CHARACTERISTICS OF ASYMMETRIC *PRIMULA* HYBRID ZONES, NATURE CONSERVATIONAL AND PRACTICAL IMPORTANCE OF THE HYBRIDS

PhD theses

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1. INTRODUCTION AND OBJECTIVES

In plants, several examples can be found for areas of two closely related species overlapping, and a so-called *hybrid zone* developing between them – a zone containing hybrids in a small or large number besides the parent species – in which special adaptational and speciational processes are happening. With the newly developed molecular techniques, the genetic structure of hybrid zones can be discovered, and thus, more and more researchers undertake the task of clarifying and describing the ongoing processes. The models created can help with the understanding of microevolutionary processes taking place just in front of our eyes.

The number of known and described hybrid zones in the world is a couple of hundreds, but only a few of their structures are known – except for some taxa belonging to certain genuses (eg. *Helianthus, Iris, Eucalyptus, Geum, Ipomopsis, Picea, Pinus, Quercus, Populus*).

In a wider sense the geographic region might be called a hybrid zone in which – on the basis of the data in the literature - hybrids occur within the spreading area of the parent species. In this sense the hybrid zone spreads from Ireland to Hungary (Eastern-Bakony) over Scotland, England, France, Italy, Southern and Eastern Austria (Lüdi in Hegi 1926, Meusel et al. 1978). Significant hybrid occurrences may develop in habitats, whose environmental circumstances are favourable for this. These habitats containing hybrids in significant numbers – and usually of extreme rather than of intermediate nature – are the most suitable for studying the phenomenon of hybridisation. Therefore, *in a special sense*, these suitable habitats can be considered hybrid zones, i. e. the scenes of local speciation and adaptation processes.

Of the hybrids frequently occurring in plants, my research have been focused on such a taxon which, due to the small probability of joint occurrence of the parent species having different habitat preferences, is rare in nature, and its appearance in mass is a unique phenomenon. This taxon is the *Primula veris* x *P. vulgaris*, whose seed parent species is very heterogeneous in itself, several subspecies and varieties of it are listed in taxonomical literature, and sometimes these cannot be sharply differentiated from each other. One of my objectives was to collect and summarise the known pieces information on *Primula veris* x *P. vulgaris* hybrids and on hybrid zones, in relation to this.

In Hungary – *P. brevistyla DC., P. austriaca Wettst.* – hybrid forms (species) between *Primula veris* L. and *P. vulgaris* Huds. are only known in the extended region of the Bakony Hills. I examined the circumstances of the occurrences of *Primula* hybrids in the Bakony and Keszthelyi Hills, classified the occurrences, and determined the vegetation types in which the hybrids occur and in which their occurrences may be expected.

I examined the characteristics of such an aboundant occurrence in detail, the most significant hybrid occurrence known in Hungary with nearly a thousand hybrid individuals.

Already during my first visit of the study site I noticed that hybrid *Primulas* occur in large numbers within characteristic relief conditions in a 1,5-2 km long and about 50-60 m wide narrow, strip-like area on the Zörög Hill, therefore I first used the term *hybrid zone* for the Zörög Hill *Primula* population (Cservenka 2000). One of my first observations was that the habitat of the hybrids differs from its parent species, and their occurrences are strongly influenced by relief and light supply in relation to this. During my research done since 1997, the occurrences of hybrids in the Zörög Hill have been mapped, the characteristic conditions of occurrences have been determined from data of forest management plans (vegetation type, closure of foliage, soil type), and during predictive mapping the importance of relief variables (height above sea level, exposure (N-S, E-W), slope, surface-curvature) have been assessed.

During the evaluation of indication spectra of the coenological surveys carried out in different habitats of the parents species and the hybrids, I tried to reveal the characteristics of the hybrid zone which directly determine the spreading of the hybrids (species composition, cover and competition).

During studying the morphological and genetic structures of the populations, I got a picture of the direction of microevolutionary processes taking place in the *Primula* hybrid zone.

As the parent *Primula* species are well known medicinals, I tried to reveal whether – and how – the hybrid feature appears in the flavonoide composition of the flower.

I tried to find the answers for the following questions:

1. In what circumstances and vegetation types do *Primula* hybrids occur in Hungary?
2. Which environmental factors play a role in the development and maintenance of the *Primula* hybrid zone on the Zörög Hill?
3. How can the (nearly extreme) habitat of the hybrids occurring in the above zone be characterised from a phytocoenological aspect? Will the assessment performed with ecological indicator values show any difference between the habitat of the hybrids and that of the parent species?
4. On the basis of which morphological features can the hybrids be unambiguously differentiated?
5. What is the genetical pattern of the hybrids and the parents species? What is the relation between phenotypic variability and genetic pattern? Are the parent species uniform/stable? Can any sign of introgression be seen in their populations?
6. What is the flavonoid-spectrum of the parent species? Do hybrid features appear in the flavanoide composition?
7. On the basis of the observed features, which hybrid zone model can be best used for the explanation of the structure of the *Primula* hybrid zone? What conclusions can be drawn from this?
8. How can the *Primula* hybrids and the hybrid zone be evaluated on the basis of the results from the aspect of nature conservation?

2. MATERIALS AND METHODS

2.1. Examination of the occurrences of *Primula* hybrids in the Transdanubian Hills; the Zörög Hill

The cowslip (*Primula veris L.*) is a species of Eurasian, the primrose (*Primula vulgaris Huds.*) is of Atlantic-Mediterranian occurrence (Soó 1970). Areas of the parent species overlap in large areas in Europe (Meusel et al. 1978), but they hybridise in this zone only in some places. Hungarian occurrences are reported from the Keszthelyi Hills by Nagy and Dános (1979), Szabó (1987) Óvári and Bérces (1999), from the Northern Bakony and Lower Bakony by Polgár (1935), Rédl (1942), Nagy and Dános (1979), Mészáros and Simon (1999) and by Bauer (2001).

