Pea aphid wing plasticity variation has a multigenic basis

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**Conflict of Interest**

The authors declare no conflicts of interest.

**Abstract**

Phenotypic plasticity, the ability of a single genotype to produce a range of phenotypes in response to environmental cues, can exhibit genetic variation like any trait. Discovering the genetic basis of plasticity and plasticity variation is critical to understand how populations will respond to the ongoing environmental challenges brought about by, for example, climate change. Here, we investigate the genetic basis of the pea aphid (*Acyrthosiphon pisum*) wing plasticity variation. In this species, genetically identical, highly fecund wingless and dispersive winged individuals are produced by pea aphid mothers in uncrowded versus crowded environments, respectively. We focus specifically on the genetic basis of the propensity to produce winged individuals in response to crowding. We crossed a low to a high plasticity line and examined plasticity variation in backcross progeny (F1 x low parent), finding that differences between lines had a strong genetic component and that multiple loci likely to contribute to this variation. Transcriptional profiling revealed a candidate gene, *yellow-h*, which was found within a genomic locus contributing to plasticity variation. Overall, we provide novel information about the genetic basis of an ecologically-relevant trait and contribute to the growing literature recognizing the importance of understanding the genetic basis of plasticity variation.

**Keywords**: genetic variation, phenotypic plasticity, polyphenism, genetic cross

## INTRODUCTION

Phenotypic plasticity is the ability of a genotype to produce different phenotypes in response to different environments (Pigliucci et al. 2006). Like all traits, plasticity can exhibit phenotypic variation and that variation can have a genetic basis. Studies across diverse species, from water fleas (Boersma et al. 1998) to beetles (Moczek 2002) to bryozoans (Harvell 1998) have demonstrated genetic variation for environmental responsiveness. Because of this variation, plasticity as a trait can evolve by natural selection, and changes in environmental responsiveness can in turn be important for adaptation and diversification (DeWitt et al. 1998; West-Eberhard 2003; Pfennig et al. 2010).

Polyphenism is a subcategory of phenotypic plasticity where only two or three distinctive morphs are produced by a single genotype. Some of the best known polyphenisms are found in insects, such as the castes of social insects and the dimorphic horns of dung beetles (Simpson et al. 2011), but examples can be found across the different realms of life (West-Eberhard 2003). In polyphenic systems, the alternative morphologies are encoded by the genetic and developmental program to produce environment-matched morphs. In contrast, the *propensity* to produce one morph compared to its alternative is responsive to environmental conditions. That propensity, or the sensitivity of the switch mechanism, can exhibit genetic variation such that some genotypes are more responsive to the environment than others. The molecular genetic basis of the central switch mechanism has been deciphered in several polyphenic systems (e.g., Kijimoto et al. 2012; Ragsdale et al. 2013; Xu et al. 2015), but much less is known about the genetic basis of its variable sensitivity (Fawcett et al 2018 provides a rare example). In this study, we examine the genetic basis of this variable sensitivity in pea aphids (*Acyrthosiphon pisum*).

Aphids have long been a model system for studying plasticity (e.g., Wadley 1923; Kennedy and Stroyan 1959; Dixon 1973). Of interest here is their wing polyphenism, in which the same genotype can produce the dramatically different phenotypes of winged or wingless morphs (Braendle et al. 2006). The winged and wingless morphs are optimized for dispersal versus reproduction, similar to flight capable and incapable morphs found in an array of insect taxa (Zera and Denno 1997). In pea aphids, high density brought about by a crowded host plant causes mothers to produce genetically identical offspring that

grow up to be winged, while a low-density environment results in pea aphid mothers producing wingless daughters (Sutherland 1969).

Although the wing polyphenism is dimorphic in that it comprises winged and wingless aphids, there is a great deal of variation in natural populations for the propensity to produce winged offspring when an aphid mother experiences crowded conditions (Lamb and MacKay 1979; Weisser and Braendle 2001; Grantham et al. 2016; Parker and Brisson 2019; Parker et al. 2021). For example, Parker and Brisson (2019) assayed the wing plasticity response -- as measured by the percent of winged offspring produced by pea aphid mothers of a single genotype when crowded -- of 192 lines collected from two adjacent alfalfa fields in Ithaca, NY. They found an entire range of variation, from lines that produced almost no winged offspring to lines that produced nearly all winged offspring. Our goals for this study were to (1) perform an initial investigation into the genetic architecture of the pea aphid wing plasticity variation; and (2) use transcriptional profiling to identify potential candidate genes contributing to line variation for this phenotype.

## MATERIALS AND METHODS

***Aphid lines and crosses***. The low parent (LP) produces few winged offspring after crowding treatment, while the high parent (HP) produces many winged offspring after crowding (Fig. 1A). We crossed females of LP to males of HP to produce an F1 line, then backcrossed females of the F1 line to males of LP to create backcross offspring (Fig. 1B). We do not expect maternal versus paternal roles to be significant; both of the paternal line's X chromosomes get passed on to offspring (Jaquiery et al. 2013), and although maternally-inherited endosymbionts may impact wing plasticity (Leonardo and Mondor 2006) in our crossing scheme all offspring lines inherited LP's endosymbionts (and mtDNA), so these do not contribute to the variation among lines.

