

Developmental noise and phenotypic plasticity are correlated in *Drosophila simulans*

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Abstract

Non-genetic variation is the phenotypic variation induced by the differential expression of a genotype in response to varying environmental cues and is broadly categorized into two types: phenotypic plasticity and developmental noise. These aspects of variation have been suggested to play an important role in adaptive evolution. However, the mechanisms by which these two types of non-genetic variations influence the evolutionary process are currently poorly understood. Using a machine-learning-based phenotyping tool, we independently quantified phenotypic plasticity and developmental noise in the wing morphological traits of the fruit fly *Drosophila simulans*. Utilizing a rearing experiment, we demonstrated plastic responses in both wing size and shape as well as non-zero heritability of both phenotypic plasticity and developmental noise, which suggests that adaptive phenotypic plasticity can evolve via genetic accommodation in the wing morphology of *D. simulans*. We found a positive correlation between phenotypic plasticity and developmental noise, while the correlation between the plastic response to three kinds of environmental factors that were examined (nutrient condition, temperature, and light–dark cycle) was poor. These results suggest that phenotypic plasticity and developmental noise contribute to evolvability in a similar manner, however, the mechanisms that underlie the correspondence between these two types of variation remain to be elucidated.

Keywords: developmental noise, *Drosophila*, fluctuating asymmetry, heritability, wing shape, phenotypic plasticity

Lay Summary

Non-genetic variations consist of phenotypic plasticity and developmental noise, and these variations have been suggested to influence the direction and rate of phenotypic evolution. However, the role of phenotypic plasticity and developmental noise in the evolutionary process is still poorly understood. In this study, we examined the broad-sense heritability of plasticity and developmental noise, as well as the correlation between the degree of plasticity in response to three kinds of environmental factors and between the degree of plasticity and developmental noise in wing size and wing shape in *Drosophila simulans*. We found that phenotypic plasticity and developmental noise were heritable and positively correlated with each other, while the correlation between plasticity induced by different environmental cues was poor. Our results suggest that phenotypic plasticity and developmental noise may affect the direction and rate of phenotypic evolution in a similar manner.

Introduction

Phenotypic variation is the target of selection and is produced by genetic and non-genetic causes. Evidence for the prominence of non-genetic variation in evolutionary processes is rapidly increasing in recent years (Danchin, 2013; Draghi, 2020; Ghaleb et al., 2007; Price et al., 2003). For instance, the “plasticity-first” hypothesis theorizes that non-genetic variation may take the lead in adaptive evolution (Fusco & Minelli, 2010; Pigliucci et al., 2006). Non-genetic variation can be classified into two kinds: phenotypic plasticity and developmental noise. The former is defined as an adaptive or maladaptive phenotypic response to environmental variation and reflects the responsiveness of development to external environmental conditions (Bradshaw, 1965; Scheiner, 1993). The latter is phenotypic variation within an individual, and reflects the robustness of development against microenvironmental perturbations (Geiler-Samerotte et al., 2013; Kiskowski et al.,

2019; Spudich & Koshland, 1976; Uller et al., 2018). Developmental noise is thought to be caused by spontaneous somatic mutations and changes in internal conditions of growing phenotypes such as random fluctuations in chemical and physical signaling processes. Although there is a blurred distinction between these two kinds of non-genetic variation depending on the definition of environment, causes of variation, and the level at which the variation is considered (i.e., gene, phenotype, or organism), here we define variation induced by factors outside of organisms as plasticity and variation induced by factors inside of organism as developmental noise, based on Scheiner (1993). Currently, we have a limited understanding of how these two kinds of variations influence evolutionary processes.

Phenotypic plasticity is suggested to guide adaptive evolution through genetic accommodation (i.e., plasticity-first evolution) (Badyaev, 2011; Levis & Pfennig, 2016; Levis et al., 2018; Pfennig

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et al., 2010; West-Eberhard, 2003). This process occurs when: (a) plasticity exists; (b) plasticity is heritable; (c) natural selection favors certain plastic responses, which guides the evolution of adaptive phenotypic plasticity; and (d) through this process, the pre-existing phenotypic plasticity is refined by natural selection into a functional phenotype (Levis et al., 2018). Thus, the ability of phenotypes to respond plastically and adaptively to environmental perturbations could influence the direction and rate of evolution. The rationale behind these ideas is that adaptive plasticity can first build developmental mechanisms that generate the adaptive phenotypes more often than the non-adaptive ones (e.g., adaptive developmental bias), and these mechanisms could then later become genetically determined. To study the evolutionary significance of phenotypic plasticity via genetic accommodation, this developmental bias needs to be quantified.