In order to compare them, during the spring of 2003 I visited and examined the habitat conditions of *Primula* hybrid occurrences published by Nagy and Dános (1979), and further occurrences having been found since then (Szabó 1987, Mészáros and Simon 1999, Bauer 2001, Bauer ex verb.).

On the basis of field observations, the hybrid occurrence on the Zörög Hill proved to be suitable for detailed morphological and genetical examinations due to a uniquely high number of hybrid individuals.

The botanical research of the study area – the Cuha-valley and the Zörög Hill – was started by Kitaibel, who reached the area of Csesznek in the years 1799 and 1802. He recorded floristical data from the Cuha-valley in 1799 (Gombocz 1945). Data about the Cuha-valley and some joint areas can also be found in *The Flora Work of Veszprém County* written by Pillitz (1908, 1910), but the first solid and sytematic botanical research was done by Polgár (1935). *The Flora Work of the Bakony Hills* was published in 1942 (by Rédl), in which detailed data can be found about the Cuha-valley.

The works of Fekete et al. (1961), Fekete and Komlódi (1962), Fekete (1963, 1964, 1988), moreover that of Fekete and Zólyomi (1966) significantly contributed to the presently known phytogeographic picture of the region. Research results in recent years (Bölöni and Király 1997, Bölöni et al. 1997, Bauer 2001, Galambos 2001) enriched the knowledge of the flora of the area primarily with floristical data.

2.2. Maps of local occurrences, determining the focal points of occurrences on the basis of forestry management plans

At the beginning of the research (1999), the occurrences and their sizes were surveyed on the Zörög Hill and a spot map of the occurrences of the seed parent *Primula veris* and the hybrids was prepared. This was used for predictive mapping. The mapping of *Primula vulgaris* did not seem reasonable because it occurs everywhere except in closed beech woods located on the northern and western sides of the Kis-Zörög side and its steep sourthern side, however more and less abundant occurrences can also be found in its stands.

For the determination of the focal points of the occurrences of the populations, first digital layers prepared with Arciew 3.1 GIS software from forestry management plan data (vegetation type, canopy closure, age of trees, soil types) were used.

The mapping of the populations was repeated in 2003, and a more detailed and precise point map was prepared by using a GARMIN eTrex Vista GPS istrument.

In the most abundant hybrid occurrence at the southern spur of the hill, in a one-hectar area the spatial positions of individuals of the *Primula* hybrid zone were determined with an even more precise new method using a Trimble GeoExplorer II professional GPS receiver. The first step of the measurement technique was to determine the EOV-coordinates (Unified National Projection System) of 8 such points from which all the *Primula* individuals occurring in the area could be seen. After this the area was divided into 8 sub-areas around the determined points.

From these starting points direction angles and distances were measured in the direction of each individual. I used a 0,5° precise Silva Eclipse Pro trough-compass for the measurement of the direction angle, and a ±2 mm precise Leica DISTO Classic[5] laser telemeter for the determination of the distance. Data of direction angle and distance were recorded in a minute, i.e. the taxon (*Primula vulgaris*, *P. veris* or hybrid) and its type (typical or introgressed in the case of *P. veris*; typical, vulgaris type, mixed in one, and mixed separately in the case of hybrids – see later) belonging to the given values were recorded. After field data collection, the above mentioned „raw" coordinates were adjusted with so-called differential correction with the help of the downloaded base data of the permanent GPS reference station of the Eötvös Lóránd University, Budapest. This way we determined the correct spatial postion of the points – at least in x and y direction – with decimeter accuracy.

Primula plants received individual coordinates (and further attributes important for the study) in such a way that copies of the starting points were made, and these were modified with the previously recorded offset values (direction angle and distance) by using Trimble GPS Pathfinder Office application.

2.3. Examination of the habitat preference of *Primula* hybrids on the Zörög Hill on the basis of relief

First the digital versions of the spot map about the occurrences of the species and a standard (Unified National Mapping System) EOTR topographic map of 1:10 000 scale covering the area of the Zörög Hill (11 km^2) were prepared. The further treatment and analysis of relief and occurrence data were done with GRASS 4.2 GIS software. A digital surface model of the area was prepared from digital contour-lines with block-krieger interpolation. The established database contained the digital surface model, the relief variables derived from it and the occurrences of *Primula veris* and the hybrids. Maps belonging to the database consist of cells representing a 10 x 10 m surface, in which the value of the presented variable can be found.

The full research area covers 11 km^2, so each map is described with a grid map containing 110.000 cells.Variables describing the relief were derived from the digital surface model. The basic algorithms necessary for the generation of variables were ensured by GRASS 4.2 software, but these algorithms were modified when calculating cardinal exposure and surface curvature (Aszalós 1997).

The treatment of relief and occurrence maps in a joint geographic information system database let us determine the exact relief conditions of the occurrences of the hybrids. With the so-called joint intersection procedures, the data from all the cells of two (or more) maps can be examined with a pairwise comparison of the cells belonging to the same point of the maps. The distributions of the occurrences of the species (histograms), as well as the averages, means, lowest and highest values and the standard deviation in the function of the five relief variables were generated with such procedures.