We maintained clonal lines in individual cages on fava bean (*Vicia faba*) seedlings at 20° C, with a 16H:8H light:dark cycle. To induce sexual reproduction, we moved aphids to fall-like conditions at 18° C with a 12H:12H light:dark cycle for three generations, then combined unmated females with males in a 5:2 ratio on plants (generally 5 females to 2 males, or 10 females to 4 males, per plant). We collected melanized eggs and applied an antifungal 1% calcium propionate solution before placing the eggs at 2° C for three months. Afterwards, eggs were hand-cleaned of any fungus and placed at 20° C to hatch. Each hatching foundress reproduced asexually to generate a clonal offspring line with a unique genetic identity.

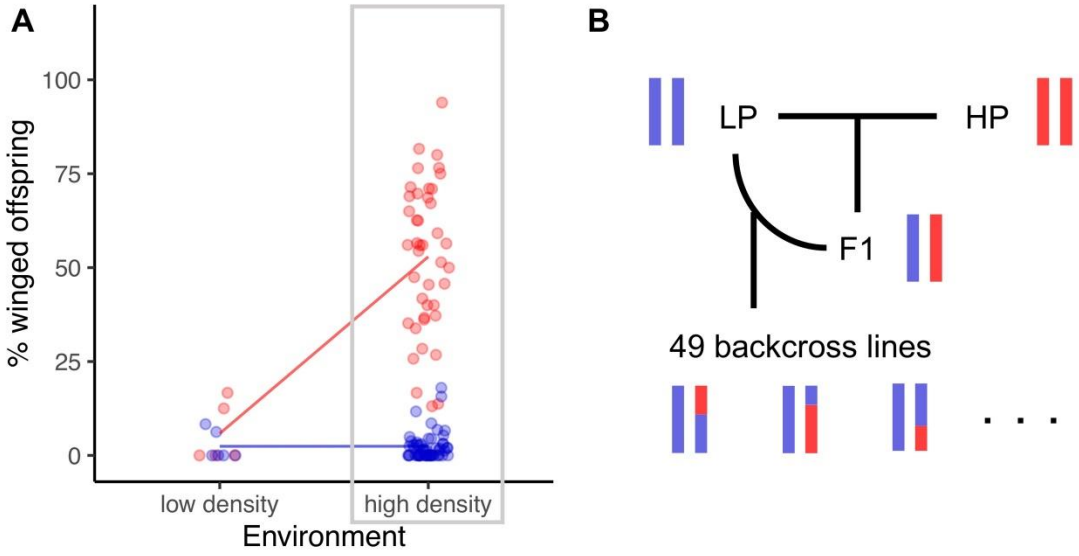


**Figure 1. Cross between parent lines with distinct wing plasticity phenotypes.** (A) Two parent lines were selected for their different wing plasticity responses: a "high plasticity" line (HP, shown in red) with a large difference in winged offspring production between low- and high-density environments, and a "low plasticity" line (LP, shown in blue) with no difference in winged offspring production between low- and high-density environments. As both lines produce few winged offspring in a low-density environment, winged offspring production in the high-density environment (gray box) is a good proxy for the strength of plasticity. (B) The selected parent lines, LP and HP, were crossed to produce an F1 line. This F1 line was then repeatedly crossed back to parent LP to produce a panel of 49 backcross lines, each with a distinct genotype.

***Crowding treatment.*** Crowding treatments were performed to assess the plasticity phenotypes of the parent lines, the F1 line, and the 49 backcross lines, using winged offspring production in crowded conditions as a proxy for the strength of plasticity (as suggested by Fig. 1A). For crowding treatment, 36 wingless adult asexual females from a given line were selected from mixed-age stock cages that could vary in density. 12 aphids were placed into each of three 35 mm petri dishes, with a dampened filter paper, to create three crowding replicates. The dishes were enclosed in a 90 mm dish with dampened filter paper to maintain humidity. After 24h, aphids were moved to fava bean seedlings (four/plant; three plants per replicate) for 24h, then adult aphids were removed, leaving any offspring. Offspring were reared for 7-10 days until wing phenotypes could be scored visually. All backcross lines were tested in three crowding replicates, as above; the F1 line was tested in six replicates and the parental lines were tested in 54 (LP) and 42 (HP) replicates respectively.

We excluded samples where fewer than two of the four adult aphids placed on a plant survived, and where fewer than eight offspring were produced on a plant. We also excluded all crowding replicates from tests where fewer than five out of the expected nine plants for a line met the survival and fecundity criteria and were unaffected by human error. The minimum number of adult aphids included in one crowding replicate was two, mean 9.46 (minimum 11 among the three crowding replicates combined), and the minimum number of offspring included in one crowding replicate was eight, mean 66.9 (minimum 70 among the three crowding replicates combined). For analysis, the wing percentage for each crowding replicate was calculated from the total number of winged and wingless offspring from that replicate, with wing asymmetric aphids treated as wingless.

Line phenotypes were compared to the parent lines, LP and HP, with Wilcoxon rank-sum tests with continuity correction and p-values were adjusted with the Benjamini and Hochberg (FDR) method (Table S1).

***Low-density baseline for parent lines***. To obtain a baseline wing percentage in uncrowded environmental conditions, we reared aphids from each parental line at a low density of 4/plant for two generations, transferring young nymphs to fresh seedlings each generation to keep population densities low. We counted the number of winged and wingless aphids in the second generation.

