

# Fluctuating asymmetry in ecological and environmental research: *Quo vadis?*

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**Handling Editor:** Mark Tjoelker

## Abstract

1. Májeková et al. (2024) demonstrated that leaf fluctuating asymmetry (FA) is not a reliable indicator of stress. I broaden the perspective on the findings by these authors.
2. High prevalence of confirmation bias could explain why as much as 39% of 131 unique entries (plant species × stress type) in the database of published studies compiled by these authors showed the significant increase in FA with stress.
3. The use of blind methods should be considered obligatory for any study addressing environmental or genetic impacts on FA.
4. Both data and conclusions from FA-related studies that did not report blinding should only be used when proof of negligible impact from confirmation bias can be provided.
5. It is essential that all measures taken against biases are described in each submitted manuscript, and that the need to check for these requirements is included in instructions for reviewers.

## KEYWORDS

bioindication, blinding, confirmation bias, developmental instability, environmental stress, fluctuating asymmetry

## 1 | IN ASYMMETRY WE TRUST?

The eco-evolutionary theory of developmental instability posits that (i) minor, non-directional deviations from perfect symmetry in morphological traits—known as fluctuating asymmetry (FA)—arise when an individual is unable to buffer against environmental or genetic stress during development and (ii) lower developmental stability, reflected by greater FA, signals an individual's 'lower quality', leading to reduced mate attraction and diminished genetic fitness (Gavrikov et al., 2023; Møller & Swaddle, 1997; Polak, 2003; Waddington, 1942). This theory is straightforward, intuitively appealing, internally coherent, and thus compelling; it meets all the

criteria that Fagerström (1987) outlined as characteristics of a successful ecological theory. Additionally, an unjustified belief in the simplicity of protocols used to quantify FA has led to promoting FA as a handy tool for ecological and environmental studies that can be used even by schoolchildren (Malafeeva & Marina, 2010; Zakharov et al., 2001).

One of the very first studies of FA (Van Valen, 1962) yielded controversial results and several subsequent papers (Wiener & Rago, 1987; Willig & Owen, 1987) expressed concerns regarding some studies that have supported developmental instability theory. Nevertheless, two papers by Parsons (1990a, 1990b), which non-critically summarized the supportive evidence, caused a boost in

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FA-related studies: the number of publications mentioning FA increased from 27 during 1986–1990 to 222 during 1991–1995 and to 724 during 2016–2020 (ISI Web of Science core collection, 4 September 2021).

Further accumulation of disconfirming evidence and the appearance of several critical papers, including one named 'Waltzing with asymmetry' (Palmer, 1996), did not diminish the popularity of studies testing developmental instability theory or using FA as a stress index in applied environmental research. Indeed, the suggestion 'to abandon the search for a general link between FA and environmental stress' (Bjorksten et al., 2000, p. 165) was ignored by the disciples of this theory. A meta-analysis of the heritability of developmental stability by Møller and Thornhill (1997), which concluded that FA is closely associated with fitness, was accompanied by seven (!) critical comments, including one which classified this meta-analysis as 'a giant step backward' (Markow & Clarke, 1997). Last but not least, a paper concluding that herbivory caused an increase in FA in plants, which was based on fabricated data (as demonstrated by Vogel et al., 2004) and therefore retracted (Møller & de Lope, 2001), was cited at least by 29 scientific publications after its retraction (Google Scholar, 22 June 2024), including Lobregat et al. (2018) and Gouveia-Barrocas and Gonçalves (2023). These examples illustrate the strong belief many scientists have in the developmental instability theory and in its practical applications.

