The distribution of cytotypes of *Vicia cracca* in Central Europe: the changes that have occurred over the last four decades

Rozšíření cytotypů *Vicia cracca* ve střední Evropě: co se změnilo během posledních 40 let

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The formation and maintenance of polyploids (via the development of various reproductive barriers) rank among the central questions of studies on polyploid evolution. However, the long time scale of most evolutionary processes makes the study of the dynamics of diploid-polyploid groups difficult. A suitable candidate for a targeted comparative study is *Vicia cracca* (*Fabaceae*), which in the late 1960s was subjected to a detailed cytotype screening in Central Europe. Re-sampling the original localities offers a unique opportunity to assess changes in the ploidy structure of the populations, which should reflect the cumulative effect of all the evolutionary forces acting on the plants. Using flow cytometry, the DNA ploidy levels of more than 6,500 individuals of *V. cracca* collected at 257 localities in Austria, the Czech Republic, Germany and the Slovak Republic were estimated. Three different cytotypes (2x, 3x and 4x) were detected. While tetraploids predominated in the western part of the area investigated (179 populations), the diploids had a more easterly distribution (62 populations). There is a secondary zone of cytotype contact near the boundary between the Czech and Slovak Republics. Sixteen populations (~6%) consisted of a mixture of 2x and 4x cytotypes. Triploids are very rare; only seven individuals were found in two otherwise diploid populations, indicating the existence of breeding barriers between diploids and tetraploids. The distribution of cytotypes is similar to that determined four decades ago using chromosome counts. Nevertheless, there are some discrepancies, namely the current absence of: (i) the diploid cytotype in southern Bohemia and (ii) the altitudinal segregation in the distribution of cytotypes, including two formerly recognized chromosomal races of diploids, perhaps a result of more representative sampling. Identical monoploid genome sizes (1Cx-values) of both the majority ploidy levels support an autopolyploid origin of the tetraploids.

K e y w o r d s: Central Europe, contact zone, cytotype distribution, flow cytometry, genome size, mixed-ploidy populations, ploidy, *Vicia*

Introduction

Polyploidy, i.e. the presence of more than two complete chromosome sets in a nucleus, is widely acknowledged as one of the key factors in the diversification of plants (Soltis et al. 2003). In fact, it is estimated that at least 70% of flowering plants underwent one or more rounds of genome duplication in their evolutionary history (Masterson 1994, Soltis 2005). Evolutionary processes governing the formation and maintenance of polyploids under natural conditions are therefore the focus of many current research projects (e.g., Felber-Girard et al. 1996, Ramsey & Schemske 1998, 2002, Husband 2000, 2004, Husband & Schemske 2000, Otto & Whitton 2000, Husband & Sabara 2004, Baack 2005,

Kennedy et al. 2006). Basically, the success of newly formed polyploids depends on their ability to avoid the so-called minority cytotype exclusion (Levin 1975), which results when various prezygotic and/or postzygotic breeding barriers between the polyploids and their diploid progenitors develop, such as cytotype sorting along ecological gradients (Lumaret et al. 1987, Felber-Girard et al. 1996, Husband & Schemske 1998, Johnson et al. 2003, Baack & Stanton 2005, Hülber et al. 2009), divergence in flowering time (van Dijk et al. 1992, Petit et al. 1997, Bretagnolle & Thompson 2001, Nuismer & Cunningham 2005), gametic selection (Husband et al. 2002, Kennedy et al. 2006), or selection against inter-ploidy hybrids (van Dijk et al. 1992, Hardy et al. 2000, 2001). The evolution of assortative mating in relation to behavioural mechanisms could also be an important component of the successful establishment and/or coexistence of animal-pollinated diploid-polyploid plant species (Thompson et al. 2004). Among the isolating mechanisms, the spatial segregation of different cytotypes is perhaps the most thoroughly explored both because it is easy to do using flow cytometric (FCM) techniques and it enables a wide range of challenging questions to be addressed (Petit et al. 1999, Kron et al. 2007, Suda et al. 2007a, b). In fact, knowledge of the variation in ploidy levels and their geographic distribution is one of the prerequisites for subsequent ecological and evolutionary research on polyploids (e.g. to explore questions of niche separation, phylogenetic history, etc.).

One of the intensively studied diploid-polyploid plant groups is *Vicia cracca* (*Fabaceae*). This taxon belongs to *Vicia* subg. *Cracca* (Dumort.) Peterm., which includes approximately three-quarters of the species in this genus. According to Kupicha (1976) and Leht (2005), this subgenus can be divided into 17 sections, the most speciose and most variable of which is the section *Cracca* Dumort. The aggregate species *Vicia cracca* s.l. contains several related and morphologically similar species with partially overlapping geographic ranges, including *Vicia cracca* L. s. str., *V. oreophila* Žertová, *V. incana* Gouan, *V. dalmatica* A. Kern, and *V. tenuifolia* Roth. Despite some phenotypic differentiation (e.g., in the number of leaflets, length of the inflorescence in relation to the subtending bract and the banner/claw ratio of the standard), morphological characters are of limited use for species recognition. More reliable separation of the taxa is provided by karyological markers. Whereas *V. cracca* and *V. oreophila* share the base chromosome number x = 7, in other species x = 6 (Hanelt & Mettin 1989).