***Number of loci involved in trait variation.*** We estimated the minimum number of loci contributing to trait variation (*n*) using Wright's method (Wright 1968) with some modifications and improvements (Lande 1981; Cockerham 1986; Zeng 1992; Eq. 1). Use of a single backcrossing direction in the calculation was inspired by (Lander and Botstein 1989). Wright's method assumes additive gene action or transformation of the data to produce an additive pattern; to meet this assumption, we used an $x^{1.5}$ transformation of the data to correct for dominance effects as much as possible. Zeng's (1992) equation allows us to also correct for the effects of linked loci and unequal allelic effects (Eq. 2), and we followed the example of (Caillaud and Via 2012) for the values of two parameters in this equation, $\overline{c}$ and $C\alpha$. With N representing the number of replicate measures and "B" indicating the collection of backcrosses:

$$n_e = \frac{(\overline{HP} - \overline{LP})^2 - \frac{\sigma^2_{HP}}{N_{HP}} - \frac{\sigma^2_{LP}}{N_{LP}}}{16\sigma^2_B - \frac{(\sigma^2_{HP} + \sigma^2_{LP})}{2}} \qquad \text{(Eq. 1)}$$

$$n = \frac{2\overline{c}n_e + C\alpha(n_e - 1)}{1 - n_e(1 - 2\overline{c})} \qquad \text{(Eq. 2)}$$

***RNA collection and RNAseq analyses***. Asexual female aphids from the two parent lines were reared to adulthood in uncrowded conditions and RNA was collected from head tissues for RNAseq. The uncrowded condition was selected to demonstrate the intrinsic differences between the lines that could relate to their ability or propensity to respond to a change in environment. Head tissues are appropriate for this application as mother aphids likely sense and integrate environmental cues before signaling their offspring.

Wingless adults were dissected in cold PBST between the second and third thoracic segment to ensure that the whole head was retained. Individual heads were homogenized in DNA/RNA Shield (Zymo) and RNA was extracted using the Quick RNA Miniprep Plus Kit (Zymo). The Janelia Quantitative Genomics

Support Team created the libraries and sequenced the samples on Miniseq (2x150bp, ~2M reads per replicate). Four replicates per genotype were sequenced.

We aligned reads to the pea aphid V3 genome (Li et al. 2019) using hisat2 (Kim et al. 2019). We calculated read counts using the htseq 2.0 (Putri et al. 2022) function htseq-count with overlap mode "union". We conducted differential expression analysis using edgeR 3.38.4 (Robinson et al. 2010; McCarthy et al. 2012) with default parameters except for filtering (large.n=2 and min.prop=0.5). We performed principal component analysis using limma 3.52.4 (Ritchie et al. 2015). We tested for enrichment of GO terms with Fisher's exact tests using OmicsBox 3.1.11 (BioBam) and the FatiGO package (Al-Shahrour et al. 2004).

***RFLP analysis***. We PCR amplified three loci in 25 µl reactions with 62.5 ng gDNA and 0.5 µM each primer using 2X Easytaq PCR Supermix (Transgene). The cycling protocol included 35 cycles of 30 s at 94° C, 30 s at 60° C, and 1 min at 72° C, and then a final extension (5 min at 72° C). A two-hour digest was conducted in 24.8 µl reactions with 20 µl PCR product, 0.4 µL CutSmart Buffer (NEB), 0.2 µL restriction enzyme, and 4.2µL $H_2O$. Autosome 1 locus is at 103,118,427-103,118,756 in the V3 genome (Li et al 2019), amplifies with CTAAGAACCCGGCAGATTTACC and TTACGCCTTCAATGTCTTCAGC, and cuts with MspI at 37°C. Autosome 2 locus is at 111,120,862-111,121,202, amplifies with CGTAGCGTAGAATATGACAGCG and ATTGTATACGTGGCTCTGATGG, and cuts with MspI at 37°C. Autosome 3 locus is at 37,681,959-37,682,072, amplifies with GAATTTTCGAAGGACCAAAGCC and GACATGTCTCTTAAGGTCCGTG, and cuts with TaqI at 65°C.

## RESULTS

### *Wing plasticity in the parental lines*

The two parental lines in our study exhibit low and high wing plasticity. The low plasticity parent line (LP) produces an average of ~2% winged offspring in a low-density environment with food present and does not increase winged offspring production following 24h in a high-density environment (Wilcoxon rank-sum test, p=0.687, Fig. 1A). In contrast, the high plasticity parent (HP), significantly increases winged offspring production under crowded conditions compared to uncrowded conditions (53% versus 6%; Wilcoxon rank-sum test, p=0.000461, Fig. 1A). For subsequent experiments, we only measured winged offspring production after aphid mothers experienced crowding conditions, using that as our proxy for the plasticity response (e.g., in Fig. 2).

### *The wing plasticity response is polygenic*

We crossed the LP to HP to produce an F1 line. This line was phenotypically similar to HP in its plastic response to crowding (Fig. 2A; Table S1), indicating the presence of a dominant locus or loci. We crossed this F1 line to the LP to create a backcross panel (n=49 lines) enriched for recessive alleles. We assayed the plasticity response for the parental lines many times, while the F1 was measured with six crowding replicates of 12 aphids each and each of the backcross line types were measured with three crowding replicates (Fig. 2). Among the backcross progeny, line had a significant effect on plasticity (one-way ANOVA, n=49, $p<2.2x10^{-16}$), with differences between lines explaining 73% of the variance and differences among crowding replicates within lines explaining only 27%. Thus, genetic differences account for most of the observed variation in plasticity.