2.4. Study of the habitat preference of *Primula* hybrids on the basis of coenological examinations

For the examination of habitat preference of the taxa, coenological surveys were done in April and June 2001 on the western slope of the southern spur of the Zörög Hill. I made an attempt at determining the ecological factors playing a determinative role in the development and maintenance of the hybrids and the hybrid zone with the sampling of the three different forest types and through the analysis of relative ecological indicator values (Borhidi 1993, 1995) of the taxa of the samples. In each of the three types (on the top, on the upper section and at the botton of the slope) 5 coenological surveys were done with the Braun-Blanquet method and with the use of 20x20 m quadrats. Instead of recording AD values in the field, percental covers were estimated, and later these were recalculated.

Samples were evaluated individually by the relative ecological indicator values given by Borhidi (1993, 1995) and corresponding samples were drawn in order to recognise basic differences between the examined habitat types.

2.5. Examination of morphological variability

Three-year-long examination of Primula populations: On the southern spur of the Zörög Hill where individuals of all the three populations occur relatively closely to each other, *Primula vulgaris, P. veris* and hybrid individuals were marked with wooden sticks. *P. vulgaris* individuals were marked at the lower third of the western side near the Cuha-creek while hybrids were marked near the chine. Individuals of islandlike populations of *P. veris* were examined on the chine of the hill. Individuals of the marked *Primula vulgaris* populations could also be monitored without shortages during the three years. This cannot be said about the hybrids and the *P. veris*, which are much exposed to trampling by game. Therefore, it was not possible to monitor all the 50 individuals marked in 1998 in the next two years because of serious damage to the marker sticks, but independently from this, the state of the populations could be assessed.

Comparison of Zörög Hill Primula populations with each other, and with other populations of the parent species: the Zörög Hill hybrid *Primula* population with characteristic morphological features was compared to the populations of its parent species, and the latter were compared to „clean" populations (free of the other parent species) originating from „clean" habitats: *P. vulgaris* – Büdöskúti-valley, Keszthelyi Hills, *P. veris* – Veszprémfajsz. In this study the populations were represented with a four-five times higher number of individuals.

Leaf morphology and distribution of seed-size: the biggest leaf was collected in June from each individual of the sampled Zörög Hill populations. The Lenght and width of the leaf-blade and leaf angle were measured. For the examination of leaf epidermis supplements skinnings were prepared by hand. The preparates were covered with watery glycerine and evaluated immediately with Image Pro Plus 3.0 (*Media Cybernetics, USA*) software.

In the same period, capsules were collected from all the three populations. The number of seeds per capsule, the number of capsules per inflorescence and the average seed number per inflorescence were recorded. Seed size was determined with COLIM image analysis software.

2.6. Study of genetic variability

In the Genetic Institute of the Biological Centre of Szeged genetic examinations are carried out since 1998 with RAPD-PCR molecular technique (Williams et al. 1990). The DNA was extracted mainly from fresh young leaves (Doyle and Doyle (1990)) and in some cases from petals (Lin and Ritland (1995)). For the examination of the genetic variability of the parent and the hybrid taxa, 57 oligonucleotide primers (*Operon Alameda Technologies Inc., USA*) were tested, of which 29 were estimable.

Molecular genetic analyses of nearly 80 individual genotypes originating from the Zörög Hill populations of *Primula veris, P. vulgaris* and their hybrid were carried out with three primers (OPAA4, OPAA10 és OPF20) which proved to be the most suitable on the basis of preliminary examinations.

2.7. Study of chemical compounds (flavonoide composition)

The characteristic intermediate colour of the hybrids refers to a flavanoide composition different from that of the parent species. The size of the sample (1-2 flowers) collected from each plant did not allow individual evaluation, therefore samples of individuals having similar morphologic features (flower colour, corolla diameter, petal width, calyx tooth lenght) were combined.

A qualitative and quantitative assessment of flavon glycosides and phenolic acids was carried out with thin-layer chomatography (Wagner 1996). The evaluation could be performed on the basis of the rutin (Rf~0.4) standard.

3. RESULTS AND CONCLUSIONS

3.1. The circumstances of Transdanubian hybrid occurrences

In the case of Transdanubian occurrences, the *P. vulgaris* frequently occurs in cool, mesophilous beech forests and hornbeam-beech forests, but it also appears in sunny and disturbed habitats: in clearances, along paths. We can find the *P. veris* primarily in sunny places, in more opened turkey oak woods of southern exposure and in thermophilous oak forests. According to Soó (1970), the forest ecotype *P. veris subsp. inflata* is close to *P. vulgaris* regarding its habitat preference. I also found its isolated populations in less closed hornbeam-oak forests but have not seen any in beech woods.

The parent species meet in the Transdanubian Mountains within characteristic relief conditions. The rocky – dolomitic – surface, the relatively big height above sea level and the western exposure are determinative factors in the occurrence of *Primula* hybrids. In order to make sure of this assumption, I visited the sites of known hybrid occurrences in the area of the Bakony and the Keszthelyi Hills. In the Keszthelyi Hills and in the Eastern Bakony, islandlike populations of *P. veris* occur in the surroundings of *P. vulgaris*, while its opposite is characteristic for occurrences in the Southern and Lower Bakony. Similar occurrences but less significant in number were observed at two sites: in the Mina-valley located to the north of Nagyvázsony and on the Láz-tető in the Keszthelyi Hills. The relief and the base rock are similar to those of the Zörög Hill in these sites. In most of the cases the hybrids can be observed sporadically in small and isolated populations in places where the relief does not have a significant role.

The previously recorded and the observed occurrences, interestingly, fell onto margins of hills. My assumption is that hybridisation between *Primula veris* and *P. vulgaris* is predominantly limited to marginal areas of dolomitic regions (350-450 m above sea level, western exposure).