One approach to quantify developmental bias is to evaluate developmental noise, a measure of phenotypic robustness against perturbations (Uller et al., 2018). Viewing organismal development as an analog to thermodynamics under equilibrium states, Kaneko, Furusawa and colleagues (Kaneko & Furusawa, 2006; Sato et al., 2003) proposed that developmental noise can be a general measure of developmental bias. Recently, this hypothesis (reviewed in Kaneko & Furusawa, 2018) received empirical support based on correlations between developmental noise and other sources of variation in gene expression patterns of *Escherichia coli* (Furusawa & Kaneko, 2015) and wing morphological traits of the sepsid fly *Sepsis punctum* (Rohner & Berger, 2023).

However, our understanding of developmental noise and its implication for phenotypic plasticity and evolution remains limited. For instance, although phenotypic plasticity and developmental noise are caused by biologically distinct processes (Scheiner, 1993; Scheiner et al., 1991), they are often lumped together as residual variation after the various types of genetic variances are estimated (Wilson et al., 2010). Moreover, the variance of developmental noise is typically measured as the difference in trait values between the left side and right side of an individual (i.e., fluctuating asymmetry, FA) (Gangestad & Thornhill, 1999; Rohner et al., 2022; Van Valen, 1962). FA is notoriously difficult to evaluate. Not only does FA require repeated measurements of all the paired traits (Palmer & Strobeck, 1986), but a reliable estimate of FA variance in a population often requires a substantial amount of samples (Houle, 1997). In this study, we overcome this logistical challenge by using a recently developed machine-learning-based method to semi-automatically measure phenotypes from digital images (Porto & Voje, 2020). With this method, we can collect big data quickly and repeatedly at a low cost.

Although non-genetic variation is not heritable, the propensity of a genotype to respond to environmental cues and the instability of a genotype in response to developmental noise are heritable (Carter & Houle, 2011; Scheiner, 1993). For example, in *Polygonum cespitosum*, an Asian annual plant, the degree of plasticity varies between populations (Matesanz et al., 2012). Some theoretical and empirical studies have shown that the degree of plastic responses to environmental cues varies among individuals within a population (Ledón-Rettig et al., 2010; Levis et al., 2018; McGuigan et al., 2011; Pigliucci et al., 2006). Similarly, the degree of developmental noise is known to vary within and between populations (Kiskowski et al., 2019). Genetic variation in the ability to produce phenotypic plasticity and developmental noise is a potentially important contributor to the ability of a population to respond to selection (e.g., evolvability, Hansen & Houle, 2008).

Here, we revisit the relationship between developmental noise and phenotypic plasticity of the wing morphological traits of

Drosophila simulans. We first estimate phenotypic plasticity by measuring the plastic response of iso-female lines to multiple environmental conditions and developmental noise by FA. We then estimate the broad-sense heritability of phenotypic plasticity and developmental noise. Finally, we evaluate the correlation between phenotypic plasticity in response to different environmental cues and between phenotypic plasticity and developmental noise. By integrating these results, we aim to advance our understanding of the biological implication of non-genetic variation in evolutionary processes.

Methods

Study species, sampling, and rearing

Drosophila simulans is a common fruit fly species in Japan. We captured adult individuals of *D. simulans* from the campus of Chiba University, Japan (35° 62' 79" N, 140° 10' 31" E) in 2020 and established iso-female lines. Each iso-female line was reared with a standard medium that was made based on that described by Fitzpatrick et al. (2007) (500 mL H₂O, 50 g sucrose, 50 g dry yeast, 6.5 g agar, 5.36 g KNaC₄H₆·4H₂O, 0.5 g KH₂PO₄, 0.25 g NaCl, 0.25 g MgCl₂, 0.25 g CaCl₂, and 0.35 g Fe₂(SO₄)·6.9H₂O) in 170 mL bottles under a 12 h light–dark cycle at 25°C. Each iso-female line was inbred over 20 generations to remove genetic variation in a line and the maternal effect.