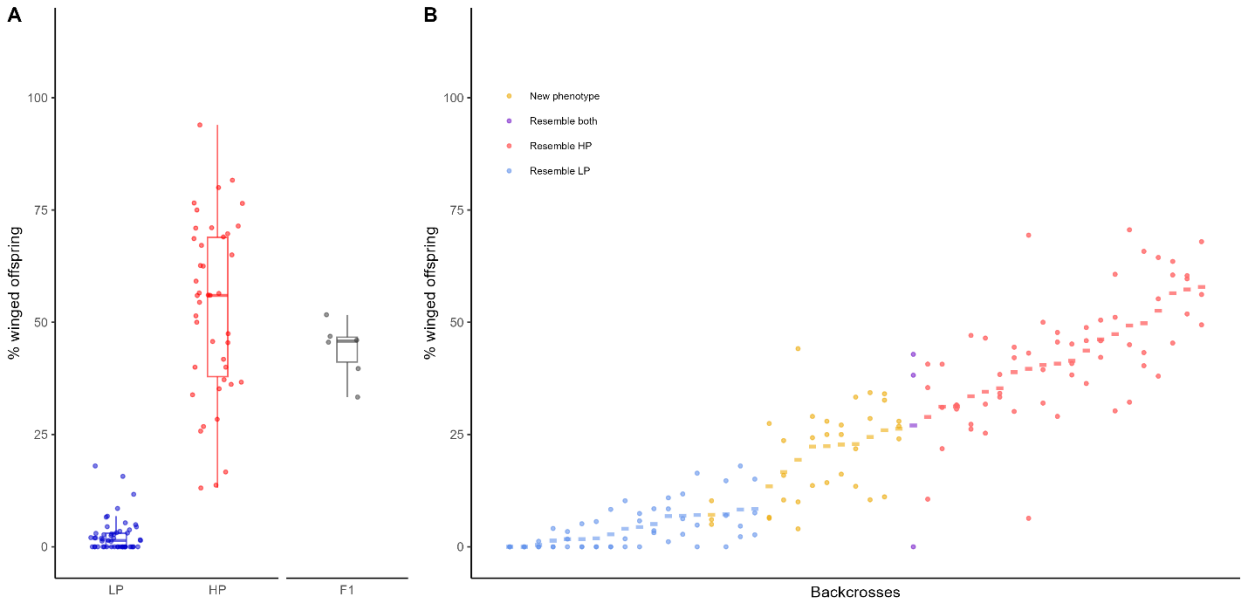


**Figure 2. Variation in the plasticity phenotype across parental and progeny generations**. (A) Shows the percentage of winged offspring in crowded conditions for the LP parent with 54 replicates (each replicate comprises offspring of 12 crowded mothers), the HP parent with 42 replicates, and the F1 offspring line with six replicates (points). (B) Shows the same type of data for backcross lines (n=49), with three replicates (points) for each backcross line. The mean for each line is also shown.

We found that plasticity variation is under polygenic control. Backcross progeny showed continuous variation for their plasticity response, rather than discrete low and high groups (Fig. 2B). A one locus, two allele, dominant-recessive genetic hypothesis does not explain the presence of intermediate phenotypes. This is true whether the parental lines were initially in a homozygous or heterozygous state. Thus, two loci or more must be involved. Moreover, the large proportion (~40%) of backcross progeny phenotypes statistically indistinguishable from the HP (Wilcoxon rank-sum tests; Fig. 2B; Table S1) indicates the presence of at least one locus with a dominant allele contributing to plasticity variation.

Using a modified version of Wright's method (Wright 1968; Lande 1981; Cockerham 1986; Zeng 1992), and transforming the data to correct for dominance effects as much as possible, we estimated that the minimum number of loci contributing to the parent lines' difference in plasticity strength is 3.47. However, our aphid populations likely violate this method's assumption that the HP has all the

increasing alleles and the LP has all the decreasing alleles; the parent lines may have heterozygous sites that were not captured in the F1 line; and dominance may not be completely controlled. For all of these cases, deviations are expected to produce an underestimate of the true number of loci. Simulations by Otto and Jones (2000) suggest that uncorrected dominance can produce an underestimate around 20% lower than the true value. We conclude that plasticity variation in this population is certainly affected by multiple loci. The true number of loci is likely higher than our specific estimate, but may still be relatively modest.. The low estimated number of loci also suggests the presence of at least one locus of relatively large effect (Lander and Botstein 1989).

***Transcriptional profiling reveals a candidate gene in linkage with plasticity variation***

To complement our genetic cross, we sought to identify genes differentially expressed between the parental lines by generating head tissue transcriptomes from each. Principal component analysis revealed separation of the parental lines on the first axis of variation (Fig. 3A). We found 105 genes significantly differentially expressed between LP and HP ($\log_2$ fold change>2 or <-2 and FDR<0.05; Fig. 3B; Table S2). A gene ontology overrepresentation analysis revealed four terms that were significantly overrepresented, all related to glyoxylate metabolic processes (Table S3). The observed differences in expression may relate to any and all phenotypic differences between LP and HP, including the lines' difference in plasticity. Therefore, we examined the list of differentially expressed genes for candidates with some possible relation to plasticity. *yellow-h* (LOC100161748; accession number XP_106656928.1; Fig. 3B) stood out given the known role of genes in the *yellow/major royal jelly protein (MRJP)* family in plasticity and caste determination in hymenopterans (reviewed in Buttstedt et al. 2014), among other functions.

