



## Body colour and genetic variation in winged morph production in the pea aphid

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### Abstract

Aphids (Homoptera: *Aphidoidea*) produce a number of different phenotypes in their life-cycle, among which are winged (alate) and wingless (apterous) morphs. Lowe & Taylor (1964) and Sutherland (1969a, b) were the first to suggest that aphid clones differ in their propensity to produce the winged morph and that in the pea aphid (*Acyrtosiphon pisum* Harris), this propensity is linked to the colour of the phenotype. We tested for the occurrence of genetic variation in winged morph production by rearing individuals from red and green clones of pea aphid under wing-inducing (crowding) and control conditions, and scored the phenotypes of their offspring. Clones differed significantly in alate production and red clones produced on average a higher proportion of winged morphs than green clones. Importantly, however, there was considerable variation between clones of the same colour. Broad-sense heritabilities of winged morph production were 0.69 (crowding treatment) and 0.63 (control). Clones also differed in the number of offspring they produced. When exposed to the crowding stimulus, aphids deferred offspring production, resulting in a higher number of offspring produced in the crowding treatment than in the control.

### Introduction

Polyphenism, i.e., the production of two or more alternative phenotypes by a single genotype, is a characteristic feature of aphids (Dixon, 1998). During the phase of parthenogenetic reproduction, most species produce a number of morphologically different phenotypes, among which are winged (alate) and wingless (apterous) morphs. Winged morphs are able to migrate and to colonise new host plants, and generally have a longer developmental time and lower fecundity than wingless morphs (Zera & Denno, 1997; Dixon, 1998). This trade-off is fundamental to most wing polyphenic insects and is thought to maintain the polyphenism (Zera & Denno, 1997). In many species, winged morphs are produced only in response to deteriorating conditions, i.e. when individuals are crowded

or feed on bad-quality plants (Bonnemaison, 1951; Lees, 1966; Dixon, 1998). Apart from intraclonal morphological variation, aphids also exhibit interclonal variation in body colour (Börner, 1952; Müller, 1961; Miyazaki, 1987). Body colour often has a genetic basis and in a number of species, several distinct colour morphs can be observed (Miyazaki, 1987). In contrast to wing polyphenism, however, little is known about the adaptive significance of aphid body colour and the selective forces maintaining colour polymorphisms (e.g., Markkula, 1963; Miyazaki, 1987; Agawa & Kawata, 1995; Losey et al., 1997; Kerns et al., 1998).

In the pea aphid, *Acyrtosiphon pisum* Harris, clones are mostly red or green and this colour difference is under genetic control (Müller, 1961, 1962). Lowe & Taylor (1964) and Sutherland (1969a, b) were the first to suggest that in aphids, clones may differ

in their propensity to produce the winged morph, and that in the pea aphid this propensity is linked to the colour of the phenotype. Both Lowe & Taylor (1964) and Sutherland (1969a, b) studied a single red and a single green clone and found that the green clone produced fewer winged morphs than the red clone. Because of the low numbers of clones investigated, however, the authors could not rule out a chance association between body colour and the propensity to produce winged morphs. In more detailed studies, Lamb & MacKay (1979, 1983) applied a standardised crowding stimulus to a large number of pea aphid clones and showed the existence of genetic variation for the propensity of producing winged morphs, but did not test for differences among clones of different body colour.

In this study, we analysed variation in the propensity to produce winged offspring in seven clones of pea aphid to address the following questions: (1) Are there any genetic differences in winged morph production among clones? (2) Do clones differ in their plastic response to changed conditions, i.e. an increase in the degree of crowding? (3) Is the variation observed linked to the body colour of the clones?

