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A case for a joint strategy of diversified bet hedging and plasticity in the pea aphid wing polyphenism

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Phenotypic plasticity and diversified bet hedging are strategies for coping with variable environments. Plasticity is favoured when an organism can predict future conditions using environmental cues, while bet hedging is favoured when predictive cues are not available. Theoretical analyses suggest that many organisms should use a mixture of both strategies, because environments often present both scenarios. Here, we examine if the pea aphid wing polyphenism, a well-known case of plasticity, is potentially a mixture of plasticity and bet hedging. In this polyphenism, asexual females produce more winged offspring in crowded conditions, and wingless offspring in uncrowded conditions. We find that pea aphids use plasticity to respond to crowding and we find considerable genetic variation for this response. We further show that individual aphids produce both winged and wingless offspring, consistent with the variability expected in a bet hedging trait. We conclude that the pea aphid wing polyphenism system is probably a mixture of plasticity and bet hedging. Our study adds to a limited list of empirical studies examining mixed strategy usage, and suggests that mixed strategies may be common in dispersal traits.

1. Introduction

Phenotypic plasticity and diversified bet hedging are two strategies that have evolved in response to environmental heterogeneity [1]. Adaptive phenotypic plasticity produces different phenotypes from a single genotype in response to cues that predict future environmental conditions [2]. Diversified bet hedging also results in multiple phenotypes produced from a single genotype, but differs from plasticity because no predictive cues are used; the different phenotypes are *always* produced [3–5]. Bet hedging is favoured in fluctuating environments that *lack* predictive cues about the future [3–5].

Phenotypic plasticity and bet hedging are often discussed as alternative strategies for coping with environmental variability [6]. However, most environments consist of both predictable and unpredictable changes and theoretical models suggest that environmental cues with varying levels of predictive power should favour a joint strategy (also known as coin-flipping plasticity) [7–9]. In a joint strategy, a response to an environmental signal induces a phenotypic change, while the magnitude of this change differs between individuals [1]. While there is a growing body of theoretical work and simulation studies focused on scenarios where a mixed strategy is favoured [8–10], few empirical investigations have addressed this issue ([11,12]; see also references within [8]).

Here, we test for the usage of a joint strategy in the wing polyphenism of pea aphids (*Acyrthosiphon pisum*). During the summer, parthenogenetic females produce genetically identical winged and wingless daughters based upon environmental conditions experienced by the mother [13]. In optimal conditions, females

Table 1. Pea aphid genotypes tested, collection site and endosymbionts present.

genotype	collection site	*Regiella*	*Rickettsia*	*Spiroplasma*
MA 1	Berkley, MA	Y	N	N
MA 2	Berkley, MA	N	Y	N
NY 1	Rochester, NY	N	N	Y
NY 2	Ithaca, NY	N	N	Y
NY 3	Ithaca, NY	N	N	N
NY 4	Rochester, NY	N	N	N
NY 5	Rochester, NY	N	N	Y
NY 6	Ithaca, NY	N	N	Y
NY 7	Rochester, NY	Y	N	N
NY 8	Rochester, NY	N	N	Y

produce wingless daughters with increased fecundity, while high population density and insufficient food cause the production of dispersal-capable, winged daughters (reviewed in [14]). While multiple studies have shown that pea aphids respond to these cues in a plastic manner, the magnitude of the response is variable and genotypes never produce 100% winged offspring [14,15]. These previous observations could be solely due to plasticity, or due to a combination of plasticity and bet hedging. If the response were purely plastic, we would expect individuals within a genotype to respond similarly. However, if the same conditions were to elicit different responses within a genotype, this would be strong evidence for bet-hedging. Our purpose here, therefore, was to take a closer look at the wing polyphenism, with special attention to the variance of response both among genotypes and among individual aphids within a genotype to consider the possibility that this well-known polyphenism is actually a mixture of plasticity and bet hedging.

