C. R. Geoscience 345 (2013) 350–359

Contents lists available at SciVerse ScienceDirect

Comptes Rendus Geoscience

www.sciencedirect.com

Stratigrahy, sedimentology (Palaeoenvironment)

Estimating variation in stomatal frequency at intra-individual, intra-site, and inter-taxonomic levels in populations of the *Leonardoxa africana* (Fabaceae) complex over environmental gradients in Cameroon

Estimation de la variation de la fréquence stomatique aux niveaux intra-individuel, intra-site, et inter-taxonomique chez des populations du complexe Leonardoxa africana *(Fabaceae) selon des gradients environnementaux au Cameroun*

Walter Finsinger [a,*,1], Thibaut Dos Santos [b,1], Doyle McKey [b,c]

[a] *UMR 5059 CNRS/UM2/EPHE, Centre de bio-archéologie et d'écologie (CBAE), Institut de Botanique, 163, rue A.-Broussonnet, 34090 Montpellier, France*
[b] *UMR 5175 CNRS, Centre d'écologie fonctionnelle et évolutive (CEFE), 1919, route de Mende, 34293 Montpellier cedex 05, France*
[c] *Institut universitaire de France, 103, boulevard Saint-Michel, 75005 Paris, France*

ARTICLE INFO

Article history:
Received 29 August 2012
Accepted after revision 21 June 2013
Available online 2 August 2013

Keywords:
Stomatal frequency
Phenotypic plasticity
Variance-component analysis
Leonardoxa
Central Africa

ABSTRACT

Variation of stomatal frequency (stomatal density and stomatal index) includes genetically-based, potentially-adaptive variation, and variation due to phenotypic plasticity, the degree of which may be fundamental to the ability to maintain high water-use efficiency and thus to deal with environmental change. We analysed stomatal frequency and morphology (pore length, pore width) in leaves from several individuals from nine populations of four sub-species of the *Leonardoxa africana* complex. The dataset represents a hierarchical sampling wherein factors are nested within each level (leaves in individuals, individuals in sites, etc.), allowing estimation of the contribution of different levels to overall variation, using variance-component analysis. SI showed significant variation among sites ("site" is largely confounded with "sub-species"), being highest in the sub-species localized in the highest-elevation site. However, most of the observed variance was accounted for at intra-site and intra-individual levels. This variance could reflect great phenotypic plasticity, presumably in response to highly local variation in micro-environmental conditions.

Mots clés:
Fréquence stomatique
Plasticité phénotypique
Variance-component analysis
Leonardoxa
Afrique centrale

RÉSUMÉ

La variation de la fréquence stomatique (densité de stomates [SD] et index stomatique [SI]) inclut des variations potentiellement adaptatives et des variations liées à la plasticité phénotypique, dont le degré peut être fondamental dans la régulation et pour le maintien d'une haute « *water-use efficiency* » et donc pour faire face aux changements environnementaux. Nous avons analysé les fréquences et la morphologie (longueur et largeur des pores) de stomates de feuilles provenant de neuf populations de quatre

* Corresponding author.
E-mail address: walter.finsinger@univ-montp2.fr (W. Finsinger).
[1] These authors contributed equally to the manuscript.

1631-0713/$ – see front matter
http://dx.doi.org/10.1016/j.crte.2013.06.003

sous-espèces du complexe *Leonardoxa africana*. Le jeu de données représente un échantillonnage hiérarchisé dans lequel les facteurs sont emboîtés à chaque niveau (feuilles dans individus, individus dans site/populations, etc.). En utilisant une « variance-component analysis », on a estimé la contribution de chaque niveau à la variation globale. SI est significativement différent entre les sites et atteint les valeurs les plus élevées dans le site/sous-espèce localisé à la plus haute altitude. Néanmoins, la plus grande variance est enregistrée aux niveaux intra-sites et intra-individus. Cela pourrait être le reflet d'une grande plasticité phénotypique qui répond à une forte variation locale des conditions micro-environnementales.

