

The Polymorphism and Hybridization of the *Centaurea* Species

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Abstract. The *Centaurea* genus is one of the most complicated because it has great morphological diversity and, due to this fact, it has been little studied in scientific literature. Special attention was given to this genus when invasive species managed to conquer new territories, causing real damage in crops and pastures. However, there are few studies in Romania about its decorative and medicinal properties. This paper addresses the polymorphism problem based on herbarium samples and field observations. The results of these observations facilitate further mechanism to address this kind of research in any direction.

Keywords: transgressive species, hybrids, polyploidy level, morphological differences, involucre bracts

INTRODUCTION

The *Centaurea* genus is one of the largest genera of the family *Asteraceae* (containing between 200-700 spp.) which are barely addressed in the literature because of their taxonomic, nomenclature and species delimitation problems (Vanderhoeven *et al.*, 2002; Koutecký, 2007; Susanna and Garcia-Jacas, 2007, 2009; Vonica and Cantor, in press; Wagenitz and Hellwig, 1994). These difficulties arise from polyploidy and morphological changes which are very frequent in this group of plants.

Species of the genus *Centaurea* came to the attention of researchers partly because of their medicinal attributes but also their neophyte invasion due to their pastures and crops from the USA. Recent studies approached other issues (paleontological, genetically and morphometric) which clarified the systematic of the *Centauries* genus (Garcia-Jacas *et al.*, 2006; Koutecký, 2007). However, from the horticultural and medicinal point of view it was very little approached, therefore, we believe that the reason behind this paper is to help the knowledge of the horticultural aspects.

The spontaneous flora of Europe recognised 221 species of *Centaurea* and the Romanian flora only 54 taxa (Ciocârlan, 2009; Nyárády *et al.*, 1964; Tutin *et al.*, 2000). This difference is due to the imperfect taxa (hybrids) which were not recognised because they are considered specific to certain geographical areas (Koutecký, 2008).

Hybridisation is one of the most conflicting features of this genus. The hybrids between different species of *Centaurea* genus have been observed in all subgenera: *Centaurea*, *Lopholoma* and *Cyanus* (Fernandez-Casas and Susanna 1986; Garcia-Jacas and Susanna, 1992; Garcia-Jacas *et al.*, 1998, 2000, 2001, 2006; Haapanen, 1988; Koutecký, 2007; Mráz *et al.*, 2011; Ochsmann, 2001). In fact, these species are difficult to determine because they have a great capacity for hybridisation and they have many hybrids with species from the same section/subgenus or even with species from different sections/ subgenus.

Centaurea species pollination is highly dependent on the botanical characteristics, and in this case, the most species have an entomophilous pollination made by bees and other insects. Also, an important role for the pollination of these species has blooming period, geographical area and climatic conditions.

The identification of genetic polymorphism for *Centaurea* genotypes classification, the assessment of genetic diversity, selection of qualitative and quantitative characters and choice of parental species is very important in the hybridisation process for obtaining highly productive plants.

MATERIALS AND METHODS

The observations were made on plants that can be found in the Natural History Museum Herbarium from Sibiu, but also in the field. We checked 1360 herbarium samples (species and their hybrids) of *Centaurea* from Nyarady Herbarium. For the research observation measurements on the bracts involucre of the *Centaurea* inflorescence were made. In the field observations were made on the *Centaurea atropurpurea* Waldst. et Kit. populations from Cheile Turzii (CJ), Faget Forrest (CJ), Rosia de Secas (AB), *Centaurea scabiosa* L. populations from Zackel Hill (SB), Loamnes (SB) and species from *Centaurea phrygia* group from Ocna Sibiului (SB). The involucral bracts were taken from 15 specimens from each population.

RESULTS AND DISCUSSION

Based on the results of observations and determinations obtained from our experiment we can conclude that the hybrids with the highest morphological diversity belong to “groups”: *C. stoebe*, *C. jacea*, *C. phrygia*, *C. triumfetti*, *C. scabiosa*. The “group” name was used for these species and intermediate forms of hybrids, because they are very difficult to determine. *Centaurea scabiosa* group from *Acrocentron* section (*Lopholoma* subgenus) has many fertile hybrids with species from the same section or different sections. The problem lies in the difficulties in detecting hybrids within this section. The determination in the field is easier when the parental species have florets of different colours. Alternatively, when one of the parental species belongs to section *Chamaecyanus*, the hybrid origin is obvious from the achene characters. The fewest natural hybrids can be meeting to the *Centaurea atropurpurea* Waldst. et Kit. because the flowering period does not match with the flowering period of the other species.

From *Centaurea* subgenus, *C. stoebe* group has many morphological differentiations, probably as a result from either direct autopolyploidisation of the diploid species or allopolyploidisation. We found in the herbarium many hybrids with *C. stoebe* ssp. *stoebe* (*C. rhenana* Bor.) and *C. stoebe* ssp. *micranthos* (Gugler) Hayek (*C. bibersteinii*, *C. micranthos* Gmel.)