These sites are suitable for the development of intermediate habitat types, where the circumstances are the most favourable for hybridisation. Webb (1951) found in the case of hybrid occurrences in the Wicklow Mountains, situated to the south of Dublin, that the frequency of hybrids was higher than what was found in North England occurrences. Pugsley (1927) had similar observations, namely that frequency of hybrids was similar to that of the parent species in North Italian occurrences.

Life conditions of populations of *P. veris* occurring in atypical forest habitats are basically influenced by the available light, the amount of which is determined by canopy closure. Hybrid populations have better shade tolerance than *P. veris*, they also occur in more closed forests. The occurrence types of hybrids were classified according to habitats of *Primula veris*, where open, grassy and forest types rich in light even in the later part of the vegetational period were considered typical occurrences of *P. veris*. Occurrences of *P. veris* in more closed forests (reaching 70-100 % canopy closure during the vegetational period) were considered atypical.

According to my observations, hybrid occurrences mostly associated with the following phytocoenoses (Borhidi 2003), or their appearance may be expected in these within the overlapping areas of the parent species:

Dolomite detritus slope forest *(Primulo veris-Tilietum platyphyllae* (Isépy 1968, Borhidi 1996) – in the Bakony and Keszthelyi Hills

Pannonic mixed karstic forest (*Fago-Ornetum* Zólyomi 1950, 1958) – mainly in Keszthelyi Hills

Roof forests (*Veratro-nigrae-Fraxinetum orni* Kevey & Borhidi 2001) – mainly in the Eastern Bakony

Mountaneous (sub-pannonic) hornbeam-oak forests (*Carici pilosae-Carpinetum* Neuhäusl & Neuhäuslova-Novotná 1964, Borhidi 1996) – in the Bakony and Keszthelyi Hills

Pannonic turkey oak-sessile oak woods (*Quercetum petreaea-cerris* Soó 1963) – in Bakony

(*Asphodelo-Quercetum roboris* Borhidi & Járai-Komlódi 1959, Borhidi 1996) – in the Keszthelyi Hills, Lower Bakony

Pannonian white oak woods (*Vicio-sparsiflorae-Quercetum pubescentis* Zólyomi ex Borhidi et Kevey 1996) – Keszthelyi Hills

Pannonian karst white oak low woods (*Cotino-Quercetum pubestentis* Soó 1931, 1932) – in Keszthelyi Hills

Alder groves (*Aegopodio – Alnetum* V. Kárpáti, I. Kárpáti & Jurko 1961) – Bakonyalja

3.2. Dependence of hybrid occurrences on relief

The assumption of our research on predictive mapping performed in the years of 1999-2000 was that field occurrences of hybrids can be modelled with relief variables and its non-mapped occurrences – in the knowledge of relief conditions - can be predicted.

The hybrid populations live near breakdowns, at relatively big heights above sea-level in sparse oak forests along the chine. Therefore, according to our hypothesis, height above sea-level and surface curvature have strong predictive force in the determination of the occurrences of the taxon. Of the examined variables, height above sea-level and the east-west component of exposure turned to be the most important factors in the determination of the occurrence of the hybrid *Primula* population. Breakdowns, i.e. areas with significant curvature, are the occurrences of dry, open oakwoods, and also that of the seed parent species *P. veris*. Compared to this, the effect of height above sea-level is only secondary although its role is highlighted in this case because breakdowns can be found at a relatively big height above sea-level in the study site.

The importance of the east-west component of exposure may be more easily explained and understood: the focal points of the modelled occurrences – similarly to occurrences in the field – fell onto the western sides because of high solar irradiation (Cservenka et al. 2000, Aszalós 2003).

The *Primula veris* is the plant of dry oakwoods: for the survival of its populations it needs extra light getting to the foliage of open deciduous woods. Even if hybrids have higher shade tolerance than their mother species, their occurrences are still tied to the latter.

Parallel to the rate of canopy closure, mosaics of spatially structured populations of *P. veris* can be observed on the Zörög Hill. The demography of the patches changes according to canopy closure which proceeds according to the regeneration cycle of the forest. In my opinion, the *P. veris* settled onto the hill from eastern or southern direction due to clearances and was restricted to its chine and plateau edges as the forest grew up. It persists now in places where the amount of the available light is still enough for it, i.e. the forest could not becoma completely closed for some, in this case edafic, reason. If we compare the spot (patch) map of occurrences and the geological map of the area, it can be seen that the locations of occurrences closely match the marked occurrences of upper triassic dolomite formations appearing on the surface along the chine and plateau edges of the hill. If, besides relief factors, the occurrences of open or dry oakwoods and dolomite detritus slope forests had been present among the input factors of the applied predictive mapping (Cservenka et al. 2000, Aszalós 2003), the model would have provided appropriate prediction for the occurrence of the hybrid species even in a wider geographic region.

3.3. Habitat conditions and the characteristics of the hybrid zone

The experiences of our examinations performed at the habitat of the hybrids are basically similar to those of earlier observations of Clifford (1958), Mowat (1961) and Woodell (1965) according to whom it is necessary for the establishment of hybrid populations that one of the parents should move out from its typical habitat to a place which lets the hybrid seedlings grow up and bring flowers. This is only possible if the *Primula veris* settles in the forest in such a habitat where the coverage of the grass level is small for some reason. This is the case in almost every examined area, but the steep, rocky slopes near the plateau of the Zörög Hill having frequently been disturbed by game are typically such places. In the forest developed on the dry chine between the dolomite detritus slope forest and the thermophilous oak woods, the *P. veris* common in the nearest Turkey oakwood appears in large number and still finds favourable conditions regarding temperature and light. It also occurs in small number on the steep western slopes in the closed forest a few meters from the chine. Especially many *P. veris* individuals can be found under huge dolomite blocks facing west. These seem to serve as refugees to the *P. veris* – the forest cannot close near these blocks, the irradiation is higher and, therefore, favourable microclimatic conditions are ensured. The Atlantic-Mediterranian *Primula vulgaris*, which is very frequent in mesophilous forests of the Northern Bakony and in clearances along cuts, is less specialised here regarding its habitat preference. It is due to these factors that the two species meet with relatively high frequency on the upper third of the western slope of the Zörög-Hill thus providing the possibility for hybridisation.