Vicia cracca s. str. is widespread in the Northern Hemisphere. It is a shortly-rhizomatous, tendrillous, polycarpic perennial herb, which typically occurs in meadows, along roads and river banks, and at forest margins on rather fertile, moist soils. Sexual reproduction clearly prevails; the vegetative spread via below-ground runners is limited. The mating system has not yet been determined with a certainty (Zhang & Mosjidis 1998, Jaaska 2005). The species is supposed to be predominantly allogamous (Hanelt & Mettin 1989), although some experiments suggest that selfing is also possible (Rousi 1973). There are three different ploidy levels in *Vicia cracca*: diploid (2n = 2x = 14), triploid (2n = 3x = 21) and tetraploid (2n = 4x = 28); a few aneuploids are also reported (Rousi 1961, Chrtková-Žertová 1973a, Roti-Michelozzi 1984). There are a number of chromosome counts for Europe (Table 1, Fig. 1) that provide a representative picture of ploidy variation on a coarse scale. On the other hand, chromosome counts for countries outside Europe are rather scarce. The closely related *V. oreophila* is a tetraploid (2n = 4x = 28) and occurs locally in Central European mountain systems (Žertová 1962, Chrtková 1995).

Table 1. – Summary of previously published data on ploidy levels (chromosome counts) in *Vicia cracca*. an = aneuploid.

Country	Ploidy level	References
Afghanistan	4x	Podlech & Dieterle 1969
Austria	2x, 4x	Rousi 1961, 1962, Chrtková-Žertová 1973a
Belorussia	4x	Semerenko 1989
Bulgaria	4x	Kuzmanov 1975
Canada	4x	Ledingham 1957, Mulligan 1961, Rousi 1961, Tomkins & Grant 1978
Czech Republic	2x, 3x, 4x, an	Chrtková-Žertová 1973a, Dvořák et al. 1977
Denmark	4x, an	Rousi 1961, Chrtková-Žertová 1973b
Finland	4x	Rousi 1961, Chrtková-Žertová 1973b, Arohonka 1982
France	2x, 4x	Rousi 1961
Germany	4x, an	Rousi 1961, Chrtková-Žertová 1973b
Hungary	2x, 4x	Baksay 1954, Rousi 1961, Chrtková-Žertová 1973a
Iceland	4x	Rousi 1961
Italy	2x, 3x, 4x, an	Gadella & Kliphuis 1970, Roti-Michelozzi 1984, 1992, Roti-Michelozzi & Allione 1987
Japan	2x	Huziwara & Kondo 1963
Mongolia	2x	Měsíček & Soják 1969, 1995
Netherlands	4x	Rousi 1961, Gadella & Kliphuis 1963, Hommel & Weiffering 1979
New Zealand	4x	Rousi 1961
Norway	4x	Chrtková-Žertová 1973b
Poland	2x, 4x	Ryka 1954, Rousi 1961, Chrtková-Žertová 1973a
Russia (Asian part)	2x, 3x, 4x	Rousi 1961, Belyaeva & Siplivinskij 1975, 1977, 1981, Krasnoborov et al. 1980, Nikiforova 1984, 1990, Sokolovskaya et al. 1989, Krasnikov & Schaulo 1990, Volkova et al. 1999, Zhukova et al. 1973
Russia (European part)	4x	Efimov 1987, 1988
Slovakia	2x, 4x	Činčura 1963, 1981, Chrtková-Žertová 1973a, Dvořák et al. 1977, Hallonová 1982
Sweden	4x	Rousi 1961, Chrtková-Žertová 1973b, Lökvist & Hultgĺrd 1999
Switzerland	4x	Rousi 1961
Turkey	2x	Şahin & Babaç 1990, İnceer & Hayirliođlu 2005
United Kingdom	4x	Rousi 1961

In the present study, the current distribution of cytotypes of *V. cracca* in Central Europe was determined and compared with the disribution recorded about 40 years ago by Chrtková-Žertová (1973a) (in total, she examined 313 localities but the exact number of plants analyzed is unknown). Due to the lack of historical karyological data, similar comparative studies are rare, which makes temporal changes in cytotype distribution a largely unexplored phenomenon. Our aim was to locate ploidy-mixed populations, which are a prerequisite for subsequent detailed investigations of the evolutionary forces determining cytotype co-existence, assessing inter– and intra-cytotype interactions (competition, siring success), etc. Specifically, the following questions are addressed: (i) What is the current distribution of the different cytotypes of *V. cracca* at landscape and microgeographic scales? (ii) What changes in ploidy distribution/frequency have occurred over the last four decades? (iii) How frequent are populations heterogenous in terms of ploidy level and which cytotypes are involved? (iv) What is the frequency of individuals with intermediate ploidy levels and to what extent has inter-cytotype gene flow occured?

