Parasitoids induce production of the dispersal morph of the pea aphid, *Acyrthosiphon pisum*

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In animals, inducible morphological defences against natural enemies mostly involve structures that are protective or make the individual invulnerable to future attack. In the majority of such examples, predators are the selecting agent while examples involving parasites are much less common. Aphids produce a winged dispersal morph under adverse conditions, such as crowding or poor plant quality. It has recently been demonstrated that pea aphids, *Acyrthosiphon pisum*, also produce winged offspring when exposed to predatory ladybirds, the first example of an enemy-induced morphological change facilitating dispersal. We examined the response of *A. pisum* to another important natural enemy, the parasitoid *Aphidius ervi*, in two sets of experiments. In the first set of experiments, two aphid clones both produced the highest proportion of winged offspring when exposed as colonies on plants to parasitoid females. In all cases, aphids exposed to male parasitoids produced a higher mean proportion of winged offspring than controls, but not significantly so. Aphid disturbance by parasitoids was greatest in female treatments, much less in male treatments and least in controls, tending to match the pattern of winged offspring production. In a second set of experiments, directly parasitised aphids produced no greater proportion of winged offspring than unparasitised controls, thus being parasitised itself is not used by aphids for induction of the winged morph. The induction of wing development by parasitoids shows that host defences against parasites may also include an increased rate of dispersal away from infected habitats. While previous work has shown that parasitism suppresses wing development in parasitised individuals, our experiments are the first to demonstrate a more indirect influence of parasites on insect polyphenism. Because predators and parasites differ fundamentally in a variety of attributes, our finding suggests that the wing production in response to natural enemies is of general occurrence in *A. pisum* and, perhaps, in other aphids.

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Inducible defences are known from a variety of animals and plants (Schultz 1988, Harvell 1990, Tollrian and Harvell 1999). Such defences are likely to be of value if attack by natural enemies is spatially or temporally intermittent, the defence is costly and natural enemies can be perceived as a non-fatal cue (Levins 1968, Maynard-Smith 1972, Lloyd 1984, Riessen 1992, Frank 1993, Adler and Karban 1994, Astrom and Lundberg 1994, Padilla and Adolph 1996). Most examples come from plant-herbivore or predator-prey systems (Tollrian and Harvell 1999). Inducible defences also play a role in the interaction between parasites and their hosts, the majority of examples being immune responses to parasite attack (Carton and Nappi 1997, Grundler et al. 1997, Jarosz and Glinski 1997, Frost 1999). However, induced changes in morphology, which are frequently reported from plant-herbivore and predator-prey interactions, are virtually unknown from host-parasite systems.

One simple defensive measure which can be taken against natural enemies is to escape from them. This is often a simple behavioural response allowing escape from the immediate vicinity of a natural enemy. Recently, it has been demonstrated that such escape responses can also involve the development of a specialised dispersal morphology, under conditions analogous for the development of other types of induced defences. When pea aphids, *Acyrthosiphon pisum* (Harris), were exposed to predatory ladybirds or products thereof, individuals increased the proportion of winged morphs among their offspring (Dixon and Agarwala 1999, Weisser et al. 1999). *Acyrthosiphon pisum*, like many aphids, can produce a wingless, non-dispersive morph or an winged dispersal morph during asexual reproduction: production of winged offspring had previously been shown to be induced by crowding and low plant quality for *A. pisum* and many other aphid species (Lees 1967, Sutherland 1967, 1969a, b, Forrest 1970, Shaw 1970, Dixon and Glen 1971, MacKay and Lamb 1996). The aphid dispersal morph is itself costly to produce, since winged morphs take longer to develop than apterous individuals and are less fecund (Noda 1960, MacKay and Wellington 1975, Wratten 1977, Dixon 1998) and, additionally, dispersal itself is undoubtedly costly (Ward et al. 1998). There is also some evidence of both spatial and temporal variation in the occurrence of the numerous natural enemies of aphids (Weisser 2001). Increased production of winged morphs by *A. pisum* in response to ladybird predators, constituted the first demonstration of the production of a dispersal morph in response to natural enemies, and provides one of the few examples of an enemy-induced morphological change in a terrestrial ecosystem.

For many insects including aphids, parasitic Hymenoptera are major natural enemies (Godfray 1994, Quicke 1997). These parasitoids differ from predators in their mode of action in a number of important ways, in particular in their effect on host population dynamics and their interaction with host physiology (Hassell 1978, Godfray 1994, Quicke 1997). In aphids attacked by parasitoids, individuals are not killed immediately and can continue to reproduce for a period after parasitism (Starý 1970). Because of the short generation times of aphid parasitoids, extinction of aphid colonies can occur on a very short timescale (Tamaki et al. 1970, Völkl 1992, Weisser 2000).

Because of the advantages of dispersing from infected colonies, we hypothesised that aphids respond to the presence of parasitoids with an increased production of winged morphs. We tested this hypothesis in two sets of experiments. In the first set of experiments, aphids were exposed as colonies on plants to the parasitoid *Aphidius ervi* Haliday. In the second set of experiments, we compared the proportion of winged offspring produced by aphids which has been experimentally parasitised with that of unparasitised aphids.