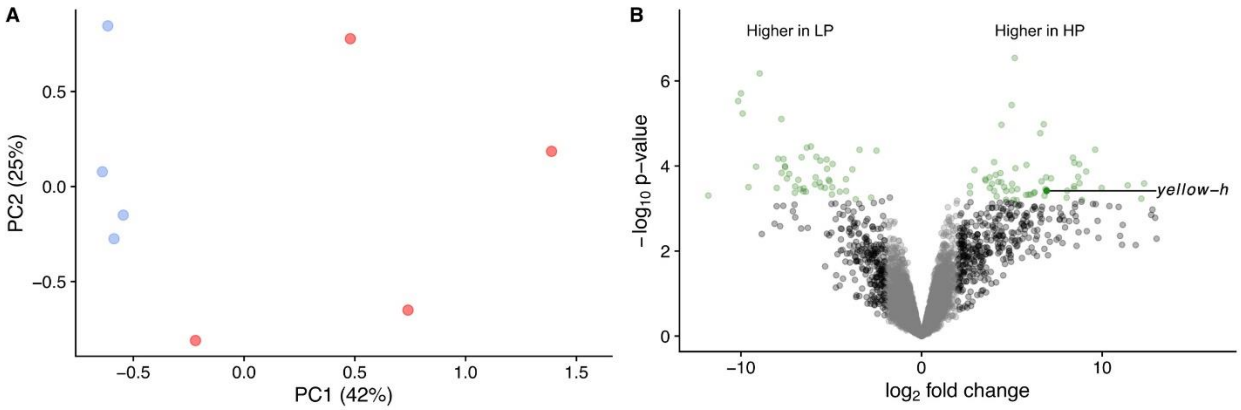


**Figure 3. The parental lines exhibit gene expression differences**. (A) Principal component analysis clustering of four LP (blue points) and four HP (red points) head transcriptomes on the first two principal components. Each transcriptome is represented by a single point. (B) Volcano plot showing differential expression between LP and HP. Genes that were significantly differentially expressed ($log_2$ fold change > 2 or < -2 and FDR < 0.05) are highlighted in green; genes that had either $log_2$ fold change > 2 or < -2, or FDR < 0.05, but not both, are shown in black.

A restriction fragment length polymorphism assay targeting a nearby SNP to the *yellow-h* gene (~2 Mb distant; closer to *yellow-h* than to any other significantly differently expressed gene) to the *yellow-h* gene, on autosome 1, showed a significant relationship between genotype and wing percentage among backcross progeny (Wilcoxon rank-sum test, $p=1.37x10^{-5}$; Fig. 4), while randomly chosen loci on autosomes 2 and 3 showed no relationship (Wilcoxon rank-sum tests; autosome 2: p=0.98; autosome 3: p=0.90; Fig. S1). We conclude that the region of autosome 1 containing the *yellow-h* locus and other linked loci causes differences in the wing plasticity phenotype.

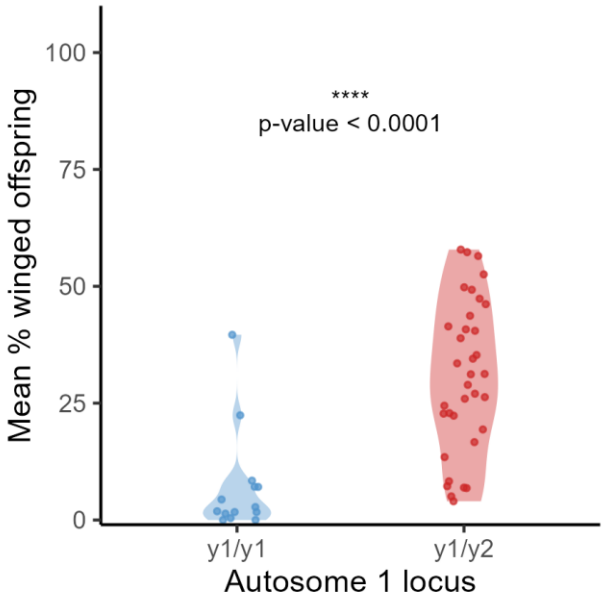


**Figure 4. A restriction fragment length polymorphism marker on autosome 1 falls within a region causing wing plasticity differences.** Average percent winged offspring for backcross lines that possess different genotypes at a SNP near *yellow-h* on autosome 1. Different genotypes are denoted on the x-axis.

## DISCUSSION

Here, we performed an initial investigation into the genetic architecture of pea aphid wing plasticity variation, an ecologically-relevant trait that balances the proportion of highly fecund (wingless) to dispersive (winged) individuals in pea aphid populations (Weisser and Stadler 1994; Zera and Denno 1997). We found a strong genetic contribution to plasticity variation and inferred that multiple loci contribute to the trait, consistent with the multigenic nature of plasticity variation found generally in animal and plant systems (El-Soda et al. 2014; Jin et al. 2023; Lafuente et al. 2024).