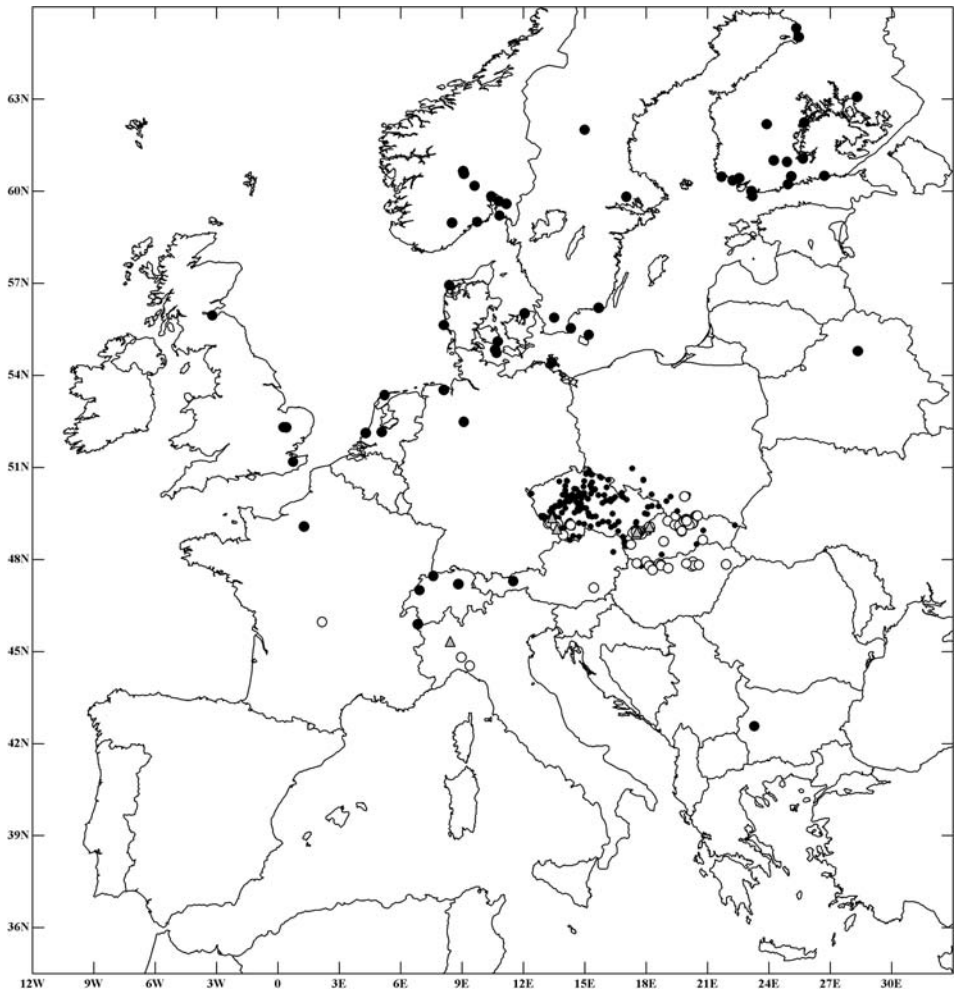

Fig. 1. – The distribution of plants of *Vicia cracca* with different ploidy levels in Europe based on published chromosome counts (see Table 1 for references). ○ diploid, ● tetraploid, △ mixed (2x + 4x) population. Triploids are not shown. The symbol size for tetraploids was reduced in areas for which there are many records of chromosome number in order to improve legibility.

Material and methods

Plant material

Plants were collected in the Czech Republic (4,323 individuals, 163 populations), the Slovak Republic (1,895 individuals, 62 populations), Austria (313 individuals, 30 populations) and Germany (23 individuals, two populations) during 2005–2008. A total of 6,554 plants from 257 populations were collected. The details of the locality, including the geographic coordinates (WGS 84) and altitude, and the number of plants analyzed, their DNA

ploidy levels and the collector(s) are listed in Appendix 1. At each locality, one leaf from each of 1–156 plants (~25 on average) was collected, put into a plastic bag and delivered to the Laboratory of Flow Cytometry, Průhonice, Czech Republic for ploidy analysis. There was at least a distance of 5 m between plants, which avoids the sampling of sibs. The fine-scale distribution of cytotypes was mapped in one mixed-ploidy population with a similar frequency of 2x and 4x individuals (no. 189, CZ – Horní Suchá). Plants were collected from a 200 × 100 m plot, and once again, the distance between plants was at least 5 m. Distributional maps were prepared using DMAP for Windows, ver. 7.2e (Alan Morton, Windsor, UK).

Flow cytometry

DNA ploidy levels (Suda et al. 2006) of *Vicia* were estimated using flow cytometry. Young, intact leaf tissue of the analyzed plant(s) and an appropriate amount of leaf tissue of the internal reference standard (*Pisum sativum* 'Ctirad', Doležel et al. 1998; 2C-value set to 8.84 pg following Greilhuber et al. 2007) were co-chopped using a sharp razor blade in a plastic Petri-dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20) (Otto 1990, Doležel et al. 2007). The crude suspension was filtered through a 0.42 µm nylon mesh to remove tissue debris and then incubated for at least 30 minutes at room temperature. Isolated nuclei were stained with 1 ml of Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12\ H_2O$) supplemented with AT-selective fluorochrome 4',6-diamidino-2-phenylindole (DAPI) and ß-mercaptoethanol at final concentrations of 4 µg/ml and 2 µl/ml, respectively. Immediately after staining, the relative fluorescence intensity of at least 3,000 particles was recorded with a PA-II flow cytometer (Partec GmbH, Münster, Germany) equipped with a mercury lamp for UV excitation. Resulting histograms were evaluated with FloMax software (Partec GmbH, Münster, Germany) and DNA ploidy levels determined on the basis of the sample/standard ratio. The *Vicia* tissue was processed within 1–5 days of collection. Usually, bulk samples from up to 10 *Vicia* plants (one leaflet from each plant) were measured. Our previous trial analyses confirmed the reliability of such a protocol (i.e., the lack of endopolyploidy and the low mitotic activity in the *Vicia* tissues selected for flow analyses). In addition, good congruency between the number of nuclei in particular peaks and the number of analyzed individuals with different ploidy levels allowed us to estimate the proportions of the cytotypes in mixed samples with a high accuracy. Only histograms with coefficients of variation (CVs) of G_0/G_1 peaks of both the bulked sample and the standard that were below 3.5% were considered. If the quality of analyses did not meet this criterion, all plants from the bulked sample were re-analyzed separately (in order to detect potential between-plant differences in fluorescence intensity).