Strains and wing collection

Six iso-female lines were randomly chosen and used for our experiments. The degree of phenotypic plasticity was quantified by comparing wing morphology and wing size of individuals reared under seven combinations of three environmental factors. These combinations consisted of three nutrient conditions (high, intermediate, or low), three light–dark cycle conditions (10 h light/14 h dark, 12 h light/12 h dark, or 14 h light/10 h dark), and three temperature conditions (20°C, 23°C, or 26°C). In the present study, high, intermediate, and low nutrient media were composed of a 0%, 40%, and 80% reduction, respectively, of the active yeast and sucrose concentrations of the standard medium. For each environmental condition, one of the three environmental factors varied from the standard condition (12 h light/12 h dark, 23°C, intermediate nutrient). Since the standard condition was shared three times, the total number of environmental conditions was seven. For each iso-female line, 32 eggs were put into the vial and reared under each of the seven conditions from eggs to adults. Two days after the first adult emerged in a vial, all female adults were collected. The left and right wings of the females were separated from their bodies and placed directly onto a glass slide. A glass cover was placed over the wings and the cover glass and glass slide (i.e., dry mount) were glued to flatten the wings.

Analysis of wing morphology and wing size

We took pictures of the wings with a CMOS camera (Leica MC190 HD, 10 million pixels) of the stereoscopic fluorescence microscope (Leica M165 FC) under constant light conditions where the wings are lit up by the tracing stand under the glass slide. To evaluate the variation in wing morphology, we measured the x–y coordinates of 12 landmarks placed at the vein intersections of the wings (Figure 1A) following Houle et al. (2017). To place landmarks in acquired images, we used the machine-learning program, “ml-morph” (Porto & Voje, 2020). First, we built a training set based on 125 wings that were manually landmarked, then used ml-morph to place the landmarks on 250 new images, which were

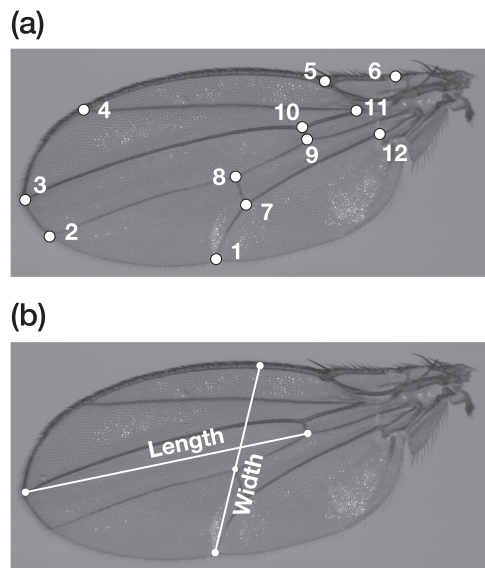


Figure 1. Picture of the left wing, and illustration of wing morphology (A) and wing size (B) measurements.

trained by the first training set, and any erroneous landmarks were manually corrected. Landmark data from 250 images were used to build the second training set as new teaching data. Next, we used *ml-morph*, trained by the second training set, to place landmarks on 1,800 images and manually corrected any errors. Finally, we built the third training set using the 1,800 landmarks data, trained *ml-morph* with the third training set, and used this *ml-morph* to obtain the landmarks. Note that the images used to build the training set included not only the images used in this study but also wing images of other *Drosophila* species that are not presented in this study. With these procedures, we improved the accuracy of the landmarking. Using the training sets based on 1,800 images, we automatically landmarked, and then manually corrected any wing coordinates that were found to be incorrect by *ml-morph*. This procedure allowed us to repeatedly measure all specimens twice. All right wings were horizontally flipped. In total, we landmarked both left and right wings from 410 individuals (820 images).

All statistical analyses were conducted using R 4.1.2. We standardized all wing coordinates using the generalized Procrustes analysis (GPA) with respect to size, rotation, and translation, which translated the original coordinates data to a common coordinate system, by using the “*geomorph*” package in R (Adams & Otárola-Castillo, 2013). To evaluate the variation in wing size, we also measured the wing length and width based on the method described by Lack et al. (2016) using ImageJ version 2.1.0 (<https://imagej.nih.gov/ij/>). For the wing length measurement, we measured a straight line drawn from the intersection of the anterior cross-vein and L4 longitudinal vein, to where the L3 longitudinal vein intersects the wing margin. For the wing width, we measured a straight line from the intersection of the L5 longitudinal vein and posterior wing margin, passing through the intersection of the posterior cross-vein and L4, and terminating at the anterior wing margin (Figure 1B).

