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Extreme developmental instability associated with wing plasticity in pea aphids

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A key focus of evolutionary developmental biology is on how phenotypic diversity is generated. In particular, both plasticity and developmental instability contribute to phenotypic variation among genetically identical individuals, but the interactions between the two phenomena and their general fitness impacts are unclear. We discovered a striking example of asymmetry in pea aphids: the presence of wings on one side and the complete or partial absence of wings on the opposite side. We used this asymmetric phenotype to study the connection between plasticity, developmental instability and fitness. We found that this asymmetric wing development (i) occurred equally on both sides and thus is a developmental instability; (ii) is present in some genetically unique lines but not others, and thus has a genetic basis; and (iii) has intermediate levels of fecundity, and thus does not necessarily have negative fitness consequences. We conclude that this dramatic asymmetry may arise from incomplete switching between developmental targets, linking plasticity and developmental instability. We suspect that what we have observed may be a more widespread phenomenon, occurring across species that routinely produce distinct, alternative phenotypes.

1. Introduction

How phenotypic variation is generated by developmental systems is a key question in evolutionary developmental biology. Typically, developmental processes produce a target phenotype [1] that is specified by a combination of genetics and environment. Some phenotypes are highly canalized, meaning that despite perturbations in the genetic, outside or internal environment, the target phenotype is largely achieved [2,3]. But developmental noise may still lead to variation around a mean phenotype. This type of random phenotypic variation is often referred to as developmental instability (DI) [4] and can be observed through subtle asymmetries in bilateral characters. These subtle departures from symmetry are often quantifiable and measured as the difference between the left and right sides, referred to as fluctuating asymmetry [5,6]. In a highly canalized trait that successfully attains its target phenotype, fluctuating asymmetry measures hover around zero, while traits with high developmental noise show larger values.

When a developmental environment changes, the target phenotype can change. This is the case for organisms that display adaptive phenotypic plasticity, wherein a single genotype can produce one or more phenotypes in response to environmental conditions during development [7]. These alternative phenotypes can themselves each be highly canalized, with different target phenotypes that each match their respective environments. But depending on how stable the developmental processes are, these environment-specific developmental targets can also display DI and thus asymmetries.

Phenotypic plasticity and DI are often considered in tandem because both contribute to phenotypic variation (e.g. [8]). In particular, plasticity may result in increased DI and consequently introduce an evolutionary cost, thus limiting

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the more widespread phylogenetic occurrence of plasticity [9]. The rationale underlying this connection is that in plastic organisms, the genotype must produce more than one phenotype. Because of this increased complexity, developmental mistakes may happen. Some studies have demonstrated a general positive correlation between the degree of plasticity of a trait and the extent of DI in that same trait [8], while others have not [10], indicating that the relationship between phenotypic plasticity and DI may be trait specific. And DI does not always lead to negative fitness consequences. If there are no negative fitness consequences, then there is no evolutionary cost to DI.

Here, we investigate the connection between phenotypic plasticity, DI and fitness by interrogating DI and fitness in a textbook case of phenotypic plasticity: the wing dimorphism of the pea aphid (*Acyrthosiphon pisum*). Pea aphids, like many aphid species, are asexual and viviparous during the spring and summer months. During this part of their life cycle, two different winged and wingless morphs are present in the population. Adult females can produce both morphs, and because their asexual reproduction occurs without recombination, these morphs are genetically identical (reviewed in [11]). Females produce winged daughters in a high-density environment and wingless daughters in a low-density environment [12]. The traits associated with winged or wingless morphs are a result of the trade-offs between dispersive and reproductive life-history traits, respectively (reviewed extensively in [13]). Trade-offs emerge from the fact that each aphid has a finite amount of resources that can be spent on development and reproduction.

During a previous study examining natural variation for the propensity to produce winged offspring in response to environmental cues by individual lines of pea aphids [14], we observed the appearance of a striking wing-asymmetry phenotype: aphids missing fore- and hindwings on one side but not the other. We therefore set out to investigate the link between the pea aphid wing phenotypic plasticity and DI. We describe the wing-asymmetry phenotype, characterize it as a DI and explore its fitness effects. Our results indicate that there is a genetic basis to this tendency to develop asymmetric wings and there is little to no fitness cost associated with this curious phenotype. Our results suggest a more general connection between DI and plasticity: that DI in organisms with non-continuous plasticity (i.e. polyphenisms) can manifest as dramatic developmental asymmetries in bilateral traits because of instability in the switch to different target phenotypes between sides of individuals. Moreover, although seemingly maladaptive, they can, in fact, have no observable fitness consequences.

