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Review Article

Shape asymmetry — what's new?

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Studies of shape asymmetry have become increasingly abundant as the methods of geometric morphometrics have gained widespread use. Most of these studies have focussed on fluctuating asymmetry and have largely obtained similar results as more traditional analyses of asymmetry in distance measurements, but several notable differences have also emerged. A key difference is that shape analyses provide information on the patterns, not just the amount of variation, and therefore tend to be more sensitive. Such analyses have shown that apparently symmetric structures in animals consistently show directional asymmetry for shape, but not for size. Furthermore, the long-standing prediction that phenotypic plasticity in response to environmental heterogeneity can contribute to fluctuating asymmetry has been confirmed for the first time for the shape of flower parts (but not for size). Finally, shape analyses in structures with complex symmetry, such as many flowers, can distinguish multiple types of directional asymmetry, generated by distinct direction-giving factors, which combine to the single component observable in bilaterally symmetric structures. While analyses of shape asymmetry are broadly compatible with traditional analyses of asymmetry, they incorporate more detailed morphological information, particularly for structures with complex symmetry, and therefore can reveal subtle biological effects that would otherwise not be apparent. This makes them a promising tool for a wide range of studies in the basic and applied life sciences.

Introduction

The study of asymmetry in the external structure and internal organs of organisms has a long history, but the application of new techniques or conceptual approaches continues to produce novel insights in this field. Since the methods of geometric morphometrics gained widespread use in the 1990s, investigators have applied them to study asymmetry [1–3], and by now a large body of research on the asymmetry of shape exists [4].

Most studies on asymmetry have focused primarily on fluctuating asymmetry (Figure 1A), the random differences between sides usually attributed to developmental noise [5–7], and often used to assess phenotypic effects of various stressors on organisms [8–13]. Other types of asymmetry include directional asymmetry (Figure 1B), the systematic differences among the repeated parts in different positions (most prominent in the asymmetries of internal organs of most animals, including humans), and antisymmetry (Figure 1C), where most individuals are clearly asymmetric but differ in the directions of their asymmetries [4,14,15]. Contrary to studies using distance measurements, analyses of shape have found directional asymmetry almost ubiquitously, even in structures that superficially appear to be symmetric [4,16]. Antisymmetry seems to occur occasionally and under somewhat special circumstances, both for traditional trait measurements and shape and manifests itself in a bimodal distribution of left-right asymmetries [4,17,18].

For the most part, analyses of shape asymmetry have produced results that are similar to those from more traditional analyses of various distance measurements [15,19], but there are a few clear exceptions to this trend. In particular, directional asymmetry, which only occurs occasionally for distance measurements [15,20,21], is nearly ubiquitous in analyses of shape, at least in animals [1,4,16]. Also, studies of asymmetry using shape data have been able to trace the ontogenetic origins of asymmetry in unprecedented detail [22] and to identify several factors that influence asymmetry but had

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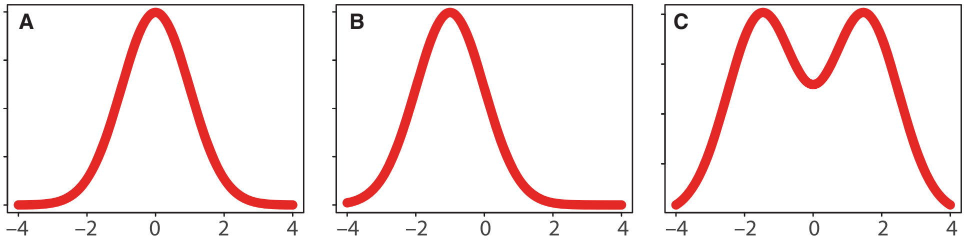

Figure 1. Types of asymmetry for a quantitative trait.