## Materials and methods

We used three red and four green clones of *A. pisum*, originally descending from single parthenogenetic females collected in Central Europe. The origins of these clones were as follows: 'Bayreuth Green' (BG) and 'Bayreuth Red' (BP), collected 1997 in Bayreuth, Germany; 'London Green' (LG) and 'London Red' (LP), collected 1997 in Sunningdale near London, U.K.; 'Norwich Green' (NG) and 'Norwich Red' (NP), obtained 1997 from Prof. A.F.G. Dixon's Laboratory in Norwich, U.K.; 'Berkshire Green' (SG), collected 1996 in Silwood Park, Ascot, U.K. Aphids were reared on a dwarf form of broad bean, *Vicia faba* L. (variety The Sutton, Nickerson-Zwaan Ltd, Roswell, Lincolnshire LN7 6DT, UK) potted in a commercial growing medium (TKS<sup>®</sup> 2, Floragard VertriebsGmbH, D-26129 Oldenburg, Germany). To prevent mixing of clones, all aphids were caged by enclosing the host plant in a transparent cellophane bag (width 185 mm, length 390 mm, Armin Zeller GmbH, Aarwangenstr. 32, 4900 Langenthal, Switzerland). Aphids and plants were kept in constant temperature chambers under long-day conditions (L16:D8) at  $20 \pm 1^\circ\text{C}$ .

Aphids from all clones were reared for one generation at low density (5–10 individuals per plant) to avoid possible effects of unequal rearing conditions and grand-maternal effects on the outcome of the experiment. From these low density-culture, 18 apterous adults per clone were transferred singly to 18 bean plants. After 24 h, two new-born offspring from each adult were transferred as a pair to 18 new plants. On the day when all individuals had started to reproduce and their phenotype (winged or wingless) could be determined, all replicates with both aphids being apterous were used for the experiments ( $N=109$ ). One of these second generation individuals was exposed to a crowding treatment: the aphid was placed for 24 h in a petri-dish ( $\varnothing$  5 cm) together with five adult pea aphids of a differently-coloured clone (crowding treatment). Small pieces of filter paper soaked in water were added to the petri-dishes to avoid desiccation of the animals. The second aphid of each pair remained on the plant (control treatment). After 24 h individuals from the crowding and the control treatments were transferred to new plants and any offspring produced during this period were discarded. In the crowding treatment, hardly any offspring were produced in the petri-dishes. For the next 48 h adults were allowed to reproduce on the new plants. All offspring were reared to maturity to determine their phenotype (winged or wingless). In the pea aphid, winged and wingless morphs are determined just before birth (Sutherland, 1969a, b), so the phenotypes of these offspring were already determined when the individuals were transferred to new plants.

Data were analysed using the SAS statistical package (v.6.12, SAS Institute 1989). Percentages of winged offspring were arcsin-transformed for analysis. Data was analysed using two way nested ANOVA with the factor 'clone' nested in colour. The number of offspring produced by an individual was used as a weight variable. We used SAS procedure GLM with the option 'test' to let SAS calculate the appropriate error terms for the analysis.

Phenotypic variance,  $V_P$ , may be partitioned into environmental  $V_E$  and genetic components,  $V_G$ , such that  $V_P = V_E + V_G + \text{Cov}_{GE}$ . The genotype-environment covariance  $\text{Cov}_{GE}$  is zero in a randomised environment (Falconer, 1989). In clonal organisms,  $V_G$  can be estimated from the among-clone variance component, and  $V_E$  from the within-clone variance component. We used the SAS-procedure VARCOMP to estimate the variance components and