2. Results

(a) The wing-asymmetry is a developmental instability

Wing-asymmetric aphids show missing or deformed forewings and/or hindwings on one side of the body, compared to their symmetric, two-winged sisters (figure 1*a*). Our first goal was to characterize the wing-asymmetry phenotype. For this, we focused on three lines that consistently produced wing-asymmetric aphids (lines 507, 509 and 584). We collected a total of 88 wing-asymmetric aphids from the three lines. Of these, 44 had missing or deformed wings on the right side, while 44 had missing or deformed wings on the left side, indicating that the deformity occurs with equal frequency on both sides ($\chi^2 = 0$, $p > 0.99$; figure 1*b*). The lack of direction to this asymmetry indicates that this phenotype is a result of DI (i.e. the inability of developmental processes to produce a wild-type, target phenotype that is the same on both sides of a bilateral individual, which therefore renders it asymmetric) [15].

We also scored the severity of the deformity on the affected side using a score of 0, 1, 2 or 3, from most to least abnormal (see Material and methods). The forewing was much more likely to be reduced or absent than the hindwing: 69 of the 88 aphids examined had no forewing at all (figure 1*c*), with 36 of those aphids having a small or shrivelled hindwing. However, we observed no occurrences of the inverse pattern of a small or deformed forewing in the absence of any hindwing development (figure 1*c*). Overall, the degree of deformation of the forewing was positively correlated with the degree of deformation of the hindwing (Spearman's rho = 0.55, $p < 0.001$). We also did not observe cases where fore- and hindwings were defective on opposite sides. Therefore, the development of the hindwing appears to be coupled with that of the forewing.

To determine if variation in other, non-wing related structures was correlated with variation in wing asymmetry, we focused on a character known to differ between winged and wingless asexual female aphids: the number of secondary rhinaria. Secondary rhinaria are olfactory sensilla located on the third and fourth antennal segments of adult aphids that are used to detect volatile compounds: winged females have many and wingless females have few (figure 2*a*) [16]. As expected, we found that winged aphids had many more rhinaria than wingless aphids (figure 2*b*). We further found that among wing-asymmetric aphids, the side with the missing or deformed wing was not more likely to be accompanied by a lower rhinaria count relative to the other side (Mann–Whitney *U*-test; $p = 0.23$). Therefore, wing-asymmetry does not correlate with rhinaria trait variation. Furthermore, because rhinaria are not asymmetric, the asymmetry is not a whole-body phenomenon.

The wingless females and rhinaria we examined for figure 2*b* were females whose mothers had never been crowded and who developed on uncrowded plants. When we examined rhinaria from wingless females derived from crowded mothers, we observed that some of them had rhinaria counts that were almost, but not quite, as high as those from winged aphids (figure 2*c*). This suggests that these two categories of wingless females might be qualitatively different, and more differences between them might be found if more characters were examined.

(b) The wing developmental instability has a genetic basis

Our second goal was to determine if the wing developmental instability has a genetic basis. We examined 192 genetically unique aphid lines as described in [17]. We used a 24 h crowding treatment to induce adult, wingless mothers to produce winged offspring as in [14]. Because wing-asymmetric aphids are a subset of winged aphids, we only considered lines further if they produced a large number of winged females, at least 45% winged offspring out of a minimum number of 120 offspring over three crowding replicates. Seventeen of the 192 lines fit these criteria and are presented here.