2. Material and methods

Aphids were reared on *Vicia faba* plants covered with cages in climate-controlled rooms at approximately 19°C, 35% humidity, and 16 L : 8 D cycle. Aphids were maintained for three generations at low population density (approx. six females per plant) prior to the start of experiments. We allowed low-density adults to produce nymphs for 24 h; this was the pre-crowding treatment. Adults were then crowded for 24 h in groups of 10 in 35 mm Petri dishes containing moist filter paper to prevent desiccation. From the 40 crowded adults, 10 were moved to a single plant for each genotype; the remaining 30 were discarded. Females larviposited for four consecutive 24 h intervals and then were discarded. Offspring from each 24 h interval were moved to new plants to minimally disturb the adult females, which remained on the same plant for the 4 days. Rearing conditions should not affect offspring phenotypes because morph determination in the pea aphid is pre-natal [16]. The phenotype, winged or wingless, of each offspring was counted as adults (phenotypes cannot be visibly distinguished until third instar, and are easiest to phenotype as adults) for each genotype and time point (pre-crowding and four 24 h intervals post-crowding). This experiment was repeated three times.

We used three genotypes to investigate the offspring phenotypes of individual aphids, rather than groups, produced in response to crowding. Aphids were crowded as above and placed individually (as compared to the last experiment, where they were placed in groups of 10) on plants for 24 h. Offspring were phenotyped as adults. This experiment was performed in triplicate.

To determine if facultative bacterial endosymbionts affected wing induction (*Regiella* is thought to have an effect [17]), we tested each aphid genotype for the presence of *Serratia*, *Hamiltonella*, *Regiella*, *Spiroplasma*, *Rickettsia*, *Rickettsiella*, *Wolbachia* and X-type, with *Buchnera* as a positive control. Diagnostic PCRs were run for each symbiont using published primers [18] and repeated for confirmation (table 1).

We used the lme4 package to make generalized linear models (GLM, quasibinomial, link logit function) and generalized linear mixed models (GLMM, binomial, link logit function) in R v. 3.2.5 [19,20]. Models were compared with an ANOVA using a Wald's χ^2 statistic [21]. The effect sizes of independent variables were calculated as marginal R^2 values [22].

3. Results

(a) Genotypic variation for the polyphenism exists in natural populations

Genotype significantly impacted the proportion of winged offspring in the pre-crowding treatment (GLM, $\chi^2_{9,29} = 114$, $p = 1.5 \times 10^{-8}$), explaining 71% of the variance (figure 1). All genotypes produced more winged offspring after experiencing the crowding cue. The effect differed markedly among genotypes, with genotype and treatment (pre-crowding versus day 1 post-crowding) significantly affecting the response (GLM; genotype, $\chi^2_{9,50} = 473$, $p = 2.1 \times 10^{-12}$; treatment, $\chi^2_{1,49} = 1695$, $p < 2.2 \times 10^{-16}$; genotype × treatment, $\chi^2_{9,40} = 66$, $p = 0.32$). Genotype also had a large effect in the post-crowding treatment, explaining 69% of the variation. Finally, some genotypes had greater variance between replicates than others (ANOVA; genotype, $F_{9,30} = 3.9$, $p = 2.4 \times 10^{-3}$, genotype accounts for 54% of the variance).

(b) Individuals produce a mixture of winged and wingless offspring

We examined the proportion of winged offspring produced by *individual* females within genotypes, with the prediction that they would produce both winged and wingless offspring if they bet hedge. We counted the phenotypes of the offspring produced by 30 individual females for three genotypes from