In knapweed, the floral capitulum contains approximately 40 central hermaphroditic disc flowers and they may or may not include a ring of sterile ray flowers. According to Koutecký, (2007), ray flowers are present in *C. jacea* group *sensu stricto* and absent in *C. nigra* group, whereas *C. thuillieri* group is polymorphic for this trait. Hybrids of these two species can be differentiated through the presence of this ring of sterile ray flowers. Another notable feature distinguishing diploids and tetraploids is the pappus of the achenes, which is more developed in diploids than tetraploids (Hardy and Vekemans, 2001). The hybrids usually are morphologically intermediate between the parental plants. The extent of

hybridization between particular plants depends largely on their ploidy levels. Plants of the same ploidy level can cross easily and their hybrids, and are often fertile and capable of backcrossing (formation of extensive hybrid swarms and introgression are possible). In opposite, plants differing in ploidy levels hybridise rarely and their hybrids are almost sterile.

In Europe knapweeds (*Centaurea jacea* L. *sensu lato*) constitute a very polymorphic polyploid complex with two ploidal levels, diploid (2x) and tetraploid (4x) (Gardou, 1972; Koutecký, 2007). From this group many hybrids were described as non-hybrid species. The latest, these being described as hybrids. The greatest number of hybrids was found in *Centaurea jacea* group. *Centaurea jacea* L. is an insect pollinated species, and self-pollination experiments have shown that it is self-incompatible (Hardy and Vekemans, 2001; Koutecký, 2007), Morphological characters are well defined only to the diploid species which have different geographical area. From Central Europe were found for *Jacea* subgenus, more than seven specific and subspecific taxa. Flora Europaea describes only three species from this subgenus, namely: *Centaurea phrygia* L., *C. stenolepis* A. Kern and *C. indurata* Janka (reference). The difference in number is given by the introgressive forms between two species that have morphological diversity. In the herbarium were found many hybrids with species from *Jacea* group such as: *C. jacea* L., *C. pannonica* (Heuff.) Simonk., *C. banatica* Roch (*C. rocheliana* (Heuff.) Dostal, *C. nigrescens* W. but also with species from *Phrygia* group (*Leptanthus* section).

To *Centaurea phrygia* group, has many species with a great morphological diversity of the involucre bracts and due to this fact chaos in determination results (Fig. 1).

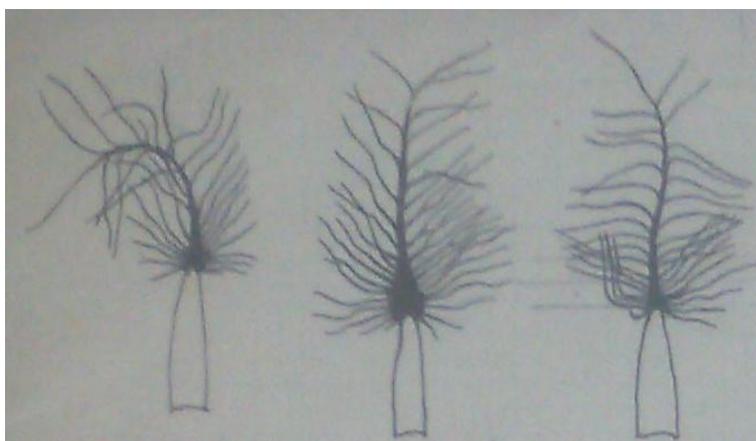


Fig.1. Morphological diversity of the involucre bracts to *Centaurea pseudophrygia*

In the herbarium can be observed many hybrids between these species which differ slightly from one another, forming a more or less continuous transition from one species to the other (Fig. 2), with each of the intermediate populations being one step. It is probable that this pattern originated from introgressive hybridisation. In the herbarium, there are many species from Romania described as non-hybrid species. Actually there are fewer species because Koutecký (2007) observed that there are two levels of ploidy (2x, 4x), separated only by geographical area. However, he also observed the existence of triploid populations which appeared due to introgressive hybridisation between *C. pseudophrygia* C.A.Mey and *C. stenolepis* A. Kern and between *C. oxylepis* (Wimm. & Grab.) Soo (4x) and *C. phrygia* L. (2x) (Koutecky, 2008).



Fig. 2. Hybridisation between diploid and tetraploid species
 a. *Centaurea phrygia* L (2x); b. *Centaurea X extranea* Beck.;
 c. *Centaurea nigrescens* Willd. (4x)

Previous studies of hybridisation within the *Jacea* section have demonstrated a strong reproductive barrier between diploids and tetraploids: hybridisation of plants sharing the same chromosome number is frequent and the hybrids are generally fertile, whereas hybridisation between the ploidy levels is limited and the hybrids are usually sterile.

It can be observed that the inflorescence of the *Centaurea* species do not contain only the seeds of the same species, because one inflorescence contains 20-60 flowers or more. The inflorescence is visited by insects all day long which bring pollen from different species, which is why the flowers of the same inflorescence can be pollinated in a different way.