A timely publication questioning the indicative value of FA (Májeková et al., 2024) significantly contributes to a balanced perspective on the use of FA in ecological and environmental research. Years ago, I argued that the current level of knowledge does not allow the practical use of plant FA for bioindication (Kozlov, 2017), and I was pleased to see that Májeková et al. (2024) fully supported my opinion. These authors carefully discussed the possible reasons behind the lack of a clear effect of environmental stress on FA in plant leaves but did not explain (1) why as much as 39% of 131 unique entries (plant species  $\times$  stress type) in their database of published studies showed the significant increase in FA with stress. Answering this question is crucial to understanding (2) how the research methodology should be changed to minimize the risk of false positive conclusions. Finally, it remains unclear (3) which fraction of data accumulated over decades of intensive research addressing the presumed link between FA and stress can be used for future generalizations. My comments addressing these issues aim to broaden the perspective on the findings by Májeková et al. (2024).

## 2 | INTERACTIONS BETWEEN DATA AND THEORIES

In ecology, evolutionary biology and environmental science, data are often regarded as 'hard facts' that provide a solid foundation for building objective knowledge (Fagerström, 1987). Although physicists and philosophers of science have long challenged this view (e.g. Bostrom, 2003; Feyereabend, 1975; Kuhn, 1970), many biologists continue to uphold it (Lortie, 2021; Markowetz, 2017), even in light

of substantial evidence that theories heavily shape data generation, processing and interpretation (Haila, 1988).

Two types of evidence indicate that established theories can shape research data in their favour. First, unintentional confirmation bias—the inclination to favour information that aligns with existing beliefs and hypotheses (Rosenthal, 1976)—can undermine data neutrality, making findings appear more compatible with prevailing theories than they truly are. Second, publication bias, which can manifest in various ways (e.g. authors choosing not to submit studies with contrary results or journals more frequently rejecting such submissions), encourages an accumulation of data that supports the dominant theory (Fanelli, 2012; Jennions et al., 2013). This selective data accumulation reinforces the theory's influence, further directing future research toward similar outcomes (Gavrikov et al., 2023). However, the existence of positive feedback loops between theory and data, resembling the positive loops between investors' perceptions and economic fundamentals in financial markets (Soros, 2013), in eco-evolutionary and environmental research domains remains unclear. If these loops exist, they may easily lead to a rapid deviation of scientists' views from the real situation.

Long ago, Fagerström (1987, p. 258) noted that 'many ecological theories are in fact retained—and rightly so—although they are demonstrably wrong'. This surprising statement was generalized and developed by Barseghyan (2015), who stated that a theory is rejected only when other theories that are incompatible with that current theory are accepted. Arguably, scientists aiming to develop a new theory, modify an existing one, or narrow its scope should first separate the wheat from the chaff. This involves (i) identifying reliable data within a pool of evidence and (ii) assessing the actual balance between supporting and opposing studies.

## 3 | DISCOVERY OF BIASES IN PUBLISHED STUDIES OF FA

The task (ii) specified above is challenging because available (i.e. published) data may not be representative of actual findings. More than two decades ago, Palmer (1999) found that selective reporting is widespread in studies of FA and sexual selection, confirming the existence of a positive feedback loop between theory and data, that is, the theory's effect on data collection. However, other mechanisms exist that act in a similar way.

I began exploring the effects of pollution and herbivory of plant FA in the early 1990s and first published several supportive studies (e.g. Kozlov et al., 1996; Zvereva et al., 1997). Then the absence of pollution effects on FA in birches (Valkama & Kozlov, 2001) came as a big surprise. Next, only 3 of 134 correlation coefficients based on data on plant FA collected by our team around 18 industrial polluters statistically confirmed an increase in FA with pollution load (Kozlov et al., 2009). Several other 'negative' results obtained by our team in the late 1990s–early 2000s remained unpublished until recently (Zverev & Kozlov, 2021a, 2021b). At the same time, a meta-analysis of published data (a total of 25 effect sizes) demonstrated a

strong and significant increase in FA near industrial polluters (Kozlov et al., 2009). Similarly, meta-analysis by Beasley et al. (2013) found a large overall effect of environmental stress on FA across 179 effect sizes extracted from 53 publications.