The diagrams show the distribution of left-right differences in a quantitative trait such as a length measurement taken on both sides. (**A**) 'Pure' fluctuating asymmetry. The mean asymmetry is zero (i.e. no directional asymmetry) and individual differences are small random deviations from perfect symmetry. (**B**) Directional asymmetry (combined with fluctuating asymmetry). Directional asymmetry is a population property and denotes the mean asymmetry (shift of the mean or mode of the distribution away from zero). Because fluctuating asymmetry is ubiquitous, the diagram shows a distribution of individual asymmetry values around the mean. (**C**) Antisymmetry. Individual asymmetries follow a bimodal distribution because population contains a mix or 'left-sided' and 'right-sided' individuals. Figure from [4].

not been demonstrated with other methods [23,24]. Combined with some conceptual innovations [6,7], these developments establish studies of morphological asymmetry in a new context that is linked to other fields of inquiry such as phenotypic plasticity and the evolution of body plans. This review focuses on several areas where studies of shape asymmetry have produced results that differ from those obtained for traditional distance measurements and outlines the perspectives offered by this new and broader context.

Quantifying shape variation and asymmetry

Shape is defined as all the geometric features of an object except for its size, position and orientation [25]. To characterize the shape of biological structures, morphometric analyses mostly use configurations of landmarks, points that can be located precisely and correspond unambiguously among all the specimens included in a study. Shape variation can be extracted from landmark coordinates by using a Procrustes superimposition, which standardizes position, size and orientation of configurations so that only shape variation remains, and usually a projection to the shape tangent space [25,26]. The resulting shape information can then be further analysed using the standard methods of multivariate statistics to address specific research questions.

Asymmetry is the deviation from perfect symmetry, and to quantify asymmetry, the underlying symmetry of a biological structure needs to be understood. Symmetry can be defined as the repetition of parts in different positions and orientations, and possibly reflected to their mirror image. Simple examples are our left and right hands or the left and right halves of our face, both involving two mirrored copies, but more complex symmetries also exist, for instance in flowers, sea urchins or some unicellular algae, where more parts are repeated and arranged in specific patterns [4,27–29]. Two types of symmetry can be observed in biological structures: matching symmetry (Figure 2A), where the repeated parts are separate from each other but can be superimposed to match each other (as the left and right hands can be matched by putting the palms of both hands together), and object symmetry (Figure 2B), where the parts are attached to each other in a fixed arrangement (such as the left and right halves of our face, where the plane of symmetry runs through the middle of the face) [30,31]. In some instances, investigators have a choice between these types of symmetry [27]: in flowers, for instance, a study might investigate the object symmetry of the whole flower [32,33], or it might focus on the variation among the different petals separately and use the method for matching symmetry [24,34]. In addition, the parts of a structure with complex symmetry may each be symmetric in themselves, for instance if the parts of a complex structure each have bilateral object symmetry [24,27,34–36].

Morphometric studies investigating asymmetry combine the Procrustes approach with the considerations about repeated parts for a specific type of symmetry. For matching symmetry, the analyses use separate landmark configurations of the repeated parts, which may be reflected as appropriate (Figure 2C) [1–3]. The subsequent analyses with matching symmetry use the shape space of the landmark configuration for the repeated parts [4,27]. For object symmetry, multiple copies of the entire landmark configuration are included, each transformed according to the arrangement of repeated parts (Figure 2D) [30,31,37]. In this case, the component

