Understanding intraspecific variation in genome size in plants

Co víme o vnitrodruhové variabilitě velikosti genomu u rostlin

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Intraspecific variation in genome size makes it possible to study ongoing processes of genome size evolution. Although there are over 200 papers on intraspecific variation in genome size, there is still limited understanding of this phenomenon, especially as many of these papers are based on weak methodology and therefore report biased or false evidence of the extent of intraspecific variation. In this paper the recent progress in understanding the spatio-temporal dynamics of intraspecific variation in genome size caused by the gradual accumulation of mutations is reviewed. The results of the case studies on *Microseris douglasii*, *Zea mays*, *Silene latifolia*, *Hordeum spontaneum* and *Lolium* hybrids, and in particular that on *Festuca pallens,* are discussed. The variation in genome size that occurs within species is caused mainly by differences in the content of repetitive DNA, in particular it is a consequence of the dynamics of transposable elements. Variation may be induced and maintained polytopically. We assume that it is probably more frequent in groups of young radiating species. Even in the initial stages, the variation in genome size generated within a population seems to be restricted by selection, which is also important in stabilizing genome size within species. The long-term persistence of the variation within a population and its further accumulation may be enhanced by gametes with different genome sizes, produced by the segregation of unequally sized homeologous chromosomes. Over large geographical scales and across contrasting environmental gradients, the distribution of genome sizes within species may be influenced by the nucleotype effect, with smaller genomes being more successful at higher latitudes and altitudes and under stressful conditions. However, the small differences in genome size within species seem generally to be of minor importance relative to other components of plant fitness that may be selectively favourable under particular environmental or habitat conditions. The processes generating variation in genome size may be associated with phenotypic variation. While the shift in the genome size of a population through selection enables adaptive evolution of genome size in a newly arising species, the spatio-temporal variation in genome size within an ancestral species allows for a rapid multiple genome size divergence of related species through random drift in genome size (founder effect, bottleneck effect) during range fragmentation, hybridization and/or polyploidization.

K e y w o r d s: chromosome segregation, DNA content, genome size evolution, inheritance, microevolution, natural selection, nucleotype effect, phenotypic effect, spatio-temporal variation

Introduction

Understanding the evolution of genome size is important in many scientific disciplines, from systematics to evolutionary biology, which study genome size and its biological and evolutionary consequences across a variety of taxa, to evolutionary genomics and molecular biology, which focus upon the processes that result in the evolution of genome size at the molecular level. Recently, substantial advances were made in understanding inter-species relationships in genome size and the major mechanisms responsible for the diversification of genome size among species (SanMiguel & Bennetzen 1998, Grover et al. 2004,

Bennetzen et al. 2005, Vitte & Panaud 2005, Zuccolo et al. 2007). However, there is little knowledge of the early phases of genome size divergence, that occurs before two populations may be recognized as separate species with different genome sizes, or of the population dynamics of genome size. This knowledge is essential for linking descriptive genome size data and molecular mechanisms through processes of microevolution, and for providing a synthetic view of genome size evolution. We assume that substantial progress in understanding these processes will be provided primarily by studies of species that show variation in genome size, since this may be interpreted as an indicator of ongoing genome size diversification.

The variation in genome size within species may be due to chromosomal variations, e.g. polyploidy, aneuploidy, presence of supernumerary chromosomes (B-chromosomes) and sex chromosomes, or various chromosomal rearrangements, e.g. frequently accompanying hybridization and polyploidization events. Important variation in genome size is also generated by mutational processes at the molecular level, namely the activity of transposable elements, length polymorphism in various repeat sequences (both forming the substantial portion of heterochromatic regions), genomic duplications, ectopic recombinations, etc. While chromosomal variation typically originates during mitosis or meiosis and causes sudden and large changes in genome size, mutations at the molecular level may appear at different stages of ontogeny and cause a gradual increase in variation.

This review focuses on recent advances in the understanding of intraspecific variation in genome size caused by gradual mutational processes (sometimes termed "unorthodox" variation; Greilhuber 1998) with reference to particular species. The species were selected on the basis of the reliability of the method used to determine variation in genome size and whether it was possible to interpret the variation from an ecological or evolutionary perspective. Particular attention was paid to the results of our ongoing research on *Festuca pallens*.

Brief historical survey

The first karyological studies indicated that chromosome number, total chromosome length and nucleus size are highly species specific (Tischler 1921–1922). It also became evident that if there are differences in genomic parameters among taxa the origin of these differences through microevolution is probably associated with the existence of intraspecific variation. The differences in the number of supernumerary chromosomes and heterochromatin knobs in maize (Kuwada 1925, Longley 1927, 1938) probably provided the first indication of intraspecific variation in genome size. However, exact measurements of genome size only became possible in the early 1950s with the introduction of Feulgen densitometry (Swift 1950) and made more easily determinable due to the application of flow cytometry (Heller 1973, Galbraith et al. 1983).

Since the first studies on flax (*Linum usitatissimum*; Evans et al. 1966, Evans 1968) over 200 studies reporting intraspecific variation in genome size in more than 80 species have been published. In the late 1980s and early 1990s, with the increasing number of studies on genome size, the idea of a plastic genome became increasingly popular and the concept of intraspecific variation in genome size became widely accepted (Cavallini & Natali 1991, Ohri 1998). In the most extreme cases, genome size differs on average

1.71-fold between juvenile and adult leaves of *Hedera helix* (Schäffner & Nagl 1979), up to 2.88-fold among individuals of *Collinsia verna* (Greenlee et al. 1984) and up to 1.48-fold between leaves of the same *Helianthus annuus* individual (Michaelson et al. 1991b). This "golden age" of studies on intraspecific variation in genome size came to an end when a series of papers on the methodology published by Greilhuber and co-workers criticized several contemporary estimates of genome size (Greilhuber 1986, 1988, 1998, 2005, Greilhuber & Ebert 1994, Greilhuber & Obermayer 1997, Temsch & Greilhuber 2000).