In addition, genome sizes (C- and Cx-values in picograms of DNA; Greilhuber et al. 2005) were estimated for eight diploids + two tetraploids from mixed population no. 171 and one diploid + one tetraploid from population no. 173. In these measurements, the intercalating fluorochrome propidium iodide (PI) together with RNase IIA (both at final concentrations 50 µg/ml) were used and the samples were excited by a 532 nm solid state laser (model Cobolt Samba; Cobolt AB, Stockholm, Sweden) housed in the Partec CyFlow instrument. All samples were measured three times on different days to avoid potential random fluctuations in the flow cytometer readings, and a fluorescence intensity of 5,000 particles was recorded.

Chromosome numbers

To confirm the reliability of the ploidy estimates, FCM results were supplemented by conventional chromosome counts. Actively growing root tips of germinating seedlings were pre-treated with 0.002 M 8-hydroxyquinoline for 2 hours, fixed overnight in a 1:3 mixture of cold acetic acid and 96% ethanol, macerated for 80 s in 1:1 concentrated (37%) HCl : 96% ethanol at room temperature, and stained with acetocarmine. Chromosomes were observed at 1,000-fold magnification using an Olympus BX61 microscope (Olympus Corp., Tokyo, Japan) equipped with an immersion objective. Four diploids (i.e., three plants from population no. 189 and one plant from population no. 230) and four tetraploids (i.e., one plant each from populations 68, 84, 124 and 189; see Electronic Appendix 1) were analyzed. At least three weĺl-spread chromosome plates were counted for each individual.

Statistical analyses

The spatial segregation of diploid and tetraploid plants in the mixed population was tested by determining the ploidy level (i.e., same vs. different) of the nearest neighbour for all mapped individuals. Deviations from a random distribution were assessed using the Fisher's exact test (S-Plus 6.2 Professional; Insightful Corp., Seattle, WA, USA). Differences in altitudinal ranges were analyzed using the Mann-Whitney U test available in statistical package NCSS 2000 (Kaysville, UT, USA).

Results

FCM analyses yielded high-resolution histograms with low CVs and negligible background noise. Three different DNA ploidy levels were recorded among 6,554 plants from 257 Central European populations (Fig. 2). Mean relative fluorescence values ± standard errors (SE) for individual cytotypes were as follows: 2x – 0.710±0.008; 3x – 1.071±0.005; and 4x – 1.415±0.017. While diploids and tetraploids were abundant and accounted for 34.5% (= 2259 individuals) and 65.4% (= 4288 individuals) of all the individuals sampled, respectively, triploid plants occurred very rarely; only seven individuals (= 0.1%) were found. At the population level, 60 populations (= 23.3%) consisted solely of diploids, 179 populations (= 69.7%) solely of tetraploids and 18 populations (= 7.0%) were ploidy-mixed. Diploids and tetraploids co-existed in 16 populations while the co-existence of 2x and 3x cytotypes was documented in two adjacent populations in the Slovak Republic (nos. 224 – Vlkolínec and 226 – Lúčky; see Electronic Appendix 1). Karyological data confirmed the FCM results and all DNA diploids and DNA tetraploids counted had 14 and 28 somatic chromosomes, respectively.

The ploidy distribution in the area investigated was far from random and the cytotypes showed a distinct longitudinal segregation (Fig. 3A). While tetraploids prevailed in the west, the diploids were more easterly distributed. Diploid-tetraploid contact zone occurs near the boundary between the Czech and Slovak Republics. The cytotype turnover along the contact zone is quite abrupt; no diploids were detected west of the contact zone and only a few tetraploids east of the contact zone, where they often formed mixed populations with diploids (Fig. 3A). The cytotype distribution at a fine spatial scale in mixed popula-