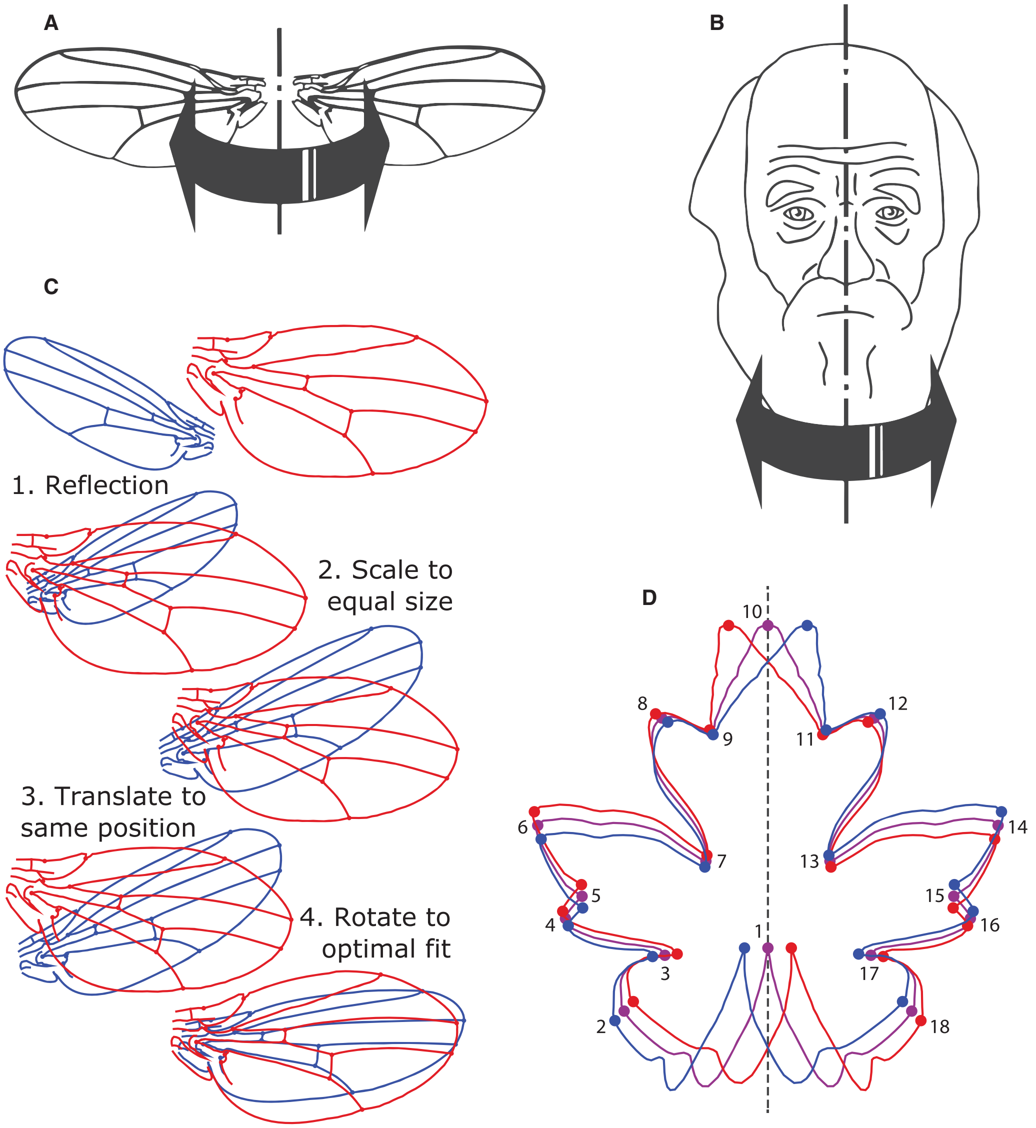

Figure 2. Matching symmetry and object symmetry.
(**A**) Matching symmetry. The repeated parts, two fly wings in this example, are separate from each other with the axis of symmetry passing between them (from [31]). (**B**) Object symmetry. The repeated parts, here the two halves of the face, are joined and the axis of symmetry runs through the centre of the combined structure (from [31]). (**C**) Procrustes superimposition for matching symmetry. This involves reflection of structures from one side (here the left, in blue), scaling, translation and rotation to an optimal fit. In practice, all landmark configurations in a sample are included in a single Procrustes superimposition simultaneously (modified from [4]). (**D**) Procrustes superimposition for object symmetry. The original landmark configuration (blue) and a reflected copy (red) are superimposed; their consensus is a perfectly symmetric shape (purple). In practice, all landmark configurations in a sample are included in a joint Procrustes superimposition simultaneously (from [4]).

of completely symmetric shape variation and one or more components of asymmetry occupy distinct subspaces of the shape space of the landmark configuration for the whole structure [4,27]. This overall approach provides a powerful and flexible method for analysing even complex types of symmetry [4,27,28], for instance to investigate asymmetries in flowers or unicellular algae [24,29,32,33,35].

