Relationships of stomata size and genome size in Neottiae

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Abstract – Genome size is sometimes considered as an adaptive trait especially to environmental factors like climate or latitude. Relationships between cell size and DNA content has been reported for many species including plants. Cell size is also influenced by DNA endoreplication, which increases cell DNA amount. In plants, stomata guard cells exhibit some features including the lack of endoreplication. Consequently, guard cells are the most suitable cell type in plants to analyze such relationships. European genera and species of Neottiae exhibited variation of their 2C genome size. Guard cells have been thus measured in different Neottiae plants species. In *Epipactis*, several stands distributed in a limited geographic area and along an altitudinal gradient have been sampled. A general and positive significant relationship is thus obtained between DNA content and guard cell size within Neottiae. Variation among plants was less significant within *Epipactis* species. Only in *E. palustris*, a significant relationship was reported between DNA content and elevation. Relationships between cell size and genome size has been validated in Neottiae but not a general link to environmental conditions like elevation.

INTRODUCTION

Plants exhibit a large range of variation for genome size including among related species. This variation has been shown to be linked with the duration required for DNA replication and cell division rhythm and also to cell size (Cavalier-Smith, 1978). These cellular traits could be related to adaptation for environmental conditions. Consequently, genome size could be an adaptive trait mostly through variable amount of repeated sequences. The objectives of the study is thus to check relationships between cell size and DNA amount and then to test adaptive potential of genome size variation (to a large range of elevation). The study is carried out in Neottiae species that show a range of variation for 2C DNA content. Stomata are suitable to investigate cell size (no DNA endoreplication).

MATERIALS AND METHODS

Leaf collection: plant species listed in Table 1 (average of 5 plants per stand).

Cell size: measurement of length, width and area of stomata (Figure 1-a) with light microscope using Leica application suite (> 50 stomata per plant on lower leaf epidermis).

Variation among plants, stands and species have been analysed by hierachical analysis of variance with R.

Genome size is available for most Neottiae species (Prat *et al*., 2014).

Table 1. Species collected for measuring stomatal size.

Species	Number of stands
Cephalanthera damasonium	2
Cephalanthera longifolia	3
Epipactis atrorubens	9
Epipactis helleborine	5
Epipactis microphylla	1
Epipactis palustris	7
Epipactis placentina	1
Neottia ovata	1

RESULTS

Stomatal size showed a significant variation for all investigated parameters among plants, stands and species (*p-value* < 0.01).

Stomatal width and area are positively correlated with genome size (Table 2, Figure

2); the most significant relationships is for stomatal area and genome size, especially within genus *Epipactis*. Stomatal length, width and area are highly significantly correlated (Table 2).

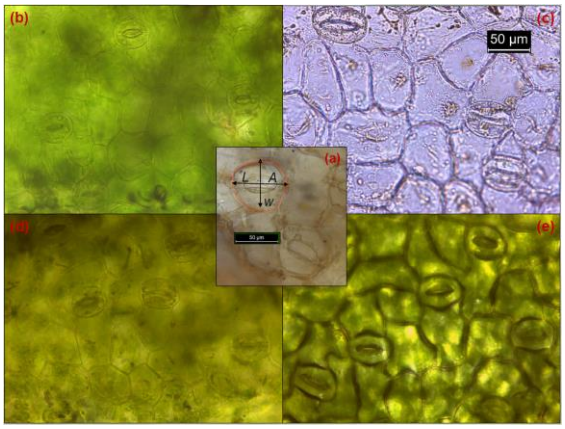

Figure 1. Lower epidermis of *Neottia nidus-avis* (a) with measured stomatal parameters (*L*: stomatal length; *w*: stomatal width; *A*: stomatal area), of *Cephalanthera longifolia* (b); of *Epipactis atrorubens* (c); of *E. helleborine* (d) and of *E. palustris* (e).

Stomatal size is not affected by elevation (Figure 3) in spite of a slightly negative relationships reported for *Epipactis* (r = -0.19 NS).

Table 2. Correlations in some Neottiae species between stomatal size and other traits.

	Stomatal length	Stomatal width	Stomatal area	Genome size
Stomatal width	0.66***			
Stomatal area	0.85***	0.95***		
Genome size	0.35	0.50**	0.51*	
Elevation	0.01	0.21	0.17	-0.01

Genome size and elevation are not related: the most positive relationship has been observed for *Epipactis palustris* (Figure 4).

CONCLUSION

Stomatal size varies according to plants, stands and species.

Genome size and stomatal size are significantly related in investigated species, particularly in *Epipactis* while *Cephalanthera* exhibited smaller stomata than expected according to their genome size.

Environmental factor, elevation in the present study, was not significantly related to stomatal size nor genome size. Only non significant trends have been observed.