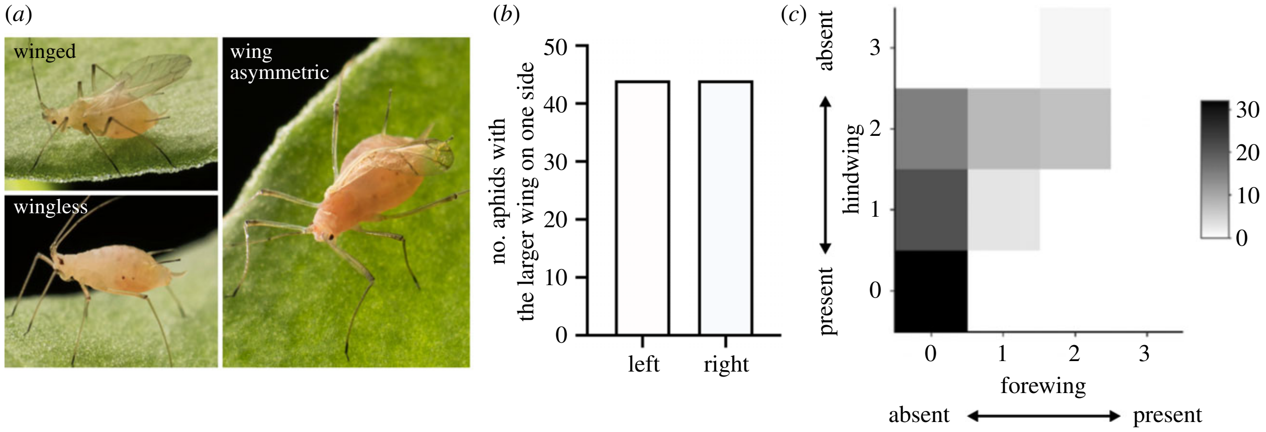

Figure 1. Characteristics of the wing-asymmetry phenotype across individuals. (*a*) Winged, wingless and wing-asymmetric aphids (photos by Adam Fenster); the wing-asymmetric aphid is an example of one completely missing fore and hindwings on its left side. (*b*) The affected side of wing-asymmetric aphids from all three lines combined. $n = 88$. (*c*) The co-occurrence of forewing (*x*-axis) and hindwing (*y*-axis) asymmetries. By definition, in all cases the forewing was either absent or deformed (a score of 0, 1 or 2; see Material and methods for a full explanation). The key indicates the number of aphids. $n = 88$. (Online version in colour.)

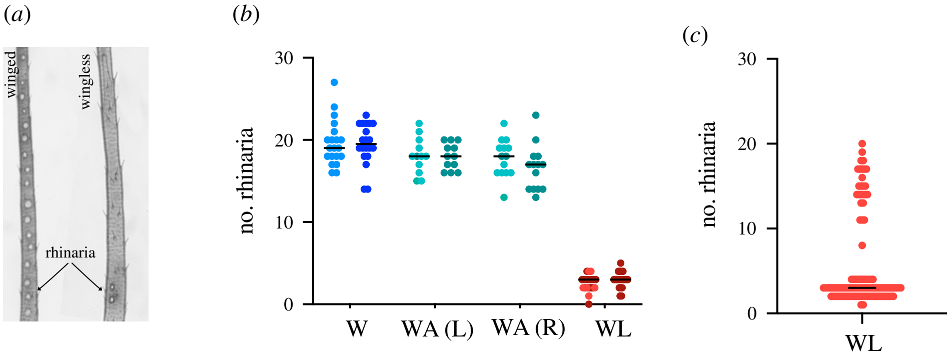

Figure 2. The number of secondary rhinaria on the third antennal segment of different morphs. (*a*) Photos of typical antennae plus rhinaria (small clear circles; 17 shown for the winged and 2 for the wingless) for winged and wingless aphids. (*b*) Rhinaria counts for winged (W), wing-asymmetric (WA), and wingless (WL) aphids. $n = 20$ for each morph. Left (on the left, lighter colour) and right (on the right, darker colour) sides were counted for each individual. Of the WA aphids, the deformed side is shown in parentheses, with L = left and R = right. For W and WA aphids here, their mothers were subjected to crowding; the WL aphids were maintained at low density for several generations prior to collection and thus never experienced high density. (*c*) Rhinaria counts of wingless females whose mothers were crowded. Given that we observed no differences between left and right sides, only data for the left antennae are shown ($n = 106$). All data are from line 509. (Online version in colour.)

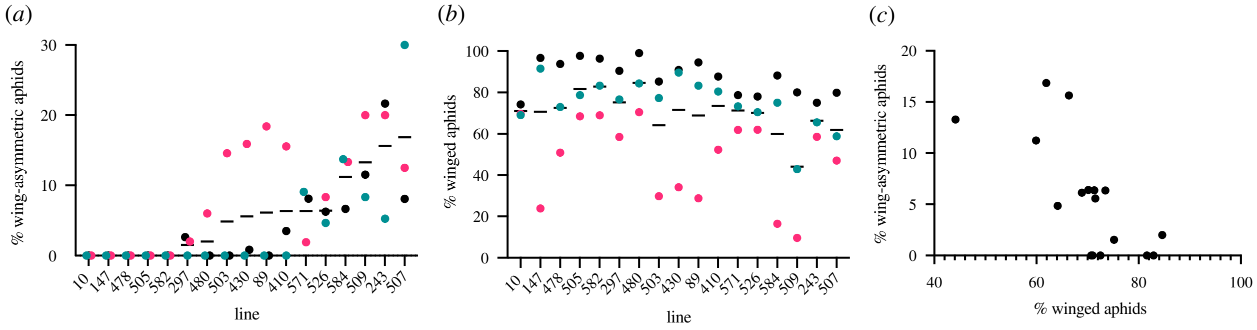

Figure 3. Lines differ in their production of wing-asymmetric aphids. (*a*) The percentage of winged aphids that were wing-asymmetric for the 17 lines, shown for the three replicate crowding treatments (replicates represented in three colours, with means shown as a bar). The lines are ordered by the average number of wing-asymmetric aphids they produced across all replicates. (*b*) The percentage of winged offspring produced for these same lines across the three replicates, presented in the same order as (*a*). (*c*) The percentage of wing-asymmetric offspring produced by each of the 17 lines (averaged across the three replicates) relative to the average percentage of winged offspring produced by that line in response to crowding. (Online version in colour.)