A broad range of analyses can be performed with shape data. To quantify fluctuating and directional asymmetry and to provide statistical tests, a generalization of the two-factor, mixed-model ANOVA originally devised for assessing left-right asymmetry of distance measurements [15,38] is available for shape asymmetry with any type of symmetry [3,27,31]. The magnitude of fluctuating asymmetry for individuals can be quantified as the shape distance between the left and right sides (and similar measures are conceivable for object symmetry and complex symmetries), adjusted for any directional asymmetry in the data [4]. This measure can be followed over different growth stages [22,39] or related to a variety of factors such as genetic, environmental, performance or socioeconomic measures [8,9,40–45]. Patterns of covariation in fluctuating asymmetry of shape have been used to investigate the developmental origins of integration in morphological structures [46–48], based on the reasoning that covariation of fluctuating asymmetry between traits indicates direct developmental interactions between them [4,49].

Fluctuating asymmetry: not just developmental instability

The usual interpretation of fluctuating asymmetry is that it is an expression of developmental instability, an imprecision in the control of developmental processes [5,14]. Even though each pair of structures on the left and right sides of an individual develop under control of the same genome and in the same environment, such imprecision causes them to differ slightly, and these differences are manifest as asymmetry. In this context, it is useful to use the concept of the target phenotype, the phenotype expected for a structure and a particular genotype and environment in the absence of any variation or random noise [6]. Using this notion, developmental instability can be redefined as the deviation of actual phenotypes from the target phenotype [6,7]. The concept of the target phenotype also is useful to connect studies of fluctuating asymmetry to other aspects of development and phenotypic variation [6,7].

The exact origin of the random noise in the developmental processes that generates the deviations from the target phenotype is unclear, but many molecular and cellular processes operate at variable rates (or even switch on or off) and can therefore produce variability over time and among cells [50]. Because many cellular and developmental processes have nonlinear dynamics, these processes may amplify or attenuate such random variation and may produce statistical interactions with other factors, such as genotype and environmental conditions [5,7,51]. A key point is that the origin of variation, and possibly also the potential for buffering to reduce variation, originates from the cellular mechanisms that collectively constitute the developmental processes generating the observable phenotype. Variation and buffering are therefore intrinsic parts of the development of the structure under study, and do not rely on an outside source of variation.

Not all fluctuating asymmetry, however, originates in this way from developmental noise, and therefore is an indicator of developmental instability. An important assumption in the discussion above is that the left and right sides (or more generally, all repeated parts) share the same target phenotype, which in turn implies that they must share the same genotype and environment. This is a standard assumption that is required if fluctuating asymmetry is to be interpreted as developmental instability [5]. If this assumption is violated, genotypic differences (such as somatic mutations) or environmental differences between sides might produce an additional contribution to fluctuating asymmetry. This possibility has been discussed theoretically for a long time [4,6,32,52–54], but direct evidence for it has not been available. Freeman et al. [55] conducted an experiment in which growing pumpkin leaves were wrapped in aluminium foil on one side for 24 h to exclude all light, and found that this treatment had a lasting effect on growth and asymmetry of the leaves. It has not been clear, however, whether less drastic microenvironmental heterogeneity, as it occurs in nature, would also be sufficient to cause such an effect.