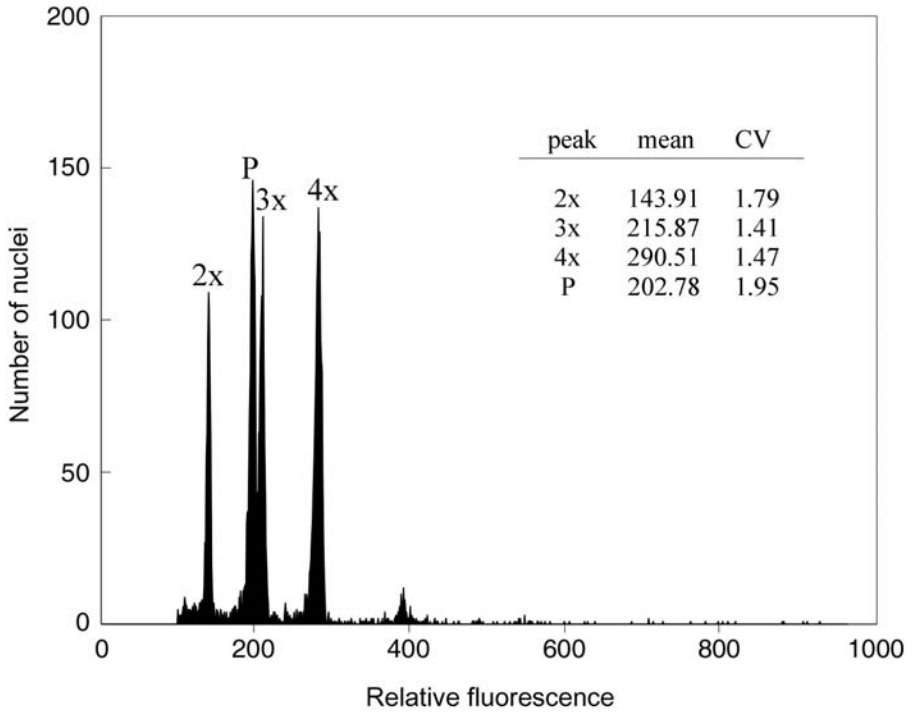

Fig. 2. – Flow cytometric fluorescence histogram of three ploidy levels (2x, 3x and 4x) of *Vicia cracca* together with the internal reference standard (*Pisum sativum* 'Ctirad'; P). Nuclei of all plants were isolated, stained with DAPI and analyzed simultaneously.

tion no. 189 (Horní Suchá, NE Moravia) also significantly deviated from random (P = 0.009). Both diploids and tetraploids preferentially clustered with plants of the same ploidy level (Fig. 4).

The altitudinal ranges of both majority cytotypes were recorded because previous studies report some differentiation and recognize two chromosomal races (lowland and mountain) within diploids. We found significant differences (P = 0.006) between the altitudinal distribution of diploid (mean = 440 m a.s.l.) and tetraploid (mean = 526 m a.s.l.) populations. However, because these results may be influenced by unbalanced sampling across different altitudinal zones, we refrain from commenting on these differences. In fact, there was little ploidy segregation among five altitudinal zones, as defined in increments of 250 m (Fig. 5). All zones harboured both 2x and 4x cytotypes and most also mixed-ploidy populations. A cumulative frequency of populations along the altitudinal gradient confirmed similarities between both ploidies and did not reveal any discontinuity that would correspond to different altitudinal variants within any cytotype (Fig. 6).

Mean 2C-values±SD for diploid and tetraploid cytotypes were estimated to be 5.76±0.05 pg and 11.51±0.02 pg, respectively. Monoploid genome sizes (1Cx-values ±SD) were 2.88±0.03 pg and 2.88±0.01 pg for diploids and tetraploids, respectively (non-significantly different using the Mann-Whitney U test; $Z = -0.277$, P = 0.782).