The number of wing-asymmetric aphids varied greatly among the lines, from 0% of the winged offspring being wing-asymmetric, to as many as 30% (figure 3*a*). Although there were differences among replicates, some lines consistently produced wing-asymmetric aphids while others did not (figure 3*a*). These differences among lines suggest that the wing developmental instability has a genetic basis. Furthermore, all 17 of these lines produce relatively high proportions of winged offspring when crowded (figure 3*b*), which suggests that the higher observed numbers of wing-asymmetric aphids

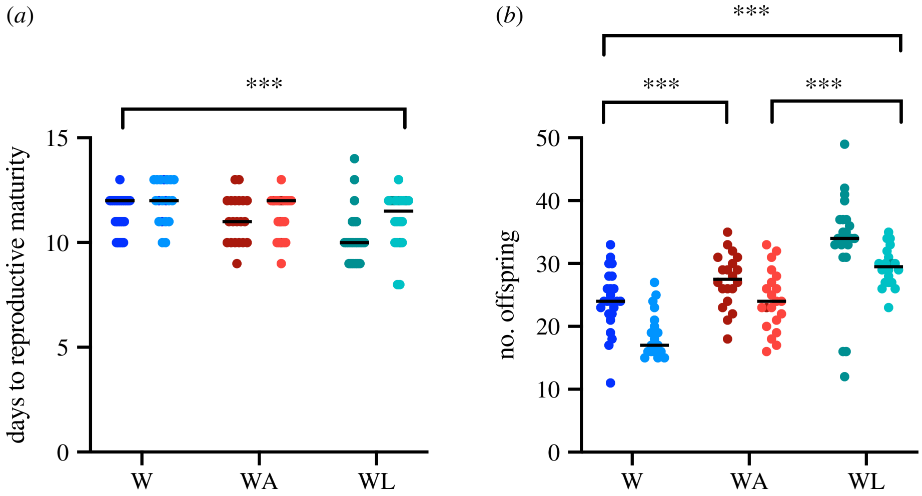

Figure 4. Fitness attributes of winged (W), wing-asymmetric (WA) and wingless (WL) aphids. (*a*) The days to reproductive maturity across morphs. (*b*) The number of offspring produced in the first 5 days once reproductive maturity is achieved. The 507 (darker colour, on the left for each morph) and 584 (lighter colour) represent two genetically unique lines used to replicate these experiments. For both panels, points are samples within genetic lines and the line shown is the median. ***$p < 0.001$. (Online version in colour.)

in some lines is not a trivial consequence of those lines producing more winged aphids. In fact, the propensity to produce winged offspring when crowded had a negative correlation with wing-asymmetric aphid production (figure 3*c*).

(c) Fitness differences associated with unstable wing development

To determine whether or not there are fitness costs associated with the unstable wing development phenotype, we measured two aspects of fitness: time to reproductive maturity and fecundity (number of offspring produced over the first 5 days of reproductive maturity). For time to reproductive maturity, the lines and phenotypes, but not the interaction term, were significantly different (quasi-Poisson generalized linear model (GLM); line: $F = 5.5$, 1 d.f., $p = 0.021$; phenotype: $F = 8.3$, 2 d.f., $p < 0.001$, interaction: $F = 0.9$, 2 d.f., $p = 0.41$). In particular post-hoc tests revealed that winged females spent more time developing than wingless females ($z = 4.1$, $p < 0.001$; figure 4*a*). This is consistent with previous studies in other aphid species that have shown that wingless females attain reproductive maturity earlier than winged females (e.g. [18]). The reproductive output across the first 5 days of offspring production also differed by phenotype (figure 4*b*; quasi-Poisson GLM; $F = 38$, 2 d.f., $p < 0.001$). Wingless aphids produced significantly more offspring than wing-asymmetric aphids ($z = 4.7$, $p < 0.001$), and wing-asymmetric aphids produced significantly more offspring than winged aphids ($z = 4.0$, $p < 0.001$). These data show that fitness differed among aphid morphs.

3. Discussion

Our study aimed to examine the connections between phenotypic plasticity, developmental instability (DI) and fitness. Overall, we (i) find that the DI has a heritable basis, (ii) discover that fitness differed among aphid morphs, and (iii) suggest that later developmental switching between winged and wingless target phenotypes leads to more DI, and that the resulting dramatic asymmetry might be a common phenomenon among plastically polymorphic (i.e. polyphenic) species.

