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Bohemian populations of *Orchis mascula* – where did they come from?

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Orchis mascula s. l. (Orchidaceae) is a group of several probably closely related subspecies (or microspecies). The geographic range of the group covers almost the whole Europe, but only some of the types are relatively widespread within particular regions. One of the subspecies named *Orchis mascula* ssp. *signifera* and the nominal subspecies *Orchis mascula* ssp. *mascula* occur also in the Central Europe. The delimitation between these two types is based on the morphology of the flower perianth segments (aristate-acuminate in ssp. *signifera* vs. obtuse, acute or shortly acuminate in ssp. *mascula)* and relative length of the middle lobe of the labellum (up to twice as long as the lateral lobes in ssp. *signifera* vs. not more than 1½ times longer in ssp. *mascula).* Czech specimens of *Orchis mascula* had been in general classified as ssp. *signifera* (although not typical) until discovery of a small population displaying many diagnostic characters of ssp. *mascula* in Krušné hory Mts on the western border of Bohemia in 2004. Many other populations occurring in the north-western part of Bohemia have been claimed as ssp. *mascula* since then although no detailed morphometric or molecular analysis has been made.

We examined Bohemian populations of *Orchis mascula* in order to check their assignment to either of the subspecies. Specimens from almost all North-Western Bohemian populations were collected together with typical ssp. *signifera* from Moravia and Austria and typical ssp. *mascula* from Western Europe (The Netherlands, Belgium, Spain, North France) The labellum and the left perianth segment of a flower in lower part of the inflorescence was taken from each plant for morphometric analyses avoiding any damage to the rest of the plant. We used an analysis of outlines based on elliptic Fourier functions to analyze variation in shape of perianth segments and sliding semi-landmark based thin plate spline method to analyze variation in shape of labellum.

Our results more or less confirmed the importance of the diagnostic characters as the major variation gradients generally corresponded to shape changes of the traits traditionally used for subspecies delimitation although the discrimination was rather poor in case of shape of the labellum and observed differences between the subspecies seemed to be almost completely blurred by

intra-population variability. The North-Western Bohemian populations were found to be morphologically intermediate between the typical specimens of the subspecies but slightly more proximate to ssp. *mascula*. Other two Bohemian populations from Krkonoše Mts (Giant Mts) and Orlické hory Mts, i. e. north-eastern part of Bohemia were clearly assigned to ssp. *signifera*. Our results indicate that the geographical extent of ssp. *mascula*-like populations in the Czech Republic is wider than was originally proposed by other authors reaching from Krušné hory Mts. to the Ještěd Massif near Liberec. The observed pattern in morphological variation could be a result of a post-glacial migration of *mascula* type from the west and *signifera* type from the east. These types could have met in the region of the Bohemian Massif hybridized and made a transitional zone within which more or less intense gene flow occurs. Possibility of the migration of rather Atlantic ssp. *mascula* to Western Bohemia is supported by a number of oceanic taxa that occur in this region and have apparently immigrated in it during the Holocene.

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Artikel/Article: Bohemian populations of Orchis mascula- where did they come from? 361-362