Materials and methods

Experimental animals and plants

Two clones of *A. pisum* were used in the experiments described: SG, a green clone from Sunningdale, England and BP, a red clone from Bayreuth, Germany. These clones were used by Weisser et al. (1999) in their experiments with ladybirds, and had been maintained in the laboratory since mid-1997. Aphids were reared and experiments conducted on a dwarf variety of the broad bean, *Vicia faba* L. (The Sutton, Nickerson-Zwaan Ltd., UK).

Aphidius ervi were mixed stock descended from two sources, wild-collected parasitoids collected from *A. pisum* in the vicinity of Bayreuth, Germany and parasitoids from a commercial supplier (Katz Biotech Services, Welzheim, Germany). They had been reared in the laboratory on *A. pisum* hosts for a number of generations.

Experiments with free ranging *Aphidius ervi* in aphid colonies

For the experiments, clones were maintained as a number of segregated "lines" maintained on separate plants. A line was used for one replicate per experiment, providing a single foundress aphid as ancestor to all the aphids within a matched replicate (Fig. 1). This foundress was placed on a plant for 1–2 days until she had given birth several times. Her progeny were grown on the plant, and 8–15 of these grandparents, depending on the experiment, were transferred to a new plant at the fourth instar or adult stage, remaining there until they had given birth to sufficient progeny (the parental aphids) for use in a single experimental replicate (typically after 3 days). At the late fourth instar or early adult stage, the parental aphids, all the grandchildren of a single aphid and reared on the same plant, were divided and used for control and experimental treat-
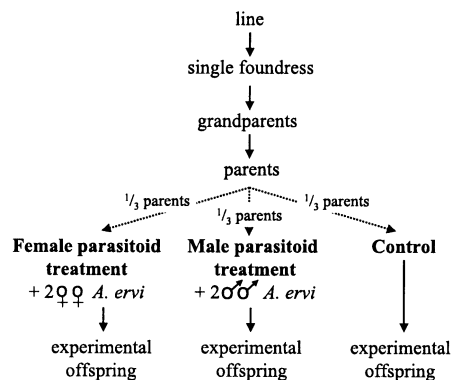

Fig. 1. Experimental approach for each replicate in experiments with free ranging *A. ervi* in aphid colonies.

Fig. 2. Diagrammatic summary of the three experiments with free ranging *A. ervi* in aphid colonies, to illustrate the differences between experiments. Aphids remained on the same plant for the entire duration of a single horizontal arrow (↔), different arrows indicate when aphids were moved to a new plant. Thus for experiment 1B, aphids remained on the same plant from the beginning of day 1 until the end of day 3 and were then moved to a new plant. A parasitoid shape (🐝) above an arrow indicates that parasitoids were present in the male and female parasitoid treatments for the entire duration of the time for which aphids were on the plant. Similarly, a crossed-out parasitoid (✳) indicates that parasitoids were not present in these two treatments. A vertical arrow (↑) indicates that dropping behaviour was recorded.

Experiment 1A: clone SG
25 last instars /adults per plant
(offspring of 15 grandparents).
Dead parasitoids replaced on day 4.

Experiment 1B: clone SG
15 young adults per plant
(offspring of 10 grandparents).
Dead parasitoids replaced beginning of day 4.

Experiment 2: clone BP
15 last instars per plant
(offspring of 8 grandparents).
Dead parasitoids replaced beginning of days 3 and 5.
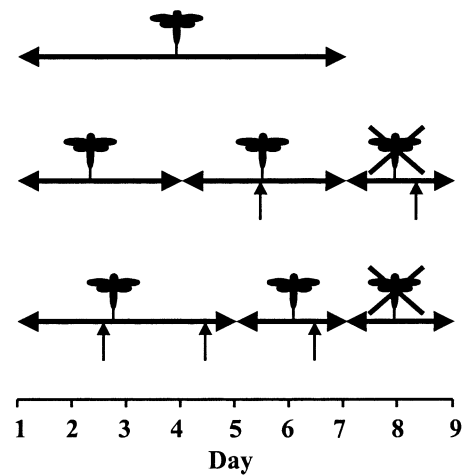
ments (Fig. 1). A split brood design, using aphids of the same ages, was thus used.

Within each replicate, plants were matched for age and size. All rearing and experiments were conducted on individual potted bean plants (pot diameter 10 cm) within air-permeable cellophane bags (18.5 × 39 cm). During the preparative and experimental periods all plants were kept at 20 ± 1°C and 75% R.H., although some plants with offspring were transferred to a greenhouse at non-constant temperature. The matched nature of the experiments and the fact that *A. pisum* wing morph determination occurs before birth (Sutherland 1969a) ensure that no bias was introduced into our results by this procedure. All grandparental and parental aphids were unwinged.

For the experiment, bagged plants with the parental aphids were randomly allocated to one of three treatments within each replicate: control, male parasitoid treatment or female parasitoid treatment. For each of the parasitoid treatments, two parasitoids, both male or both female depending upon treatment, were added to the bag. Two CO_2-anaesthetised parasitoids were placed together in a 0.5 cm^3 gelatine capsule. When they became active, they were introduced into the bag confined in one half of the capsule. The parasitoids were able to escape into the bag from the capsule's open end. To control for disturbance to the aphids, in control treatments an empty half gelatine capsule was placed in the bag. Bags were periodically checked for parasitoids and, if parasitoids were found to be dead they were replaced

in further gelatine capsules. In such cases empty gelatine capsules were added to the other treatments within a replicate.