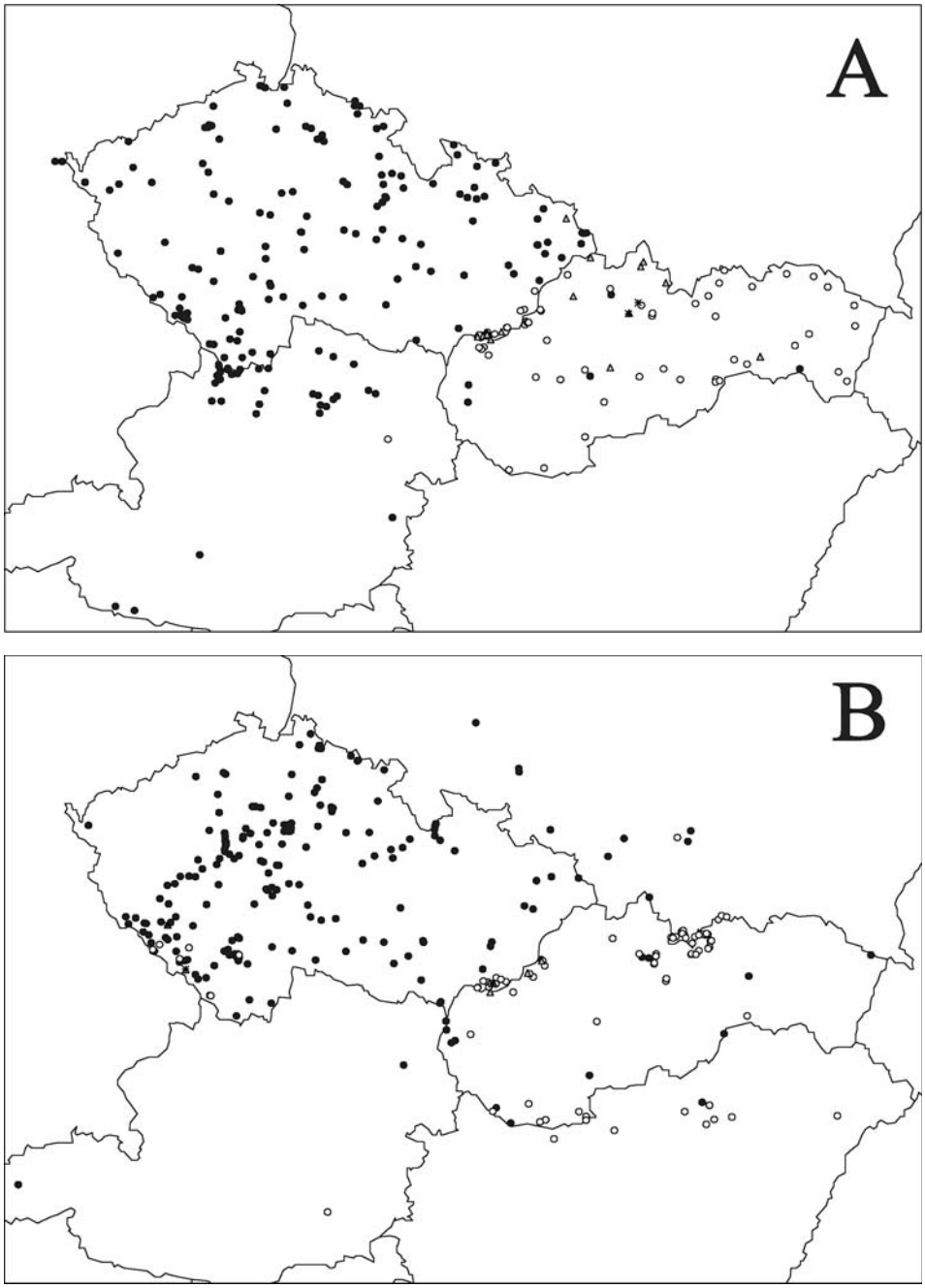

Fig. 3. – The distribution of cytotypes of *Vicia cracca* in the area investigated. (A) Recent map based on flow cytometric results; (B) Historical map constructed on the basis of chromosome counts published about four decades ago (cf. Chrtková-Žertová 1973a). ○ diploid, ● tetraploid, △ mixed (2x + 4x) population, ∗ triploid.

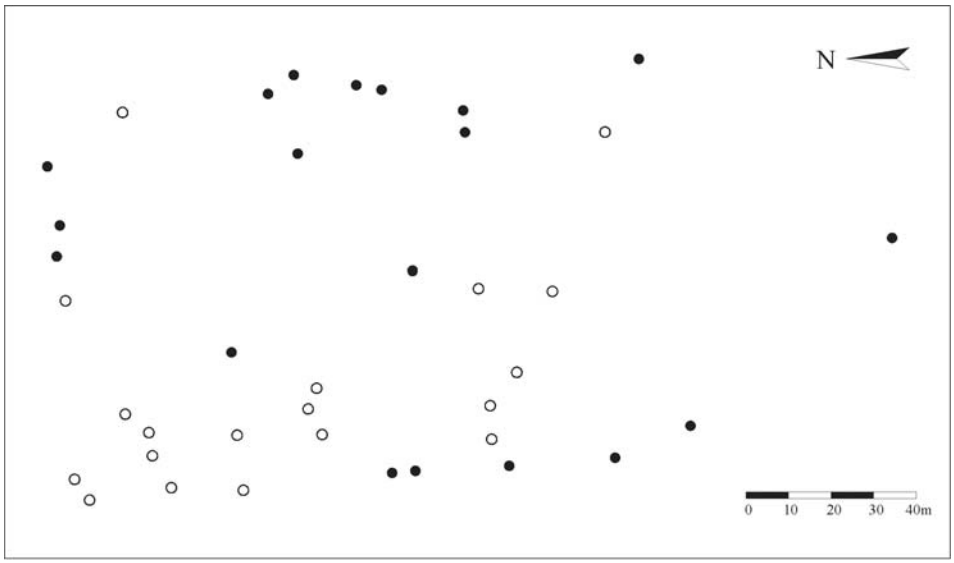

Fig. 4. – Fine-scale distribution of of *Vicia cracca* cytotypes in a mixed-ploidy population at Horní Suchá, CZ (no. 189; see Electronic Appendix 1). Open and full circles designate diploids and tetraploids, respectively.