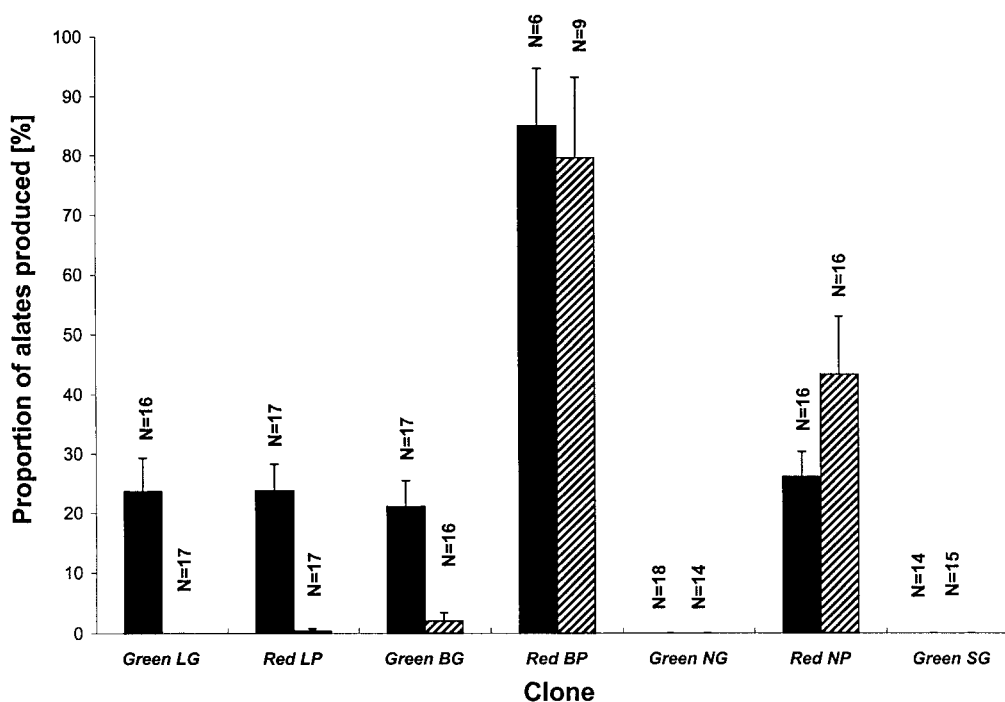


Figure 1. Proportion of winged morphs among offspring born during 48 h after treatment of different clones. Solid bars: aphids crowded for 24 hours in a petri-dish. Hatched bars: aphids that remained on plant during the 24 h (control). See text for explanations.

calculated broad-sense heritabilities ( $h^2 = V_G/V_P$ ) following Lynch & Walsh (1997).

## Results

Two green clones (NG and SG) produced no winged morphs either in the control treatment or the crowding treatment (Figure 1). One green clone (LG) produced winged morphs only when crowded while the remaining four clones produced winged morphs in both treatments. Both treatment and colour had a significant effect on the proportion of winged offspring produced (Table 1, Figure 1). The interaction between treatment and clone was significant, indicating that clones differed in their response to the treatments (Table 1). In the clone NP, aphids in the control produced a higher proportion of winged offspring than those in the crowding treatment, but the difference was not significant when a separate t-test on this data was performed ( $F_{1,30}=1.47$ ,  $P=0.2349$ ).

To test the response of clones to crowding directly, differences were calculated between the percentage of winged offspring in the crowding treatment and the percentage of winged offspring in the control, for each pair of aphids that descended from the same mother

and grew up on the same plant. As expected from the analysis presented above, there were significant differences between clones in their response to the crowding stimulus, but body colour was not significant (ANOVA with clone nested in body colour, Colour:  $F_{1,5.2} = 0.73$ ,  $P = 0.43$ , Clone[Colour]:  $F_{5,90} = 9.86$ ,  $P < 0.0001$ ,  $N = 97$  pairs of aphids for the seven clones analysed).

Broad-sense heritability estimates were 0.69 for the crowding treatment and 0.63 for the control, emphasising the presence of considerable genetic variation in winged offspring production in pea aphids.

Clones differed significantly in the number of offspring they produced but no effect of colour was found (Figure 2, Table 2). Control individuals produced fewer offspring than aphids exposed to the crowding stimulus (Figure 2, Table 2).

## Discussion

Lamb & MacKay (1979, 1983) were the first to systematically test for differences in winged offspring production between clones of pea aphid. Lamb & MacKay reared field-collected individuals for two generations under standard laboratory conditions be-

Table 1. Statistical analysis of arcsin-transformed percentage data (procedure GLM of SAS). The number of offspring produced by an individual was used as a weight variable

Source of variation	DF	Error term	F	P
Colour	1	$0.874 * MS [clone (colour)] + 0.126 * MS [Error]$	7.27	0.0426
Treatment	1	$0.882 * MS [clone * treatment (colour)] + 0.118 * MS [Error]$	2.09	0.2058
Clone (Colour)	5	$MS [clone * treatment (colour)]$	4.19	0.0708
Colour*Treatment	1	$0.874 * MS [clone * treatment (colour)] + 0.126 * MS [Error]$	0.24	0.6436
Clone*Treatment (Colour)	5	$MS [Error]$	8.31	0.0001
Error	194	$MS [Error] = 1.04$		