Evidence that this is indeed the case comes from a study of asymmetry in the flowers of *Iris pumila* [24]. In the temperate zone and higher latitudes, solar irradiance predominantly comes from one direction (e.g. on the northern hemisphere, from the south) and therefore produces a gradient affecting all plants in a location in similar ways (up to localized effects such as shading by neighbouring plants, etc.). Because such a gradient affects parts differentially according to their orientation (Figure 3A,B), it is possible to use statistical analyses to search for systematic effects on morphological traits if the compass orientations of parts are recorded. For *Iris pumila*, such effects of compass orientation were found in three different flower parts for shape (Figure 3C,D), but not for size [24]. This is clear evidence that phenotypic plasticity in response to heterogeneity of environmental factors can produce asymmetry of morphological structures. In most such studies, compass orientation is not recorded and therefore this component of asymmetry is normally included in the estimate of fluctuating asymmetry, along with any asymmetry from phenotypic plasticity in response to smaller-scale heterogeneity (e.g. shading) and the asymmetry due to developmental instability. Because only this single species has been examined to date [24,34], it is impossible to gauge how widespread this phenomenon may be. Likewise, quantifying how much fluctuating asymmetry originates from phenotypic plasticity in response to environmental heterogeneity is very difficult.

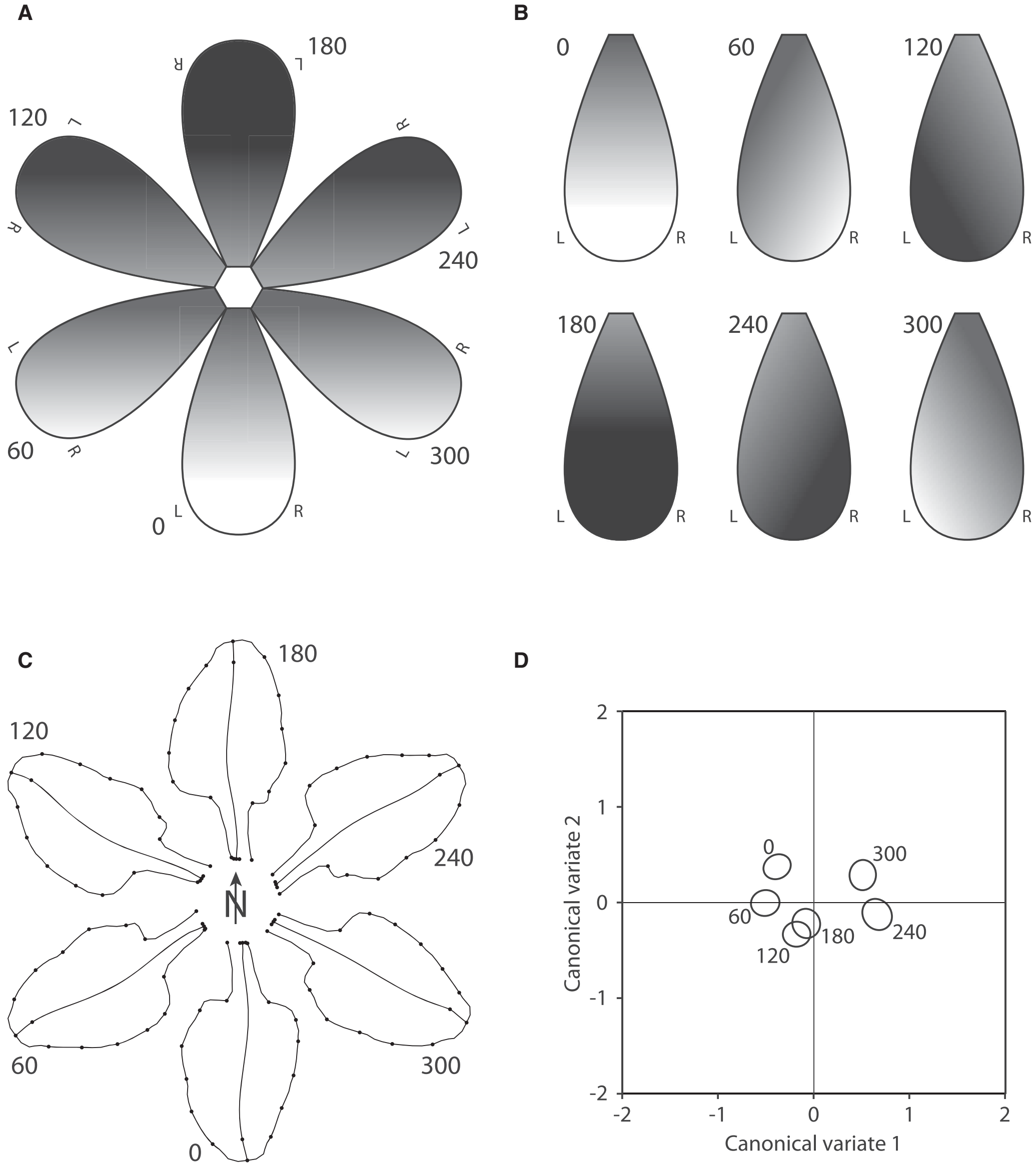

Figure 3. Environmental heterogeneity as a source of asymmetry.
(**A**) A complex structure, such as a flower, in an environmental gradient (shading). Numbers indicate the orientation of each part relative to the direction of the gradient (from bottom to top). Note that the level and direction of the gradient are distinct for each part. (**B**) The parts from panel (**A**) disassembled and arranged parallel to one another. The distinctive patterns imposed by the gradient mean that a reaction norm can translate these differences into morphological differences, and therefore can produce asymmetry in the whole structure. (**C**) The means for the asymmetric component of shape of the standards (petals) in flowers of *Iris pumila* according to their compass orientations. As floral organs were collected, the compass orientation for each was recorded to the nearest 60°. The diagrams show the mean shape for each orientation, amplified 15-fold to make the variation more easily visible. Notice the 'pinwheel symmetry' resulting from the subtle but consistent asymmetries of all parts. Shape differences among orientations indicate phenotypic plasticity in response to a gradient, most likely solar irradiance. (**D**) A multivariate ordination of the shape differences between compass orientations. The plot is from a canonical variate analysis of the asymmetric component of shape (cf. panel **C**) and shows the 95% confidence ellipses for the mean shapes, most of which appear clearly distinct of each other. Figure compiled from [24].