The slow germination of hybrid seeds acts against the survival of seedlings (Woodell 1959), so the joint occurrence of the parent species is not enough in itself for either the establishment or the stable existence of hybrid populations. On the steep western slope of the Zörög Hill an almost nudumlike hornbeam-oakwood could be recognised; the focal point of the occurrences of hybrids can be found here. The intermediate feature of the relatively narrow (some ten meters wide) zone rich in hybrids is well represented by the light, humidity and temperature requirement spectra compared to those of the adjacent areas (Bauer and Cservenka 2002). Besides the assessment of ecological indicator values, we also tried to reveal other factors playing a role in the long-term survival of the hybrid population, from which a community structural factor, the low ground coverage of the grass level and therefore the resulting weak competition proved to be the most important (Cservenka and Bauer 2002). The habitat which is quite dry and sunny before shooting stays nudumlike in the later vegetational period due to the strongly closing canopy. Plants of the grass level occur thread by thread not causing competitive disadvantage to the *Primula* hybrids, which seem to have weaker adaptational ability in habitats typical of the parent species. Our observations also correspond to those of the earlier researches (Clifford 1958, Woodell 1965): namely, hybrids may be over-represented in hybrid zones by the virtue of living longer, but this can also be explained by the open possibility for backcrossing ensured by the

about three-week long overlapping flowering period of the parents and their better adaptation ability to the special circumstances. This is supported by the fact that there are areas within the zone in which only hybrids occur. This is shown by the highest relative frequencies of the *Primula* hybrids experienced on the western slopes of the Zörög-Hill and the significant relative negative correlation found between these values and the cover of the grass-level.

3.4. Phenotypic variability

Flower morphological characteristics of individuals of the parent and the hybrid populations were being examined on the Zörög Hill for three consecutive years, between 1998 and 2000. Lenght of scape, pedicels, tube, calyx, calyx tooth and corolla diameter were measured, the colour and throat pattern of flowers was evaluated. I found that individuals of the seed parent *P. veris* show high phenotypic variability.

Former botanists, both Polgár and Borbás reported the hybrids of both *P. veris* subspecies (*ssp. veris* and *ssp. inflata*) in the area. Morphological characteristics of the *P. veris* subspecies seem to be mixed in the area, they cannot be distinguished on a morphological basis. The leaves of *P. veris* individuals are usually lanceolate, abruptly narrowing to petiole, the calyx is as long as the tube or a little longer (1,8-1,9 cm). The corolla diameter, petal width, calyx and pedicel length proved to be quite variable. The pollen donor *P. vulgaris* shows much smaller phenotypic variability.

Among the examined flower morphological characteristics, hybrids could be best distinguished from their parents (showing intermediate feature) on the basis of the colour and size of the corolla (corolla diameter and petal width), the length of calyx tooth, scape and pedicel on scape. They also differ from their parents in leaf-shape: their leaves are characteristically oval and gradually narrowing to petiole. Besides typical hybrid forms having scape, several other nothomorphs (treated as varieties in the taxonomical literature) being in a transition series between the two species could be observed within the zone (Cservenka 2001).

Hybrid individuals found in the hybrid zone are very variable: on the basis of the presence of scape and its length „typical", „*vulgaris* type" (basal flowers with intermediate flower characteristics – *P. x radiciflora?*) and „mixed" forms (*P. wiesbaurii?*) could be obviously distinguished. The latter appears in two different forms: flowers with similar flower characteristics appear in inflorescence (short scape, long pedicels) and on (basal) pedicels within one individual or on separate individuals very close to each other (Cservenka et al. 2002). The proportion of *vulgaris* type and mixed individuals in the hybrid population is approximately 2-3 %. Hybrids with nearly as long pedicels as the scape and with bigger, pale yellow colour flowers (*P. x flagellicaulis*?) and *P. veris* like (*P. x gaisbergensis*?) also occur.

It is very hard to give an exact morphological description about the hybrids due to intrapopulation heterogeneity. The phenotypic features in many cases do not convince us of the hybrid origin of certain individuals. This refers to individuals standing at the ends of the transition series close the the parent species, from which they differ slightly. According to the taxonomical literature the *Primula veris* L. x *P. vulgaris* Huds. = *x P. variabilis,* ***P. brevistyla,*** *P intermedia; Primula veris* <u>*ssp. inflata*</u> L. x *P. vulgaris* Huds. = *P. x austriaca* forms are closer to *P. veris*, they have scapes. I could not found unambiguos reference to „*vulgaris types*" (hybrids with intermediate flower characteristics but no scape) and to „*mixed types*" (having inflorescence and basal flowers at the same time) in those *P. veris* sub-species which occur in Hungary.

Regarding the nomenclature of *Primula* hybrids, the work of Wright Smith and Fletcher (1947) was found to be the most detailed. Their classification was used by Soó (1970) for the names of *Primula* hybrids. In the works of the above mentioned authors, in the description of *P. veris (and ssp. veris) x P. vulgaris* hybrids, some reference can be found to individuals more similar to *P. vulgaris* observed by Nagy (1978) and by myself (2000). The hybrid types described in Austro-Hungary, the x *P. radiciflora* and x *P. sanctae coronae* Lange et Mortensen, are evidently closer to *P. vulgaris*. Wright Smith and Fletcher (1947) note that these types differ from each other in the degree of toothing of calyx, but they do not mention the presence or

absence of scape. The x *P. flagellicaulis* is more or less intermediate between the two parents: it has a scape as long as the leaves, and the pedicels are as long as the scape or even longer.

