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Genome size variation in gymnosperms under different growth conditions

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Running title: Genome size in wild and cultivated gymnosperms.

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Abstract

Genome is structurally and functionally influenced by ecological factors during adaptive processes. Several natural factors can cause this, and here we present the effects of different growth conditions on the genome size. Genome size assessments were carried out by flow cytometry for a set of 19 taxa considering for each three plant conditions: i) wild plants growing in their natural habitats used as control group; ii) potted plants, and, iii) bonsai plants. Our results show a large variation in the genome size of bonsai and potted plants in respect of their wild representatives. The most important conclusion is that 1C*x* values measured in potted plants can be up to 8.48% inferior and in bonsai plants up to 26.83% higher than the values assessed for the respective wild individuals. In the case of *Juniperus thurifera*, this divergence largely exceeded the genome size variation previously estimated along the natural geographical range of the species. Such deviation from expected values could be interpreted as a genuine genome size variation or either resulting from biochemical or/and DNA compactness changes triggered by growth conditions. In any case, the present results evidence plant response to human-induced environmental changes, thus making the current approach potentially interesting for the prediction of climate change influence on plants and for applied aspects.

Keywords: bonsais; genome size; gymnosperms; potted plants; wild plants.

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1. Introduction

Plants display an astonishing plasticity for which genomic processes are being revealed as largely implicated. Amongst them, transposable elements' activity is thought to be a major source of genetic innovation and a powerful agent of adaptive change, besides accounting for an important part of genome size variation across plants (Chénais *et al.* 2012, and references therein). Genome is structurally and functionally influenced by ecological factor, which dramatically orientate its dynamics during adaptive processes (Nevo 2001, 2004). Amongst those affecting genome size, the soil and altitude constraints are of special relevance (Temsch and Greilhuber 2001; Johnston *et al.* 2005; García-Fernández *et al.* 2012; Pustahija *et al.* 2013). In turn, moderate changes in climate conditions and phosphorus availability were found not correlated with genome size changes (Pellicer *et al.* 2010). Genome size studies centered in the effect of the environmental changes in wild plants are scarce (e.g. Bureš *et al.* 2004 in *Ceratonia siliqua* L.; Suda *et al.* 2007 in *Senecio* L.). In the present context of climatic change, there is a need to indentify to which factors the plant genome is responsive, and to decipher how and how fast changes in ecological conditions might affect genome dynamics.

Aiming to provide some elements of response to these questions, we propose here to use bonsais as model plants. Bonsais illustrate one of the extreme-most examples of organism plasticity in long-lived beings, and as such, they represent interesting models for studying adaptation processes during lifespan. The drastic morphological constrains applied on the plant are compensated by deep cares (careful nutrient supplying, optimal temperature and humidity, avoidance of ageing by frequent branch pruning; Juanjo Pardo, personal communication). Bonsais provide a system to study the effect of growth conditions as they can be compared with their corresponding

wild and potted plants. Wild plants are growing in their natural habitats or planted in the ground in gardens without any special treatments. The potted plants are morphologically constrained and may be submitted to nutrient and water limitation, reduced space, and exposition of roots to unfavourable temperatures. In this work, we evaluated the response of genome size to these different growing conditions. We compared the C-values assessed in bonsais, plants growing in the wild and plant growing in pot, for 19 gymnosperm taxa belonging to four families (Table 1). We chose gymnosperms to start this investigation since they constitute a phylogenetically very well characterised group (Chaw *et al.* 1997; Lu *et al.* 2014), they are less diverse than angiosperms at morphological, systematic, physiological and genomic levels (Zahn *et al.* 2005 and references therein), and they are relatively slow in reflecting adaptation at genomic level (Romo *et al.* 2013).

2. Materials and Methods

Genome size was assessed in the Centres Científics i Tecnològics, Universitat de Barcelona, following the protocol described in Romo *et al.* (2013). *Pisum sativum* L. 'Express long' and *Triticum aestivum* L. 'Chinese spring' (2C = 8.37 pg and 30.90 pg, respectively, Marie and Brown 1993) were used as internal standards in order to cover the range of DNA contents. Nuclear DNA amount was estimated in five individuals of wild populations of each species, whereas only one individual (two repeats, as in the other cases) of the bonsai and potted representatives was analyzed, because they do not constitute natural populations and, especially in bonsais, it is difficult to find more than one individual from each condition. In two species of *Juniperus* L., *J. phoenicea* L. and *J. sabina* L., different parts of the bonsai plants were measured in order to detect some differences in the genome size values, if any. We proceeded to statistical analyses with

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Stata 10.1 (Stata Corporation, Texas, USA) and R 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria), carrying out Friedman and two-way ANOVA tests in order to compare the genome size of the same species through the three states. Although the sample is small, we carried out the ANOVA test because of its robustness and of the fact that the residuals' distribution is almost normal. A non-parametrical test (Friedman) was also carried out to compare the results obtained from both tests.

3. Results and Discussion

The results obtained are summarized in Table 1 and Fig. 1. Half-peak coefficients of variation (HPCV) both of standards and target plants were always lower than 5%, indicating fair measurements. Complementarily to the focus of the present work, the first genome size estimate is provided here for *Podocarpus neriifolius* D. Don, according to the Kew gymnosperm C-values database (Murray *et al.* 2012).