Currently, the accuracy of over 50 studies documenting intraspecific variation in genome size is thought to be questionable because of the methodology used, or completely refuted when re-investigated. The main causes of false or unrealistic intraspecific variation in genome size are namely (i) failure to use internal standards, (ii) unrecognized taxonomic heterogeneity in the plants studied (Greilhuber & Speta 1985, Greilhuber & Obermayer 1997), (iii) inaccuracies in the Feulgen reaction (mainly when hot hydrolysis was used), and (iv) the presence of metabolic compounds, such as tannins, flavonoids and anthocyanins, which affect DNA staining (Greilhuber 1986, 1988, 1998, Noirot et al. 2000, 2003, 2005, Price et al. 2000, Loureiro et al. 2006, Walker et al. 2006, Greilhuber et al. 2007, Bennett et al. 2008). The numerous reports of intraspecific variation in genome size were subsequently doubted or refuted, for instance, in *Linum usitatissimum* (Evans et al. 1966, Evans 1968, Durrant & Jones 1971, Joarder et al. 1975 vs Greilhuber & Schweizer in Greilhuber 1998), *Pisum sativum* (Cavallini & Natali 1990, 1994, Cavallini et al. 1993 vs Greilhuber & Ebert 1994, Baranyi & Greilhuber 1995, 1996), *Helianthus annuus* (Cavallini et al. 1986, 1989, 1996, Michaelson et al. 1991b, Natali et al. 1993, Price & Johnston 1996, Price et al. 1998 vs Greilhuber 1998, Price et al. 2000), *Hedera helix* (Kessler & Reches 1977, Nagl 1979, Schäffner & Nagl 1979, Nagl et al. 1983 vs Polito & Alliatta 1981, König et al. 1987), *Arachis hypogaea* (Singh et al. 1996 vs Temsch & Greilhuber 2000), *Glycine max* (Graham et al. 1994, Rayburn et al. 1997 vs Greilhuber & Obermayer 1997) and *Aegilops squarrosa* (Furuta et al. 1975 vs Eilam et al. 2007). In the late 1990s, these results undermined the general acceptance of the concept of a plastic genome and resulted in scepticism about the validity of any reports of intraspecific variation in genome size.

The study of intraspecific variation in genome size became popular again when the methodology used to detect the variation was improved (Greilhuber 2005) and when flow cytometry was widely adopted as an analytical method (Doležel & Bartoš 2005, Doležel et al. 2007, Greilhuber 2008). The most recent best practice includes (i) verification of any variation by the double-peak or bimodal peak that results from measurements of two co-chopped samples (Michaelson et al. 1991a, Doležel & Göhde 1995, Greilhuber 2005), (ii) the consistent use of an internal standard for all measurements (preferentially only one individual), (iii) repeating the measurements on different days and in different seasons, using different instruments, or alternative fluorescent dyes (Šmarda & Bureš 2006, Walker et al. 2006, Šmarda et al. 2008b), and (iv) checking for the presence of metabolic compounds that affect DNA staining and fluorescence intensity (Price et al. 2000, Loureiro et al. 2006, Walker et al. 2006, Greilhuber et al. 2007, Temsch et al. 2008).

Since 2005, the existence of intraspecific variation and differences in genome size has been clearly documented by double peaks for two accessions of *Dasypyrum villosum* (Greilhuber 2005), Romanian populations of *Festuca rupicola*, *F. vaginata*, *F. polesica*

and *F. pallens* (Šmarda 2006), among and within populations over the whole natural range of *F. pallens* (Šmarda & Bureš 2006, Šmarda et al. 2007b, 2008a), among Central European populations of *Koeleria macrantha* and *K. tristis* (Pecinka et al. 2006), within *Bituminaria bituminosa* (Walker et al. 2006), within diploid, tetraploid and hexaploid cytotypes of *Senecio carniolicus* in the Alps and Carpathians (Suda et al. 2007b), within *Curcuma longa* (Leong-Škorničková et al. 2007), within hybrids of *Hieracium brachiatum* (Suda et al. 2007a), within *Juncus biglumis* (Schönswetter et al. 2007), within two races of *Lagenaria siceraria* (Achigan-Dako et al. 2008), and two races of *Picris hieracioides* in Europe (Slovák et al. 2009). Temsch et al. (2008) also show by double peaks the apparent reduction in genome size in epidermal cells of corollas in *Dahlia variabilis*.

Case studies

Microseris douglasii

The most complex intraspecific variation in genome size in wild taxa is that recorded using Feulgen densitometry in species of the annual *Microseris* (*Asteraceae*). The genome size of these species is reported to vary over 1.20-fold among individuals and 1.14-fold among populations of *M. douglasii* and *M. bigelovii* (Price et al. 1980, 1981a, 1981b), of which both species have the same constant number of chromosomes (2n = 18). Individuals grown in an environmental chamber from achenes collected from wild *M. douglasii* populations in one year vary up to 1.27-fold in genome size, although intrapopulation variation in genome size was generally low even among individuals that were morphologically distinct and also among potential *M. douglasii* and *M. bigelovii* hybrids (Price et al. 1981a, 1986). The mean DNA content significantly differs among populations, and more interestingly, significantly differs also within a population in different years. As the mean genome size of a population positively correlates with geographical and temporal changes in annual precipitation and soil depth, Price et al. (1981a, 1986) conclude that the spatio-temporal pattern observed in genome size in a population results from selection for smaller genome sizes in dry and/or time-limited environments. This hypothesis was later tested also at the interspecific level by Castro-Jimenez et al. (1989), who compared the physiological response to drought stress of *M. bigelovii* and *M. laciniata*. As the physiological performance of *M. bigelovii* (small genome, 2C = 2.6 pg) is considerably better than that of *M. laciniata* (large genome, 2C = 6.8 pg), Castro-Jimenez et al. (1989) argue for the validity of the hypothesis. However, as these species belong to different phylogenetic lineages (Lohwasser et al. 2004) and have different life-cycles and breeding systems (annual, predominantly inbreeding *M. bigelovii* versus perennial and outcrossing *M. laciniata*) the validity of this comparison is doubtful. These differences may be more associated with differences in genome size than ecophysiological performance (cf. Bennett 1971, 1972). Despite the vague nature of the evidence, later studies provide convincing evidence of large genomes generally missing in extreme environmental conditions across the Californian flora (Knight & Ackerly 2002). The ecological, evolutionary and phenotype constraints on taxa with large genomes were consequently documented worldwide (Vinogradov 2003, Knight et al. 2005).

The F_1 progeny of crosses between *M. douglasii* plants that differ in genome size may also vary in genome size and be significantly smaller or larger than the parental midpoint

(Price et al. 1983). Surprisingly, the genome sizes of the F_2 progeny obtained from self-breeding F_1 intraspecific hybrids may differ even among the capitula on a single plant (Price et al. 1983). Price et al. (1983) therefore assumed that (i) some portion of the DNA in the F_1 hybrids may be unstable and can undergo spontaneous and unpredictable change, and (ii) any changes in this unstable DNA that occurs in the postzygotic and postembryonic phases may give rise to a plant that is chimeric in DNA content and the production of variable F_2 progeny when self-pollinated. However, as yet no plants of *Microseris* with a chimeric DNA content have been identified and the molecular basis of the "unstable DNA" remains to be experimentally tested.