On the other hand, the description of hybrid types listed under *Primula x austriaca* Wettst., the x *P. gaisbergensis* Pax (close to *P. veris subsp. canescens*; has no basal flowers; its flowers are small, semi-nodding and can be found on a scape longer than the leaves), the *x P. richteri* Pax (flowers can be found on the scape, the bottom of the leaves is greyish) and the *x P. wiesbaurii* Pax (the bottom of the leaf blade is greyish, the flowers are basal or they can be found on a short scape) refers to hybrid types closer to *P. veris*. The description of *x P. wiesbaurii* Pax matches one type of the „mixed" hybrid found by Cservenka (2001), but no reference was found to the other type in which inflorescence and basal flowers can be observed on the same plant (Cservenka and Mihalik 2001).

In the hybrid zone I recognised an individual which matches the description of *Primula vulgaris var. caulescens*. The difference between this plant and typical *P. vulgaris* individuals was only that besides the basal flowers, a short scape developed, on which three flowers could be seen on long pedicels. I also believe that this feature cannot appear in clean *P. vulgaris* populations (Miller Christy 1922); this might be a segregated feature. Regarding flower characteristics, this form completely looks like *P. vulgaris*, unlike mixed hybrids (*P. x wiesbaurii?*), which have intermediate flowers.

The fact that hybrid forms classified as belonging to both *P. veris* subspecies occur in the hybrid zone (relatively close to each other) also suggests that *Primula veris ssp. veris* and *ssp. inflata* subspecies described earlier in the area cannot be distinguished from each other (see Länger and Saukel 1993).

Because of doubts experienced during the differentiation of *Primula veris* subspecies and due to the sometimes quite different judgement of their spreading areas I used the general name *Primula x brevistyla* for the hybrids in my publications (though other forms also appear). While hybrid forms form a transitional series between the two parents, the hybrid species can also be treated as a *nothospecies* and hybrid forms as *nothomorphs* (Szabó T. ex verb.).

On the basis of the above, the transitional series can be described with the following hybrid forms:

Primula vulgaris → *P. x radiciflora* → *P. vulgaris var. caulescens* → *P. x wiesbaurii* (*P. x sanctae-coronae?*)

→ *P. x flagellicaulis* → *P. x brevistyla* → *P. x richterii* → *P. x gaisbergensis* → ***Primula veris*** (*P. x austriaca*)

Neither the examined populations of the parent species nor that of the hybrid could be distinguished clearly on the basis of the throat pattern that former researchers (Mowat 1961, Woodell 1965) found characteristic to the species.

The species significantly differ from each other both regarding the number of seeds developed in the capsules and seed size (Mihalik et al. 2001., Kálmán et al. 2003). Most seeds were found in the capsules of *P. veris* (38 in average), while somewhat less were developed in the capsules of *P. vulgaris* (32 in average). No difference was observed between number of capsules developed on hybrids and *P. veris* individuals; ratio of empty capsules was also similar.

Majority (84,8 %) of *Primula veris* seeds fell in the 0,5-1,0 mm^2 surface category, while seeds of *P. vulgaris* (55 %) and the hybrids (43,4 %) predominantly were classified in the next, 1,0-1,5 mm^2 category.

Standard deviation of the size of hybrid seeds is almost twice than that of the parent species. The ratio of seeds having bigger surface than 2,0 mm^2 in case of hybrids is about 10 %, while this value is less than 1 % in case of the parent species (0,9 and 0,6 %). Large hybrid seeds are usually round and flat, these are probably not viable (Valentine 1955).

The Zörög Hill populations of the parent species significantly differred in many characteristics from clean populations originating from other places of the Transdanubian Mountain Range.

In the case of *Primula vulgaris*, this might be caused by the different environmental circumstances (phenotypic plasticity), but – while hybrids frequently occur mixed with its seed parent *P. veris*, or in its nearest neighbourhood – in the case of the Zörög Hill population of *P. veris* this might be the result of introgression. This would explain the its high morphological variability and its significant differences form the clean population.

3.5. Genetic variability

In the Zörög Hill population of *Primula veris* the introgression assumed only on the basis of phenotypic variability was confirmed during genetical examinations (Cservenka and Mihalik 2003). The determination of genetic differences among individuals and between populations of natural *Primula veris* x *Primula vulgaris* hybrids and their parent populations was done with RAPD molecular markers. Of the preliminary tested 57 oligonucleotide primers, 29 proved to be suitable for further examinations. The ratio of polimorphic bands was high, of the gained 208 fragments 167 showed polimorphism. While the pollen donor *Primula vulgaris* was separated from the hybrids in 44 % of all cases, the *P. veris* was clustered together with the hybrids in most of the cases (87,5%). The above seem to suggest that the genetic similarity between hybrids and seed-parent *P. veris* is higher than between hybrids and the pollen donor species. *Vulgaris* type hybrids were separated from the other hybrids in the majority of cases and clustered together with *P. vulgaris*. These forms are probably backcrosses. The close relationship of the parent species is also proved by the fact that 186 fragments were gained with 13 oligonucleotide primers of which 74 appeared in both species (40%). This is indicated by the high average value (1,53) of Nei-Li indices (1979) reflecting genetic similarity.

For the examination of intra- and interpopulation genetic variability, the RAPD-PCR reaction was carried out with 3 primers which proved to be the most suitable on the basis of preliminary examinations (OPAA4, OPAA10 and OPF20). 42 evaluable fragments could be gained with the three primers of which 32 proved to be polimorphic (76,2%).