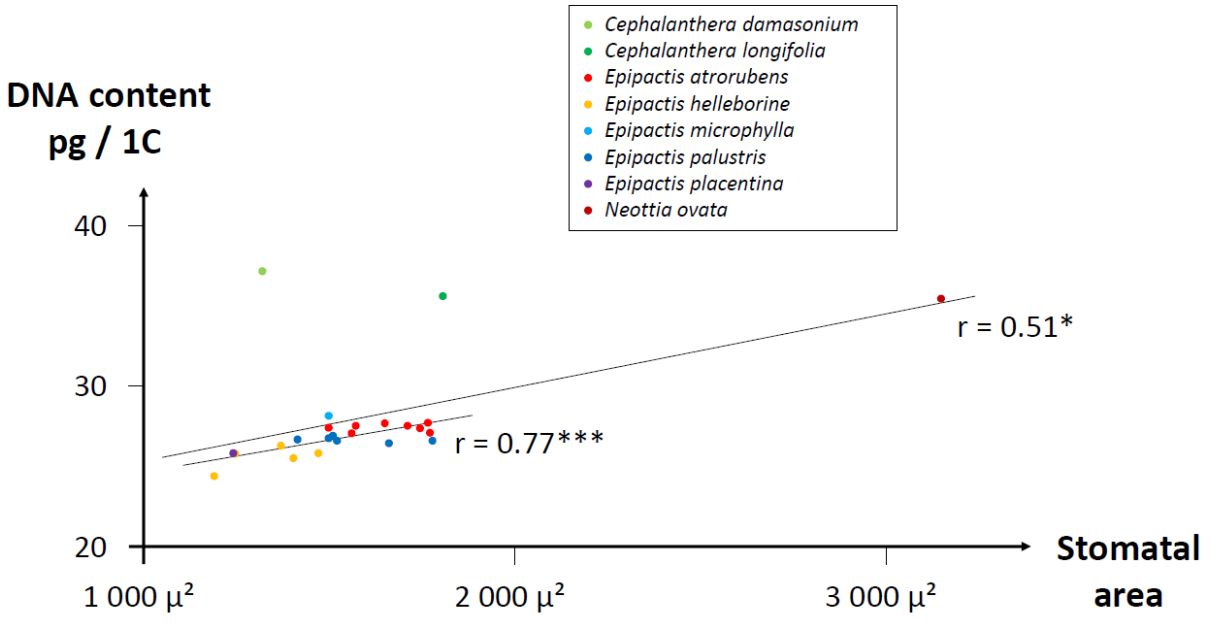

Figure 2. Relationship between stomatal area and genome size in Neottiae.

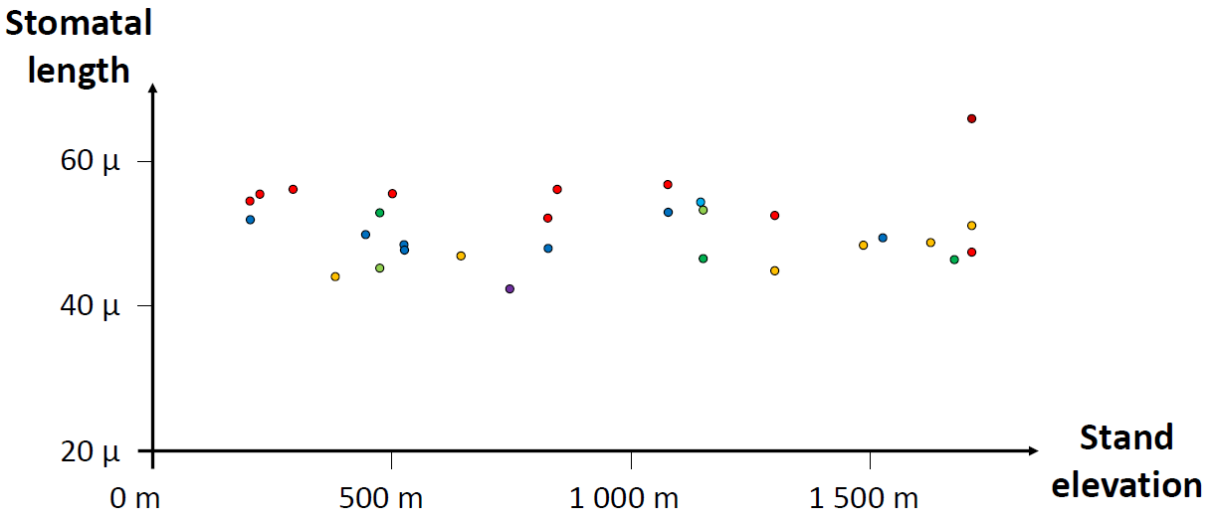

Figure 3. Relationship between stomatal length and stand elevation in Neottiae (see colour code in figure 2).

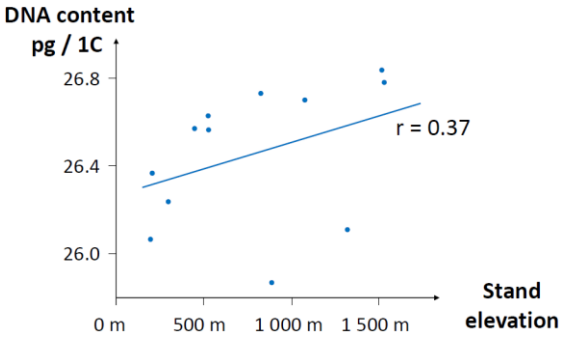

Figure 4. Relationship between genome size and elevation in *E. palustris.*

References

Cavalier-Smith T. 1978. Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. *J. Cell Sci*., 34: 247-278.

Prat D., Brown S.C, Gévaudan A. 2014. Evolution des Neottieae, apport de la cytométrie en flux. Actes 16e colloque de la Société Française d'Orchidophilie, Blois. *Cah. Soc. Fr. Orchid.*, 8: 125-133.