Although the pattern of intraspecific variation in genome size in *Microseris* is still one of the best known cases, the results mentioned above should be treated with caution and tested using the recently improved methodology. It should be pointed out that (i) the Feulgen microdensitometry in leaf epidermis used by Price and co-workers is no longer used in such studies (Greilhuber 2008) and (ii) the environmentally correlated variation in genome size reported in many other species is caused by the presence of fluorescence inhibiting cytosolic compounds (Greilhuber 1998, Price et al. 2000).

Zea mays

A well-known and widely accepted example of intraspecific variation in genome size is that described for maize (*Zea mays*) and several other *Zea* species (cf. Poggio et al. 1998: Table 1). Genome size in maize is affected by the presence of supernumerary (B) chromosomes (Poggio et al. 1998, Rosato et al. 1998), which may in extreme cases (with 2n = 20A + 34B) increase its genome size 2.55-fold (Jones & Rees 1982: 22). Another source of the genome size variation is the amount of heterochromatin observed as variation in the number of heterochromatin knobs and C-bands or DAPI-bands (McClintock 1929, Longley 1938, Laurie & Bennett 1985, Rayburn et al. 1985, Porter & Rayburn 1990, Tito et al. 1991, Poggio et al. 1998). The heterochromatin mostly consists of various repetitive DNA sequences, namely retrotransposons (Peacock et al. 1981, Ananiev et al. 1998, Messing et al. 2004, Lamb et al. 2007). The dynamics of retrotransposons in maize has an effect on the mosaic colour pattern of maize seeds, which led to the discovery of retrotransposons (McClintock 1950, 1951, 1953). Later studies documenting massive amplification of retrotransposons in maize provided evidence that the transposons have played a major role in the evolution of genome size (SanMiguel et al. 1996, 1998, SanMiguel & Bennetzen 1998, Bennetzen et al. 2005).

Up to a 1.36-fold variation in mean genome size among several maize populations without B-chromosomes determined using Feulgen densitometry is reported by Poggio et al. (1998). A 1.28-fold difference between two maize inbred lineages without B-chromosomes that was indicated by a double-peak in a simultaneous flow cytometry measurement is recorded by Michaelson et al. (1991a). Generally, in populations with small genomes, genome size is correlated with the number of B-chromosomes, whereas in populations with large genomes, the effect of B-chromosomes may be masked by variation in repetitive DNA and plants with B-chromosomes do not necessarily have a larger DNA content than those without B-chromosomes (Poggio et al. 1998). In both North and South America cultivars and lineages at high altitudes frequently have small genomes (Laurie & Bennett 1985, Rayburn et al. 1985, Rayburn & Auger 1990, Poggio et al. 1998, Rosato et al. 1998). The

unimodal relationship between genome size and altitude is recorded for maize populations in New Mexico (low altitudes) and Arizona (high altitudes) (Rayburn 1990) and documented for 401 Californian species (Knight & Ackerly 2002). Cultivars of North American maize with small genomes occur much more frequently at high latitudes (Laurie & Bennett 1985, Rayburn et al. 1985), experience a shorter growing season (Bullock & Rayburn 1991), grow faster and are higher yielding (Biradar et al. 1994). In addition to the important agronomic consequences, these findings confirm the nucleotype effect of genome size at the intraspecific level. This effect may be a consequence of the fact that the amplification of large genomes requires more energy and a longer period of time, which may put them at a selective disadvantage in time-limited environments with short vegetation periods such as at high latitudes and altitudes or in dry areas (low altitudes with high temperatures). The nucleotype effect, e.g. the time required to complete a mitotic cycle (Evans & Rees 1971), duration of meiosis (Bennett 1971) or minimum generation time (Bennett 1972), was originally documented using among-species comparisons (Cavalier-Smith 1978).

As in *Microseris*, attention was paid to the intraspecific heritability of genome size in maize. Using flow cytometry and internal standards, Michaelson et al. (1991a) revealed that the genome size of F_1 plants from crosses between two inbred lines with different genome sizes varied around the parental mean. A detailed analysis of the F_1 progenies from several crosses of different maize inbred lines revealed a much more complex pattern of inheritance, with the genome size of the F_1 progeny frequently significantly lower or higher than parental means, or very variable even when the parents had very similar genome sizes (Rayburn et al. 1993). These unstable F_1 progeny principally originated from crosses between related maize inbreds, while crossing of unrelated lines resulted in F_1 hybrids with a stable genome size close to the parental mean and higher heterotic responses (Biradar & Rayburn 1993a). The existence of both stable and unstable F_1 progenies accords with the results of intraspecific crosses in *Microseris douglasii* (Price et al. 1983, see above). However, both Rayburn et al. (1993) and Biradar & Rayburn (1993a) used external standards and their results may therefore have been biased by technical errors and/or the presence of DNA staining inhibitors (cf. Greilhuber & Obermayer 1997, Price et al. 2000, Noirot et al. 2003, Doležel & Bartoš 2005, Greilhuber et al. 2007). Similarly, results supporting the existence of intraplant variation in genome size and differences in heterochromatin condensation obtained using this method (Biradar & Rayburn 1993b, 1994) should be considered carefully.

Silene latifolia

The 1.02–1.05-fold differences in genome size in dioecious *Silene latifolia* revealed using flow cytometry is due to the presence of a large Y sex-chromosome in males (Costich et al. 1991, Meagher & Costich 1994, Doležel & Göhde 1995, Vagera et al. 2004). Statistically significant levels of variation in genome size are also found among individuals and populations of the same sex (Meagher & Costich 1994, 1996, 2004, 2008). The extent of this variation is reported by Meagher & Costich (1994). In six North American populations they document a constant 1.039-fold difference between sexes and 1.058-fold sex-independent difference among the two most different populations. The difference between sexes match those documented by the double peaks (Doležel & Göhde 1995; 1.039-fold), however, the difference within sexes remains to be tested in this way.

The genome size of *S. latifolia* is repeatedly reported to have a phenotypic effect upon flower size, which increases independently of sex with decreasing genome size (Meagher & Costich 1994, 1996, 2004, 2008, Meagher et al. 2005, Meagher & Vassiliadis 2005). The negative correlation between genome and flower size is also found in several related dioecious and hermaphroditic taxa (Meagher & Costich 2004, 2008). The comparison of flow cytometry measurements obtained using two different dyes, intercalating propidium iodide (PI) and PI together with GC-selective chromomycin A3 (CA3), revealed that the main differences in genome size is probably due to variation in AT-rich regions, which are assumed to consist of repetitive DNA (Meagher & Costich 1996, 2004, 2008, Meagher & Vassiliadis 2005).