Our first major result is that we have demonstrated that pea aphid wing DI, which yields asymmetric wings, has a genetic basis, as evidenced by the differences among pea aphid genotypes (figure 3*a*). Some aphid genotypes routinely produce wing-asymmetric aphids while others never do. DI has been observed to have a genetic basis in other systems as well (reviewed in [19]). In contrast with most other studies, the wing DI observed here is dramatic and easily observable, while DI is usually inferred from relatively minor, quantitative differences between sides as measured by fluctuating asymmetry [20]. For example, the differences in wing shape between the left and right side of *Drosophila melanogaster* individuals are subtle and only quantifiable with precise geometric-morphometric analyses [21]. Here we have asymmetries that can be seen by the eye. The obvious difference between the fly example and the pea aphid wing-asymmetry is that flies have a single developmental target when they are developing a wing, while aphids have one of two alternatives depending on the environment. This observation—that such a dramatic asymmetry accompanies developmental plasticity—shows that the two can go hand in hand.

The mechanisms underlying DI are generally unknown, with the exception of a few studies that have identified genes that contribute to DI (e.g. [22,23]). Here, we observe an inverse relationship between wing inducibility and wing-asymmetry (figure 3*c*). These two traits therefore seem linked at the genetic level, such that the same gene(s) or linked gene(s) control the propensity to produce winged offspring in response to a high-density environment and the production of wing-asymmetric aphids. This intriguing result warrants future investigations.

It's unclear how the aphid wing-asymmetry develops at the mechanistic level. The phenotype is reminiscent of the first *wingless* gene mutants in *Drosophila melanogaster,* which displayed fully winged, one-winged and wings absent animals, with similar changes to halteres (wg^1) [24]. Later studies showed that the mutation was a small deletion in an enhancer that drove expression in the wing and haltere [25]. The link between lower expression and the variable phenotypes suggests that wg^1 mutants produced a near-threshold amount such that too little resulted in no wing/haltere, and enough resulted in the opposite [26]. And importantly for this study, this threshold could be met or missed independently on each side of the body. A similar mechanism could be acting in the wing asymmetry. In the aphids, however, the asymmetry is not off or on like it was observed in *Drosophila*. Rather, a gradation of phenotypes is observed (figure 1*c*) that suggests a more complicated basis than the action of a single, key gene.

We also show that extreme DI results in fitness differences, but not necessarily the expected ones. Upon first glance, wing-asymmetric aphids might be assumed to be highly maladaptive. But in fact, we show that wing-asymmetric aphids produce more offspring than winged aphids. Thus, if an environment has changed from high to low density, there is a benefit to switching from winged to wingless even if a seemingly maladaptive wing asymmetry accompanies the switch. This lack of cost under these circumstances may be why the wing-asymmetric aphids exist at relatively high frequency in some lines (figure 2*a*). This is an important observation because it informs the debate regarding the costs and limits of plasticity. Developmental constraints are often thought to limit the ubiquity of plasticity [27]. One possible constraint often mentioned is DI, although the literature on whether or not DI actually results in fitness costs is mixed (reviewed in [28]). In the pea aphid case, we found no evidence for a cost, at least with respect to fecundity.

Finally, we suggest that the extreme asymmetry associated with this plastic wing dimorphism may be one example of a more widespread phenomenon. First, consider how aphid wings develop. All viviparous female aphids are born with wing buds, regardless of their future phenotype [29,30]. Wingedness is also the ancestral state, since aphids evolved from winged ancestors [31]. Therefore, regardless of pre- or post-natal wing morph determination, the winged state can be diverted into the wingless pathway, but not vice versa [29]. This is presumably because it is possible to degrade wings and wing musculature and return that energy to embryo production, while it is less feasible to add wings and wing musculature past a certain developmental time point. Wingless, asexual pea aphid females appear to cease wing bud growth as first instar nymphs [32,33]. Other species can divert from winged to wingless even later [34]. Thus, switching from a winged-morph developmental target to a wingless one is common among aphid species.

We hypothesize that the wing-asymmetric pea aphids described here have incompletely transitioned from winged to wingless morphs postnatally. They started life as winged individuals but switched to the wingless developmental trajectory. Our experiments did not directly address this, but two ideas support this hypothesis. First, species with earlier morph determination yield morphs more dedicated towards reproduction versus dispersal relative to species with postnatal wing determination [13]. In other words, the earlier a target phenotype is determined, the better that morph develops. A later transition likely leads to less conversion from winged to wingless structures and physiology, and this can be associated with some DI. Second, we observed possible evidence of wingless aphids that have incompletely transitioned, in the form of wingless aphids with intermediate rhinaria counts (figure 2).

