



A novel alternative to *F*-tests for ecological studies



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ABSTRACT

Differences or similarities in the variance of fitness traits are crucial in several biological disciplines, e.g. ecological, toxicological, developmental and evolutionary studies. For example the variance of traits can be utilized as a biomarker of differences in environmental conditions. In the absence of environmental variability, the differences of the variance of a trait can be interpreted as differences of the genetic background. Several tests and transformations are utilized when testing differences between variances. There is, however, a biological tendency for the variance to scale proportionally to the square of the mean (scaling effect) which can considerably bias the results of the tests. We propose a novel method which allows for a more precise correction of the scaling effect and proper comparisons among treatment groups and between investigations. This is relevant for all data sets of distributions with different means and suggests the reanalysis of comparisons among treatment groups. This correction will provide a more reliable method when using bioindicators.

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1. Introduction

1.1. Phenotypic variance as biomarker

Biologists utilize biomarkers (defined as functional measures of exposure to various stressors) in the attempt to monitor the impacts of environmental stressors on biological systems (Adams et al., 2001; Depledge and Galloway, 2005; Beasley et al., 2013). The environmental conditions can affect the development of a trait and for example developmental instability (DI) can occur when environmental stress affects the buffering capacity of the processes that provide stability to an organism's development (Lens et al., 2000). Harsher environmental conditions are supposed to increase DI (Graham et al., 1993).

Developmental instability at the population level can be estimated as the phenotypic variance of morphological characters (σ^2p) (Pertoldi et al., 2006a), or as the average fluctuating asymmetry of morphological characters (FA) (Palmer and Strobeck, 1986).

The estimates of DI using σ^2p as its estimator may be blurred by genetic variation (σ^2g) unless that genetically identical individuals are utilized (monoclonal strains), however also the presence of environmental variability (σ^2e) can produce a bias (Lajus et al., 2003).

If genetic variation is present, then $\sigma^2p = \sigma^2g + \sigma^2e + G \times E + \text{covGE} + \text{DI}$ (Andersen et al., 2006, 2008; Pertoldi et al., 2001a,b), where $G \times E$ is the genotype–environment interaction and covGE is the genotype–environment covariance. The genetic variation term (σ^2g) can then be further subdivided in $\sigma^2g = \sigma^2a + \sigma^2d + \sigma^2i$, where σ^2a , σ^2d and σ^2i are the additive, dominant and interaction components (Pertoldi et al., 2006b,c).

Fluctuating asymmetry has been suggested as a more reliable estimator of DI as it can be measured at the individual level (measured as the difference in length between the right and left side of a trait). In addition there is no genetic and environmental variation between the right and left side of the traits (Palmer and Strobeck, 1986).

Several investigations have utilized the variations of the mean and variances (σ^2p and FA) of morphometric traits in various species to infer environmental differences as a biomarker; ranging from plants, e.g. in Scots pine (*Pinus sylvestris*) (Kozlov et al., 2002), winged sumac (*Rhus copallinum*) (Duda et al., 2004) and seagrass (*Halophila ovalis*) (Ambo-Rappe et al., 2008), to invertebrates, e.g.

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a species of springtail (*Folsomia candida*) (Kristensen et al., 2004), cabbage aphid (*Brevicoryne brassicae*) (Gorur, 2006), rough woodlouse (*Porcellio scaber*) (Godet et al., 2012), and sea urchins (*Arbacia lixula* and *Paracentrotus lividus*) (Savriama, 2015), to vertebrates, e.g. Eurasian treecreeper (*Certhia familiaris*) (Helle et al., 2011).

A meta-analysis conducted by Beasley et al. (2013) tested the hypothesis that FA is a reliable biomarker of environmental stress in insects. The main conclusion was that environmental stressors explained 36% of the variation of effect on FA across studies. The authors also discussed the finding that larger effects on FA were found in controlled laboratory experiment, where the environmental variance is minimized, compared to studies conducted under natural conditions. However, empirical studies supporting its general adequacy for monitoring species or populations are generally lacking or contradictory (Vøllestad et al., 1999; Woods, 1999; Gilligan et al., 2000).

2. Taylor's Power law and F-test

The one-tailed *F*-test is the most common way to compare variances (σ^2) and it has been used to generate hypotheses (Hallgrímsson and Hall, 2005). The null-hypothesis (H_0) for the *F*-test is that there is no difference between two variances (σ_1^2 and σ_2^2) and the alternative hypothesis (H_A) is that the larger variance is significantly different from the smaller variance. There is however a tendency (which should be taken into account) for the σ^2 to scale proportionally to the square of $\bar{\mu}$ (Lewontin, 1966):

$$\sigma^2 = z \cdot \bar{\mu}^2, \quad (1)$$

where a measure of individual level variability is estimated by z , and b is the scaling exponent which is equal to 2 (Pertoldi et al., 2007, 2008). Following a log transformation, Eq. (1) becomes:

$$\log \sigma^2 = \log z + b \cdot \log \bar{\mu} \quad (2)$$

The regression of $\log \sigma^2$ (dependent) on $\log \bar{\mu}$ (independent) gives a line with an intercept equal to z and a slope of 2 (Taylor, 1961). When σ^2 scales proportionally to the square of $\bar{\mu}$ it is quite common to take the logarithm of all observations and thereafter to compare the two σ^2 values using an *F*-test (the so called log-test) or to calculate the coefficient of variation (CV), which is σ divided by $\bar{\mu}$, i.e. $CV = \sigma/\bar{\mu}$ (Neves et al., 2012). The CVs can then be compared with a naïve test, which compares the CVs with an *F*-test in the same way as the variances are compared (Levy and Siegal, 2008; Geiler-Samerotte et al., 2013). Other modifications of the *F*-test exist, for example the approximate *F*-test, the likelihood ratio test, Miller's test, Bennett's test and the Wald test. All these tests (with the use of approximations and/or transformations of the data) take into account potential violations of the assumptions required by the *F*-test, like non-normally distributed data, small sample size and scaling of $\bar{\mu}$ with σ^2 (Hallgrímsson and Hall, 2005). The *F*-test is also utilized when deciding what kind of tests should be used when for example testing correlations or differences between means and medians (for example Welch test or Mann–Whitney *U*-test). Unequal sample variances are common in ecology and evolutionary studies, together with other violations like small sample size and deviations from normal distribution, however the scaling effect remains a major problem which has not been resolved and which is often ignored (Ruxton, 2006).

3. Methods and results

3.1. Mathematical justification

The aim of this paper is to show that all studies where *F*-tests have been used to calculate differences in population means, potentially harbor misleading conclusions. Even the log-transformation

and the use of the CV can produce a biased effect as this log-transformation and the way of scaling the data with the mean (CV) can only be correctly used if $b=2$ (σ^2 must scale proportionally to the square of $\bar{\mu}$) (Taylor and Woiwod, 1980). $b=2$ is the expected value for pure statistical reasons as it is an intrinsic property of the σ^2 formula.

But we should bear in mind that $b=2$ is rather the exception than the rule. As an example, Fisher (1937) already noted that σ^2 in many morphological traits has been found to scale proportionally to $\bar{\mu}$ itself, rather than the square of $\bar{\mu}$ which is indicating that $b<2$. Furthermore, we propose a new method for a more accurate correction of the scaling effect.

3.2. Derivation of mathematical alternative

The procedure has been derived and tested in the following way:

- A Log-transformation of the two variances, to be compared by an *F*-test (σ_1^2 and σ_2^2).
- B Log-transformation of the two means ($\bar{\mu}_1$ and $\bar{\mu}_2$) in which we assume that $\bar{\mu}_2 > \bar{\mu}_1$.
- C Estimation of the slope (b) of the line using the values: ($\log \bar{\mu}_1$, $\log \sigma_1^2$) and ($\log \bar{\mu}_2$, $\log \sigma_2^2$) in Eq. (2) $\log \sigma^2 = \log z + b \cdot \log \bar{\mu}$.
- D Which therefore becomes

$$b = [\log(\sigma_2^2) - \log(\sigma_1^2)] / [(\log \bar{\mu}_2) - (\log \bar{\mu}_1)] \quad (3)$$

the relationship between mean and variance is b and is expected to be 2 due to the scaling effect.

- E Estimation of the slope of the line by subtracting the value of 2 (the expected slope due to the scaling effect) in Eq. (2) $\log \sigma^2 = \log z + b \cdot \log \bar{\mu}$, which therefore becomes:

$$\log \sigma^2 = \log z_C + (b - 2) \log \bar{\mu}, \quad (4)$$

This allows an estimation of the corrected values of the log-variance (σ_{2C}^2):

$$\log \sigma_{2C}^2 = \log z_C + (b - 2) \log \bar{\mu}_2, \quad (5)$$

where $\log \sigma_{2C}^2$ is the corrected values of the log-variance and $\bar{\mu}_2$, is the mean at which the variance $\log \sigma_{2C}^2$ is estimated. $\log z_C$ is the corrected intercept which is different from the intercept of Eq. (2). The line described by Eq. (4) therefore has a new intercept ($\log z_C$), which passes through the ($\log \bar{\mu}_1$, $\log \sigma_1^2$) and the ($\log \bar{\mu}_2$, $\log \sigma_{2C}^2$). $\log \sigma_{2C}^2$ can be estimated from the corrected slope: $(b - 2)$ by substituting in Eq. (3); $b = [\log(\sigma_2^2) - \log(\sigma_1^2)] / [(\log \bar{\mu}_2) - (\log \bar{\mu}_1)]$ and resolving for $\log \sigma_{2C}^2$ which is $\log \sigma_{2C}^2$, Eq. (3) becomes:

$$\log \sigma_{2C}^2 = (b - 2)(\log \bar{\mu}_2 - \log \bar{\mu}_1) + \log \sigma_1^2 \quad (6)$$

These equations can be combined to a reestimated variance based on the original values as

$$\sigma_{2C}^2 = e^{\left(\frac{\log(\sigma_2^2) - \log(\sigma_1^2)}{\log(\bar{\mu}_2) - \log(\bar{\mu}_1)} - 2 \right) \times (\log(\sigma_2^2) - \log(\sigma_1^2)) + \log(\sigma_1^2)} \quad (7)$$

The entire graphical procedure is explained in Fig. 1a–d.

4. Statistical computation

Code for estimating the corrected variances in *R* is available in Appendix 1. These estimates cannot readily be compared with the variance of the population with the smallest mean since their ratio does not follow the *F*-distribution. We therefore suggest estimating a *P*-value by a simulation approach with code also found in Appendix 1. For these simulations we compare the reestimated variance with reestimations for a large set of simulated distributions with the same sample sizes as the empirical test

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