Lolium hybrids

Lolium is an evolutionarily young genus that comprises eight closely related diploid species (2n = 14). The four inbreeding species have 1.4-fold larger genomes and individual chromosomes than the four outbreeders, which is probably due to the amount of repetitive DNA (Rees & Jones 1967, Hutchinson et al. 1979). Despite this difference, taxa from both groups may be successfully crossed, and the homeologous chromosomes, which significantly differ in size, regularly pair and segregate during meiosis (Gupta & Rees 1975, Hutchinson et al. 1979, Jenkins 1985). Therefore, the F_1 hybrids are frequently fertile and produce progeny when intercrossed, or backcrossed with parental species with either larger or smaller genomes (Hutchinson et al. 1979). As expected, the F_1 progeny resulting from crossing *Lolium* species with different genome sizes have genome sizes equal to the average genome size of their parents. By intercrossing F_1s, or backcrossing the F_1s with their parents, progeny that varies in genome size is produced due to the segregation of homeologous chromosomes of different sizes during meiosis (Gupta & Rees 1975, Hutchinson et al. 1979). When intercrossed, the genome sizes of the progeny varied over the whole range between that of the two parental species and when backcrossed, the genome sizes ranged from that of the F_1s to that of the backcrossed parental species (Gupta & Rees 1975, Hutchinson et al. 1979).

Due to the difference in genome sizes and fertility of the various generations (F_2, BF_2 and others) of *Lolium* interspecific hybrids the pattern of genome size in these hybrids may be used as a model for studying the dynamics of genome size variation within species. Consequently, the variation in progeny that results from crossing two plants with the same genome size (as discussed above for *Microseris* and maize) may be due to the size differences of homeologous chromosomes within or between crossed plants rather than de novo variation at the molecular level.

Although Hutchinson et al. (1979) assessed a number of morphological characters in various *Lolium* interspecific hybrids that varied in genome size, they found no phenotypic effect of genome size. Such an effect was later documented by Sugiyama et al. (2002) in *Lolium perenne*. Sugiyama et al. (2002) used flow cytometry and found up to 1.04-fold differences in the mean DNA content of 15 perennial rye-grass cultivars. Genome size of these 15 cultivars is positively correlated ($P < 0.1$) with seed size, cell size, root mass and leaf size (Sugiyama et al. 2002). These correlations accord with the nucleotype effect of DNA content recorded for many species (namely, the significant effect of DNA content on cell size and consequently upon the size of organs; Bennett 1972, 1973, Edwards &

Endrizzi 1975, Cavalier-Smith 1985). As in maize, these findings indicate that the nucleotype effect may be exhibited even at the intraspecific level.

As in many other studies, unfortunately, the differences in genome size documented by Sugiyama et al. (2002) were not verified by double-peaks. Scepticism is increased by the low statistical significance of the correlation because of the small number of cultivars with extreme genome sizes. Although Sugiyama et al. (2002) document statistically significant differences in genome size between herbage and turf cultivars ($P < 0.1$), no such difference is revealed when recalculating the original data set after correcting for pseudoreplication ($P = 0.132$; t test using population means cited by Sugiyama et al. 2002: Table 1).

Hordeum spontaneum

This winter annual species was studied genetically because it is considered to be a wild progenitor of cultivated barley (*Hordeum vulgare*; Nevo et al. 1986). The first evidence that there is about 1.13-fold variation in relative genome size in 12 accessions of *H. spontaneum* from Israel is reported by Kankanpää et al. (1996). Turpeinen et al. (1999), using flow cytometry with PI staining, record a 1.05-fold difference in mean genome size among 10 populations in Israel. They report a significant positive correlation between population genome size and mean January temperatures, and population genome size and the length of the midgrowth period. Unfortunately, the extent of the observed variation was not verified by double peaks in either of these studies.

It was simultaneously shown that genome size within nine worldwide *H. spontaneum* accessions is positively correlated with the number of copies of *BARE-1* retrotransposons, which are important in determining the genome size in the genus *Hordeum* (Vicient et al. 1999). Studying selected *H. spontaneum* accessions, Vicient et al. (1999) revealed that genome size is correlated with evaporation and alluvium soil type, and found a trend towards an increased number of *BARE-1* copies in plants from hot and dry desert conditions. In agreement with Turpeinen et al. (1999), Vicient et al. (1999) assume that a large genome size is associated with higher aridity and desert conditions. Although the nucleotype effect generally suggest that plants with a large genome are at a disadvantage in time-limited and stressfull environments due to the increased replication cost at mitosis and meiosis of the high DNA content (see example in maize), the above studies indicate that this effect may be effectively masked by the activity of retrotransposons and possibly also other molecular processes.

Further research into the dynamics of *BARE-1* retrotransposons at microclimatically contrasting sites in Evolution Canyon, Lower Nahal Oren, Israel, revealed that there is an increasing number of *BARE-1* copies in plants from the upper, extreme slopes of the canyon and indicate that there is a possible induction of retrotransposon activity in stressful environments (Kalendar et al. 2000). The differences in the number of copies of retrotransposons documented by Kalendar et al. (2000), however, is not enough to cause detectable shifts in total genome size and flow cytometry measurements (with PI dye) did not show significant differences in genome size among the study sites (Kalendar et al. 2000). In parallel to recording the number of copies of the internal parts of *BARE-1* retrotransposons, both Vicient et al. (1999) and Kalendar et al. (2000) recorded the number of copies of Long Terminal Repeats (LTRs; the intact *BARE-1* retrotransposon consists of an internal part that is surrounded on both sides by one LTR) and the frequency of solo

LTR formation. Solo LTR is a common effect of retrotransposon elimination by unequal homologous recombination (Vitte & Panaud 2003) and in this case the proportion of the overall ratio of LTRs (solo + intact) : internal parts is higher than 2 : 1. By finding a higher ratio of solo LTRs in smaller genomes, Vicient et al. (1999) and Kalendar et al. (2000) indicated that the higher number of retrotransposons observed is not only due to the intensity of their amplification, for instance in stressful environment, but also due to their slower loss via recombination. Based on studies on *Laupala* (Orthoptera), *Arabidopsis*, rice and other taxa, it was suggested that rather than genome amplification, genome loss is the determinant of the genome size (Petrov et al. 2000, Devos et al. 2002, Ma et al. 2004, Bennetzen et al. 2005,Vitte & Bennetzen 2006).

Festuca pallens

Knowledge of intraspecific variation in evolutionarily-derived fine-leaved fescues of the *Festuca* sect. *Festuca* has recently increased. To date, there is intraspecific variation (≥ 1.036-fold) indicated by double peaks in four species: *Festuca pallens*, *F. polesica*, *F. rupicola* and *F. vaginata* (Šmarda 2006). Detailed knowledge of intraspecific variation in genome size is provided by recent studies on *F. pallens* s.l. This allogamous taxon occurs in Central Europe, from eastern France to southern Romania, and mainly grows in relict rocky habitats in river valleys, mountain gorges and karstic landscapes. There are two ploidy levels in *F. pallens* s.l., diploid (2n = 14) and tetraploid (2n = 28), which can be treated as two closely related species if a narrow taxonomic concept is applied (Šmarda et al. 2007a).