Therefore, DI may present itself most radically in plastically dimorphic species. Alternative phenotypes (i.e. polyphenisms) are often called threshold traits [35], in contrast with quantitative traits. Quantitative traits vary in small ways with increases or decreases of various developmental signals, while threshold traits do not change unless the signal level is at or near the threshold. As noted by Palmer [36,37] when discussing left-right asymmetries, for some traits, thresholds can be achieved or missed independently on each side of an organism. He notes that another Hemipteran, the firebug *Pyrrhocoris apterus*, also has a wing dimorphism that sometimes yields extreme wing-asymmetry [38], suggesting that this phenomenon might be common among wing-dimorphic insects. Similarly, dimorphic mites where an individual develops with or without armoured legs can be asymmetric, with one armoured leg and one unarmoured leg [39]. These examples suggest a more common phenomenon of extreme asymmetry associated with developmentally plastic dimorphisms of bilateral traits.

4. Material and methods

(a) Aphid lines

The 17 lines of pea aphid (*Acyrthosiphon pisum*) used here were initially collected from two alfalfa fields outside of Ithaca, NY [17]. Lines were reared on fava bean seedlings (*Vicia faba*) under long-day conditions (16 : 8 L : D photoperiod) in an 18 ± 1.5°C, 35–45% relative humidity incubator to ensure the aphids stayed in their asexual cycle. Prior to any experiments, lines were kept at low density (seven or fewer aphids per plant) for at least three generations to prevent grandmaternal and maternal effects on wing phenotype. Aphids reared on the same plants were divided randomly for all following experiments.

(b) Winged offspring production

To induce production of winged and wing-asymmetric aphids, wingless adult females from the low-density stock were subjected to a combined crowding and starvation treatment. Ten adult asexual wingless females were collected from a stock within 3 days of becoming reproductively mature and placed in a 32.5 mm × 15 mm Petri dish (Fisher) with a piece of damp Whatman paper for 24 h. They were then placed individually on 60 mm × 15 mm Petri dishes with a *Vicia faba* leaf inserted into 9 ml 2% bacto agar and left to produce offspring for 24 h. The adult females were removed after 24 h and the offspring produced were reared until they reached adulthood (11 ± 2 days), at which point the phenotype (winged, wingless or wing asymmetric) was recorded.

(c) Phenotype characterization

Once the winged, wingless and wing-asymmetric offspring reached adulthood, they were placed individually on leaf plates to test their time to reproductive maturity and their fecundity. The time to reproductive maturity was defined as the number of days between their birth and the birth of their first offspring. Individuals were checked every 24 ± 2 h for offspring production. We recorded the number of offspring each individual produced per day during the first 5 days of reproductive maturity.

Winged aphids are characterized by a set of forewings and hindwings as well as a protruding muscle on the back. To describe the phenotype of the wing-asymmetric aphids, the affected side (the side lacking a full set of wings) was scored using a two-part system in which the forewing and hindwing were independently assigned a number from 0 to 3 denoting the severity of the deformity. A score of 0 indicated the total absence of any muscle or wing development, 1 indicated the presence of a small protrusion where the wing would be, 2 indicated the presence of a small, deformed wing (often shrivelled), and 3 indicated the presence of a normal wing. The affected side, right or left, was also recorded.

(d) Antennae collection

Winged, wingless and wing-asymmetric aphids from both the crowding and rearing density experiments were collected and put into a 75% ethanol solution to preserve their antennae. Forceps were used to remove the antennae from the base of the head, leaving the pedicel and distal segments intact. A drop of paraffin oil was placed on a microscope slide and the antenna was placed in the droplet, then covered by a cover slide. Clear nail enamel was used to secure the cover slide. Right and/or left antennae were collected for winged, wingless and wing-asymmetric aphids. We used a Leica DM5000 B microscope at 10 × and 20 × magnifications to photograph and record the number of rhinaria.

(e) Statistical analysis

Whether wing-asymmetry was found more frequently on the right or left side of an aphid's body, the correlation between the degree of wing deformation on the fore- and hindwings, and differences in rhinaria counts were analysed using StatPlus. Data on the number of days to reproductive maturity were examined using a quasi-Poisson generalized linear model (GLM) implemented in R v. 3.5.0 (R-core team). Aphid line (507 and 584) and phenotype (winged, wing-asymmetric and wingless) were modelled as fixed effects, and minimal models were derived by first removing the interaction term between these two factors, then phenotype, then line, and statistical significance of each factor was determined by comparing models with ANOVA and F-tests. Post-hoc tests examining differences between treatments were conducted in the multcomp package v. 1.4-13 [40]. Fecundity data were analysed in the same way using a quasi-Poisson GLM.

Data accessibility. All supporting data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.0rxwdbrxf [41].