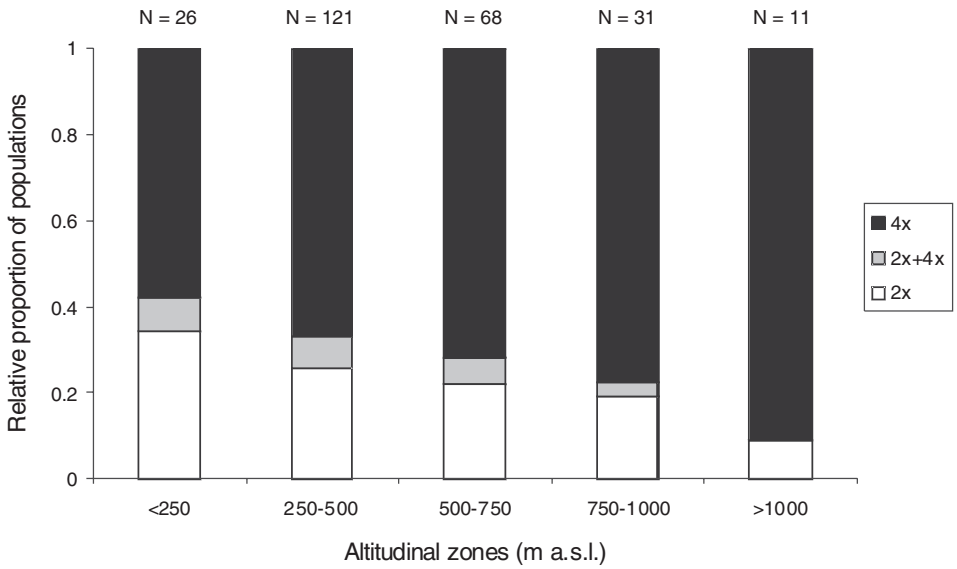

Fig. 5. – Histogram of the relative proportions of the different cytotypes of *Vicia cracca* recorded in each of five altitudinal zones (arbitrarily defined by increments of 250 m). White, grey and black columns designate diploid, mixed (2x + 4x) and tetraploid populations, respectively. The total number of populations within each altitudinal zone (N) is shown above the column.

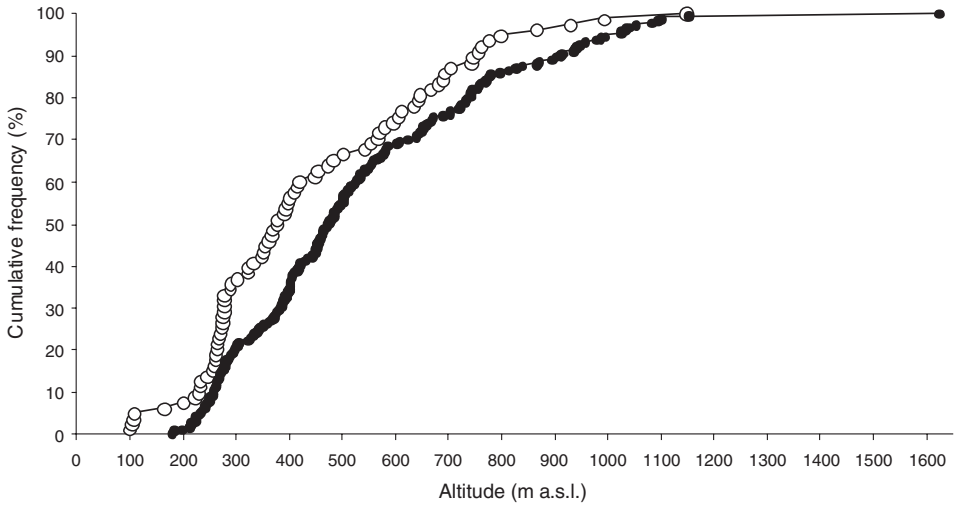

Fig. 6. – The increase in the cumulative frequency of ○ diploid and ● tetraploid populations of *Vicia cracca* with increase in altitude.

Discussion

Using flow cytometry, we determined DNA ploidy levels in a representative number of plants of *V. cracca* from four Central European countries and characterized the cytotype distribution pattern. Such an investigation would have been difficult without FCM. This high-throughput technique has several advantages over other methods of measuring ploidy level, high speed and reliability in particular, which makes it possible to survey ploidy variation over large spatial scales using large population samples. Recent cytogeographic studies thus routinely gather DNA ploidy data from >1,000 plants (e.g., Burton & Husband 1999, Baack 2004, Šafářová & Duchoslav 2010). In terms of sample size, the present study is possibly based on a higher number of karyologically-checked individuals than any other study.

Flow cytometric screening of 6,554 *Vicia* plants together with eight confirmatory chromosome counts revealed the presence of three ploidy levels – diploid, triploid and tetraploid. All these cytotypes are already recorded from Central Europe (see Table 1). The even ploidy levels (i.e. 2x and 4x) accounted for 99.9% of all samples and only seven triploids were detected in two otherwise diploid populations. The current distribution of cytotypes recorded here is similar to that recorded about four decades ago using chromosome counts of cells in root-tip squashes (Chrtková-Žertová 1973a). Perhaps the most prominent discrepancy is the current absence of the diploid cytotype from southern Bohemia. Although Chrtková-Žertová (1973a) reports at least eight populations with diploids (Fig. 3B), we failed to find any diploids even after an intensive search of the same localities (cf. Fig. 3A). At least two non-exclusive scenarios can be invoked to explain the observed pattern: (i) As *V. cracca* occurs almost exclusively in man-made habitats, ecological succession or changes in local management might have affected the community composition and caused the extinction of diploids. (ii) Diploids may have disappeared

because of frequency-dependent selection, traditionally called minority cytotype exclusion (Levin 1975). According to this theory, the plants with the rare ploidy level experience a transmission disadvantage as a consequence of the high proportion of hybrid matings and will eventually be outcompeted. The effect of this process is more limiting in obligate out-crossers, while its role decreases with autogamy, asexuality, and/or the development of other inter-ploidy reproductive barriers (Kao 2007). *Vicia cracca* is regarded as a predominantly allogamous species (Hanelt & Mettin 1989), although there is little experimental support for this. Nonetheless, at least facultative selfing is possible as shown by crossing experiments (A. Eliášová, unpubl.; see also below). It is unlikely that the chromosome counts in the historical paper are erroneous because of the relative simplicity of karyological surveys and the good agreement between the results of Chrtková-Žertová (1973a) and our ploidy estimates in other parts of the area investigated.