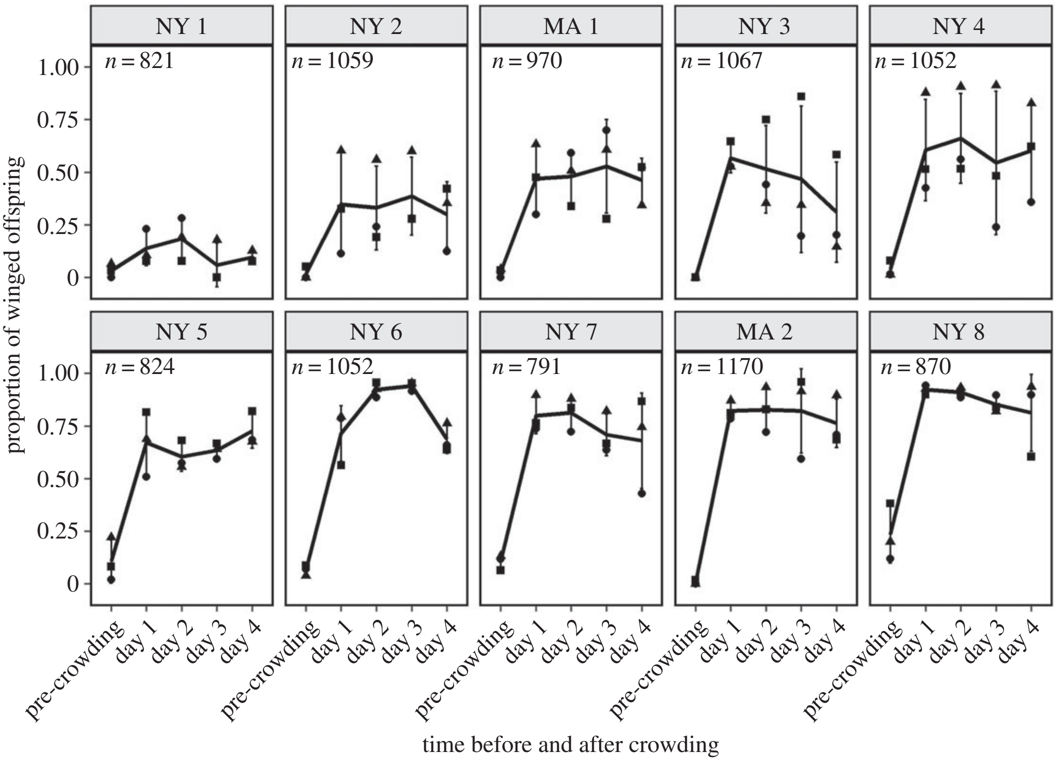

Figure 1. Pea aphids respond to crowding via plasticity, by increasing the proportion of winged offspring. For each of the 10 genotypes (MA 1–2, NY 1–8), the winged offspring produced for the 24 h before and 4 days after a 24 h crowding treatment is illustrated. The genotypes are ordered from lowest to highest, by the proportion of winged offspring they produced on day 1. The lines indicate the mean and the points (circles, squares and triangles) differentiate the three experimental replicates (the offspring from the same sets of 10 females over time). Sample sizes indicate the number of offspring counted per genotype.

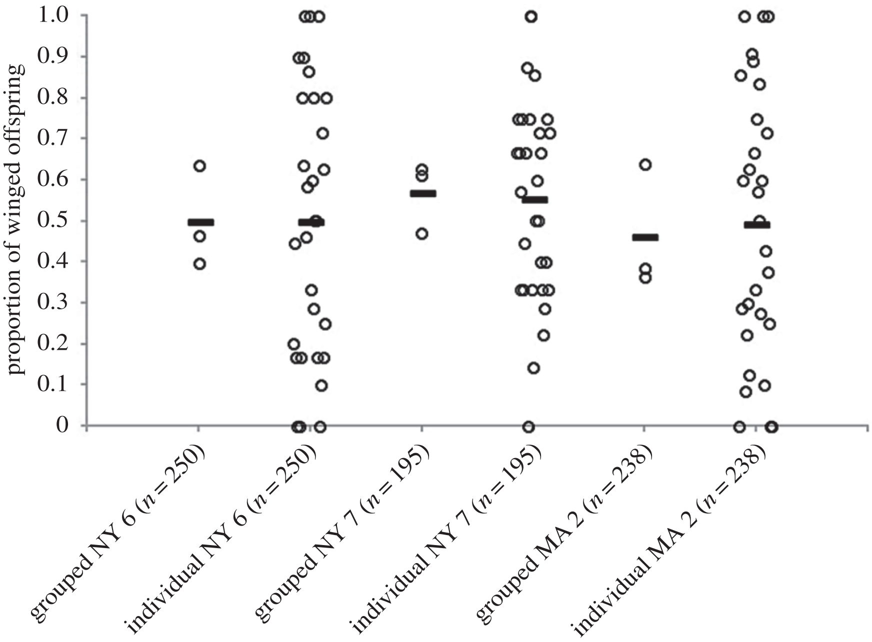

Figure 2. Individual females almost always produce a mixture of winged and wingless offspring. Shown are the proportions of winged offspring produced by three genotypes in the first 24 h post-crowding. 'grouped' = offspring of sets of 10 adult females ($n = 3$), and 'individual' = those same offspring, broken down by individual females ($n = 30$). The bar shows the mean.

the first 24 h after crowding. Quite strikingly, we observed that individuals produced a broad distribution of winged offspring proportions, ranging from 0 up to 1 (figure 2). We found that they were significantly over-dispersed from the expected binomial distribution (χ^2 test of homogeneity; MA 2, $\chi^2_{28} = 94.8$, $p = 3.4 \times 10^{-9}$; NY 6, $\chi^2_{29} = 102$, $p = 3.7 \times 10^{-10}$; NY 7, $\chi^2_{29} = 47.8$, $p = 0.015$).