The difference between the findings of our team and the outcomes of meta-analysis conducted by us appeared too large to attribute it solely to the publication bias. Therefore, we suggested that published data had also been affected by research bias, that is, the tendency to explore organisms and conditions in which the researcher has a reasonable expectation of detecting statistically significant effects (Gurevitch & Hedges, 1999). This bias may, in particular, manifest itself by selecting plant species that are most sensitive to pollution and by selecting polluters with the most severe environmental impacts (Kozlov & Zvereva, 2009). However, this explanation was not satisfactory, as a substantial part of our data was collected from species sensitive to pollution (e.g. conifers) and near polluters with extreme environmental impacts (e.g. Sudbury, Norilsk and Monchegorsk smelters). Nevertheless, we were unable to provide a better explanation at that time.

#### 4 | CONFIRMATION BIAS MAY BE RESPONSIBLE FOR MANY OF SUPPORTIVE FINDINGS

A decade later, we identified another suspect: confirmation bias, also known as observer or expectancy bias. This bias is defined as the tendency of humans to seek out evidence and interpret it in a manner that confirms their existing ideas and hypotheses (Nickerson, 1998; Rosenthal, 1976). While well-documented in psychology and cognitive science, confirmation bias is poorly known to ecologists and environmental scientists (but see Kozlov et al., 2014; Marsh & Hanlon, 2007; van Wilgenburg & Elgar, 2013).

Confirmation bias attracted our attention because it primarily results from automatic processes occurring unintentionally (Hergovich et al., 2010). It provides a mechanistic explanation for the presumed positive feedback loop between the strength of a theory and the proportion of data supporting it. This hypothesis, as applied to developmental instability theory, was confirmed in two different ways.

First, we experimentally demonstrated that scientists familiar with this theory who believed that the plant leaves they were measuring originated from a heavily polluted site reported significantly higher values of FA compared to scientists who believed that the same leaves were collected from an unpolluted site. Thus, scientists who expected to find high FA in some samples produced data confirming their expectations (Kozlov & Zvereva, 2015).

Second, we re-measured samples used in two published studies. The first study (Kozlov et al., 1996) found an increase in leaf FA of mountain birch (*Betula pubescens* var. *pumila*) near the copper-nickel smelter in Monchegorsk relative to distant (unpolluted) sites. The second study (Pignataro et al., 2023) reported an increase in the wing FA of a butterfly (*Morpho helenor*) in forest edge habitats relative to forest interior habitats. Both studies did not apply blinding; original

measurements were conducted by co-authors aware of both sample origin and the hypothesis being tested. Blind re-measurements disproved both conclusions (Kozlov, 2024; Kozlov & Zverev, 2018), suggesting that the likelihood of false positive discoveries within the published data concerning the impacts of environmental stress on FA of plants and animals is higher than previously assumed. In other words, many supportive evidence may be erroneous.

Therefore, my answer to question (1) posed above is: many studies have found an increase in FA with increased stress (or putative stressor) due to imperfect methodology that did not include measures preventing the impact of confirmation bias on research data.

#### 5 | FIGHTING CONFIRMATION BIAS

Confirmation bias can be easily avoided by using a blind method, where the person conducting measurements is not aware of the origin of the samples being measured or the hypothesis being tested (Noseworthy et al., 1994; Saltaji et al., 2018). The study by Májeková et al. (2024) confirmed the importance of blinding: none of their original data sets, obtained by blind measurements of plant leaves, demonstrated a significant association between FA and a putative stressor. Furthermore, an analysis of data compiled by Májeková et al. (2024) demonstrated that only 8.3% of published studies based on blind measurements showed a positive association between FA and stressor, whereas studies using non-blind measurements yielded 43.9% confirmatory results. This difference in proportions appeared highly significant ( $\chi^2_1 = 10.6, p = 0.001$ ).