Three experiments were carried out (Fig. 2):

Experiment 1A
Wing induction was tested in clone SG. Parental aphids were left on the plants for 6 days.

Experiment 1B
Wing induction was again tested with clone SG. In this experiment, aphids were moved between plants at the beginning of days 4 and 7, in order to avoid overcrowding of the offspring, which had occurred in Experiment 1A. When the aphids were moved the second time, the parasitoids were removed, in order to examine the possibility that the presence of parasitised adults might induce the production of winged offspring in other adults or that parasitised adults themselves might produce more winged offspring.

Experiment 2
Wing induction was tested in clone BP. It was originally intended to move parental aphids to new plants every two days, since BP exhibits a much stronger wing-induction response to crowding than SG (Weisser and Braendle 2001). However, on day 3, it was found that numbers of aphid offspring were low, and as a consequence aphids were only moved to new plants at the beginning of day 5, along with para-

sitoids, as well as at the beginning of day 7, without parasitoids.

While experimental offspring were growing to adulthood, a number of plants became stressed and began to die as a consequence of the numbers of aphids on them. If this occurred within a replicate, aphids were transferred to new plants in all treatments for that replicate. This was achieved by cutting the old plant at the base, with the aphids on it, and transferring it to a new pot with a fresh plant. Since winged offspring are maternally induced (Sutherland 1969a), this should not have affected the proportion of winged offspring produced. Any aphids which fell off the plant during this process were also transferred using a paintbrush. At adulthood, all experimental offspring were harvested from the plant and frozen for later counting.

Numbers of offspring and the proportion of winged offspring were determined for all groups of offspring in all experiments. For the female parasitoid treatments of Experiments 1B and 2, the number of dead parasitised aphid-mummies were counted, but these not used in obtaining the proportion of winged individuals, since wing production is often suppressed in parasitised aphid nymphs (Christiansen-Weniger and Hardie 1998, 2000). At counting, the morph of any remaining nymphs was determined under a binocular microscope. Winged fourth instar *A. pisum* are easily differentiated from unwinged fourth instars by the occurrence of wing buds.

In experiments 1B and 2, the extent to which aphids dropped from plants in the male and female parasitoid treatments and in the control was determined. The dropping behaviour of *A. pisum* in response to natural enemies (see Losey and Denno 1998), or its consequences, might potentially be related to the production of winged offspring, since dropped aphids receive tactile stimulation in a similar manner to aphids which are crowded (Sutherland 1969a, Weisser et al. 1999, Weisser 2001, Sloggett and Weisser in press). A simple measure of dropping behaviour is the instantaneous number of aphids which are not on the plant. Although this does not measure aphid dropping in the strict sense, since many dropping aphids will fall from one part to another part of the plant, it is likely to be proportional to the amount of dropping occurring. Thus the plants were examined in situ and aphids which were walking in the pot or on the bag were counted for each plant for all treatments and replicates. Plants were not moved prior to or during counting to avoid any disturbance to the aphids on the part of the experimenter. In Experiment 1B dropping behaviour in clone SG was examined on day 5, when the parasitoids were present and on day 8, when they were absent. In Experiment 2, measures of BP clone dropping behaviour were taken every two days whilst the parasitoids were present.

Do aphids use parasitism of the self as a cue for the production of winged offspring?

One possible cue that aphids could use for the production of winged offspring would be if they themselves were parasitised. Under such conditions it is likely to be adaptive for the remaining offspring produced by the female after parasitism to be winged. Two similar experiments, using SG and BP, were carried out to test this hypothesis.

For each replicate, a single adult foundress was placed on a plant for one to two days until it had produced a few offspring. These offspring were then reared until the fourth instar on the plant. For each replicate one of these nymphs was then parasitised by placing it in a gelatine capsule with a female parasitoid until the parasitoid had struck it with its ovipositor. Only one assumed oviposition per aphid was allowed: in a few cases parasitoids were able to attack the aphid more than once. Another nymph from the same plant was placed in a similar gelatine capsule with no parasitoid for the same length of time.

These aphids were then transferred to separate plants and allowed to produce offspring until the parasitised aphid mummified or until their first offspring showed signs of reaching adulthood. Parasitism status was recorded in all cases as were the numbers of offspring and proportion of winged offspring for each aphid. For both SG and BP, in one paired replicate the growing offspring had to be transferred to new plants before adulthood.

Statistical analysis

Statistical analysis was carried out using SPSS 10.0 for Windows. Analysis was for related samples, due to the split brood experimental design, using matched treatments in each replicate (except in the case of BP dropping behaviour: see below). Nonparametric statistics were used in the analysis of proportions of winged offspring (Siegel and Castellan 1988), as winged morph production data was not normally distributed. The Friedman ranking test for related samples, with post hoc comparisons, was used for analysing the proportion of winged offspring produced in experiments 1A, 1B and 2. Data for total offspring production in these experiments was analysed using Analysis of Variance, with treatment comprising a fixed factor and line as a random factor: a Student-Newman-Kreuls post hoc test was carried out on significant data (Zar 1999). Dropping behaviour, data for which was also not normally distributed, in Experiment 1B was also analysed using Friedman tests. However, for BP, it was found that in a number of cases both parasitoids had died when measures of dropping behaviour were taken in parasitoid treatments. Since data from these replicate treatments was excluded it was not possible to produce an
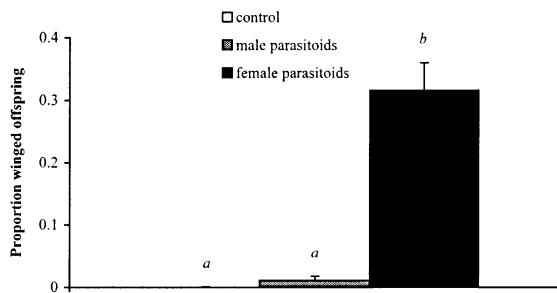
Fig. 3. Proportion of SG clone winged offspring in experiment 1A. For all data, n = 10 matched replicates. Data with the same letter does not differ significantly under the Friedman post hoc test.