Authors' contributions. R.E.H., C.L.H., E.T.H. and A.M. carried out the laboratory work; R.E.H., C.L.H., B.J.P. and J.A.B. designed the study, participated in data analysis and drafted the manuscript. J.A.B. coordinated the study. All authors revised the manuscript, gave final approval for publication and agree to be held accountable for the work performed therein.

Competing Interests. The authors have no competing interests.

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References

1. Nijhout HF, Davidowitz G. 2003 Developmental perspectives on phenotypic variation, canalization, and fluctuating asymmetry. In *Developmental instability: causes and consequences* (ed M. Polak), pp. 3–13. New York, NY: Oxford University Press.
2. Waddington CH. 1942 Canalization of development and the inheritance of acquired characters. *Nature* **150**, 563–565. (doi:10.1038/150563a0)
3. Stearns SC, Kawecki TJ. 1994 Fitness sensitivity and the canalization of life-history traits. *Evolution* **48**, 1438–1450. (doi:10.1111/j.1558-5646.1994.tb02186.x)
4. Klingenberg CP. 2019 Phenotypic plasticity, developmental instability, and robustness: the concepts and how they are connected. *Front. Ecol. Evol.* **7**, 56. (doi:10.3389/fevo.2019.00056)
5. Waddinton CH. 1957 *The strategy of the genes*. London, UK: George Allen Unwin.
6. Van valen L. 1962 A study of fluctuating asymmetry. *Evolution* **16**, 125–142. (doi:10.1111/j.1558-5646.1962.tb03206.x)
7. Bradshaw AD. 1965 Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* **13**, 115–155. (doi:10.1016/S0065-2660(08)60048-6)
8. Tonsor SJ, Elnaccash TW, Scheiner SM. 2013 Developmental instability is genetically correlated with phenotypic plasticity, constraining heritability, and fitness. *Evolution* **67**, 2923–2935. (doi:10.1111/evo.12175)
9. DeWitt TJ, Sih A, Wilson DS. 1998 Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77–81. (doi:10.1016/s0169-5347(97)01274-3)
10. Scheiner SM, Caplan RL, Lyman RF. 1991 The genetics of phenotypic plasticity. III. Genetic correlations and fluctuating asymmetries. *J. Evol. Biol.* **4**, 51–68. (doi:10.1046/j.1420-9101.1991.4010051.x)
11. Wilson ACC, Sunnucks P, Hales DF. 2003 Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). *Biol. J. Linnean Soc.* **79**, 115–135. (doi:10.1046/j.1095-8312.2003.00176.x)
12. Sutherland ORW. 1969 The role of crowding in the production of winged forms by two strains of the pea aphid, *Acyrthosiphon pisum*. *J. Insect Phys.* **15**, 1385–1410. (doi:10.1016/0022-1910(69)90199-1)
13. Zera AJ, Denno RF. 1997 Physiology and ecology of dispersal polymorphism in insects. *Ann. Rev. Ent.* **42**, 207–230. (doi:10.1146/annurev.ento.42.1.207)
14. Parker BJ, Brisson JA. 2019 A laterally transferred viral gene modifies aphid wing plasticity. *Curr. Biol.* **29**, 2098–2103.e5. (doi:10.1016/j.cub.2019.05.041)
15. Palmer AR. 1994 Fluctuating asymmetry analyses: a primer. In *Developmental instability: its origins and evolutionary implications* (ed. T.A. Markow), pp. 335–364. Dordrecht, The Netherlands: Kluwer.
16. Pickett JA, Wadhams LJ, Woodcock CM, Hardie J. 1992 The chemical ecology of aphids. *Annu. Rev. Entomol.* **37**, 67–90. (doi:10.1146/annurev.en.37.010192.000435)
17. Chung SH, Parker BJ, Blow F, Brisson JA, Douglas AE. 2020 Host and symbiont genetic determinants of nutritional phenotype in a natural population of the pea aphid. *Mol. Ecol.* **29**, 848–858. (doi:10.1111/mec.15355)
18. Ishikawa A, Miura T. 2009 Differential regulations of wing and ovarian development and heterochronic changes of embryogenesis between morphs in wing polyphenism of the vetch aphid. *Evol. Dev.* **11**, 680–688. (doi:10.1111/j.1525-142X.2009.00375.x)
19. Leamy LJ, Klingenberg CP. 2005 The genetics and evolution of fluctuating asymmetry. *Annu. Rev. Ecol. Syst.* **36**, 1–21. (doi:10.1146/annurev.ecolsys.36.102003.152640)
20. Palmer AR, Strobeck C. 1986 Fluctuating asymmetry: measurement, analysis, patterns. *Annu. Rev. Ecol. Syst.* **17**, 391–421. (doi:10.1146/annurev.es.17.110186.002135)
21. Klingenberg CP, Zaklan SD. 2000 Morphological integration between developmental compartments in the *Drosophila* wing. *Evolution* **54**, 1273–1285. (doi:10.1111/j.0014-3820.2000.tb00560.x)
22. Debat V *et al.* 2011 Developmental stability: a major role for cyclin G in *Drosophila melanogaster*. *PLoS Genet.* **7**, e1002314. (doi:10.1371/journal.pgen.1002314)
23. Clarke GM, Yen JL, McKenzie JA. 2000 Wings and bristles: character specificity of the asymmetry phenotype in insecticide-resistant strains of *Lucilia cuprina*. *Proc. R. Soc. Lond. B* **267**, 1815–1818. (doi:10.1098/rspb.2000.1215)
24. Sharma RP. 1973 Wingless—a new mutant in *Drosophila melanogaster*. *Drosophila Inf. Serv.* **50**, 134.
25. Schubiger M, Sustar A, Schubiger G. 2010 Regeneration and transdetermination: the role of wingless and its regulation. *Dev. Biol.* **347**, 315–324. (doi:10.1016/j.ydbio.2010.08.034)
26. Bejsovec A. 2018 Wingless signaling: a genetic journey from morphogenesis to metastasis. *Genetics* **208**, 1311–1336. (doi:10.1534/genetics.117.300157)
27. Auld JR, Agrawal AA, Relyea RA. 2010 Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc. R. Soc. B* **277**, 503–511. (doi:10.1098/rspb.2009.1355)