Dimension reduction and measurement of variance

After GPA, the shape data consisted of 20 dimensions. To reduce this to a smaller number of effective morphological dimensions, we performed principal component (PC) analysis on the averaged

coordinate data, in which the original coordinates data were averaged by the iso-female lines and rearing environmental conditions. The degree of phenotypic plasticity of each iso-female line for wing morphology was defined as the standard deviation of the left-wing PC scores among the seven rearing conditions. In addition, to evaluate the possibility of introducing a spurious correlation due to using only one wing, we also analyzed the right-wing PC scores and the average of left- and right-wing PC scores. In the results shown in the main text, we report the first five PCs, whose contribution was greater than 5% of the total phenotypic variance (Supplementary Figure S1). This cut-off value was chosen because if all traits (PC) have even contributions, the contribution per trait (PC) is expected to be 5% (total dimensions of shape data are 20 due to GPA). We however performed all analyses using the full-rank (20 dimensions) data to confirm that our results are robust against the number of dimensions to retain. When performing multiple comparisons, *P*-values were adjusted by applying the Bonferroni correction. The developmental noise was assessed using FA which was the difference between the right- and left-wing PC scores. The degree of developmental noise was then evaluated as the standard deviation of FA among individuals (Goswami et al., 2015; Klingenberg, 2019; Rohner et al., 2022). We evaluated the phenotypic plasticity and developmental noise of the wing length and wing width using the same approach.

Correlation between plasticity under different environmental conditions

To examine the correlation among the strength of plastic responses to each environmental factor, we used Pearson’s correlation test. For wing morphology, we used the left-wing PC scores (analyses based on the right-wing PC scores and the average of left- and right-wing PC scores are also performed and will be presented in supplementary material). PC1–PC5 were analyzed and addressed as independent wing morphology traits. Each strength of plastic response was defined as the standard deviation of the left-wing PC scores across each rearing condition. For wing size, the left-wing length and the left-wing width were used, and the strength of plastic response was evaluated in the same manner. We then applied the Pearson’s correlation test between the strength of plastic response to each environmental factor.

Broad-sense heritability

Following a previously described method (Becker, 1964; Scheiner & Lyman, 1989), we estimated the broad-sense heritability of phenotypic plasticity as

$$H_{pi}^2 = \frac{\sigma_G^2 \times E}{\sigma_P^2},$$

where σ_P^2 is the total phenotypic variance and $\sigma_G^2 \times E$ is the genotype–environment interaction. We used the first five PCs (PC1–PC5) to estimate the heritability of phenotypic plasticity in wing morphology. To estimate σ_G^2 , σ_E^2 , and $\sigma_G^2 \times E$, we decomposed the variance into respective components of interest using the “VCA” package in R. In this model, the variance components of associated with iso-female line, environment, and their interaction represent σ_G^2 , σ_E^2 , and $\sigma_G^2 \times E$, respectively. The PC scores used for the estimations were derived from the PC analysis performed on the standardized coordinates of the left wings. First, we calculated the heritability of each trait from its respective PC scores and then we calculated the average heritability of the five independent traits and considered them to be the heritability of the phenotype plasticity in wing morphology. Heritability of the

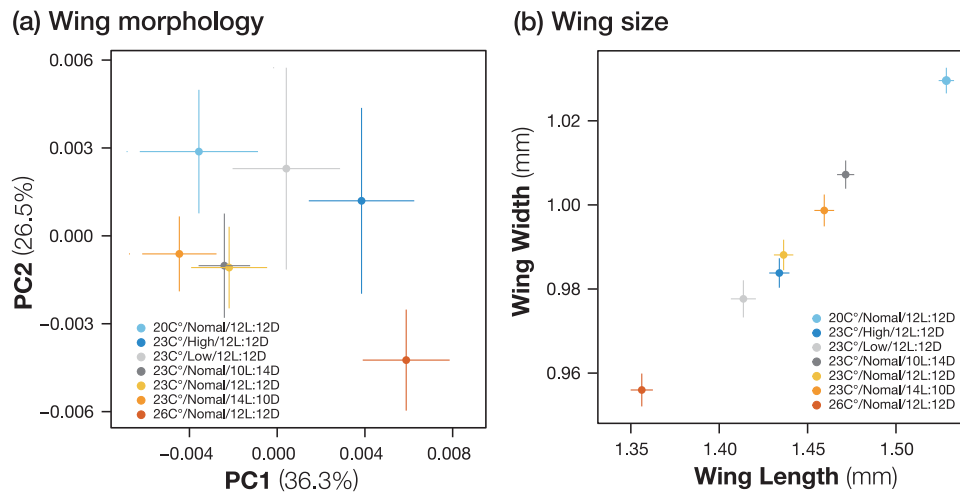


Figure 2. The variation in wing morphology (A) and wing size (B) of *Drosophila simulans* among the different rearing environmental conditions. The color of points and error bars represent the environmental conditions to which the individuals were exposed. Points are the mean and error bars are the standard error of the mean.

phenotypic plasticity in wing length and width was estimated using the same procedure.