Results of the ANOVA test show a significant difference in genome size between bonsai, wild and potted plants ($P = 0.02$, Fig. 1). No significant differences have been found in the Friedman test. We do not find genome size differences among all the studied species of Cupressaceae ($P = 0.417$ for the Friedman test and $P = 0.06$ in the ANOVA) neither among the Pinaceae ones ($P = 0.819$ and $P = 0.617$, in Friedman and ANOVA, respectively). The most striking result is the one obtained for the genus *Juniperus*. Significant differences among the three growth conditions have been found in both tests in the species of this genus ($P = 0.015$ and $P = 0.008$, Friedman and ANOVA, respectively). We also note the genome size increase in the bonsai state of *Taxus baccata* L. compared to the other two states (see Table 1 and Fig. 1). Although the variation was not always in the same direction and therefore we cannot establish a general pattern for all the studied species, the most important conclusion is that the

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changes in ecological conditions can modify the genome size producing either a decrease up to 8.48% (in potted plants) or an increase up to 26.83% in 1C*x* values (in bonsais) as compared with respective wild individuals. No differences between leaves located at different parts of plants have been found in the two species tested for this point, indicating that genome size is homogenous in the plant crown.

The case of *Juniperus thurifera* L. is of special interest for several reasons. This species exhibits a particularly clear trend of segregation of genome size values from the potted plant to wild and bonsai ones (Fig. 1). Furthermore, genome size has been previously assessed all around the natural distribution of this taxon, showing a remarkable homogeneity among populations, with 2C values ranging from 39.84 to 42.65 pg (Romo *et al.* 2013). The present data establish 2C = 37.67, 41.16, 45.99 pg for the potted, wild and bonsai plants, respectively. The potted-wild-bonsai system in this species displays a 1.22-fold range of genome size values (representing a 1C*x* increment of 22.09%) considerably higher than the 1.07-fold range (+7.05% 1C*x*) estimated along the natural distribution of the species, and therefore not directly attributable to natural intraspecific variability. The 1C*x*-value increase observed in bonsai *J. thurifera* might result from a relaxed selection on genome, as evoked for the shift to parasitism (Piednoël *et al.* 2012) or from epigenetic changes occurring especially in large genomes containing a high proportion of transposable elements (Wicker *et al.* 2001, 2005). Alternatively, such deviant 1C*x*-values could result from a response of bonsai and potted individuals to their grow conditions affecting aspects such as biochemical profile (e.g. cytosolic compounds, especially phenolics) or DNA packaging (e.g. degree of heterochromatin and chromatin compactness), which have been seen to interfere in the binding of DNA stain (Doležel *et al*. 2007 and references therein).

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Should the hypothesis of genuine genome size variation being verified, our results suggest that, besides their role as a filter for natural selection (Nevo 2011), changes in growth conditions could act upstream for sculpting the genome during lifespan, and that this effect would be enough important to be detected by flow cytometry. Genome size has been related with characters constrained by environmental conditions, such as cell size, generation time, ecological tolerances and reproductive traits (e.g. Weiss *et al.* 1975; Lewis 1980; Otto and Whitton 2000; Petrov 2001), and with stress tolerance and environmental adaptation (Boyko *et al.* 2010; Baack *et al.* 2005). Recent studies show that genome irregularities may be transmitted not only during vegetative propagation but also by sexual reproduction in *Vanilla* Mill. (Lepers-Andrzejewski *et al.* 2011).

4. Concluding remarks

Gymnosperms are plants showing usually a slow reactivity at genomic level despite their ability to colonize a wide array of unfavourable habitats (e.g. Romo *et al.* 2013). The fastness of apparent genome size changes observed in this study on bonsai and potted plants, might respond to the stronger modulation of human-induced changes in growth conditions, well over what is found in nature. *Juniperus thurifera* exhibits an especially highest reactivity, which makes its potted-wild-bonsai system particularly adequate for carrying out further studies. Considering the effect of growing conditions on potted plant genome, our findings recommend to take into account the possibility of a genome size alteration when using nursery-propagated individuals in restoration programs.

The extent to which other taxa -out of gymnosperms- show this trait awaits further investigations. Indeed, if this finding is confirmed for other organisms, it could

have implications for understanding harmful effects of environmental factors and their putative relationship with certain diseases in animals, including humans. In another field, it is known that some genome size changes can be caused by the activation and deactivation of transposable elements (Tenaillon *et al.* 2011); their role in the currently-described variation may be a good subject for deeper studies in future works.

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Figure caption

Fig. 1. Comparison of 1C-values in Mbp of pot-growing (green), wild (black), and bonsai (red) plants. Values for wild plants have been considered as zero in order to show the variation from them of those of potted and bonsai plants. **Cupressaceae-** *1: Cupressus sempervirens, 2: Cryptomeria japonica, 3: Juniperus communis, 4: J. oxycedrus, 5: J. phoenicea, 6: J. sabina, 7: J. thurifera, 8: Taxodium distichum,* **Pinaceae-** *9: Cedrus atlantica, 10: C. deodara, 11: Larix decidua, 12: Picea abies, 13: Picea glauca, 14: Pinus halepensis, 15: Pinus pinea 16:Pinus sylvestris, 17: Pinus uncinata,* **Podocarpaceae-** *18: Podocarpus neriifolius,* **Taxaceae-** *19: Taxus baccata.*