Though hybrids could be distinguished well and were also clearly separated from the parent species, they are genetically much closer to *P. veris* than to *P. vulgaris* (Cservenka et al. 2002). This is reflected by the small diversity values received between the hybrid and *P. veris* populations.

In the population of the pollen provider *Primula vulgaris*, much smaller pattern diversity values were gained than in the case of the other parent and the hybrid population. This may partly originate from the occurrence of the small number of *P. vulgaris* individuals in the hybrid zone and partly from the fact that only certain genotypes could adapt to the dry habitat less optimal for *P. vulgaris* and these take part in the maintenance of the hybrid zone.

That the values of genetic variability calculated for the *Primula veris* population are much higher than those of the *P. vulgaris* population may also be the sign of ongoing introgression.

On the basis of the results of my morphological and genetic studies, I can only partly agree with Clifford (1958), whose opinion is that hybridisation and introgression in the case of Primulas rather act upon the stabilisation of intermediate forms than upon the modification of the parents. The pattern and genetic diversity of hybrids originating from the hybrid zone proved to be higher than those of hybrids originating from small populations.

3.6. Flavonoide composition

The characteristic intermediate flower colour of hybrids refers to a flavonoide composition different from the parent species. With thin layer chromatography we could detect all the components characteristic to the parent species, but hybrids contain significantly more hyperoside (20-40%) than the parent species (Cservenka and Sztefanov 2002). Regarding the amount of flavonoides and their composition, hybrids are closer to *P. vulgaris*, and this also appears in the colour of their flower.

3.7. Evaluation of interspecific hybridisation between *Primula veris* and *P. vulgaris* and of the hybrid zone

The effect of hybridisation on evolution has central importance from the point of view of the speciation of species. The limitations of parent species can be well studied through their plant hybrids. In hybrid zones the frequency of introgression and its role in adaptation can be estimated. It is characteristic for all hybrid *Primula* occurrences that in their immediate vicinity one of the parents is present in a smaller number; therefore, the chance for its fertilisation by the other species is obviously higher. The asymmetric feature of the zone does not only arise from the asymmetric spatial arrangement of the parent species but also from the fact that hybrids result from non-reciprocal hybridisation as it was proved in the 1950's (Valentine 1955, Woodell and Valentine 1961-62). In the vicinity of hybrid abundances the *P. veris* is prevailingly dominant. Within the studied hybrid zone the ratio of the parent species and their hybrids is 1:3:10-12 (*vulgaris*:hybrids:*veris*). Contrary to this, in the wider surroundings of the hybrid zone the pollen provider *P. vulgaris* is very frequent. However, its occurrence in the zone is very low compared to the other parent species, which suggests *genetic disequilibria* in the *Primula* hybrid zone. Regarding habitat requirements, hybrids are also closer to *P. veris*. Similar hybridisation phenomena have been observed in the case of *Geum* (Taylor 1997) although the hybrid zone in this case seems symmetric due to reciprocal crossing.

The Evolutionary Novelty Concept (Arnold 1997), which combines the characteristics of bounded hybrid superiority, the tension zone, and the mosaic models finely describes the processes which take place in the *Primula* hybrid zone.

The appearance of hybrids at a certain height (clinality), the heterogeneous, mosaic genetic and spatial structure (probably as the result of exogeneous selection), the high values of genetic variability experienced, the ongoing introgression and the stable presence of backcrossed individuals (probably due to endogeneous selection) all support the fact that there are local adaptational processes occurring in this habitat. The final result, the „product" is not known yet, as for the development of evolutionarily new, stable genotypes a much longer time is needed. However, it is for sure that hybrid genotypes have higher fitness in extreme, intermediate habitats. *Primula* hybrids also support Rieseberg's (1997) observation, according to which homoploid hybrid species are outcrossing without exception, are characteristically perennials, and their habitat is of extreme rather than intermediate nature between those of the parents.

Nagy and Dános (1979) already reported significant hybrid abundance at the site at the end of the 70's and the presence of hybrids here (and in the region) was known even much earlier. The large number of hybrids and the high genetic variability observed in the zone suggest that a quite stable (or growing) hybrid population lives on the Zörög Hill, theoretically providing a chance for the development of stable new genotypes well-adapted to extreme conditions.

The fact that we can almost always find hybrids together with the parents, and that when hybrids occur in habitats similar to that of the parents they never appear in large number prove their weaker adaptation ability in these habitats.

In the case of larger hybrid occurrences, some kind of transitionality can always be observed. One thing is important: in these places both parent species get into a suboptimal situation, they „open up", which probably roots in smaller genetic variability (only certain genotypes tolerate the suboptimal situation, the allele frequency probably changes in such circumstances). *Hybridisation* is – as a matter of fact – *a „marginal phenomenon"*; a phenomenon which can be mostly realised in spatial (topographic) sense at the margins of hills, at a smaller scale at the margins of local spreadings, and in an even smaller scale in transitional habitats, as in ecotones (Zólyomi 1987). It is a marginal phenomenon in a genetic sense as well, one, which can be realised in differing genotype frequencies due to suboptimal habitat conditions. „Clean" populations of the parent species and their populations living in the hybrid zone differ significantly from each other in many morphological characters. This difference is probably not only morphological but also appears in the genetic structure.

Populations of the parents species living in suboptimal circumstances at the margin of local occurrences probably have more plastic genotypes which are more susceptible to introgressive hybridisation. It is especially true in the case of small *P. veris* populations occurring in the vicinity of hybrid occurrences, which genom seems to be "diluted" due to introgression. However, the habitat suboptimal for the parents ensures optimal conditions for the hybrids. The high humber of hybrid individuals and the high genetic variability experienced in the hybrid population suggest that a stable hybrid population lives on the Zörög Hill, whose conditions theorethically allow for the development of new, stable genotypes well-adapted to special habitats.