We defined the broad-sense heritability of developmental noise as the portion of the total FA variance that can be explained by the difference among iso-female lines. The equation used is as follows:

$$H_{no}^2 = \frac{\sigma_G^2}{\sigma_p^2},$$

where σ_p^2 is the total FA variance among individuals and σ_G^2 is the between-line variance of FA. To evaluate these variance components, we used VCA as described earlier. FA was estimated based on the raw (i.e., signed) difference between the right- and left-wing PC scores, and we used PC1–PC5 as the estimation of the heritability of plasticity. Heritability of developmental noise in wing length and width was estimated in the same manner.

Correlation between plasticity and developmental noise

To investigate the relationship between the degree of phenotypic plasticity and developmental noise, we used Pearson's correlation test and a linear mixed-effects model from the "lme4" package (Bates et al., 2015). This model was fitted using the maximum likelihood with "lmerMod." In the model evaluating the relationship between the wing morphological traits, we included \log_{10} of plasticity as the response variable and \log_{10} of developmental noise as the predictor variable. We included trait identity (PC1–PC5) as the random effect because here our primary focus was not wing morphology itself (each trait) but an overall tendency for those traits to vary in response to different sources of variation. The observations were weighted with the eigenvalue of each PC. In the model evaluating the relationship in wing size, we constructed the same model with trait identity (wing length or wing width) as the random effect.

Results

Variation in the strength of plastic response

For wing morphology, we identified the top five PC axes, whose contribution was greater than 5% (PC1: 36.3%, PC2: 26.5%, PC3: 10.6%, PC4: 7.7%, and PC5: 6.3%, Supplementary Figure S1). We found

a significant effect of the rearing condition on wing morphology in PC1 but not in PC2 (Figure 2A; PC1: $F_{6,30} = 4.215$, adjusted- $P < 0.05$; PC2: $F_{6,30} = 1.779$, adjusted- $P = 0.686$). In contrast, both wing length and wing width were clearly dependent on the environmental conditions to which the different iso-female lines were exposed (Figure 2B; wing length: $F_{6,34} = 15.014$, adjusted- $P < 0.001$; wing width: $F_{6,34} = 12.981$, adjusted- $P < 0.001$).

The strength of plastic responses among the environmental factors was not always correlated (Figure 3, Supplementary Table S1). Although the strength of plastic response to the nutrition and light–dark cycle conditions had a significant positive correlation ($r = 0.47$, $P < 0.01$), the strength of plastic response between temperature and nutrition conditions ($r = 0.28$, $P = 0.14$) and between light–dark cycle and temperature conditions ($r = 0.04$, $P = 0.83$) were not significantly correlated. However, it is noteworthy that the lack of statistical significance in these analyses may partly be attributable to the small sample size and generally small effect sizes of the plasticity in wing morphological traits (Figure 2). A general lack of correlation between the plastic responses under different environmental conditions is further supported by our results for wing length and wing width, where no significant correlations between the strength of plastic response to each environmental factor were found (wing length: between temperature and light–dark cycle, $r = 0.31$, $P = 0.55$; between temperature and nutrition, $r = 0.22$, $P = 0.67$; between light–dark cycle and nutrition, $r = 0.30$, $p = 0.56$, wing width: between temperature and light–dark cycle, $r = 0.10$, $P = 0.86$; between temperature and nutrition, $r = 0.57$, $P = 0.24$; between light–dark cycle and nutrition, $r = -0.25$, $P = 0.63$).

Broad-sense heritability of phenotypic plasticity and developmental noise

The broad-sense heritability (H^2) of phenotypic plasticity and developmental noise in wing shape was 5.9% (PC1: 0.019; PC2: 0.184; PC3: 0.059; PC4: 0.031; and PC5: 0; mean \pm standard error (SE): 0.059 ± 0.033) and 1.5% (PC1: 0.006; PC2: 0.046; PC3: 0.002; PC4: 0.021; and PC5: 0; mean \pm SE: 0.015 ± 0.009), respectively (Figure 4A). In addition, H^2 of the phenotypic plasticity and developmental noise in wing size was 2.5% (length: 0.031; width: 0.019; mean \pm SE: 0.025 ± 0.006) and 0.7% (length: 0.015; width: 0; mean \pm SE: 0.007 ± 0.007), respectively (Figure 4B).