Directional asymmetry of shape is widespread

Studies of asymmetry based on length measurements have found directional asymmetry to occur occasionally in some traits, but it is generally considered to be somewhat unusual [15]. Directional asymmetry often has been perceived as a nuisance in studies of fluctuating asymmetry and is sufficiently rare so that some authors have recommended excluding traits with directional asymmetry from study [20]. In contrast, when the methods of geometric morphometrics started to be used to investigate asymmetry of shape, analyses consistently found subtle but statistically significant directional asymmetry for shape, but not for size, in structures as different as mouse molar teeth and the wings of bees and flies [1,2,16]. Since then, dozens of other studies in a wide variety of animals have obtained similar results, whereas only remarkably few analyses failed to find directional asymmetry of shape (for a review, see ref. [4]). Accordingly, subtle directional asymmetry of shape seems to be near ubiquitous in animals, even in structures that superficially appear symmetrical.

In plants, however, the results seem to be more heterogeneous, with some studies finding directional asymmetry in leaf or flower shape [23,24,32], others not [10,41,42,56], and some with mixed results in different samples [45,57]. A particularly interesting type of directional asymmetry in plants is leaf asymmetry induced by phyllotactic patterning, in which systematic asymmetries in leaves are produced by interactions among leaf primordia in the meristem, most likely mediated by gradients of the plant hormone auxin [23]. Depending on the arrangement of the primordia and therefore on phyllotaxis, different types of predictable asymmetry may emerge, which can either produce asymmetry in a single direction or pairs of mirror-image leaves in a wide range of plants [23,58,59] and therefore may give rise to directional asymmetry or antisymmetry. It is conceivable that a mechanism of this kind may explain observations of bimodal distributions in the asymmetric component of shape variation [18].

Whereas the widespread occurrence of directional asymmetry in plant leaves appears to be a consequence of the developmental mechanisms of leaf initiation and growth [23,58,59], it is less clear why directional asymmetry is also prevalent in animals. The internal organs of most animals are systematically asymmetric, even though their body plans may be bilaterally symmetric at least superficially, and these asymmetries share some developmental mechanisms across the Bilateria [60–62]. Because the developmental mechanisms that produce asymmetric internal organs are partly the same that generate apparently symmetric external structures, it is conceivable that the morphogenetic processes building those structures receive left-right signals, but that the expression of left-right differences is usually suppressed to a low level (presumably by selection, if more symmetric structures function better and therefore provide a fitness advantage).