analysis for related samples. Thus, a Kruskal-Wallis test for unrelated samples was used for analysing BP dropping data.

For experiments testing whether aphids use parasitism of the self as a cue for the production of winged offspring, the proportion of winged offspring produced was analysed using the Wilcoxon signed ranks test for paired data (Siegel and Castellan 1988, Zar 1999). The Wilcoxon test was also used to compare total offspring production in the experiment with BP, where it was suspected such data was not normally distributed. For the experiment with SG a paired t-test was used to compare total offspring production data. Tests were two-tailed.

Results are presented as mean $\pm$ standard error in all cases.

Results

Experiments with free ranging *Aphidius ervi* in aphid colonies

Experiment 1A
The number of offspring recorded reaching adulthood

was lowest in the female parasitoid treatment; there was also a significant effect of line (control, 365.9 ± 23.7; male parasitoids 376.3 ± 39.0; female parasitoids, 181.5 ± 38.7. ANOVA: treatment, $F = 20.0$, 2 df, $P < 0.001$, post hoc test: female treatment differs from other two treatments; line, $F = 4.0$, 9 df, $P = 0.006$) although this does not necessarily imply that the initial number of offspring was lower, as aphid-mummies were not included in counts. The proportion of winged offspring was significantly higher in the female parasitoid treatment than in either of the other two treatments, with the other two treatments not differing significantly from each other (Friedman test, $F_r = 18.2$, 2 df, $P < 0.001$; Fig. 3).

Experiment 1B
For all periods, when parasitoids were present in experimental treatments, the numbers of aphid offspring produced were significantly lower in the female parasitoid treatment. This was the case despite parasitised aphid offspring being included in counts, in contrast to experiment 1A, and in spite of the numbers of remaining adults on the plants trending in the opposite direction (Table 1). There was also a significant effect of line, except on days 7–8 (Table 1). The lower numbers of offspring in the female parasitoid treatments are probably linked to lowered fecundity of parasitised parental aphids. In the female treatments, parasitism rate of offspring, determined when the majority of aphids would be expected to have mummified, was $24.6\% \pm 7.3\%$ (range 0.0%–73.6%) on days 1–3 and $52.8\% \pm 6.3\%$ (range 16.3%–89.4%) on days 4–6. Four female replicates from days 1–3 were unparasitised, possibly due to early death of the parasitoids (parasitism rate with these replicates excluded: $36.8\% \pm 7.8\%$).

In both periods when parasitoids were present, a significantly higher proportion of winged offspring occurred in the female parasitoid treatments than in the

Table 1. Numbers of SG clone offspring obtained in experiment 1B, with analysis of variance, and numbers of parental aphids remaining at each stage of the experiment. For all data n = 12 paired replicates. Within each experimental period data with the same superscript letter does not differ significantly under the Student-Newman-Keuls post hoc test.

|	Treatment	Adults	Offspring	Analysis of variance
Days 1–3	Control	15.0 ± 0.0	361.2 ± 12.0^a	Treatment: $F = 7.5$, 2 df, $P = 0.003$
	Male parasitoids	15.0 ± 0.0	349.1 ± 12.7^a	Line: $F = 6.7$, 11 df, $P < 0.001$
	Female parasitoids			
	– unparasitised		244.4 ± 29.1	
	– parasitised		74.0 ± 21.2	
	– total	15.0 ± 0.0	318.4 ± 16.1^b	
Days 4–6	Control	14.8 ± 0.1	380.2 ± 7.0^a	Treatment: $F = 24.5$, 2 df, $P < 0.001$
	Male parasitoids	14.8 ± 0.1	375.7 ± 9.5^a	Line: $F = 2.3$, 11 df, $P = 0.045$
	Female parasitoids			
	– unparasitised		145.1 ± 24.1	
	– parasitised		141.5 ± 14.9	
	– total	15.0 ± 0.0	286.6 ± 18.8^b	
Days 7–8	Control	14.6 ± 0.7	232.5 ± 7.7^a	Treatment: $F = 7.0$, 2 df, $P = 0.004$
	Male parasitoids	14.6 ± 0.7	227.0 ± 4.9^a	Line: $F = 1.2$, 11 df, NS
	Female parasitoids	14.9 ± 0.3	182.5 ± 16.2^b	
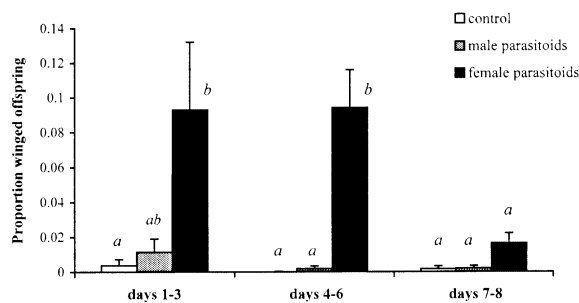
Fig. 4. Proportion of SG clone winged offspring over the three experimental periods of experiment 1B. For all data n = 12 matched replicates. Within each time period data with the same letter does not differ significantly under a Friedman post hoc test.