We also found that although the pea aphid lines examined here differed in their plasticity response, each line always produced a mix of winged and wingless offspring. In particular, we noted that even though the LP line has lost its plasticity response [*i.e.*, no statistical difference in the percentage of

winged offspring produced when comparing the crowded and uncrowded conditions (Fig. 1A)], it retains the ability to produce both winged and wingless offspring. Grantham et al. (2016) showed that pea aphids generally exhibit plasticity in that they respond to crowding by producing more winged offspring, but also produce both wing morphs as a bet-hedging strategy. This LP line, which is a wild isolate, may still be bet hedging, then, despite losing the ability to respond to the environment. And, while the LP line may exhibit some amount of bet hedging and/or other variability, we note that the HP line appears to exhibit far more, as evidenced by wide variation in the percentage of winged offspring produced in response to crowding (Fig. 2A). Work in a number of taxa has shown that the tendency for variability in a given trait may actually vary across genotypes and could itself be considered as a trait with a genetic basis (e.g., Ansel et al. 2008; Shen et al. 2012). While this is an intriguing possible explanation for the differences we observe between LP and HP, much greater data depth and a better understanding of possible technical variation in phenotyping will be needed to fully investigate the nature of non-plasticity-related variability in this system.

Given the numbers of offspring lines we were able to obtain, a traditional QTL analysis of plasticity variation with anonymous genetic markers across the genome would likely have only identified large regions and not been powered to detect loci with small to moderate effects, if any (e.g., Lander and Botstein 1989). Instead, we examined gene expression data from the two parent lines with an eye towards gene annotations with some connection to plasticity, anticipating that gene expression differences between the parental lines would relate both to the interesting difference in plasticity, and to other differences between the two lines. We explored genetic variation in offspring lines near one gene of interest, *yellow-h*, using a simple RFLP assay. We found that variation at the assayed locus does associate with wing plasticity variation in the offspring lines. This locus is likely several million bases large given the low number of recombination events in our population, but *yellow-h* is the nearest gene to the RFLP marker site that is differentially expressed in the heads of the parental lines, and is a promising candidate for generating variation in plasticity.

*yellow-h* is a member of the *yellow/MRJP* gene family found in insects and few other eukaryotes (Ferguson et al. 2011). Family members are most well known for their role in melanin production in *Drosophila melanogaster* (Brehme 1941; Biessmann 1985). The pea aphid parental lines do differ in

coloration (red versus green), but this color polymorphism is due to carotenoids, not melanin (Moran and Jarvik 2010), and body color in offspring lines is unrelated to plasticity strength (Gregory et al.). We therefore do not expect that expression of *yellow-h* is related to pigmentation. The *yellow-h* gene product could, however, potentially modulate dopamine levels. Xu et al. (2011) showed that *yellow/MRJP* family proteins can bind biogenic amines such as dopamine with high affinity. Dopamine is of great interest in the pea aphid plasticity, since maternal titer levels correlate negatively with winged offspring production (Vellichirammal et al. 2016) and manipulating maternal dopamine levels changes winged offspring production accordingly (Liu and Brisson 2023). Here, we observed higher transcript levels of *yellow-h* in HP, the line that produces many winged offspring. *yellow-h* could be binding dopamine and making it less available, thus increasing the wing percentage. This would imply that LP has decreased plasticity by not making the *yellow-h* protein, leaving more dopamine circulating. Future functional studies (for example, employing CRISPR/Cas9) should determine whether *yellow-h* does indeed play a causal role in modulating and varying the strength of pea aphid wing plasticity.

**Funding**

This work was supported by the National Science Foundation (IOS 1749514 to JAB and GRFP 1939268 to RMHD).

**Acknowledgements**

RNA sequence data was generated by David Stern and Alys Cheatle Jarvela at the Janelia Research Campus. We thank Kevin Deem for assistance with RNAseq data processing. We also thank the team of undergraduate students who assisted with live aphid work - Abigail Seaton, Sean Lee, Charlotte Irwin, and Mayesa Khan - as well as other members of the lab who also provided occasional assistance.

**Data Availability**

RNAseq data is available via the NCBI Sequence Read Archive (PRJNA1172746). Phenotype and RFLP genotype data and analysis scripts are available via Dryad.

## REFERENCES CITED


Al-Shahrour, F., R. Díaz-Uriarte, and J. Dopazo. 2004. FatiGO: a web tool for finding significant associations of Gene Ontology terms with groups of genes. Bioinformatics 20:578-580.

Ansel, J., H. Bottin, C. Rodriguez-Beltran, C. Damon, M. Nagarajan, S. Fehrmann, J. François, and G. Yvert. 2008. Cell-to-cell stochastic variation in gene expression is a complex genetic trait. PLoS Genet 4:e1000049.

Biessmann, H. 1985. Molecular analysis of the yellow gene (y) region of Drosophila melanogaster. Proc Natl Acad Sci U S A 82:7369-7373.

Boersma, M., P. Spaak, and L. De Meester. 1998. Predator-mediated plasticity in morphology, life history, and behavior of Daphnia: the uncoupling of responses. Am Nat 152:237-248.

Braendle, C., G. K. Davis, J. A. Brisson, and D. L. Stern. 2006. Wing dimorphism in aphids. Heredity 97:192-199.

Brehme, K. S. 1941. The Effect of Adult Body Color Mutations upon the Larva of Drosophila Melanogaster. Proc Natl Acad Sci U S A 27:254-261.