Direction-giving factors and levels of directional asymmetry

For structures with bilateral symmetry, there is only one kind of asymmetry: left-right asymmetry. Accordingly, directional asymmetry is a difference in the target phenotypes of the left and right sides, and few questions seem to arise about the causes of that difference. In contrast, structures with complex symmetry can simultaneously have multiple types of asymmetry, including multiple types of directional asymmetry [27]. For instance, there might be left-right and dorsal–ventral (or abaxial–adaxial) asymmetries in such structures [32]. The corresponding differences in target phenotypes may also have different causes, which have been called 'direction-giving factors' [34]. In structures with complex symmetries, such as many flowers, different kinds of directional asymmetry with different direction-giving factors can exist simultaneously, which may make it possible to investigate these factors empirically.

These ideas have been demonstrated in a study of flowers of *Iris pumila* [34], which have complex symmetry with floral organs arranged in whorls of three, and each part is also bilaterally symmetric (Figure 4). For each flower, the study recorded the compass orientations of floral parts, which differ in their relation to large-scale environmental gradients such as solar irradiance and were previously shown to contribute to shape asymmetries of floral organs [24]. Additionally, the study recorded the orientations of flower organs relative to the two bracts subtending each flower, providing information on the anatomical orientation of each part (Figure 4). These factors are usually not recorded in studies of floral asymmetry, and any directional asymmetry of those types (differences in target phenotypes in response to compass or anatomical orientation) would contribute to the estimate of fluctuating asymmetry [24,34]. In addition, the study considered the bilateral symmetry of each flower part—consistent directional asymmetries of all parts in each whorl can produce a 'pinwheel symmetry' of the whole flower (Figure 3C) and may originate from aestivation, the way developing flower organs are rolled up in the bud [24,34]. Of these three types of directional asymmetry, two were found by analyses of shape in *Iris pumila* flowers [34]: directional asymmetry in relation to compass orientation and directional