For a proper understanding of the hybrids polymorphism and an overview, it has been observed that some genetic characteristics are transmitted from parental species, and these have a maximal degree of transmission (M) or a minimal degree ($m = 0$). The parental species were noted with "AM" and "BM", and the end of the series as "Am=0" and "Bm=0". So, as far as the transmission of the hereditary features reduces, "M" (maximum) is decreasing and for the other parental species, this feature "m" (minimum) increases. For a parental species, the genetic character "M" reduced in the same proportion with the increasing of "m" for the other parental species (Nyárády, 1962).

The intermediate steps of hybridisation (AxB) between species (A) and species (B) can be noted as A= AM/ Bm; AM-1/ Bm+1; AM-2/ Bm+2; AM-3/ Bm+3;...;AxB;...;BM-3/ Am+3; BM-2/ Am+2; BM-1/ Am+1; BM/ Am= B.

This equation was simplified by noting AM-1/ Bm+1= A₋₁B or BM-2/ Am+2= AB₋₂, reaching to form A; A₋₁B; A₋₂B; A₋₃B;...;AxB;...;AB₋₃; AB₋₂; AB₋₁; B (Fig. 3).

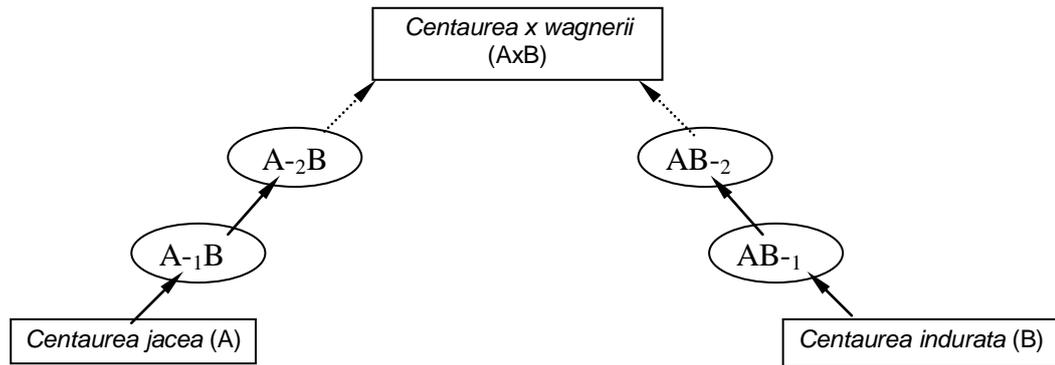


Fig. 3. Hybridisation between *C. jacea* and *C. indurata*

In addition, Nyarady (1962) clarified the problem of heredity from theoretical point of view. When the hybrid flowers (AxB) are pollinated with the pollen of one of the parental species (A) or (B), the plant will be growing from this seed and it will take the ($Ax<B$) formula (Fig. 4). When the plant was hybridised with the (B) pollen, the next generation will take the ($A>xB$) formula. Nyarady's notes help us to understanding the polymorphism of the introgressive species, but this does not help us very much. For understanding the hybridisation complex mechanism of *Centaurea* species, we need to describe other aspects regarding this mechanism.

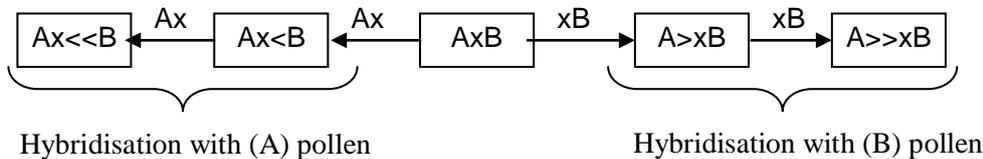


Fig. 4. Hybridisation of the (AxB) hybrid with (A) or (B) pollen

CONCLUSIONS

The large numbers of introgressive plants such as hybrid individuals from *Centaurea* genus are realities that appear in every year and multiply, creating chaos in species determination. Plant identification is hidden by the morphological variation into population and transgressive steps from hybrids. Major taxonomic problems are commonly encountered in this genus and these derived from:

- unclear morphological differences of the individual plants;
- unclear relations between morphological variation and chromosome number;
- geographical area misreporting.

Usually, none of these problems is regarded as a determination key, but they report only a small change in the primary diagnosis, creating a large number of unidentified plants.

The large number of morphological variations, which can found in some groups of *Centaurea*, hampers their recognition and their name because you cannot give to each discovered plant a scientific name. So, in describing these species, the genetic analysis, geographical distribution, the pollen morphology and the plants morphology (length of involucre bracts/ fimbria, the number of fimbria) are taken into account. All of these features

are often most important and discriminatory for *Centaurea* species determination. Due to lack of clear differences in qualitative characters, only a few features are used in species determination.

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