Buttstedt, A., R. F. Moritz, and S. Erler. 2014. Origin and function of the major royal jelly proteins of the honeybee (Apis mellifera) as members of the yellow gene family. Biological reviews of the Cambridge Philosophical Society 89:255-269.

Caillaud, M. C. and S. Via. 2012. Quantitative genetics of feeding behavior in two ecological races of the pea aphid, Acyrthosiphon pisum. Heredity 108:211-218.

Cockerham, C. C. 1986. Modifications in estimating the number of genes for a quantitative character. Genetics 114:659-664.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution 13:77-81.

Dixon, A. F. G. 1973. Biology of Aphids. Edward Arnold Ltd., London.

El-Soda, M., M. Malosetti, B. J. Zwaan, M. Koornneef, and M. G. M. Aarts. 2014. Genotype × environment interaction QTL mapping in plants: lessons from Arabidopsis. Trends in Plant Science 19:390-398.

Fawcett, M. M., M. C. Parks, A. E. Tibbetts, J. S. Swart, E. M. Richards, J. C. Vanegas, M. Cenzer, L. Crowley, W. R. Simmons, W. S. Hou, and D. R. Angelini. 2018. Manipulation of insulin signaling phenocopies evolution of a host-associated polyphenism. Nature Communications 9.

Ferguson, L. C., J. Green, A. Surridge, and C. D. Jiggins. 2011. Evolution of the insect yellow gene family. Mol Biol Evol 28:257-272.

Grantham, M. E., C. J. Antonio, B. R. O'Neil, Y. Z. Zhan, and J. A. Brisson. 2016. A case for a joint strategy of diversified bet hedging and plasticity in the pea aphid wing polyphenism. Biol. Letters 12.

Gregory, L. E., R. M. H. Driscoll, B. J. Parker, and J. A. Brisson. Impacts of body color, symbionts, and genomic regions on the pea aphid wing plasticity variation. (submitted)

Harvell, C. D. 1998. GENETIC VARIATION AND POLYMORPHISM IN THE INDUCIBLE SPINES OF A MARINE BRYOZOAN. Evolution 52:80-86.

Jaquiery, J., C. Rispe, D. Roze, F. Legeai, G. Le Trionnaire, S. Stoeckel, L. Mieuzet, C. Da Silva, J. Poulain, N. Prunier-Leterme, B. Segurens, D. Tagu, and J.-C. Simon. 2013. Masculinization of the x chromosome in the pea aphid. PLoS genetics 9:e1003690-e1003690.

Jin, M., H. Liu, X. Liu, T. Guo, J. Guo, Y. Yin, Y. Ji, Z. Li, J. Zhang, X. Wang, F. Qiao, Y. Xiao, Y. Zan, and J. Yan. 2023. Complex genetic architecture underlying the plasticity of maize agronomic traits. Plant Communications 4:100473.

Kennedy, J. S. and H. L. G. Stroyan. 1959. Biology of aphids. Annual Review of Entomology 4:139-160.

Kijimoto, T., A. P. Moczek, and J. Andrews. 2012. Diversification of doublesex function underlies morph-, sex-, and species-specific development of beetle horns. Proceedings of the National Academy of Sciences of the United States of America 109:20526-20531.

Kim, D., J. M. Paggi, C. Park, C. Bennett, and S. L. Salzberg. 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nature Biotechnology 37:907-915.

Lafuente, E., D. Duneau, and P. Beldade. 2024. Genetic basis of variation in thermal developmental plasticity for Drosophila melanogaster body pigmentation. Molecular Ecology 33:e17294.

Lamb, R. J. and P. A. MacKay. 1979. Variability in migratory tendency within and among natural populations of the pea aphid, *Acyrthosiphon pisum*. Oecologia 39:289-299.

Lande, R. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99:541-553.

Lander, E. S. and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Leonardo, T. E. and E. B. Mondor. 2006. Symbiont modifies host life-history traits that affect gene flow. Proceedings of the Royal Society B-Biological Sciences 273:1079-1084.

Li, Y., H. Park, T. E. Smith, and N. A. Moran. 2019. Gene Family Evolution in the Pea Aphid Based on Chromosome-level Genome Assembly. Molecular Biology and Evolution.

Liu, X. and J. A. Brisson. 2023. Dopamine mediates the pea aphid wing plasticity. Biol. Letters 19:20230024.

McCarthy, D. J., Y. Chen, and G. K. Smyth. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Research 40:4288-4297.

Moczek, A. P. 2002. Allometric plasticity in a polyphenic beetle. Ecological entomology 27:58-67.

Moran, N. A. and T. Jarvik. 2010. Lateral Transfer of Genes from Fungi Underlies Carotenoid Production in Aphids. Science 328:624-627.

Otto, S. P. and C. D. Jones. 2000. Detecting the undetected: Estimating the total number of loci underlying a quantitative trait. Genetics 156:2093-2107.

Parker, B. J. and J. A. Brisson. 2019. A laterally transferred viral gene modifies aphid wing plasticity. Current Biology 29:2098-2103.e2095.

Parker, B. J., R. M. H. Driscoll, M. E. Grantham, J. Hrcek, and J. A. Brisson. 2021. Wing plasticity and associated gene expression varies across the pea aphid biotype complex. Evolution.

Pfennig, D. W., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting, and A. P. Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol. Evol. 25:459-467.