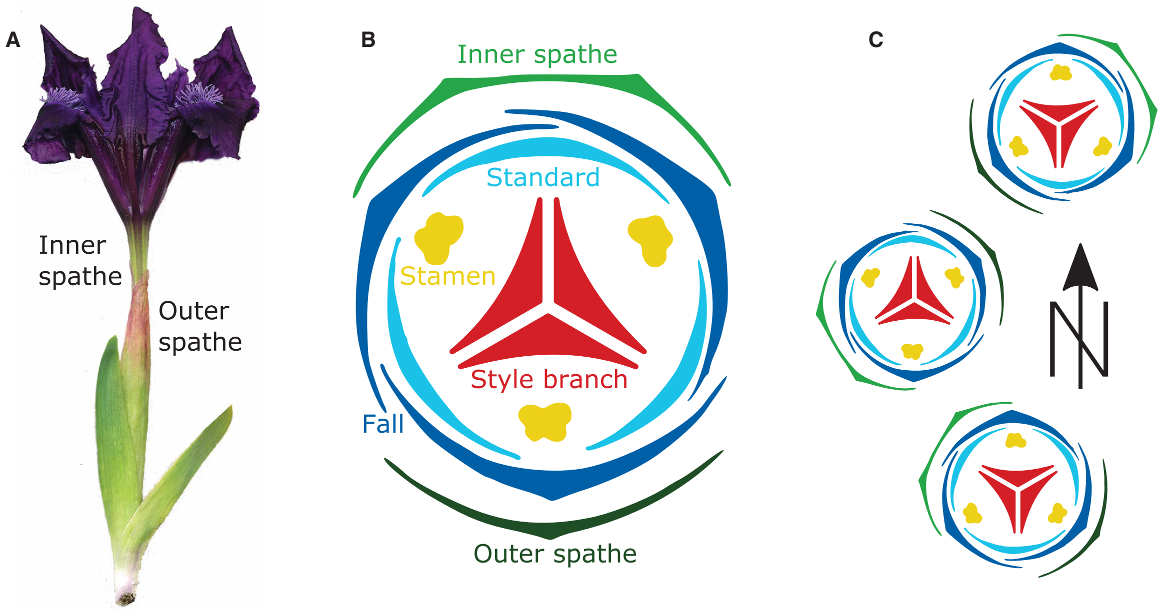

Figure 4. Complex symmetry and direction-giving factors in *Iris* flowers.
(**A**) A flower of *Iris pumila*, including the two spathes that envelop the lower parts of the flower and two leaves. (**B**) The ground plan of an *Iris* flower. The flower itself (consisting of falls, standards, stamens and style branches) is radially symmetric, whereas the two spathes provide a clear anatomical orientation. Note also that each flower organ is bilaterally symmetric in itself (about an axis of symmetry running through the middle of that organ and the centre of the flower). (**C**) The study design for investigating multiple types of directional asymmetry and the respective direction-giving factors. For each flower, the compass orientations of the flower parts and of the spathes are recorded, so that for each flower organ the compass orientation and the anatomical orientation relative to the spathes is known. Figure from [34].

asymmetry of individual flower parts, but no significant directional asymmetry in relation to anatomical orientation. Finally, no directional asymmetries at all were found for the size of floral organs [34].

Shape appears to be a sensitive indicator for differences in intrinsic and extrinsic factors that can produce systematic differences in target phenotypes, and therefore directional asymmetry. That *Iris pumila* flowers appear to show no directional asymmetry in relation to anatomical orientation may be because flowers of this species grow in a terminal position (Figure 4A) and therefore may not have a clear adaxial–abaxial (dorsal–ventral) polarity [34]. In contrast, adaxial–abaxial polarity is a direction-giving factor associated with marked directional asymmetry in flowers of many other taxa [28,32,63] and is a defining feature of bilaterally symmetrical (zygomorphic) flowers [64–66]. Similarly, in many other plants, there are differences in target phenotypes among repeated structures according to their positions along the shoot axis, both for leaves [67,68] and flowers [69]. From the perspective of the analysis of symmetry and asymmetry, these are instances of translational symmetry [27] and are therefore formally equivalent to serial homology of parts seen in many animals along the anterior–posterior axis [70–73]. This means that, in principle, diverse aspects of morphological variation are conceptually related because they can be conceptualized as directional asymmetry: the target phenotypes for repeated parts vary as a function of their location in the organism [34]. This insight opens new avenues for investigation in morphometrics, evo-devo, and comparative morphology.

Summary

- Analyses of shape asymmetry have become widespread as the methods of geometric morphometrics have been accepted as standard tools for quantifying phenotypic variation. Most have focussed on fluctuating asymmetry as a measure of developmental instability that can be correlated with measures of stress, genetic characteristics such as heterozygosity, or individual performance, and such studies have tended to yield results that are broadly compatible with traditional studies of distance measurements.

- A notable difference, since the first studies of shape asymmetry, is that studies in animals have consistently revealed subtle but statistically significant directional asymmetry, which has been unusual in traditional studies. Results for plants tend to be more varied.

- Analyses of shape asymmetry in *Iris* flowers have provided the first empirical confirmation of the long-standing hypothesis that phenotypic plasticity in response to environmental heterogeneity, and not just developmental instability, contributes to fluctuating asymmetry.

- Studies in structures with complex symmetry can distinguish multiple types of directional asymmetry that are produced by distinct direction-giving factors (which may also contribute to left-right asymmetry in bilaterally symmetric structures).

- The differences in results are most likely because analyses of shape asymmetry not only record the magnitude, but also the patterns of asymmetry and therefore are more sensitive than traditional analyses of distance measurements.

Competing Interests

The author declares that there are no competing interests associated with this manuscript.

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