoids, quickly rose to very high levels and then decreased again due to a lack of hosts (figure 1*b*,*c*).

The clones' relative frequencies at the end of the experiment revealed strong differences in competitive ability in the absence of parasitoids (figure 1*d*). Three clones increased substantially, two remained close to their starting frequency and five decreased, one nearly to extinction (5.7). The clones' final frequencies in this treatment were positively related to their mean F_i' (rate of increase), although this relationship was not statistically significant (Spearman's $\rho = 0.624$, $p = 0.054$). The outcome of competition in the presence of *D. rapae* was similar (figure 1*e*). Only two clones' frequencies differed significantly from those in the absence of parasitoids (table 1). Clone 5.15, found to be resistant to both parasitoids by von Burg *et al.* (submitted), strongly benefited from presence of *D. rapae*, while 7.10, the clone known to be most susceptible to *D. rapae*, decreased significantly. The outcome of clonal competition in the presence of *A. colemani* was strikingly clear (figure 1*f*). The remaining aphid populations consisted almost exclusively of the resistant clone 5.15, all others were nearly or completely extinct.

4. DISCUSSION

Our experiment clearly demonstrated that the presence of parasitoids can affect the relative fitness of different clones of the aphid *M. persicae* and thus alter the outcome of intraspecific competition within just a few generations. The two parasitoid species had very different effects: the presence of *D. rapae* caused only minor shifts in the relative success of the 10 clones, but the presence of *A. colemani* basically led to the fixation of a single resistant clone. However, this outcome cannot be interpreted as evidence for parasitoid-specific defence mechanisms in aphids. In *M. persicae*, there is a strong positive genetic correlation between resistance to *D. rapae* and resistance to *A. colemani* (von Burg *et al.* submitted). The outcome thus rather reflects the fact that *A. colemani* is the more infective of the two parasitoids on *M. persicae*. With low aphid numbers exposed to many wasps, selection in the *A. colemani* treatment was chiefly for increased resistance, while the very high aphid densities permitted by the limited impact of *D. rapae* made the selective regime in this treatment much more similar to the treatment without parasitoids (figure 1*a*,*b*). This favoured a similar set of clones, particularly those with a high rate of increase. Still, *D. rapae* did have a significant effect on the most and the least resistant clone.

To summarize, we find strong effects of parasitoids on the outcome of competition among aphid clones in experimental populations. This suggests that temporal or spatial variation in parasitoid densities in the field may contribute to the maintenance of host clonal diversity, but we acknowledge that experimental cages are a very simplified representation of the natural environment and thus limit the ability to generalize. On the other hand, natural aphid populations experience variation in many additional environmental variables (e.g. temperature, host plants, predators), providing even more scope for genotype × environment interactions to maintain diversity.

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