The first evidence of intraspecific variation in relative genome size in the diploid *F. pallens* came from flow cytometry measurements on two geographically distant populations from Romania (up to 1.092-fold; Šmarda 2006). A subsequent study revealed high intraspecific variation in both ploidy levels, at various geographical scales, ranging from the intrapopulation level to the entire distribution range (Šmarda & Bureš 2006). In agreement with the best practices for determining intraspecific variation in genome size (Greilhuber 2005, Doležel et al. 2007, Greilhuber et. al 2007), the presence and extent of intraspecific variability were documented by the existence of double peaks in measurements of co-chopped samples. We also assume that the individual measurements were minimally biased by seasonal differences in the concentrations of metabolic compounds. This is indicated by (i) the same genome size recorded for the same plants in different seasons and (ii) high correlation between the genome size ratios obtained from individual samples (each with an internal standard) and those from the double-peak measurements (Šmarda & Bureš 2006, Šmarda et al. 2008b). Measurements using different dyes, i.e. AT-selective DAPI and intercalating propidium iodide (PI), were also strongly correlated (Šmarda & Bureš 2006, Šmarda et al. 2008b).

Within the natural distribution range, similar maximum differences in relative genome sizes were found in diploids (up to 1.170-fold) and tetraploids (up to 1.164-fold). Both ploidy levels revealed similar geographical patterns, with larger genomes prevailing in the southeastern part of their range and only occasionally occurring in deep river valleys further to the northwest (Šmarda & Bureš 2006, Fig. 1). In addition to a possible correlation with various macroclimatic variables, this pattern may be interpreted as a consequence of surviving the glaciation and of post-glacial migration. During the last glaciation maxi-

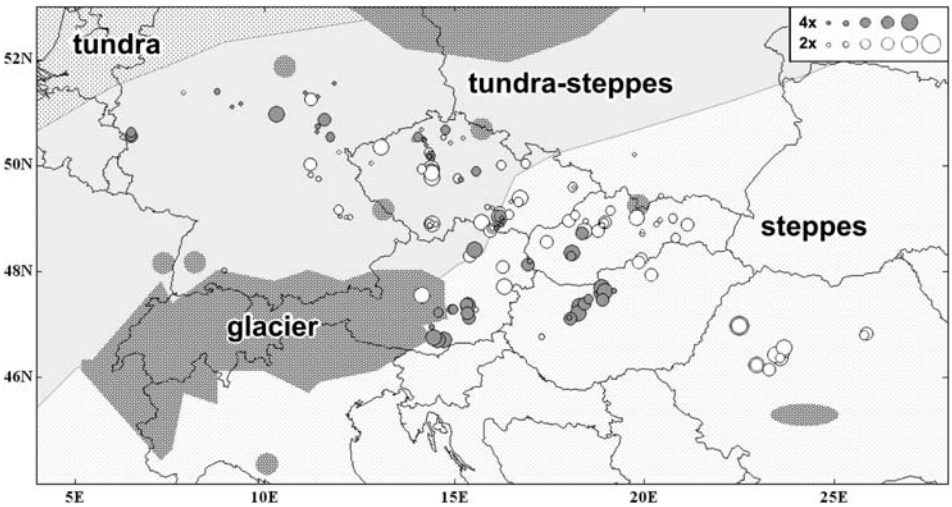


Fig. 1. – Spatial pattern of relative genome sizes within the natural range of *Festuca pallens* (modified after Šmarda & Bureš 2006). Symbol size (6 degree scale) indicates the relative DNA content; that of tetraploids was divided by two. Empty symbols are for diploids, dark symbols for tetraploids. A map of the vegetation cover in Europe at the maximum of the last glaciation 20,000 bp (Lang 1994) is superimposed. The high incidence of plants with larger genomes in the southern and eastern refugial parts of the natural range covered with periglacial steppes during the last glaciation, is of particular interest. The distribution of the variation in genome size in diploids and tetraploids is similar.

mum, which occurred about 20,000 years ago (Lang 1994), *F. pallens* may have survived in periglacial steppes (but not in periglacial tundra) and was distributed continuously throughout the southeastern part of its current range. However, more to the northwest, the steppes probably only occurred in small patches confined to suitable landforms, such as deep and narrow river valleys. This hypothesis is supported by the high frequency of diploids with large genomes at relict sites (Šmarda & Bureš 2006). However, it is unclear whether the present spatial pattern of genome sizes (i) resulted mainly due to a higher migration ability of plants with smaller genomes (re)colonizing new habitats in northwestern Europe in post-glacial periods or (ii) is a result of later selection operating on established young populations (e.g. by macroclimatic factors and nucleotypic effect). The tetraploids may be divided into three geographical types (races) with weak morphological differences: Alpine type in the south-eastern Alps, Pannonian type in the Pannonia region and Scabrifolia type in Bohemia and Germany (Pils1981, Šmarda et al. 2005, 2007a). The differences in genome size of these three types and the correlation of their genome sizes with those of the geographically close diploid populations indicate that the tetraploids may have originated independently from local diploid populations of a certain genome size in different parts of the distribution range. As in *F. pallens*, Slovák et al. (2009) assume that the spatial pattern of genome size within two races of *Picris hieracioides* in Europe reflects the glacial history and postglacial migrations, rather than an effect of in-situ environmental selection.

For both ploidy levels of *Festuca pallens* genome size varies between and within several populations along two transects across deep river valleys and one across a chain of