28. Relyea RA. 2002 Costs of phenotypic plasticity. *Am. Nat.* **159**, 272–282. (doi:10.1086/338540)
29. Johnson B, Birks PR. 1960 Studies on wing polymorphism in aphids. 1. The developmental process involved in the production of the different forms. *Entomol. Exp. Appl.* **3**, 327–339. (doi:10.1111/j.1570-7458.1960.tb00461.x)
30. Heie OE. 1987 Paleontology and phylogeny. In *Aphids: their biology, natural enemies, and control* (eds AK Minks, P Harrewijn), pp. 367–391. Amsterdam, The Netherlands: Elsevier.
31. Miyazaki M. 1987 Forms and morphs of aphids. In *Aphids, their biology, natural enemies and control* (eds AK Minks, P Harrewijn), pp. 163–195. Amsterdam, The Netherlands: Elsevier.
32. Tsuji H, Kawada K. 1987 Development and degeneration of wing buds and indirect flight muscles in the pea aphid (*Acyrtosiphon pisum* (Harris)). *Jpn. J. Appl. Entomel. Zool.* **31**, 247–252. (doi:10.1303/jjaez.31.247)
33. Ishikawa A, Hongo S, Miura T. 2008 Morphological and histological examination of polyphenic wing formation in the pea aphid Acyrthosiphon pisum (Hemiptera, Hexapoda). *Zoomorphology* **127**, 121–133. (doi:10.1007/s00435-008-0057-5)
34. Müller CB, Williams IS, Hardie J. 2001 The role of nutrition, crowding, and interspecfic interactions in the development of winged aphids. *Ecol. Ent.* **26**, 330–340. (doi:10.1046/j.1365-2311.2001.00321.x)
35. Nijhout HF. 1999 Control mechanisms of polyphenic development. *Bioscience* **49**, 181–192. (doi:10.2307/1313508)
36. Palmer AR. 2012 Developmental origins of normal and anomalous randon right-left asymmetry: lateral inhibition versus developmental error in a threshold trait. *Contrib. Zool.* **81**, 111–124. (doi:10.1163/18759866-08102006)
37. Palmer AR. 2016 What determines direction of asymmetry: genes, environment or chance? *Phil. Trans. R. Soc. B* **371**, 20150417. (doi:10.1098/rstb.2015.0417)
38. Socha R, Nedvéd O, Zrzavý J. 1993 Unstable forewing polymorphism in a strain of *Pyrrhocoris apterus* (Hemiptera: Pyrrhocoridae). *Ann. Entomol. Soc. Am.* **86**, 484–489. (doi:10.1093/aesa/86.4.484)
39. Radwan J, Unrug J, Tomkins JL. 2002 Status-dependence and morphological trade-offs in the expression of a sexually selected character in the mite, *Sancassania berlesei*. *J. Evol. Biol.* **15**, 744–752. (doi:10.1046/j.1420-9101.2002.00444.x)
40. Hothorn T, Bretz F, Westfall P. 2008 Simultaneous inference in general parametric models. *Biom. J.* **50**, 346–363. (doi:10.1002/bimj.200810425)
41. Hammelman R, Heusinkveld C, Hung E, Meinecke A, Parker B, Brisson J. 2020 Extreme developmental instability is associated with the pea aphid wing plasticity. Dryad Digital Repository. (doi:10.5061/dryad.0rxwdbrxf)