1. Introduction

The need to understand how species respond to environmental variation has become critical as environmental changes have demonstrable ecological effects in many natural systems (Parmesan, 2006; Walther et al., 2002). Determining how individuals base key life-history decisions on environmental cues is important to predict how environmental changes will influence them. Phenotypic plasticity, defined as the ability of a single genotype to modify its phenotype under heterogeneous environmental conditions (Houston and McNamara, 1992), is fundamental to a plant's ability to cope with environmental change at various temporal and spatial scales.

Stomata regulate the permeability of leaves of terrestrial plants to gases (gas phase conductance), so that the leaves can absorb CO_2 for photosynthesis without losing excessive water vapour (Raven, 2002). There is at present sufficient evidence to suggest that perennial terrestrial plants regulate their gas exchange (and thus CO_2 uptake and water loss) by varying the opening of the stomatal pores (Young et al., 2006) and by changing the stomatal frequency on leaves (Gagen et al., 2011; Hetherington and Woodward, 2003). Thus, to optimize their resource use and evade costs of having excess stomata, plants could be expected to adjust stomatal frequency (Roth-Nebelsick, 2007). Amongst several environmental factors, atmospheric CO_2 has often been observed to have a strong influence in the regulation of stomatal frequency (Chen et al., 2001; Hetherington and Woodward, 2003; Wagner et al., 1996; Woodward, 1987). However, most scholars agree with the view that responses are species-specific (Marchi et al., 2004; Tognetti et al., 2000) and include cases in which species do not respond at all or respond to increasing atmospheric CO_2 by increasing, rather than decreasing, stomatal frequency (Hetherington and Woodward, 2003).

Although strong plastic (developmental) responses have been observed in stomatal frequency (stomatal density and stomatal index [SI]) (Royer, 2001), there is clear evidence of genetic control of the CO_2 response (Jordan, 2011). Thus, in *Arabidopsis thaliana*, the HIC gene codes for CO_2 responses in both stomatal density and index (Gray et al., 2000). The latter evidence strongly suggests that stomatal frequencies are determined (at least to some extent) by the genotype and implies that plants can regulate their stomatal frequency by phenotypic plasticity, allowing them to adapt to changing environmental conditions. For example, it is often observed that plants have higher stomatal density when growing at lower CO_2 concentrations (Hetherington and Woodward, 2003; Woodward, 1987) or at higher elevation (where the partial pressure of CO_2 is presumably lower) (McElwain, 2004). The negative relationship between CO_2 concentration (or partial pressure) and stomatal frequency has often been used in palaeoecological investigations to reconstruct temporal changes of atmospheric CO_2 concentrations using (sub)fossil leaf cuticles collected from sedimentary archives (Beerling, 1999; Beerling and Chaloner, 1993; McElwain and Chaloner, 1995; McElwain et al., 2002; Steinthorsdottir et al., 2013). However, such reconstructions are based on the assumption that environmental changes at smaller scales (i.e., among individuals or within individuals of the same population) are negligible compared to the stomatal-frequency changes caused by varying CO_2 concentrations (Finsinger and Wagner-Cremer, 2009). Although estimates of the stomatal-frequency variation at intra-population and intra-individual level might be necessary to fully account for uncertainties in paleo-CO_2 reconstructions, the analysis of modern leaves and of these sources of variation is rarely conducted (Chen et al., 2001).

Here we aimed to compare stomatal frequency within the *Leonardoxa africana* complex (Fabaceae–Caesalpinioideae) at different hierarchical levels: among sub-species, among populations belonging to the same sub-species, among individuals of the same population, and within individuals. We were particularly interested in investigating at which hierarchical level the greatest changes in the SI were observed and in illustrating the role of environmental variables as potential predictors for cuticle-morphology characteristics (stomatal frequency and stomatal pore size). Our investigation focuses principally on variations of SI because this variable allows a more appropriate comparison among leaves, since the SI is less biased by external factors than is stomatal density (Beerling, 1999).

2. Material and methods

Leonardoxa africana (Baill.) Aubréville (Fabaceae: Caesalpinioideae) is a species complex native to Atlantic central Africa (Fig. 1) that comprises four distinct, mostly allopatric subspecies (*L. a. africana*, *L. a. letouzeyi*, *L. a. gracilicaulis*, and *L. a. rumpiensis*). These have been described and identified based on morphological characters, particularly those related to the plants' interactions with ants (McKey, 2000).