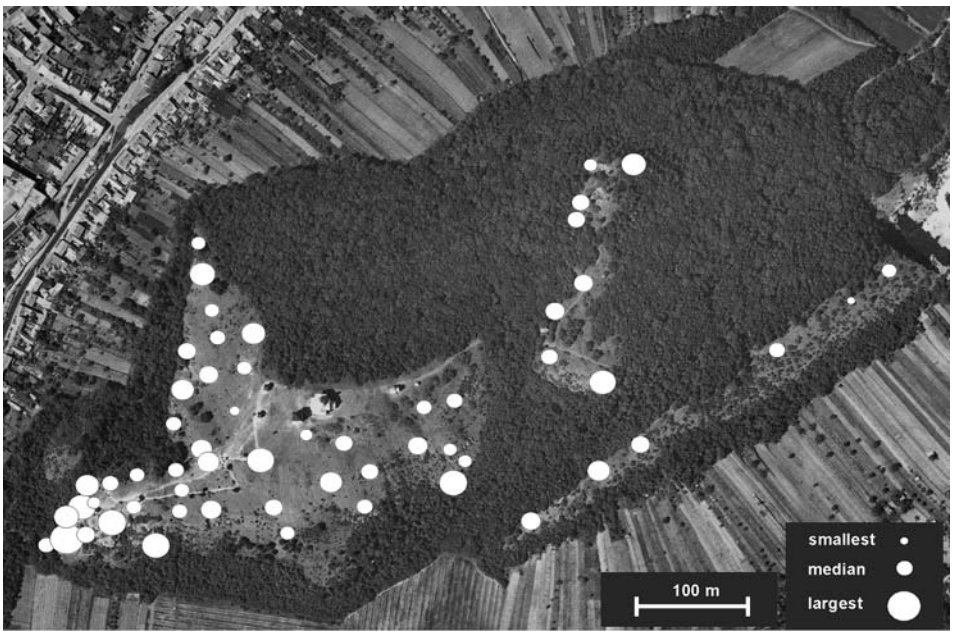


Fig. 2. – Local spatial pattern of genome size at 57 microsites (circles) in a relict tetraploid population of *Festuca pallens* at Svatý kopeček hill. The size of each circle indicates the mean genome size of three plants sampled at each microsite. Variation in genome size was distributed randomly, without any particular association with a habitat (geology, slope, inclination, vegetation cover), which argues for non-adaptivity of genome size (modified after Šmarda et al. 2007).

hills (Šmarda & Bureš 2006). Three plants from the most variable tetraploid population at Svatý kopeček hill near Mikulov (Pavlovské vrchy hills, Czech Republic) varied about 1.12-fold in genome size, while that of the remaining four populations along this transect did not vary. The high intrapopulation variation in genome size of the plants at Svatý kopeček hill was later confirmed by a more extensive sampling of 171 adult plants from the whole locality (Šmarda et al. 2007b, Fig. 2). A comparison of the genome sizes of the plants from this site revealed a continuous and positively skewed statistical distribution of genome sizes. The spatial distribution of genome sizes is random and not adapted to vegetation type or any of the other habitat properties recorded. A study of the progeny of 17 plants of *F. pallens* from the Svatý kopeček hill indicates a weak positive association between genome size and number of leaves (Šmarda et al. 2008b). This trend, however, disappears if the individual plants are randomly selected from this population (P. Šmarda et al., unpublished data). A field experiment revealed that the small differences in genome size found within this population have no or a very limited effect on seedling growth, survival and establishment (P. Šmarda et al., unpublished data). We therefore argue that the small differences in genome size found within species may generally be of minor importance with respect to other components of plant fitness, which may be selectively favourable under particular environmental or habitat conditions (P. Šmarda et al., unpublished

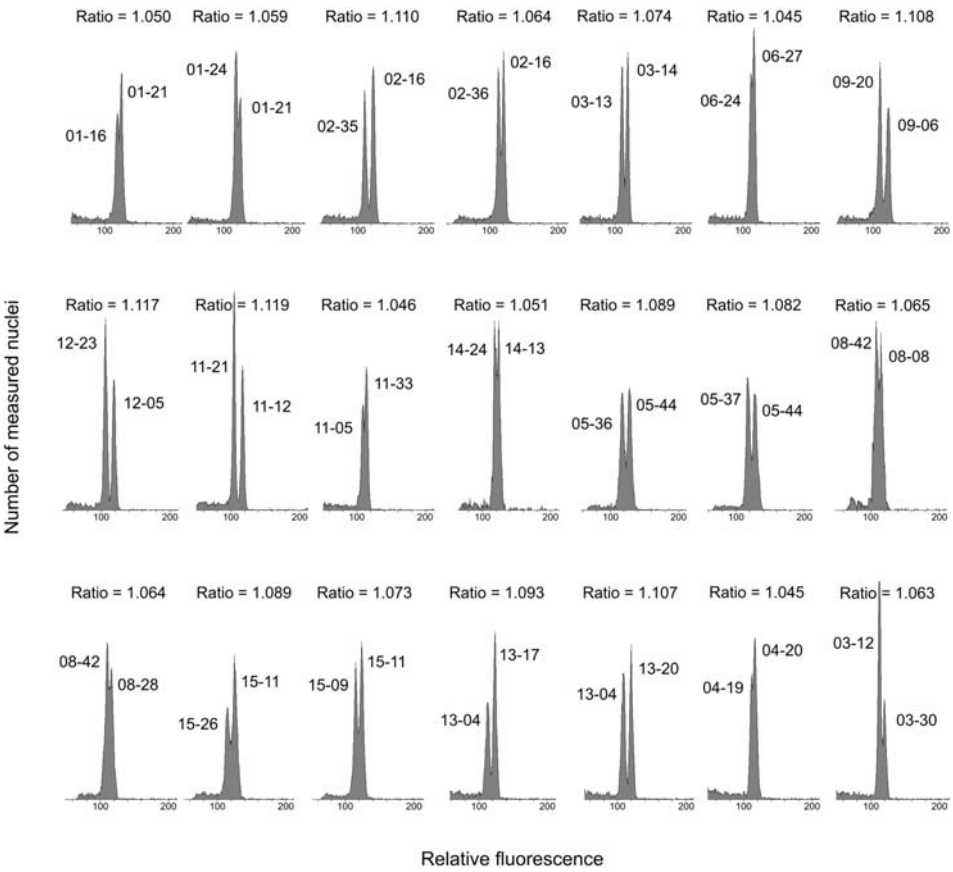


Fig. 3. – Double peaks or bimodal peaks documenting differences in genome size within the progeny of 17 *Festuca pallens* plants collected from the tetraploid population at Svatý kopeček hill. Sample designation consists of a two-digit number indicating the maternal plant, followed by a two-digit number indicating the seedling. The ratio of the sizes of the two peaks is also given (adopted from Šmarda et al. 2008b).

data). These findings support the assumption of non-adaptivity of genome size in the initial stage of genome size diversification (Gregory 2001, Bennetzen et al. 2005) and indicate that genome size divergence in closely related species could be forced or directed by other selectively important features (P. Šmarda et al., unpublished data).

The research into the dynamics and heritability of the genome size of plants from the Svatý kopeček hill revealed a high variation in the genome size in the offspring of a single plant (up to 1.119-fold), and even within seedlings grown from seeds collected from a single panicle (up to 1.117-fold; Šmarda et al. 2008b, Fig. 3). The genome sizes of the offspring generally correlate with the genome size of their mothers and the shift from the mothers' genome size towards the population mean is determined by the available spectrum of paternal genomes (supposing that the distribution of genome sizes of the pollen grains is similar to that of the whole plant population, with the maximum frequency at the median genome size; Šmarda et al. 2008b). The statistical distribution of genome sizes of

seedlings is generally similar to that for the population of adult plants. However, the seedlings differ from the adult plants, since they have both smaller and larger genome sizes, and therefore have a significantly larger range of variation in genome size (up to 1.188-fold). This difference is not a sampling artefact, but rather an effect of stabilizing selection on genome size during seedling development (Šmarda et al. 2008b, P. Šmarda et al., unpublished data). Such selection may restrict the variation in genome size that is generated within a population and thereby stabilize the genome size within species (Šmarda et al. 2008b).

