Preliminary results on climate change evidence from coprolites of Myotragus balearicus Bate 1909 (Artiodactyla, Caprinae)

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Preliminary results on climate change evidence from coprolites of *Myotragus balearicus* Bate 1909 (Artiodactyla, Caprinae)

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Abstract. Plants may vary their stomata density as a function of environmental factors, such as [CO_2], drought and temperature. Under the present atmospheric conditions, it is expected that leaves have different stomata density than they had hundreds or thousands of years ago, due to the rise of CO_2 in the atmosphere. Microhistological analyses of coprolites of the extinct *Myotragus balearicus* from Cova Estreta (Pollença, Mallorca), with a radiocarbon age of 4950 ± 38 BP (3775-3640 2σ cal BC; Wk-33010), have shown a diet including an important amount of *Buxus balearica* epidermal fragments. Three of these coprolites were used to estimate the stomata density on *Buxus balearica* epidermal fragments from this period. Additionally, three samples of the endangered *Buxus balearica*, the sole species *of Buxus* currently present on Mallorca, were collected in three different localities and leaves were examined under microscopy to determine the stomata density. A significant difference between epidermal fragments from coprolites and epidermal fragments of living plants (c^2 = 34.46, P<0.0001, L-R c^2 test), with a density average of 27.21 and 19.05 stomata/mm^2 respectively, has been recorded. The current lower density of stomata could be a plant response to climatic change in the Mediterranean islands.

Keywords. Stomata – *Buxus balearicus* – Climate change – Carbon dioxide.

Résultats préliminaires sur l'évidence du changement climatique basée sur les coprolithes de Myotragus balearicus Bate (1909)

Résumé. *Les plantes peuvent varier la densité des stomates sur la base de facteurs environnementaux, tels que le CO_2, la sécheresse et de la température. Dans les conditions météorologiques actuelles sont censés avoir une densité stomatique des feuilles différente de ce qu'ils avaient pendant des centaines ou des milliers d'années auparavant, en raison de l'augmentation du CO_2 dans l'atmosphère. L'analyse des coprolithes de la disparition microhistologique de Myotragus balearicus de Cova Estreta de (Pollença, Mallorca), avec un âge radiocarbone de 4950 ± 38 BP (3775-3640 cal BC 2σ; WK-33010) ont montré une alimentation qui comprend une quantité importante fragments épidermiques de Buxus Balearica. Trois de ces coprolithes ont été utilisées pour estimer la densité des stomates dans les fragments épidermiques de Buxus balearica cette période. En outre, trois échantillons en danger d'extinction balearica Buxus, la seule espèce de Buxus opérant actuellement à Majorque, ont été recueillies à trois endroits différents et les feuilles ont été examinés au microscope afin de déterminer la densité des stomates. Il y avait une différence significative entre les fragments épidermiques de coprolithes et de fragments de l'épiderme des plantes vivantes (c^2 = 34,46, P <0,0001, test LR c^2), avec une densité moyenne de 27,21 et 19,05 stomata/mm^2, respectivement. La plus faible densité des stomates pourrait être une réponse des plantes aux changements climatiques dans les îles de la Méditerranée.*

Mots-clés. *Stomates* – Buxus balearicus – *Changement climatique – Dioxyde de carbone.*

I – Introduction

Certain plant traits, such as leaf morphology, are sensitive to climate change effects. Some works, based on exposing plant species to varying CO_2 concentrations in controlled environment experiments, demonstrated that anincrease in atmospheric CO_2 concentration causes a reduction in stomatal density (Woodward, 1987; Woodward and Kelly, 1995). In some species, stomata density also decreased in response to drought (Giordano *et al*., 2011), although this factor has a species' dependent effect (e.g. Mehri *et al*., 2009; Guerfel *et al*., 2009). Comparative studies on stomatal density, based on herbarium material, have been used to estimate the effects of a rise in atmospheric CO_2 over the past centuries (Woodward, 1987; Peñuelas and Paoletti and Gellini, 1993). Stomatal density has also been considered as an indicator of atmospheric CO_2 concentration in studies based on plant fossil material (e.g. Beerling and Chaloner, 1992; Beerling, 1993; McElwain and Chaloner, 1995).

Herbivore coprolites are potential sources of ancient plant remains as they may contain fragments of leaf epidermis from which stomatal density can be determined. Moreover, the microscopic diagnostic features of these fragments allow for the identification of plant species. As plant species recorded on recent geological periods –such as the Holocene– can be also found in the present, comparisons of their current and fossil stomatal density are feasible. These comparisons could provide evidence of atmospheric CO_2 concentration changes.

Myotragus balearicus Bate 1909 was a ruminant artiodactyl endemic to the Eastern Balearic Islands, which became extinct more than 4000 years ago (Alcover *et al*., 1999). *Myotragus* coprolites were collected from Holocene sediments in the deposit of Cova Estreta (Pollença, Serra de Tramuntana, Mallorca). Coprolite content has shown a diet with a high amount of *Buxus balearica* Lam (Alcover *et al*., 1999; Bartolomé *et al*., 2011), which is an extant species in that area nowadays.