Relationship between phenotypic plasticity and developmental noise

There was a strong and statistically significant positive relationship between phenotypic plasticity and developmental noise (Figure 5A; $P < 0.001$, conditional $r^2 = 0.877$, Supplementary Table S2). When we evaluated all PCs together for this analysis, the positive relationship remained and even became stronger (Supplementary Figure S2; $P < 0.001$, conditional $r^2 = 0.982$). This confirms that this relationship is not driven by the choice in the number of traits to include in our analyses nor by the increased influence of measurement errors in low-ranked PCs. This positive relationship was also found in wing size (Figure 5B; $P < 0.01$, conditional $r^2 = 0.773$). Therefore, two types of non-genetic variations were tightly correlated in both wing shape and size traits of *D. simulans*.

Discussion

The biological significance of two non-genetic variations, i.e., phenotypic plasticity and developmental noise, in phenotypic evolution, has become increasingly prominent over the past decades (Furusawa et al., 2005; Price et al., 2003; Rohner et al., 2022; Uller et al., 2018; West-Eberhard, 2003) but how they affect evolvability and variability of phenotypes remains poorly understood. In this study, we quantified the plasticity and developmental noise

in wing morphological traits (size and shape) of *D. simulans* and showed that wing size and shape responded plastically to different rearing conditions. We also found genetic variation in the propensity for a genotype to produce phenotypic variation through plasticity and developmental noise. Moreover, we demonstrated a positive relationship between phenotypic plasticity and developmental noise. These results add to the increasing body of evidence supporting the biological significance of non-genetic variation. Below, we discuss our three main findings.

Wing morphology, length, and width varied depending on the environment to which the individuals were exposed. In general, the temperate populations of the fruit fly *D. melanogaster* are known to be larger than the tropical populations (David & Capy, 1988; Lack et al., 2016). Accordingly, our results showed that the wings of individuals reared under higher temperature conditions were smaller than those reared under lower temperature conditions. Unlike homeotherms, the growth rate of ectotherms generally increases as the temperature increases and drastically decreases after the temperature reaches the thermal limit (Yamahira et al., 2007). Under relatively higher temperature conditions, developmental time becomes shorter due to a high growth rate and shorter developmental times lead to small wings. This is called the “temperature–size rule” (Atkinson, 1994). Therefore, the downsized wings were thought to be derived from the decreasing developmental time. We found no evidence that the pattern of

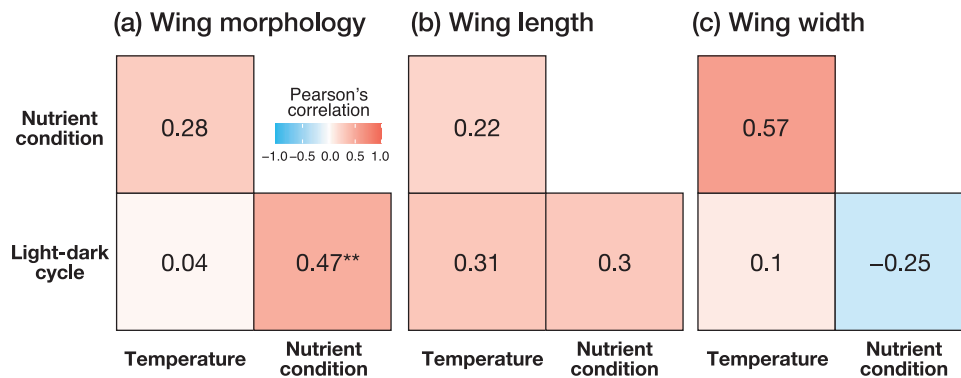


Figure 3. The correlation matrix heatmaps between the strength of plastic responses to three different environmental factors in wing morphology (A), wing length (B), and wing width (C), respectively. The value in the center of each box represents the Pearson’s correlation.