(c) No effect on the wing polyphenism from secondary endosymbionts

We tested the effect of the three facultative symbionts found in our genotypes, but none of them affected the level of wing-induction response elicited from the crowding treatment (GLMM; *Regiella*, $\chi^2_{1,28} = 0.05$, $p = 0.83$;

Spiroplasma, $X^2_{1,27} = 0.005$, $p = 0.94$; *Rickettsia*, $X^2_{1,26} = 0.76$, $p = 0.38$).

4. Discussion

(a) Pea aphids exhibit phenotypic plasticity in response to crowding

We confirmed earlier studies showing that pea aphids react to a high-density environment using phenotypic plasticity [13,23]. All 10 genotypes responded to the combination of crowding and starvation by increasing their production of winged offspring from the pre-crowding treatment (figure 1). This suggests that an experience of 24 h of crowding and starvation is predictive of future environmental deterioration in natural populations, because plasticity evolves when signals are predictive [10]. Moreover, we showed that there is genetic variation for this plasticity, consistent with previous studies [23,24]. It is notable that the slope of NY 1 (figure 1) differs from the other genotypes; however, we did not find a significant effect of slope (genotype × treatment).

(b) The wing polyphenism is probably a mixture of bet hedging and plasticity

Individual pea aphids stochastically produce different proportions of offspring phenotypes, resulting in high variability among females of a single genotype (figure 2). This stochasticity is consistent with bet hedging. Additionally, the significantly over-dispersed distribution of offspring phenotypes from individual females (figure 2) suggests that there is a biological process in this polyphenism that creates a near-flat distribution of offspring phenotype proportions.

Further rationale for calling this bet hedging can be stated in the context of Simons [6], who explicitly outlined six evidence categories for candidate bet-hedging traits. The first is the recognition of a candidate bet-hedging trait, which in this case is the production of winged and wingless offspring. The second is the identification of an unpredictable environmental factor. Crowding, starvation and interspecific interactions cause the induction of winged offspring (reviewed in [14]), but the winged offspring are not flight capable until they are adults. Thus, there is a time lag of approximately 10 days between the cue and the ability of the offspring to leave. In that time, predators can leave, plant quality can improve, or aphids can walk to better plants, so these cues are not 100% predictive of the future. The third category of evidence is that there is genotype-level candidate bet hedging. There is significantly more variability for winged offspring production in some genotypes than others (figure 1), which is evidence for a genetic basis of this trait in pea aphids. These first three categories have been sufficient to label a trait as bet hedging in other systems ([6] table S1, [12,25]). The remaining three categories (demonstrating variable fitness consequences, demonstrating that the candidate bet-hedging trait is advantageous under fluctuating selection, and showing that the degree of bet hedging matches the degree of fluctuating selection) await future studies.

In sum, we have demonstrated that pea aphids exhibit surprisingly high variability for the production of winged versus wingless offspring at many levels: in the absence and in the presence of high density cues, between genotypes, and among individual females. We conclude that pea aphids probably use a joint strategy of phenotypic plasticity and bet hedging to produce this variability. This work joins a limited number of empirical studies demonstrating mixed strategy usage. These studies have focused mostly on dormancy emergence traits, such as seed germination timing in desert annuals [26] and diapause break in killifish [12]. Our study suggests that mixed strategies may also be common in dispersal traits, such as previously shown in an amphicarpic annual plant [27] and now in the pea aphid wing polyphenism.

Data accessibility. Data are deposited in Dryad: http://dx.doi.org/10.5061/dryad.2bb5q [28].

Authors' contributions. M.E.G. and J.A.B. conceived of and designed the study, and drafted the manuscript. C.J.A., Y.X.Z. and B.R.O. carried out the study, and participated in drafting the manuscript. M.E.G. conducted data analyses. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Competing interests. The authors declare no competing interests.

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