controls (Fig. 4; Friedman test, days 1–3: $F_r = 9.9$, 2 df, $P < 0.01$; days 4–6: $F_r = 20.2$, 2 df, $P < 0.001$). In neither case did the male treatment differ significantly from the control, although on days 1–3 it did not differ from the female treatment either. A Friedman test indicated significant heterogeneity on days 7–8, after the parasitoids had been removed, although no significant differences were found in post hoc comparisons (Fig. 4; Friedman test, $F_r = 8.6$, 2 df, $P < 0.05$).

Experiment 2

Numbers of aphid offspring, parasitism and partial immunity of clone BP to A. ervi: Numbers of aphid offspring produced were significantly lower in the female parasitoid treatment, with parasitised aphids included, for all three time periods (Table 2). Again there was a significant effect of line, except on days 7–8. Numbers of adult aphids remaining also decreased most rapidly in the female parasitoid treatment.

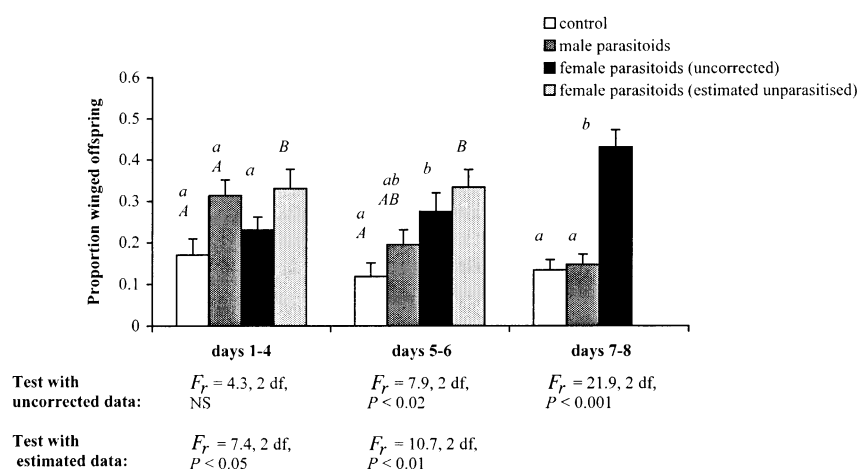In the female treatments, the mean numbers of offspring which had turned into mummies was 33.1% ±

5.8% (range 1.1%–79.7%) on days 1–4 and 18.7% ± 4.2% (range 0.0% to 58.8%) on days 5–6. A number of differences were observed in the nature of parasitism in BP relative to that in SG. Most BP parental adults were still alive at the end of day 8 whereas in the experiments with SG, many had begun to mummify at this time. Some of these adults were kept for a further week with no sign of parasitism. Upon dissection they were found to exhibit signs of parasitism (few or no embryos) or to contain poorly developed parasitoid larvae. BP offspring often developed into small adults before turning into mummies, whereas for parasitised SG nymphs, this typically occurred at the fourth instar stage (see also Pennacchio et al. 1995). Some BP mummies did not possess the typically hard covering and still contained unpupated parasitoid larvae after several days. Upon dissection, many live unmummified offspring adults were also found to contain poorly developed parasitoid larvae. It is known that the production of wings is often suppressed in parasitised aphid nymphs (Christiansen-Weniger and Hardie 1998, 2000). Thus the presence of large numbers of still-living parasitised adults potentially could lead to a gross underestimate of the number of winged offspring in the female parasitoid treatment.

To counter this effect an additional estimate of the proportion of winged offspring was made, in addition to the "uncorrected" measure using all live unmummified offspring. For each replicate, samples of unwinged adults, unwinged fourth instar nymphs and winged individuals (adults and fourth instars; no young instars were present) were dissected to obtain an estimate of the proportion of live individuals which were still parasitised. At least 20 aphids were dissected for each unwinged sample, with all individuals being dissected if there were less than 20, as was frequently the case with nymphs. 10 aphids were dissected for winged

Table 2. Numbers of BP clone offspring obtained in experiment 2, with heterogeneity analysis, and numbers of parental aphids remaining at each stage of the experiment. For days 1–6, n = 17 matched replicates; for days 7–8, n = 16 matched replicates (one replicate died before reaching adulthood). Within each experimental period data with the same superscript letter does not differ significantly under the Student-Newman-Keuls post hoc test.