Therefore, my answer to question (2) is: the use of blind methods should be considered obligatory for any study addressing environmental or genetic impacts on FA to minimize the likelihood of type I error. Considering that blinding is simple (e.g. by coding objects or not providing the measurer with information on the hypothesis tested) and imposes practically no costs, I suggest that scientific journals discontinue publication of studies in which FA was calculated from non-blind measurements.

#### 6 | THE USE OF DATA BASED ON NON-BLIND MEASUREMENTS

The strength of confirmation bias varies among observers. In our experiment, false information suggesting birch leaves were from heavily polluted sites led 3 of 17 measurers to report significantly ( $p < 0.05$ ) greater FA in these leaves compared to the presumed control. Nine of 17 measurers reported a non-significant increase in FA, and the remaining four measurers found a slight decrease in FA (Kozlov & Zvereva, 2015). These findings suggest that conclusions from studies where FA was calculated from non-blind measurements cannot be easily corrected for confirmation bias. Therefore, my answer to question (3) is that data and conclusions potentially affected by confirmation bias should either be revised (i.e. measurements should be re-done blindly) or abandoned.

This suggestion is not as stringent as it may first appear. Many studies are actually based on blind measurements, even if not explicitly stated by the authors. For instance, in FA-related studies by our team, blinding was first mentioned in 2015 (Kozlov & Zvereva, 2015), but in practice, it was applied since the mid-1990s by assigning leaf measurements to temporarily employed students who were unaware of the underlying theory and the origin of the leaves. We suspect this situation may be common for many research teams. Nevertheless, classifying a particular study as blind or non-blind requires contacting the authors, which may not always be feasible.

Certain details of the sampling protocol can unintentionally make measurements effectively blind. For example, Cornelissen and Stiling (2005) collected leaves for FA measurements from multiple trees months before assessing herbivory on the same trees. Even if leaves were measured after herbivory assessment, it is unlikely that the measurer(s) remembered the herbivory level for each particular tree. Thus, we consider the likelihood of confirmation bias affecting the relationship between FA and herbivory reported by Cornelissen and Stiling (2005) as negligible.

Finally, data biased in terms of one research hypothesis may appear unbiased concerning another hypothesis. For example, multiyear measurements of FA in birch leaves from the Monchegorsk pollution gradient (Valkama & Kozlov, 2001) may yield biased conclusions regarding the correlation of FA with pollution because the distance from each sampling site to the smelter was known to the measurers. However, the same data apparently produced unbiased conclusions regarding the correlation of FA with mid-summer temperature since we did not anticipate this association when planning the study, nor did we have temperature data available during leaf measurements.

## 7 | CONCLUSIONS

The absence of significant correlations between FA and stress consistently demonstrated by studies that use blinding (e.g. Májková et al., 2024), combined with the proven impact of confirmation bias on the outcomes of non-blind studies (Kozlov, 2024; Kozlov & Zvereva, 2015), suggests that a substantial (yet unknown) percentage of previously published evidence supporting an influential theory may not be reliable and could represent false positives. Therefore, both data and conclusions from FA-related studies that did not report blinding should be used with great caution, especially in meta-analyses and other forms of research synthesis. In practice, this means that data and conclusions aligning with the prevalent theory (i.e. reporting a positive association between FA and stress) should only be used when proof of negligible impact from confirmation bias can be provided. For forthcoming studies, it is essential that all measures taken against biases are described in each submitted manuscript, and that the need to check for these requirements is included in instructions for reviewers. These measures are already implemented by some high-impact journals, and it is suggested that other journals consider joining this initiative.

## ACKNOWLEDGEMENTS

I thank Elena L. Zvereva for fruitful discussion and commenting an earlier version of the manuscript.

## CONFLICT OF INTEREST STATEMENT

Author declares no conflict of interest.

## DATA AVAILABILITY STATEMENT

No new data were generated or analysed.

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**How to cite this article:** Kozlov, M. V. (2025). Fluctuating asymmetry in ecological and environmental research: Quo vadis? *Functional Ecology*, 39, 4–8. <https://doi.org/10.1111/1365-2435.14713>