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A comparative fluctuating asymmetry study between two walnut (*Juglans regia* L.) populations may contribute as an early signal for bio-monitoring

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Developmental stability, the ability of an individual to eliminate environmental disturbances while expressing a heritable phenotypic trait, was compared in two walnut (*Juglans regia* L.) populations, a natural and an artificial. Bilateral leaf morphometrics were used to estimate fluctuating asymmetry which refers to random deviation from perfect symmetry of bilateral traits resulting from extrinsic and intrinsic perturbations not buffered during development. Fluctuating asymmetry was used as a proxy of developmental stability. We analyzed our data from a Bayesian perspective showing that developmental stability levels are decreased in the natural population. Our results indicate that an attention may be directed towards the conservation of the natural walnut resources of the area. Fluctuating asymmetry as an indicator of developmental stability may contribute especially in the framework of comparative studies as a population biomonitoring tool.

Keywords: Signal asymmetry, Directional asymmetry, Developmental stability, Bio-monitoring, Conservation

Introduction

Adaptation of organisms to changing environments is becoming a very important issue, especially in the context of climate change. Developmental stability is the ability of an individual to eliminate environmental disturbances while expressing a heritable phenotypic trait. Developmental stability is an important notion for evolutionary and ecological studies, since it provides valuable information on the adaptation of organisms or populations to certain environments. There is evidence that developmental stability has a genetic basis (Clarke 1998), nevertheless the relationship between genes and developmental stability is still under debate (Pertoldi et al. 2006).

The lack of data and models does not allow direct estimations of developmental stability. In practice, the pattern of bilateral variation in a random sample of the population, called fluctuating asymmetry (Van Valen 1962, Palmer 1994) is widely used as an indirect developmental stability estimator. Fluctuating asymmetry refers to the random deviation from perfect symmetry of bilateral traits resulting from extrinsic and intrinsic perturbations not buffered during development (Van Valen 1962, Palmer 1994). Fluctuating asymmetry has been suggested to result from poorly co-adapted gene complexes and therefore to be useful as a measure of developmental stability (Hoelzel et al. 2002). The relation between fluctuating asymmetry and developmental stability is based on the assumption that the symmetric parts of bilateral traits are controlled by the same groups of genes and also that both sides share the same environment, during the process of development. Therefore a perfectly symmetrical phenotype can be expected. During development however, several random errors occur independently and additively in each side introducing a source of uncertainty into the phenotype development mechanism and consequently leading to random deviations from perfect symmetry. The variance of the random deviations (that is equivalent to fluctuating asymmetry) is proportional to the error rate during development. Assuming linear correlation, this variance parameter can be used as an estimator of this error rate and furthermore of developmental stability. Detailed reviews on fluctuating asymmetry are given by Palmer & Strobeck (1986, 2003), Van Dongen (2006) and Graham et al. (2010).

There is some underlying additive genetic variance associated with fluctuating asymmetry and relevant QTLs have been identified in poplars (Wu et al. 2007) and mice (Leamy et al. 2002), but the extent of its genetic basis remains unresolved (Moller & Thornhill 1997a, 1997b, Markow & Clarke 1997, Houle 1997, Van Dongen 2000b). Nevertheless, there is at least circumstantial evidence that has linked fluctuating asymmetry to environmental stress (Parsons 1990, 1992, Silva et al. 2009, Hodar 2002) and even to genetic bottlenecks (Hoelzel et al. 2002).

In this study, we compared the fluctuating asymmetry as an indicator of developmental stability in two adjacent walnut (*Juglans regia* L.) populations: an artificial and a natural. We have estimated fluctuating asymmetry by bilateral leaf morphometrics assuming that leaf asymmetry provides a reliable estimate of the developmental stability in plants and the populations that they belong to. Leaves are highly sensitive tree organs that are subject to environmental, as well as phenological growth cycles and fluctuations (Fair & Breshears 2005). Our working hypothesis is that comparative developmental stability levels estimated by fluctuating asymmetry provide a cost effective way to obtain insight with regards to population analysis and biomonitoring. Such insight may be valuable for the design of future more thorough studies.