	Treatment	Adults	Offspring	Analysis of variance
Days 1–4	Control	15.0 ± 0.0	268.9 ± 7.5[a]	Treatment: $F = 37.0$, 2 df, $P < 0.001$
	Male parasitoids	15.0 ± 0.0	263.5 ± 8.4[a]	Line: $F = 3.4$, 16 df, $P = 0.001$
	Female parasitoids			
	– "unparasitised"		133.8 ± 14.5	
	– parasitised		62.6 ± 10.4	
	– total	15.0 ± 0.0	196.5 ± 10.6[b]	
Days 5–6	Control	14.6 ± 0.1	297.2 ± 7.2[a]	Treatment: $F = 19.7$, 2 df, $P < 0.001$
	Male parasitoids	14.6 ± 0.1	294.9 ± 8.5[a]	Line: $F = 3.7$, 16 df, $P = 0.001$
	Female parasitoids			
	– "unparasitised"		198.1 ± 15.9	
	– parasitised		46.4 ± 9.7	
	– total	13.9 ± 0.3	241.8 ± 12.6[b]	
Days 7–8	Control	14.5 ± 0.2	253.6 ± 9.7[a]	Treatment: $F = 42.8$, 2 df, $P < 0.001$
	Male parasitoids	14.5 ± 0.1	248.6 ± 5.2[a]	Line: $F = 1.3$, 15 df, NS
	Female parasitoids	13.2 ± 0.4	152.3 ± 11.5[b]	

Fig. 5. Proportion of BP winged offspring over the three experimental periods of experiment 2. For days 1–6, n = 17; for days 7–8, n = 16 (one replicate died before reaching adulthood). Data with the same uppercase or lowercase letter does not differ significantly under a Friedman post hoc test. Lowercase letters refer to comparisons using all adult aphids. Uppercase letters refer to comparisons using unparasitised estimates obtained from dissection.

	days 1-4	days 5-6	days 7-8
Test with uncorrected data:	$F_r = 4.3$, 2 df, NS	$F_r = 7.9$, 2 df, $P < 0.02$	$F_r = 21.9$, 2 df, $P < 0.001$
Test with estimated data:	$F_r = 7.4$, 2 df, $P < 0.05$	$F_r = 10.7$, 2 df, $P < 0.01$	

samples. Individuals were scored as parasitised if a parasitoid larva was found during dissection: as such the estimate remains conservative since some individuals had undoubtedly been parasitised but the larva was no longer discernible. These parasitism frequency estimates were then used to estimate the number of unparasitised aphids in each class for each replicate and thus to estimate winged individuals as a proportion of unparasitised aphids, using the formula

$$E_W = \frac{N_W U_W}{(N_W U_W + N_{UW} U_{UW} + N_N U_N)}$$

where E_W is the estimated proportion of winged offspring, N_W, N_{UW} and N_N are the total numbers of winged individuals, unwinged adults and unwinged nymphs respectively and U_W, U_{UW} and U_N are respectively the proportion of dissected winged individuals, unwinged adults and unwinged nymphs which were unparasitised. Use of E_W eliminates the bias introduced in uncorrected estimates through the presence of large numbers of unwinged parasitised aphids, being an estimate of the proportion of winged offspring in the unparasitised fraction.

Parasitism in the dissected unwinged adults was 37.8% ± 5.4% on days 1–4 and 26.5% ± 5.8% on days 5–6, and in unwinged nymphs it was 72.2% ± 6.5% on days 1–4 and 49.8% ± 5.4% on days 5–6. Of a total of 313 adults and nymphs of the winged morph dissected, only one was found to be parasitised, supporting the assertion that parasitised nymphs typically develop into unwinged adults.

Production of winged offspring by clone BP

For days 1–4 there was no significant heterogeneity between treatments using the uncorrected estimate (Fig. 5; Friedman test, $F_r = 4.3$, 2 df, NS). However, with the unparasitised estimate for the female parasitoid treatment, there were significantly more winged offspring in this treatment than in the other two treatments (Fig. 5; Friedman test, $F_r = 7.4$, 2 df, $P < 0.05$). On days 5–6, the female parasitoid treatment also gave rise to more winged offspring, whether an unparasitised estimate or the original uncorrected estimate was used in comparisons (Friedman test, with uncorrected data, $F_r = 7.9$, 2 df, $P < 0.02$; with unparasitised estimates, $F_r = 10.7$, 2 df, $P < 0.01$). On days 7–8, after the parasitoids had been removed, the production of winged offspring remained significantly higher in the female parasitoid exposed aphids than in the other two treatments (Fig. 5; Friedman test, $F_r = 21.9$, 2 df, $P < 0.001$).

For the male treatment, on days 1–4, the mean proportion of winged individuals produced was close to that of the female treatment unparasitised estimate (Fig. 5), although under the median-based Friedman ranking analysis this groups with the much lower control treatment. For days 5–6 the male parasitoid treatment did not differ from either the female treatment or the control (Fig. 5). There was no difference between male treatment and control on days 7–8.

Aphid dropping behaviour in experiments 1B and 2

Differences were found between treatments in the numbers of aphids off the plants, in both experiments with SG and BP, when parasitoids were present (Table 3). The greatest numbers of aphids off the plants occurred in the female treatments. In the male treatments there were considerably fewer, but more than the controls. This pattern is discernible in all data sets, although not all post hoc comparisons exhibit significant differences and on day 6 of Experiment 2, the difference was not significant (Table 3). There was no evidence of any heterogeneity in numbers of aphids off the plants in any of the three treatments when this was recorded in the

Table 3. Dropping behaviour in experiments 1B and 2, with heterogeneity analysis. Within each experimental period data with the same superscript letter does not differ significantly under the Friedman post hoc test. * no parasitoids were present in any of the treatments.