Palynological studies on the Balearic Islands document that prior to 2880 cal BC the vegetation was very different to the of recent times (e.g., Yll *et al*., 1997). Now, the vegetation is dominated by shrub formations characteristic of xeric conditions (Yll *et al*., 1995), while prior to 2880 cal BC, it was apparently dominated by wet forests. Epidermal fragments of *Buxus balearica* contained in *Myotragus balearicus* coprolites allow us to present a paleontological proxy to relative CO_2 concentration in the atmosphere of the island c. 5700 years ago. The comparison of stomatal density of *Buxus balearica* found in the coprolites with epidermal tissues of living plants could be interpreted as possible evidence of climate change in the Mediterranean islands.

II – Materials and methods

Myotragus balearicus coprolites were collected from upper levels of the sedimentary deposits in Cova Estreta (Pollença, Mallorca) during the excavation campaigns in the nineties (Alcover *et al*., 1999). The cave is located in the Tramuntana mountains, in the North of the island, at c.350 m above sea level.

One of the coprolites was dated by the Radiocarbon Dating Laboratory of the University of Waikato (New Zealand) giving a radiocarbon age of 4950 ± 38 BP (3775-3640 2σ cal BC; Wk-33010).

Three leaves of the extant *Buxus balearica* were collected from three different sites of the Tramuntana mountains: Pollença, Escorca and Bunyola.

Three coprolites, as well as leaves samples, were used in the microhistological preparations, following the technique of Stewart (1967). The procedure includes water washing of the material, grounding in a mortar, digestion in HNO_3, water dilution, filtering at 1.0 and 0.25 mm of pore diameter, and mounting glass microscope slides. The slides were examined under a microscope at x 400 magnification. A grid of 0.2 mm square was used to count the number of stomata per unit area.

Buxus balearica is an ideal taxon for this kind of analysis as the remains of its thick cuticle are prone to be well preserved in the coprolites. Its epidermis is composed of rounded-polygonal shape cells, with thick walls. There are no trichomes in the epidermis. The stomata are only present in the abaxial face. They are circular, bigger than other epidermal cells, with two refractile C shape guard cells. These features allow their identification in the preparations. In this work, we assume that *Buxus balearica* was the only species of *Buxus* genus that *Myotragus balearicus* consumed, although there is another *Buxus* species (*B. sempervirens* L) in the Mediterranean area, with the same epidermal traits, its presence in the island has been never documented.

Several models were adjusted in data analysis. Adjust levels were submitted to verisimilitude χ^2 test (Likelihood Ratio Chi-Square). Plant or coprolite effect was firstly checked, including the sample as the only factor. Then, a model was adjusted to check possible differences between sampling sites. Finally, the model was adjusted for the entire dataset with the origin of the sample (coprolite or plant) as the only factor. All analyzes were performed using the program JMP7.

III – Results and discussion

The effect of plant or coprolite origin was not significant in any case (P> 0.07 for all models LR χ^2 test), nor was the differences between sampling sites of *Buxus balearica* (χ^2 = 2.18, P = 0.34 LR χ^2 test).

The results showed a significant difference between stomatal density in epidermal fragments of *Myotragus balearicus* coprolites and epidermal fragments of extant plants (χ^2 = 34.46, p <0.0001, LR χ^2 test), with an average of 27.21 and 19.05 stomata/mm^2, respectively (Table 1).

Table 1. Stomatal density of different tissues of *Buxus balearica*

Origin	Stomatal density (stomata/mm^2)
Coprolites of *Myotragus*	27.21a
Sant Vicenç	17.91b
Escorca	21.09b
Bunyola	19.63b

Different letters indicate significant difference (p < 0,005).

The decrease in stomatal density since middle Holocene could be due to recent increasing of CO_2 in the atmosphere, as it is suggested by other studies conducted in experiments with controlled CO_2 concentrations (Woodward, 1987; Woodward and Bazzaz, 1987; Woodward and Kelly, 1995; Ramonell *et al*., 1997; Sánchez *et al*., 2010) or with herbarium material (Woodward, 1987; Peñuelas and Matamala 1990; Paoletti and Gellini, 1993). Consequences of this change in plant physiology seem to lead to more xeric adaptations. In this sense, studies such as Ramonell *et al*., (1997) affirmed that the positive effect on stomatal density allows that the plants should live in more dry climates. In addition, Cuni *et al*., (2010) suggested that the decrease of the density stomata restricts the quantity of water steam that the plants liberate to the atmosphere.

V – Conclusions

The stomatal density found in the epidermal fragments of *Buxus balearica* from middle Holocene coprolites of the extinct *Myotragus balearicus* is greater than that found in epidermal tissues of current *Buxus balearica.* The lower density of stomata in extant *Buxus* could be the result of a plant adaptation to the current increase in carbon dioxide in the atmosphere.

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