The distinct geographic segregation of the cytotypes and narrow zone of ploidy overlap (Fig. 3) support the idea of a secondary contact zone (i.e., when two formerly parapatric cytotypes come into contact; Petit et al. 1999). For example, secondary contacts are reported in *Aster amellus* L., *Asteraceae* (Mandáková & Münzbergová 2006), *Centaurea jacea* L., *Asteraceae* (Hardy et al. 2000) and *Plantago media* L., *Plantaginaceae* (Van Dijk & Bakx-Schotman 1997). Generally, the contact zone is maintained by a balance between the dispersal rates of the cytotypes and a frequency-dependent selection against inter-ploidy hybrids. Sometimes, this tension zone is very narrow and can be just a few meters in *Ranunculus adoneus* Gray, *Ranunculaceae* (Baack 2004).

In 18 out of 257 populations (= 7.0%) of *V. cracca*, two different cytotypes were present. The majority of mixed-ploidy populations consisted of 2x + 4x cytotypes (16 populations) and all lacked intermediate ploidy levels. This finding points to the evolution of a strong reproductive barrier between cytotypes. That they are reproductively isolated was also confirmed by our reciprocal crossing experiments between diploids and tetraploids, in which only selfed plants produced seed (A. Eliášová, unpubl.). In addition, similar results are recorded for diploid plants growing among and exposed to spontaneous pollination by tetraploid plants (Rousi 1973). Generally, triploids seem to be very rare in Central Europe, only seven individuals were recorded in this study and three previously reported by Chrtková-Žertová (1973a). The occurrence of triploid plants in otherwise diploid populations indicates their genesis via a fusion of reduced and unreduced gametes of diploids (Ramsey & Schemske 1998).

Analyses of altitudinal variation were done in order to determine whether the cytotypes are segregated altitudinally and to evaluate the justification for the two diploid chromosomal races, lowland and mountain, previously recognized by some authors (Chrtková-Žertová 1973a, Chrtková 1995). Although the tetraploid populations occurred at significantly higher altitudes than those of diploids, this difference is not considered to be proof of ecological sorting but rather an artifact of the sampling procedure. In fact, both cytotypes occurred over virtually the whole altitudinal range (Figs. 5 and 6). In addition, no discontinuity in the distribution of diploid plants was detected, thus there is no support for the existence of altitudinal variants. Most probably, the sampling in previous studies was less representative and this accounts for the seeming gap in cytotype distribution.

A proportional increase in genome size, as compared to their diploid progenitors, is documented for synthetic tetraploids (Ozkan et al. 2006) and for several established natural (auto)polyploids (Brandizzi & Caiola 1998). Thus, identical monoploid genome sizes

of diploids and tetraploids of *Vicia cracca* support the autopolyploid origin of the latter cytotype, which accords with previous hypotheses (Rousi 1961, Dvořák et al. 1977).

See http://www.preslia.cz for Electronic Appendix 1.

Acknowledgements

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Souhrn

Mezi základní otázky při studiu polyploidních komplexů rostlin patří stanovení způsobu jejich vzniku a objasnění mechanismů, které umožňují dlouhodobé přežívání polyploidů (zejména evoluce reprodukčně-izolačních mechanismů mezi diploidy a polyploidy). Vzhledem k dlouhému časovému horizontu většiny takových procesů, je však zkoumání evoluční dynamiky diploidně-polyploidních skupin značně obtížné. Vhodným kandidátem pro srovnávací studii je vikev ptačí (*Vicia cracca* L., *Fabaceae*), která byla v šedesátých letech minulého století podrobena detailnímu cytogeografickému průzkumu na území střední Evropy. Cíleným odběrem vzorků na původních lokalitách se tedy nabízí jedinečná možnost posoudit potenciální změny v cytotypové struktuře populací, jež jsou výslednicí vzájemného působení různých selekčních tlaků. S použitím průtokové cytometrie jsme stanovili DNA-ploidii u více než 6 500 jedinců vikve ptačí z 257 populací z České republiky, Německa, Rakouska a Slovenska. Celkem byly detekovány tři různé cytotypy – diploidní, triploidní a tetraploidní. Zatímco tetraploidi (celkem 179 populací) převládají v západní části zkoumaného území, diploidní cytotyp vykazuje východnější rozšíření (celkem 62 populací). Sekundární kontaktní zóna mezi cytotypy byla zaznamenána v příhraniční oblasti mezi Českou a Slovenskou republikou, přičemž společný výskyt diploidního a tetraploidního cytotypu byl odhalen v 16 případech. Triploidní cytotyp je velmi vzácný (nalezeno bylo pouze 7 jedinců ve dvou jinak diploidních populacích), což ukazuje na existenci účinné reprodukční bariéry mezi majoritními ploidiemi. Aktuální rozšíření cytotypů docela dobře odpovídá situaci zjištěné tradičním počítáním chromozómů před zhruba čtyřmi desetiletími. Určité rozdíly představují zejména (i) vymizení diploidního cytotypu v jižních Čechách a (ii) nepotvrzení segregace cytotypů podél výškového gradientu (na jejímž základě byly v minulosti dokonce vylišeny dva typy v rámci diploidů). Shodná monoploidní velikost genomu (1Cx-hodnota) obou majoritních cytotypů podporuje autopolyploidní původ tetraploidů.

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