Materials and methods

Sampling

The two populations compared in this study are located in the region of Sohos, near Thessaloniki (Greece) in the area of Mt. Vertiskos at an elevation of 650-700 m (40° 49’ N, 23° 22’ E), characterized by sand-clay soils and typical Mediterranean climate. The region is renown for its walnut and features both wild populations and walnut orchards with trees that attain a height of up to 25 m. The average age of the natural population is about 60 years, however older trees can be found. The artificial population comprises of locally selected genotypes of younger age (40 years old on the average). We chose randomly 29 individuals to sample from each population at a minimum distance of 20 m from each other, avoiding sampling filial structures. Isolated trees (no other tree present at a radius of 100 m) were excluded

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Tab. 1 - Summary statistics for the posterior densities of parameters. Column Mean denotes the posterior mean. SD is the standard deviation. The last four columns show quantiles for each variable.

Parameter	Mean	SD	2.50%	25.00%	50.00%	75.00%	97.50%
DA ($\cdot 10^{-3}$)	5.57	3.45	-0.93	3.33	5.54	7.86	12.71
$\sigma_{FA\ (nat)}$	0.088	0.004	0.082	0.086	0.088	0.090	0.096
$\sigma_{FA\ (art)}$	0.079	0.003	0.072	0.076	0.079	0.080	0.085
r	1.128	0.066	1.010	1.081	1.126	1.174	1.261

from sampling. Trees exhibiting natural (infection) or anthropogenic (pruning) disturbance were excluded as well. Ten leaves per tree were randomly selected from lower branches at a height of 2 m, being approximately equally distributed on the circumference of the tree canopy. Measurements were taken after careful drainage.

Analysis

We measured the perimeters of the left (L) and the right (R) side of the leaf in a resolution of 1 mm and used the log-ratio: log(L/R) as Signed Asymmetry (SA) measure per leaf. We were interested to study developmental stability (DS) at the population level, thus fluctuating asymmetry (FA) was estimated using the pooled samples per population. We approached our analysis from a Bayesian perspective. We employed a model to infer SA and Directional Asymmetry (DA - *i.e.*, when one side is systematically larger compared to the other side), similar to the one proposed by Van Dongen (2000a, 2001, 2006 - eqn. 1):

$$Y_{ip} \sim N\left(\mu_p, \sigma^2_{FA(p)}\right)$$

where y_{ip} denotes the *i*-th SA observation taken from the population *p* (*i.e.,* natural or artificial); μ_p corresponds to the expected value of the observation. Deviation from zero indicates the presence of DA in both populations. Otherwise, in case of DA absence, μ_p should be equal to zero. Parameter $\sigma^2_{FA\ (p)}$ denotes the variance of SA in the population *p* (artificial or natural). By using a single parameter for SA per population, we assume that all the trees in each population share a common SA. We used this parameter to observe the developmental stability of the corresponding population. The prior distributions for the parameters in our model are similar to those proposed by Van Dongen (2001): $\mu_p \sim$ N(0.10) and $\sigma^{-2}_{FA(p)} \sim \Gamma(0.001, 0.001)$, where Γ denotes the Gamma distribution. In order to compare the difference in developmental stability between the two populations we estimated the posterior probability (eqn. 2):

$$P(\sigma_{FA(nat)} > \sigma_{FA(art)} | Y)$$

We applied Markov Chain Monte Carlo (MCMC) to estimate the probability in eqn. 2 and used the JAGS (Plummer 2003) software to perform this posterior simulation. From a total of 120 000 iterations, the first 20 000 were discarded as burn-in stage, while we sampled from the Markov Chain every 100 iterations in order to reduce autocorrelation between samples. Four parallel Markov Chains were simulated and convergence diagnostics were obtained using the CODA R package (Plummer et al. 2006). Further inferences were made by combining the samples from the four chains. We used parameter r = $\sigma_{FA(nat)} / \sigma_{FA(art)}$ to monitor the ratio of developmental stability values in the two populations. In addition to our Bayesian analysis, we tested the hypothesis that the variances between the two populations are equal by performing a F-test with null hypothesis H_0: $\sigma^2_{FA(nat)} = \sigma^2_{FA(art)}$ and alternative H_1: $\sigma^2_{FA(nat)} > \sigma^2_{FA(art)}$ and using the statistic $r^2 = \sigma^2_{FA(nat)} / \sigma^2_{FA(art)}$.