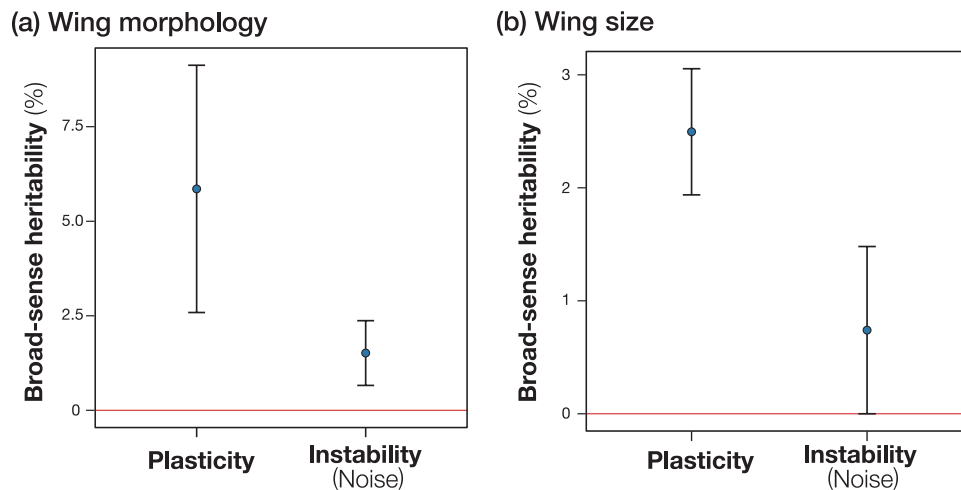


Figure 4. The broad-sense heritability of the phenotypic plasticity and developmental noise in wing morphology (A) and wing size (B). Points are the mean and error bars are the standard error of the mean.

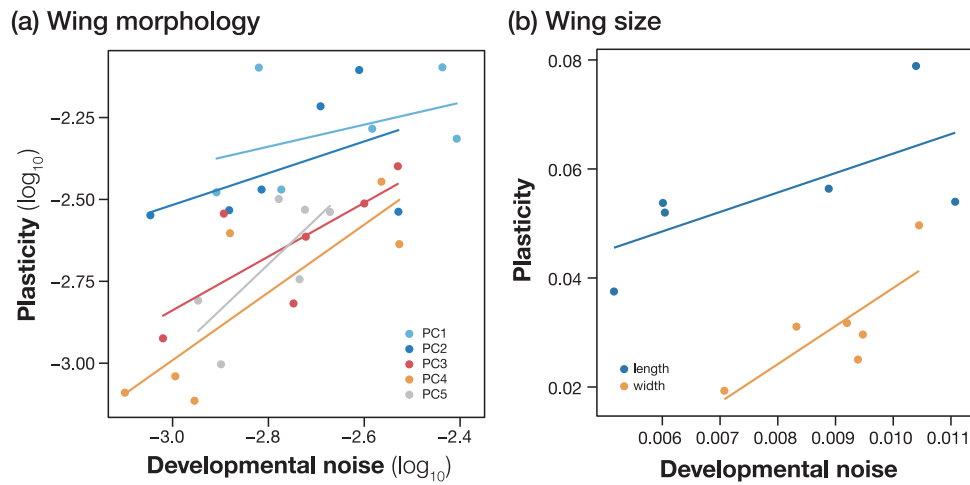


Figure 5. Relationship between the phenotypic plasticity and degree of developmental noise in wing morphology (A) and wing size (B). Each line represents simple regression lines.

plastic responses to the three environmental factors (nutrition, light cycle, and temperature) was correlated. We interpret these results as suggesting either that the actual pattern of phenotypic plasticity could not be evaluated by this range of perturbations that we introduced within one environmental factor or that the wing phenotypes can indeed respond differently to different environmental cues.

In wing morphological traits of *D. simulans*, the broad-sense heritability (H^2) of phenotypic plasticity was 5.9%, and the H^2 of developmental noise was 1.5%. These results indicate that there is a propensity for some genotypes to generate more variation in response to environmental cues or local stochastic events during development than others, and these propensities exhibit genetic variance within the fly population. Thus, when selection acts on those propensities either directly or indirectly, they should be able to evolve (Mather, 1953). Our estimates are comparable to the heritability of plasticity reported in morphological traits in *D. melanogaster* (Mackay & Lyman, 2005; Scheiner & Lyman, 1989) but are smaller than those of the behavioral traits, such as chill coma recovery time and startle response (Morgante et al., 2015). Developmental noise showed considerably lower heritability than phenotypic plasticity. Our estimates of H^2 in developmental noise were within the range of 0.7%–2.5%, which are comparable to estimates obtained from artificial selection experiment in *D. melanogaster* (Carter & Houle, 2011) and at the lower range of H^2 or the narrow-sense heritability (h^2) reviewed by Whitlock and Fowler (1997). One explanation for these low estimates in wing morphological traits is Fisher's fundamental theorem of natural selection (Fisher, 1930), which proposes that traits strongly associated with an organism's fitness will have a lower heritability. Considering the functional significance of wing morphology for flight, it is conceivable that these traits are strongly linked to fitness.