Pigliucci, M., C. J. Murren, and C. D. Schlichting. 2006. Phenotypic plasticity and evolution by genetic assimilation. J. Exp. Biol. 209:2362-2367.

Putri, G. H., S. Anders, P. T. Pyl, J. E. Pimanda, and F. Zanini. 2022. Analysing high-throughput sequencing data in Python with HTSeq 2.0. Bioinformatics 38:2943-2945.
Ragsdale, Erik J., Manuela R. Müller, C. Rödelsperger, and Ralf J. Sommer. 2013. A Developmental Switch Coupled to the Evolution of Plasticity Acts through a Sulfatase. Cell 155:922-933.
Ritchie, M. E., B. Phipson, D. Wu, Y. Hu, C. W. Law, W. Shi, and G. K. Smyth. 2015. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43:e47.
Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinform Oxf Engl 26.
Shen, X., M. Pettersson, L. Rönnegård, and Ö. Carlborg. 2012. Inheritance beyond plain heritability: variance-controlling genes in Arabidopsis thaliana. PLoS Genet 8:e1002839.
Simpson, S. J., G. A. Sword, and N. Lo. 2011. Polyphenism in insects. Curr. Biol. 21:R738-749.
Sutherland, O. R. W. 1969. The role of crowding in the production of winged forms by two strains of the pea aphid, Acyrthosiphon pisum. J Insect Phisiol 15.
Vellichirammal, N. N., N. Madayiputhiya, and J. A. Brisson. 2016. The genome-wide transcriptional response underlying the pea aphid wing polyphenism. Mol. Ecol. 25:4146-4160.
Wadley, F. M. 1923. actors affecting the Proportion of Alate and Apterous Forms of Aphids. An. Ent. Soc. Am. 16:279-303.
Weisser, W. W. and C. Braendle. 2001. Body colour and genetic variation in winged morph production in the pea aphid. Entomologia Experimentalis Et Applicata 99:217-223.
Weisser, W. W. and B. Stadler. 1994. Phenotypic Plasticity and Fitness in Aphids. European Journal of Entomology 91:71-78.
West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, New York.
Wright, S. 1968. Evolution and the Genetics of Populations, Vol. 1. Genetics and Biometrical Foundations. University of Chicago Press, Chicago.
Xu, H. J., J. Xue, B. Lu, X. C. Zhang, J. C. Zhuo, S. F. He, X. F. Ma, Y. Q. Jiang, H. W. Fan, J. Y. Xu, Y. X. Ye, P. L. Pan, Q. Li, Y. Y. Bao, H. F. Nijhout, and C. X. Zhang. 2015. Two insulin receptors determine alternative wing morphs in planthoppers. Nature 519:464-467.
Xu, X., F. Oliveira, B. W. Chang, N. Collin, R. Gomes, C. Teixeira, D. Reynoso, V. My Pham, D. E. Elnaiem, S. Kamhawi, J. M. Ribeiro, J. G. Valenzuela, and J. F. Andersen. 2011. Structure and function of a "yellow" protein from saliva of the sand fly Lutzomyia longipalpis that confers protective immunity against Leishmania major infection. The Journal of biological chemistry 286:32383-32393.
Zeng, Z. B. 1992. Correcting the bias of Wright's estimates of the number of genes affecting a quantitative character: a further improved method. Genetics 131:987-1001.
Zera, A. J. and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Ann. Rev. Ent. 42:207-230.

## LIST OF FIGURE CAPTIONS

**Figure 1. Cross between parent lines with distinct wing plasticity phenotypes.** (A) Two parent lines were selected for their different wing plasticity responses: a "high plasticity" line (HP, shown in red) with a large difference in winged offspring production between low and high density environments, and a "low plasticity" line (LP, shown in blue) with no difference in winged offspring production between low and high density environments. As both lines produce few winged offspring in a low density environment, winged offspring production in the high density environment (gray box) is a good proxy for the strength of plasticity. (B) The selected parent lines, LP and HP, were crossed to produce an F1 line. This F1 line was then repeatedly crossed back to parent LP to produce a panel of 49 backcross lines, each with a distinct genotype.

**Figure 2. Variation in the plasticity phenotype across parental and progeny generations**. (A) Shows the percentage of winged offspring in crowded conditions for the LP parent with 54 replicates (each replicate comprises offspring of 12 crowded mothers), the HP parent with 42 replicates, and the F1 offspring line with six replicates (points). (B) Shows the same type of data for backcross lines (n=49), with three replicates (points) for each backcross line. The mean for each line is also shown.

**Figure 3. The parental lines exhibit gene expression differences**. (A) Principal component analysis clustering of four LP (blue points) and four HP (red points) head transcriptomes on the first two principal components. Each transcriptome is represented by a single point. (B) Volcano plot showing differential expression between LP and HP. Genes that were significantly differentially expressed ($\log_2$ fold change > 2 or < -2 and FDR < 0.05) are highlighted in green; genes that had either $\log_2$ fold change > 2 or < -2, or FDR < 0.05, but not both, are shown in black.

**Figure 4. A restriction fragment length polymorphism marker on autosome 1 falls within a region causing wing plasticity differences.** Average percent winged offspring for backcross lines that possess different genotypes at a SNP near *yellow-h* on autosome 1. Different genotypes are denoted on the x-axis.