		Treatment	N	No. aphids off plant	Significance test
SG	Day 5	Control	12	0.83 ± 0.41^a	Friedman test:
		Male parasitoids	12	1.08 ± 0.38^a	$F_r = 7.7$, 2 df, $P < 0.05$
		Female parasitoids	12	2.58 ± 0.66^a	
	Day 8*	Control	12	0.25 ± 0.18^a	Friedman test:
		Male parasitoids	12	0.08 ± 0.08^a	$F_r = 0.7$, 2 df, NS
		Female parasitoids	12	0.17 ± 0.17^a	
BP	Day 2	Control	17	0.0 ± 0.0^a	Kruskal-Wallis test:
		Male parasitoids	14	0.07 ± 0.07^{ab}	$KW = 17.5$, 2 df, $P < 0.001$
		Female parasitoids	8	2.00 ± 0.85^b	
	Day 4	Control	17	0.12 ± 0.08^a	Kruskal-Wallis test:
		Male parasitoids	14	0.29 ± 0.13^{ab}	$KW = 12.5$, 2 df, $P < 0.005$
		Female parasitoids	17	1.24 ± 0.41^b	
	Day 6	Control	17	0.24 ± 0.14^a	Kruskal-Wallis test:
		Male parasitoids	12	0.33 ± 0.26^a	$KW = 0.7$, 2 df, NS
		Female parasitoids	14	0.50 ± 0.29^a	

absence of parasitoids at the beginning of day 7 of the SG experiment. In Experiment 2 (BP clone) there is some indication of a decline in numbers of aphids dropping off the plant as time progresses, possibly due to habituation (Table 3): as already noted, on day 6 no significant heterogeneity is found in the data, unlike days 2 and 4, although parasitoids were still present at this time.

Do aphids use parasitism of the self as a cue for the production of winged offspring?

In 16 of 18 replicates the parasitised adult SG aphids developed into mummies. In the other two replicates, the adult aphids were apparently unparasitised and continued to produce offspring. The 16 parasitised experimental replicates produced 12.7 ± 1.6 offspring compared to the control replicates' 38.1 ± 2.3 offspring (paired t-test, 15 df, $t = 13.0$, $P < 0.001$). However, of the total 203 offspring of parasitised individuals and 611 offspring of unparasitised individuals, none had wings.

In BP none of the adults had developed into mummies by the time the first offspring were reaching adulthood. As a consequence, all individuals were removed at this point and dissected. Of the 23 replicates, only 5 adults in the parasitoid treatment were found to contain parasitoid larvae.

Because of BP clone immunity to *A. ervi* (see Experiment 2), it was thus necessary to assume that all experimental adults were initially successfully parasitised and thus to use all offspring data. Because it was unclear how many "parasitised" individuals were truly parasitised, and thus our experimental offspring comprised a mixture of offspring of parasitised and unparasitised aphids, we used a non-parametric Wilcoxon test

to compare total number of offspring of experimentally parasitised individuals with controls, as well as proportion of winged offspring produced.

Experimentally parasitised offspring produced fewer offspring (57.9 ± 2.6 offspring relative to a control figure of 66.7 ± 1.37. Wilcoxon test, N = 22 non-ties + 1 tie, $T^+ = 209$, $T^- = 44$, $P < 0.01$). These offspring did not differ significantly in the proportion of winged individuals they contained, although there is a trend for unparasitised individuals to produce more winged offspring (Fig. 6; Wilcoxon test, N = 15 non-ties + 8 ties, $T^+ = 90$, $T^- = 30$, $0.10 < P < 0.05$). No obvious trend is observed if only the five replicates in which the adults were obviously parasitised when dissected are considered (2 control > parasitised, 1 parasitised > control, 2 ties).

Discussion

Since the first description of inducible spines in rotifers in the 1950s (de Beauchamp 1952), a wide range of inducible defence systems have been described. It is clear now, that inducible defences are quite widespread
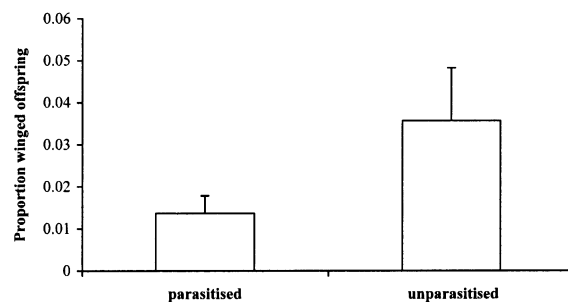

Fig. 6. Proportion of BP winged offspring obtained from parasitised and unparasitised aphids, in experiment 3.

and that more cryptic defences become visible as systems are increasingly well-studied (Tollrian and Harvell 1999). Our experiments show that in the presence of parasitoids, the pea aphid produces winged offspring that can leave a plant to escape from infected colonies. The results presented here are interesting for a number of reasons. First, this host response involves a change in morphology; second, it is an example of dispersal benefiting the host rather than the parasite. Finally, because winged offspring production in directly infected aphids is not increased, the effect of the parasitoid must be indirect.