We found two sources of non-genetic variation, i.e., phenotypic plasticity and developmental noise, to be positively and strongly correlated. We propose two hypotheses to explain these patterns, that are not mutually exclusive. First is pleiotropy where certain genes may govern both the ability to change phenotypic expression in response to external cues (phenotypic plasticity) and through local perturbations during development (developmental noise). Previous studies have suggested that genes representing hubs that stitch together genetic networks are likely to influence the overall phenotypic plasticity or robustness of organisms

(Laitinen & Nikoloski, 2019). For example, ELF3 was proposed to be a key hub gene that integrates developmental and environmental signals in response to temperature (Anwer et al., 2014; Boden et al., 2014; Box et al., 2015). Moreover, HSP-90 is known to contribute to the system's robustness or standard genetic variation or developmental noise either directly or indirectly (Mestek Boukhibar & Barkoulas, 2016). Second is canalization where both types of non-genetic variation may be produced by the same developmental machinery. Insect wings are emblematic examples of conservative characters in nature (Hansen & Houle, 2004), probably next to the body temperature in birds and mammals (Williams, 1992). Notably, the wing morphology in *Drosophila* exhibits a striking level of conservation, which is thought to have persisted for at least 40 million years. Considering the large ecological diversity in *Drosophila* while the wings are remarkably conservative, it is plausible to assume that the wing morphological traits are likely under stabilizing selection. Consequently, it is conceivable that repeated bouts of selection through the history of fly family Drosophilidae have canalized the developmental system to generate adaptive phenotypes more often than non-adaptive alternatives. This presents the possibility that prior periods of selection have shaped the developmental machinery and determined the pattern of variation in contemporary populations (Jones et al., 2007; Rohner et al., 2022; Uller et al., 2018). An interesting and relevant observation to help disentangle these two alternative hypotheses is that the correlation between two non-genetic phenotypic variations might be agnostic to underlying genetic changes (Furusawa & Kaneko, 2015). Identification of network features and molecular bases of the variation in FA will clearly be a fruitful path for understanding the biological implication of the correlation of non-genetic variation found in our study.

Recently, Rohner and Berger (2023) have shown that developmental noise is correlated with the pattern of genetic and phenotypic variation as well as with the divergence pattern among species (e.g., macroevolution) in wing morphological traits of *Sepsis* flies. Together with previous findings in *Drosophila* flies (Houle et al., 2017) and *Anolis* lizards (McGlathlin et al., 2018), these studies suggest a remarkably consistent and strong correlation between variation across multiple levels of biological organization including evolutionary divergence over tens of millions of years. Our study adds another level to these correlations: phenotypic plasticity. Interestingly, Rohner and Berger

(2023) evaluated phenotypic plasticity but did not find a correlation between plasticity and developmental noise. We propose two possible explanations for the discrepancy between these results. First, as previously noted, the plastic response in wing morphological traits was small in magnitude in *D. simulans*. The plastic variation in wing shape of *Sepsis* flies may be similarly small, making the relationship challenging to detect. Second, our sample size to estimate the developmental noise is large ($n = 87$ in Rohner & Berger, 2023, vs. $n = 410$ in our study), which allowed us to estimate the FA more precisely. The same reasoning could be applied to the other conflicting results regarding the relationship between these two non-genetic variations (Scheiner, 1993). If our arguments are correct, the correlation between plasticity and the other levels of variation should be common, but the detection of this pattern requires precise estimations. There is circumstantial evidence pointing to this possibility (Noble et al., 2019). To fully appreciate the biological significance of these non-genetic variations on the evolutionary process, further studies are required to examine the correlation between them in other organisms and traits.

Supplementary material

Supplementary material is available online at *Evolution Letters*.

Data and code availability

All raw data used in this study have been uploaded to Figshare, <https://doi.org/10.6084/m9.figshare.23615622>.

Author contributions

K.S. and Y.T. conceived and designed the study. K.S. collected the data. K.S. analyzed the data, and M.T. and Y.T. supported for analysis. K.S. drafted the manuscript. All authors reviewed the manuscript.

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