Repeated measurements of plants of *Festuca pallens* of known chromosome number indicate that variation in genome size is not caused by karyological instability, such as aneuploidy or the presence of B chromosomes (Šmarda & Bureš 2006, Šmarda et al. 2008b). We assume that the main reasons for the differences is that the amount of non-coding DNA and possibly also the activity of transposable elements varies (Šmarda & Bureš 2006, Šmarda et al. 2007, 2008a).

The high variation in genome size found even within the progeny of a single plant from the Svatý kopeček site (Šmarda et al. 2008b) indicates that variation in genome size in a population may be generated very quickly. In a panmictic population that varies in genome size this is partly caused by the differences in the genome sizes of the parental plants. Nevertheless, the progeny of such crosses should theoretically have a genome size equal to the mean of that of the parents and such a process would necessarily lead to the fast elimination of extreme genome sizes and restrict the variation within a population in few generations. In *F. pallens*, in contrast, the variation in the progeny is even higher than in the population of mature plants (Šmarda et al. 2008b) and even crosses between plants with very similar genome sizes resulted in progeny with variable genome sizes, sometimes even exceeding genome sizes of both parents (P. Šmarda et al., unpublished data). This indicates that substantial variation is generated during gametogenesis. In addition to the possible induction of this variation at the molecular level (ectopic recombination, retrotransposon proliferation/elimination), it is more likely the result of the segregation of homeologous chromosomes of different sizes. The existence of differences in the size of homeologous chromosomes is a logical consequence of crossing plants with different genome sizes, as is shown by crossing related *Lolium* (Gupta & Rees 1975, Hutchinson et al. 1979) and the *Festuca scariosa* × *F. drymeja* hybrid (Jenkins & Rees 1983) and must be the case in the panmictic population of *F. pallens* in which the genome sizes are very variable. This mechanism may in theory conserve generated variation and ensure its further accumulation, even when other molecular mechanisms act very slowly. If this is common in *F. pallens,* then it would account for the frequent polytopic occurrence of intrapopulation variation in genome size. The long-term accumulation and generation of variation may be one of the reasons for the higher frequency of large genomes in relict, long surviving populations (Šmarda et al. 2008b).

Future perspectives and challenges

The understanding of intraspecific variation in genome size is still limited by the accuracy of flow cytometry measurements, as only differences larger than approximately 1.04-fold can be unambiguously detected as double peaks (Doležel & Göhde 1995). Smaller differ-

ences may be detected by repeated measurements, but these results may be substantially biased by the physiological status of the plants and the presence of metabolic compounds that affect DNA staining (see above). Although such evidence cannot be generally taken as false, it should be carefully considered. Due to the inaccuracy of the measurements, the intraspecific variation in genome size so far recorded may only be the most striking and exceptional variation. As shown by the pilot study of *Festuca pallens* over its whole distribution range, statistically significant variation in genome size occurs in almost all the populations (Šmarda & Bureš 2006). Therefore, at least in *F. pallens,* genome size diversification is a polytopic process. At the same time, it seems that the existence of intraspecific variation in genome size is species-specific rather than a direct consequence of local ecological conditions; however, the ecological conditions and population dynamics may influence the extent of the variation.

Reliable detection of very small differences in genome size and knowledge of intrapopulation genome size dynamics is essential for understanding the early phases of the diversification of genome size among related species (see also Loureiro et al. 2010). This understanding will facilitate the detection of the effects of changes in genome size, which may help with the critical interpretation of the evolution of genome size among larger phylogenetic units where the pattern was preciously attributed to unknown historical aspects and evolutionary events. Among the most challenging questions to be addressed in the future are: How quickly and frequently is variation in genome size being generated within a population? Are the processes that generate variation spatio-temporally restricted? What are the selection forces restricting the generation of variation in genome size within a natural population and a species? What proportion of the variation actually generated in plants is restricted by selection? Is the activity of molecular processes confined to particular ontogenetic or developmental stages of an organism? Will it be possible to measure the intra-individual genome size variation assumed to exist in *Microseris* (Price et al. 1983), which may be especially important in clonal and long-lived species? What proportion of the variation is generated de novo and passively maintained in a population by the segregation of homeologous chromosomes of different sizes, as in *Lolium* hybrids (Gupta & Rees 1975, Hutchinson et al. 1979) and also assumed to occur in *Festuca pallens* (Šmarda et al. 2008b)? Is the variation in genome size influenced by the age and size of a population?

In addition to improving our knowledge of intrapopulation genome size dynamics, another important topic for future studies could be the extent of intraspecific variation in genome size in taxa from different phylogenetic units and whether this variation is related to phylogeny. Multiple reports of intraspecific variation in genome size in advanced fine-leaved fescues (*Festuca* subgen. *Festuca*; Šmarda 2006) together with the tendency of genome size to decrease during the evolution of advanced taxa within *Festuca* (Šmarda et al. 2008a) indicate that high intraspecific variation in genome size may occur particularly in young and rapidly diverging species groups. A greater intraspecific variation is likely to be recorded in frequently hybridizing genera, as in *Lolium* hybrids (discussed above) or more recently in those of *Cirsium* (Bureš et al. 2004, P. Bureš et al., unpublished data). Some phylogenetic restriction of intraspecific variation in genome size may also be assumed, based on the fact that the most convincing evidence of this phenomenon is provided by studies on grasses; however, this may also be due to the over-representation of the grass family in such studies.

Conclusions

The variation in genome size observed within a population and species depends on (i) the extent and timing of its induction, (ii) the mechanisms by which genome size is inherited, and (iii) the intensity and direction of the selection. Although all three phases are documented for some model species, the current data is mainly descriptive and insufficient for the detailed understanding of the dynamics of genome size within species and populations.

The gradual variation in genome size within species is chiefly due to differences in the content of repetitive DNA, caused mainly by the dynamics of transposable elements, as reported for example in *Hordeum spontaneum* (Kalendar et al. 2000) and maize (Messing et al. 2004). The differences in the dynamics of transposable elements are also considered to be the main reason for the variation in genome size among species (Bennetzen et al. 2005). The variation in genome size within species may be induced or maintained polytopically (cf. Šmarda & Bureš 2006). Intraspecific variation may be more frequent in groups of young radiating species, however, its correlation with phylogeny is unknown.