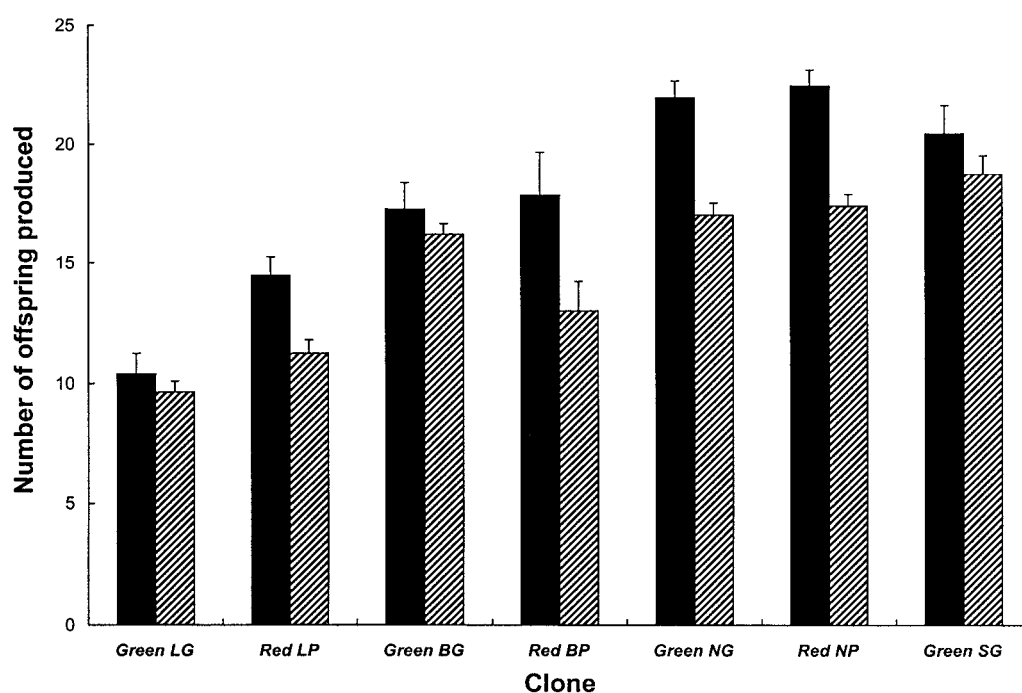


Figure 2. Number of offspring born during 48 h after treatment of different clones. Solid bars: aphids crowded for 24 h in a petri-dish. Hatched bars: aphids that remained on plant during the 24 h (control). Sample sizes as in Figure 1. See text for explanation.

Table 2. Statistical analysis of the number of offspring produced (data square-root transformed data, procedure GLM of SAS)

Source of variation	DF	Error term	F	P
Colour	1	$0.896 * MS [clone (colour)] + 0.104 * MS [Error]$	0.008	0.9306
Treatment	1	$0.905 * MS [clone * treatment (colour)] + 0.096 * MS [Error]$	27.54	0.0023
Clone (Colour)	5	$MS [clone * treatment (colour)]$	36.90	0.0006
Colour*Treatment	1	$0.874 * MS [clone * treatment (colour)] + 0.126 * MS [Error]$	4.37	0.0839
Clone*Treatment (Colour)	5	$MS [Error]$	1.69	0.1380
Error	194	$MS [Error] = 0.14$		

fore the adults of the 2nd generation were subjected to a crowding stimulus (10 individuals in a petri-dish). Their studies showed that there are significant differences between clones in the proportion of winged morphs among offspring. One potential criticism of Lamb & MacKay's (1979, 1983) studies is that the aphids crowded together in a single petri-dish are not independent replicates. The common environment will make individuals within the same petri-dish react more similar to one another, thereby decreasing the variability within clones. In our experiments, we obtained independent replicates for each clone by placing each test individual in a separate petri-dish. As in Lamb & Mackay's (1979, 1983) experiments, the possibility of maternal effects influencing the results was minimised by rearing aphids for two generations at very low densities.

Our results support Lamb & MacKay's conclusions that there is significant variation among pea aphid clones in the propensity to produce winged offspring. Variation between clones was considerable, ranging from zero percent winged offspring under crowding conditions (green clones NG, SG) to almost 80% winged offspring even under control conditions (red clone BP). Clones also differed in their response to the crowding treatment (Figure 1). While in some clones crowding resulted in a marked increase in the percentage of winged morphs among the offspring compared to the control, other clones did not show such an increase. Interestingly, this lack of response can occur both in clones with a low level of winged offspring production under control conditions (green clones NG and SG) and in those that produced a high percentage of winged morphs without any crowding (red clone BP). This variation among clones reflects genetic variation because all environmental variation was accounted for by variation among the genetically identical individuals within clones. The estimates of broad-sense heritability support the idea that variation in winged morph production is under genetic control.