3.8. Thoughts about the conservation of *Primula* hybrids

With the application of quantative, geographic and ecological criteria of rarity, Rabinowitz et al. (1986) classified 160 plant species of the British Isles. This classification can be excellently used for selecting priorities for nature conservation. If I had to try to place the *Primula* hybrids in this system, I should say that their local population size is small – not concerning a few exceptions –, their geographic spread is rather wide – though this is relative: it depends on whether the seed parent *P. veris* is treated at species or subspecies level –, and that the hybrids are unambiguously specialists regading habitat requirements. This is supported by the results of habitat indicational studies (Cservenka and Bauer 2002, Bauer and Cservenka 2002) and predictive mapping (Cservenka et al. 2000, Aszalós 2003). If I consider the geographic spread of *Primula* hybrid zones, spread then can be considered narrow. Habitats of specialists with a narrow spreading area require directed management in some cases – or at least more attention –, even if their large populations also exist (Standovár and Primack 2001). From the hybridisation of homoploid species of other genuses partly fertile F1 hybrids and backcrossed generations may also be formed, but this is a rare natural phenomenon in their case, too (*Aster* (Avers 1953), *Vaccinium* (Ritchie 1955), *Gossypium* (Hutchinson et al. 1947)).

It seems generally true for the hybrids that they usually occur in small populations; the size of these shows a declining tendency, they are specialists and colonise badly. Individuals living in the hybrid zone have adapted to the extreme habitats free of human disturbance, they can produce seeds only after several years and have only a few viable offsprings due to their hybrid origin; therefore they can be considered K-strategists. These reasons are supposedly enough for supporting their protection.

Negative or positive effects of introgressive hybridisation can only be judged cautiously. That it is not an exclusively negative process is proved by the concept of the evolutionary novelty hybrid zone model. As it was mentioned earlier, during hybridisation the population of the species which is present in a small number is exposed to introgression while in the case of closely related species, the probability of interspecific fertilisation is much higher.

In the wider neighbourhood of the occurrences of protected or strictly protected species, it is usually the (more) common parent species that is present in a higher number, so the effect is negative, indeed. If only one of the parents is protected or endangered and the other is common, the judgement will be more difficult, and genetic (and conservational) problems caused by „bad hybrids" will occur.

Nevertheless, the situation is different in the case of *P. veris x P. vulgaris* hybrids, in which the common parent species is present in a smaller number in the wider area of occurrence and the pollen provider is a protected species. Inclusion of small populations of *P. veris* is characteristic in the surroundings of *P. vulgaris*, the life conditions of which are determined primarily by the available light. At this time, this effect is positive; therefore, these hybrids can be considered „good" ones. Due to the above mentioned spatial and genetic asymmetry the occurrence of hybrids is more tightly bounded to the seed parent, therefore, the survival of isolated populations of the non-protected *P. veris*, which is becoming endangered due to canopy closure, is a matter of life and death form the aspect of the hybrids.

The *Primula vulgaris* is protected in vain (its theorethical value is HUF 2000) given that the value of the hybrid is the same although it should be much higher due to its rarity and scientific importance. One should consider that in the maintenance and survival of hybrid zones containing legally protected hybrid individuals in a big number and also of small populations, the non-protected seed parent species *Primula veris* has a most stressed role.

Similarly to the *Primula vulgaris* it seems to be true for the *P. veris* that it consists of non-equilibrium metapopulations, so the extinction-colonisation dynamics of its patchy populations is not stable, which may be an explanation for its decline in some regions of Western Europe (Grime 1978, Harrison 1991, Endels 2002). In the case of the Zörög Hill populations of *P. veris*, the special relief induces the „rescue effect", which locally prevents the complete closure of canopy on the chine and on plateau edges. Proper forest management is primarily important in the maintenance of the patch dynamics of protected or endangered species requiring half-shade or light. In turkey oak type, gradually closing forests the size and isolation of colonisable patches significantly influence the persistence of a species in a habitat due to the change of probability of successful colonisation and reduced success of reproduction of small populations (Kery et al. 2000). Valverde and Sivertown in their metapopulation model developed for *Primula vulgaris* (1997) calculated that if the size of the population falls under 5 individuals, the probablity of extinction will be very high. In the case of Transdanubian hybrid occurrences, the number of *P. veris* individuals is not far from this number. The decline of *Primula veris* is related to canopy closure; therefore, planned thinnings have to be carried out in time before the canopy would completely close. The recovery phase has to be prolonged in order to continuously ensure favourable conditions. Favourable conditions can be promoted with leaving an appropriate number of trees and tree-groups in the area.

Nagy and Dános (1979) looked into the future with anxiety even twenty years ago, hoping that the hybrid populations living on the Zörög Hill would survive until the declaration of protection for the Higher Bakony in the 1990's. At that time it seemed that the Cuha-valley would also gain protection (Kopasz 1976). The Higher Bakony Protected Landscape Area was established in 1991 but the area in question did not become protected. The expansion of protection would also be worth today while hybrids still occur in high numbers and the area is unique even at a European level. It is a step forward that the area is enrolled among the Natura 2000 sites although not because of the hybrids.

Through the examination of the spatial arrangement of genotypes and their relationship with ecological factors we may have an insight into micro-evolutionary processes responsible for maintaining hybrid zones. With my studies I wished to contribute to the knowledge of hybrid zones and to call the attention to the necessity of their protection.

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