Aphid natural enemies differ widely in their mode of action. For example, adult ladybirds, in most cases, leave a plant before all aphids have been eaten (Minoretti and Weisser 2000) whereas the predatory stages of other taxa such as syrphids or gallmidges are less mobile (Markkula et al. 1979, Chambers 1988). Because feeding behaviour, prey handling and the effect on aphid population dynamics differ among aphid predators, it is difficult to generalise the prey responses found with one aphid predator to other aphid predators. Thus, the finding that pea aphids produce winged offspring when confronted with a foraging adult ladybird does not necessarily allow us to draw firm conclusions about the generality of this response. Because the differences between parasitoids and predators are greater than those between different types of predators, however, it does now seem probable that the production of dispersive winged offspring is a general response of *A. pisum* to natural enemies.

The results presented here are conservative with respect to crowding and plant quality, other factors known to cause the production of winged offspring by *A. pisum*. Furthermore, it is improbable that the results could be explained by a parasitoid preference for parasitising unwinged morph nymphs: in the majority of SG clone controls no winged offspring were present at all, whereas winged offspring were present in nearly all the female parasitoid treatments. Even were the proportion of winged offspring to be inflated by parasitoid oviposition preferences, the actual occurrence of winged offspring in the female treatments cannot result from this phenomenon.

Our results provide a laboratory demonstration that winged offspring can potentially be produced in response to exposure to parasitoids. A strong effect of female parasitoid presence was already seen after three days of exposure, the first time when the effects were assessed. Nevertheless, in our experiments aphids were exposed to parasitoids in a confined space for several days, while in the field many aphid parasitoids often do not stay in a single aphid colonies for a prolonged period (Mackauer and Völkl 1993). Thus, a field experiment would be desirable to show the frequency at which such an effect is observed under natural conditions.

Aphids are known to disperse to new host plants as a consequence of a number of factors. In addition to host plant changes to complete the life-cycle, dispersal often occurs in response to adverse local conditions. When plants are overcrowded or plant quality is declining, the costs for an aphid clone to produce winged morphs that face a high risk of mortality probably outweigh those of staying at the current host plant. Similarly, in order for natural-enemy induced dispersal to be an adaptive strategy, the costs of dispersal have to be offset by the benefits. In this context, it would be necessary that dispersers have a high-enough chance of finding plants where the impact of natural enemies is significantly lower and thus the conditions for future reproduction are better (Weisser 2001). At present, very little is known about spatial variability in the risk posed by natural enemies (Weisser 2001, Weisser et al. in press).

The increased "background" level of winged aphids observed in the BP clone relative to SG is typical of this morph (Weisser et al. 1999, Weisser and Braendle 2001). A complicating factor in experiments with BP was the unexpected finding that this morph is apparently partially immune to *A. ervi*. Parasitised aphids typically develop into unwinged adults (Christiansen-Weniger and Hardie 1998, 2000), but dissection of aphids to estimate the number of still living parasitised adults provides a way around this problem. However, it is worth noting that on days 4–5, even with an uncorrected estimate, the proportion of winged individuals in the female parasitoid treatment is higher than in the control. The results are equivocal for males in all cases. Although in all experiments, with males the mean proportion of winged offspring was higher than that of the control, in no case was it unequivocally different.

It is clearly not the case that parasitism itself is the cue used by *A. pisum* for the production of winged offspring. No winged offspring were produced by parasitised SG clone aphids and there were no significant differences in the proportion of winged offspring produced by the BP clone aphids which had been attacked by parasitoids. The possibility that the presence of other parasitised individuals might be used as a cue for wing-induction cannot be ruled out. It is also consistent with the differences in the production of winged offspring between the male and female parasitoid treatments. With BP (experiment 2) and, more equivocally, with SG (experiment 1B) the proportion of winged offspring remained highest in the female parasitoid treatment, even after the parasitoids were removed. However, the results are not conclusive: it is equally possible that the effect of previous exposure to parasitoids might last for several further days after the parasitoids are removed. Unequivocal testing of this hypothesis would involve first parasitising aphids, as in experiment 3, and then placing them on a plant with unparasitised individuals. This would exclude the possi-

bility that aphids are continuing to respond to the occurrence of parasitoids present several days earlier.

It seems possible that production of winged morphs is mechanistically related to the dropping behaviour of *A. pisum* in the presence of natural enemies, although the pattern supporting the hypothesis here remains correlative. This dropping behaviour, as measured by the instantaneous number of aphids off the plant, was greatest in female parasitoid treatments, considerably less in male parasitoid treatments and least in controls. This closely matches the pattern of winged offspring production. How might the two be related? There are two possible non-exclusive mechanisms through which dropping and wing-induction might be linked. Dropping aphids are continuously interrupted during feeding: the mechanism could thus act through resource deprivation, analogous to that which occurs on poorer quality plants (see Sutherland 1967, 1969b but see also Sutherland 1969a). Alternatively, tactile stimulation analogous to that the aphids receive when crowded might occur as the aphids climb back onto the plant after dropping (Sutherland 1969a, Weisser et al. 1999, Weisser 2001, Sloggett and Weisser in press).

In host-parasite systems, the vast majority of known inducible defences involve complex immunological responses after invasion of the parasite. The finding that the presence of parasitoids in an aphid colony influences polyphenism emphasises that host responses to parasite attack can take a variety of forms. Because wing polyphenisms are common in many insect taxa (Zera and Denno 1997), future studies should seek to broaden our knowledge of the distribution of this induced response throughout the aphids, and perhaps even more importantly in other wing-dimorphic insects.

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