In agreement with Lowe & Taylor's (1964) and Sutherland's (1969a) hypothesis, red clones were on average more prone to producing winged offspring than green ones. However, one important result of our experiment is that there was considerable variation between clones of the same colour. For example, both under control and under crowding conditions, the red clone LP did not produce any more winged offspring than the green clones LG or BG (Figure 1). Thus, red clones produced on average more winged morphs than green clones, but the response to crowding of the dif-

ferent clones was idiosyncratic. Consequently, body colour did not significantly influence the response of clones to crowding.

Differences between aphid clones in offspring production or growth rates have been reported in a number of studies (e.g., Bournoville, 1977; Eggers-Schumacher, 1983; Simon et al., 1991; Wilhoit & Mittler, 1991; Araya et al., 1996), and our study also shows considerable differences in fecundity. At the extreme, the clone SG produced almost twice as many offspring as clone LG both in the control and after crowding (Figure 2). Body colour, however, had no significant effect on aphid fecundity. Even though the number of clones tested was rather low, the patterns suggest that there is no systematic trend such that clones of one colour are more fecund than clones of another (Figure 2).

In the 48 h following the treatment, individuals that were in petri-dishes for 24 h produced a higher number of offspring than control individuals which remained on the host plant during this period (Figure 2). Because only very few offspring were born while the aphids were kept in petri-dishes, this result indicates that aphids are able to at least partly defer offspring production when starved/crowded. Nevertheless, a period of starvation in the petri-dish results in a significant reduction in total offspring number. Although in this experiment we did not count the number of offspring produced by the control aphids in the first 24 h, results from a different experiment show that the number of offspring produced by aphids during 48 h after a one-day crowding treatment is significantly smaller than the number of offspring produced by control aphids during the entire three-day interval (W.W. Weisser & N. Minoretti, unpubl.).

Morphs of different colours can be found in a number of aphid species (Börner, 1952; Miyazaki, 1987). Little is known about the selective forces that maintain colour polymorphism (Miyazaki, 1987; Kerns et al., 1998). Losey et al. (1997) demonstrated that a red-green colour polymorphism in the pea aphid can be maintained by opposite patterns of parasitism and predation. However, despite several suggestions the relationship between morph colour and observed variation in life history traits and behaviour remains unclear (Sutherland, 1969a; Müller, 1983; Miyazaki, 1987; Agawa & Kawata, 1995; Araya et al., 1996). In our experiment, there was no clear-cut relationship between clone body colour and the propensity to produce winged offspring or aphid fecundity. When making generalisations about traits in 'red' or 'green' morphs

it is therefore important to investigate a range of clones within each colour morph.

The presence of genetic variation for wing production raises the question as to why clones differ in their propensity to produce winged offspring. Because the winged morph is mainly responsible for dispersal and the colonisation of new plants, the results imply that pea aphid clones differ in their dispersal rates. At present, it is unclear why such differences in dispersal rate should be linked to body colour. Thus, without further research it is not possible to speculate if such a relationship would be adaptive or would simply be a physiological by-product. Differences in dispersal rates between different populations have been linked to patterns of habitat persistence in other wing-dimorphic insects (e.g., Roff & Fairbairn, 1991; Roff, 1994; Denno et al., 1996). In populations occupying long-lived habitats, lower dispersal rates are expected than in populations from short-lived habitats (Southwood, 1962). In a study on the milkweed-oleander aphid, *Aphis nerii*, Groeters (1989) found significant genetic variability for winged morph production, but populations from more permanent habitats not always produced more winged morphs than populations from more temporary habitats. In another study, Lamb & MacKay (1983) suggested that peripheral or isolated populations of pea aphid lose more migrants than they receive, so over time clones that produce a large proportion of winged morphs are lost from these populations. As a result, average winged morph production in isolated or peripheral population declines compared to more centrally located populations. Population differences in dispersal rate may also be caused by a number of other factors, such as competition or inbreeding (Diekmann et al., 1999). To understand the origin and maintenance of genetic variation for wing polyphenism, future work should aim at linking the propensity to produce winged offspring in field-collected clones to other biological parameters in the habitats of these clones.

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