Results and Discussion

Summary statistics and posterior densities are shown in Tab. 1 and Fig. 1 respectively. Fluctuating asymmetry in the natural population is larger than the fluctuating asymmetry in the artificial, with a probability of 0.982. This result is also supported by the F-test (test statistic $r^2 = 1.27$) resulting to p-value = 0.021 (with the classical statistics interpretation) with confidence intervals CI = (1.047, +inf). We regard this outcome as noteworthy. Our results show that the right side of the leaf is slightly larger than the left side, but still the difference is small and additional experimentation is needed to further elucidate this finding. The natural population can be regarded as presenting reduced levels of developmental stability. It is noted that a significant amount of genetic erosion has been indicated for European *J. regia* populations and while genetic diversity parameters for northern Greek populations were higher that the average, they were notably lower than typical values reported for widespread plant species (Fornari et al. 1999). The artificial population has been long cultivated for the production of regionally renowned walnuts. Nevertheless, any positive influence of cultivation activities on the artificial popula-

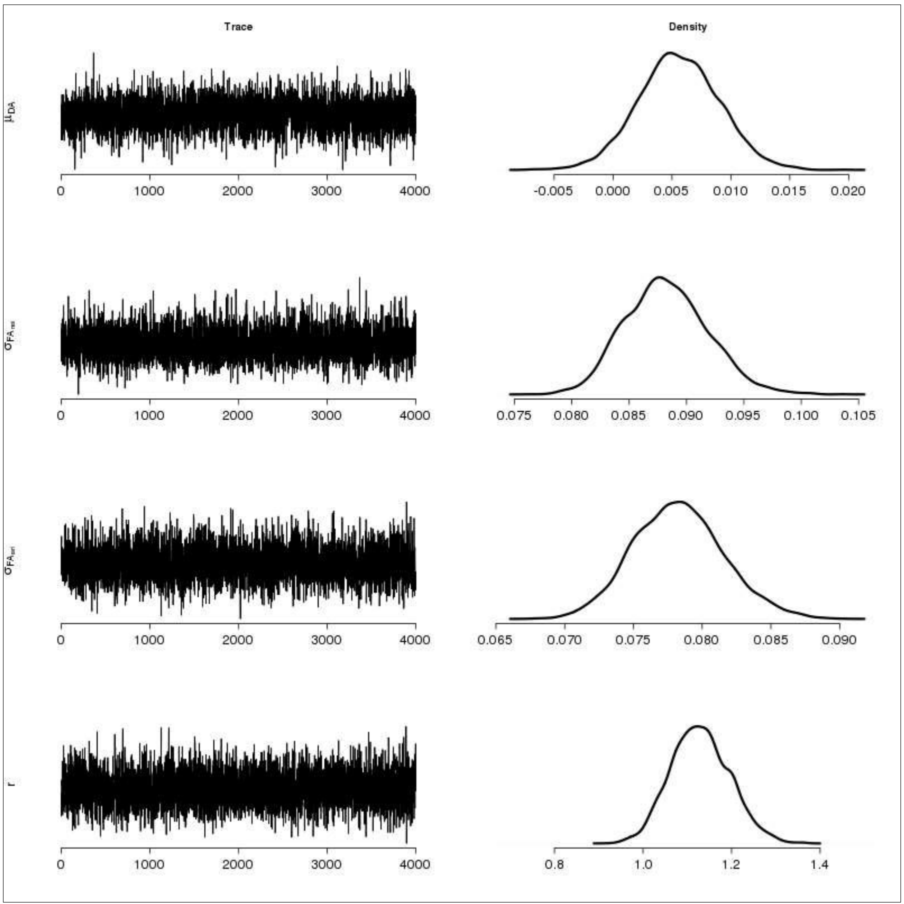

Fig. 1 - Traces and posterior densities for the parameters of interest. Plots were produced by concatenating the four MCMC chains.

tion does not apply to the adjacent natural population that may be more prone to environmental perturbations and stochastic fluctuations of effective population size. In this case, our results may call for more detailed genetic studies in order to investigate the evolutionary potential of the natural population (genetic monitoring).

We consider our findings as a potentially early warning indication for problems that may occur in the natural population under scenarios of future environmental uncertainty. Given that expression of both sides of a leaf bilateral trait is due to the same genes, then any asymmetry between the sides can be a consequence of environmental disturbance. Hence, fluctuating asymmetry could be considered as a proxy for environmental or genetic stress (Van Valen 1962, Palmer 1994). Our approach is characterized by simple data collection, while a thorough analysis can be streamlined by specific software that we plan to develop. Therefore the careful application of such an approach can present an additional tool to a battery of available applications for population biomonitoring (Leung et al. 2000, Lens & Van Dongen 2002). Natural biodiversity is extremely important and it may be vital for biomonitoring and conservation policies to take into account the contribution of simple potential "signals".

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