The variation in genome size within a population seems to be restricted by selection, which generally stabilizes genome size within species (Šmarda et al. 2008b, P. Šmarda et al., unpublished data). The long-term persistence of variation in genome size and its further gradual accumulation may be brought about by the production of variable gametes resulting from the segregation of unequally sized homeologous chromosomes at meiosis, as documented in *Lolium* hybrids (Gupta & Rees 1975, Hutchinson et al. 1979) and assumed in *Festuca pallens* (Šmarda et al. 2008b).

The processes generating the variation in genome size may be, in principle, associated with unpredictable phenotypic variation; e.g. mosaic colour of maize seeds (McClintock 1950, 1951, 1953), floral traits in *Silene* (Meagher et al. 2005, Meagher & Vassiliadis 2005), or morphological characters in *Lolium* (Sugyiama et al. 2002).

Over large geographical scales and across contrasting environmental gradients, the geographic distribution of genome sizes within species may be influenced by the nucleotype effect disadvantaging larger genomes in time-limited environments at higher latitudes and altitudes, which are limited by the duration of the period with favourable temperatures, or in very dry habitats. However, the small differences in genome size within species seem generally to be of minor importance compared to other components of plant fitness that may be selectively favourable under particular environmental or habitat conditions. Genome size divergence in closely related species, therefore, could be forced or directed also by other selectively important features (P. Šmarda et al., unpublished data). Besides adaptive evolution, the spatio-temporal variation in genome size within an ancestral species also allows for a rapid repeated divergence in the genome size of related species by random drift (founder effect, bottleneck effect), for instance during range fragmentation, hybridization and/or polyploidization (Šmarda et al. 2008b).

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Souhrn

Vnitrodruhová variabilita ve velikosti genomu je zajímavý fenomén, který umožňuje studovat právě probíhající procesy evoluce velikosti genomu. Ačkoliv bylo dosud na téma vnitrodruhové variability ve velikosti genomu publikováno více než 200 prací, naše znalosti tohoto fenoménu jsou stále velmi omezené, zejména kvůli řadě metodických nepřesností dřívějších prací podávajících zkreslené nebo zcela nepravdivé údaje o celkovém rozsahu pozorované variability. V tomto článku přinášíme přehled o současném pokroku ve znalostech časové a prostorové dynamiky vnitrodruhové variability způsobené graduálními mutačními procesy. Diskutujeme zde několik případových studií *Microseris douglasii*, *Zea mays*, *Silene latifolia*, *Hordeum spontaneum*, hybridů v rodě *Lolium* a zvláště pak výsledky našich studií *Festuca pallens*. Graduální variabilita ve velikosti genomu u druhů je zapříčiněna převážně rozdíly v obsahu repetitivní DNA, zejména pak jako důsledek dynamiky mobilních genetických elementů. Tato variabilita může být indukována a udržována polytopně. Domníváme se, že je patrně častější ve skupinách mladých a radiujících druhů. Variabilita ve velikosti genomu generovaná v populacích je už v raných stádiích omezena stabilizující selekcí, která přispívá ke stabilizaci velikosti genomu druhů. Dlouhodobé udržení variability v populaci a akumulace dalších rozdílů mohou být usnadněny existencí gamet s různě velkým genomem, vznikajících při segregaci nestejně velkých homeologních chromozomů. Napříč větším územím a na různě kontrastních ekologických gradientech může být v rámci druhu rozšíření různě velkých genomů ovlivněno nukleotypovým efektem, předpokládajícím větší úspěšnost menších genomů ve vyšších nadmořských výškách a zeměpisných šířkách a ve stresujících podmínkách. Malé rozdíly ve velikosti genomu pozorované v rámci druhu se však obecně zdají být zanedbatelné vůči dalším složkám individuální fitness, které mohou být za určitých přírodních a stanovištních podmínek selekčně výhodnější. Procesy generující variabilitu ve velikosti genomu mohou být obecně doprovázeny fenotypovou variabilitou. Zatímco posun velikosti genomu populace v důsledku selekce umožňuje u nově vznikajícího druhu adaptivní evoluci velikosti genomu, časová a prostorová variabilita ve velikosti genomu ancestrálního druhu sama o sobě umožňuje rychlý a opakovaný vznik druhů s rozdílnou velikostí genomu v důsledku náhodného driftu (efekt zakladatele, efekt hrdla láhve) při rozpadu areálu a hybridizačních či polyploidizačních událostech.

References

Achigan-Dako E. G., Fuchs J., Ahanchede A. & Blattner F. R. (2008): Flow cytometric analysis in *Lagenaria siceraria* (*Cucurbitaceae*) indicates correlation of genome size with usage types and growing elevation. – Plant Syst. Evol. 276: 9–19.

Ananiev E. V., Phillips R. L. & Rines H. W. (1998): A knob-associated tandem repeat in maize capable of forming fold-back DNA segments: are chromosome knobs megatransposons? – Proc. Natl. Acad. Sci. USA 95: 10785–10790.

Baranyi M. & Greilhuber J. (1995): Flow cytometric analysis of genome variation in cultivated *Pisum sativum* (*Fabaceae*). – Plant Syst. Evol. 194: 231–239.

Baranyi M. & Greilhuber J. (1996): Flow cytometric and Feulgen densitometric analysis of genome size variation in *Pisum*. – Theor. Appl. Genet. 92: 297–307.

Bennett M. D. (1971): Duration of meiosis. – Proc. R. Soc. London, ser. B, Biol. Sci. 178: 277–299.

Bennett M. D. (1972): Nuclear DNA content and minimum generation time in herbaceous plants. – Proc. R. Soc. London, ser. B, Biol. Sci. 181: 109–135.

Bennett M. D. (1973): Nuclear characters in plants. – Brookhaven Symp. Biol. 25: 344–366.

Bennett M. D., Price H. J. & Johnston J. S. (2008): Anthocyanin inhibits propidium iodide DNA fluorescence in *Euphorbia pulcherrima*: implications for genome size variation and flow cytometry. – Ann. Bot. 101: 777–790.

Bennetzen J. L., Ma J. & Devos K. M. (2005): Mechanisms of recent genome size variation in flowering plants. – Ann. Bot. 95: 127–132.

Biradar D. P., Bullock D. G. & Rayburn A. L. (1994): Nuclear DNA amount, growth, and yield parameters in maize. – Theor. Appl. Genet. 88: 557–560.

Biradar D. P. & Rayburn A. L. (1993a): Heterosis and nuclear DNA content in maize. – Heredity 71: 300–304.

Biradar D. P. & Rayburn A. L. (1993b): Intraplant nuclear DNA content variation in diploid nuclei of maize (*Zea mays* L.). – J. Exp. Bot. 44: 1039–1044.

Biradar D. P. & Rayburn A. L. (1994): Flow cytometric probing of chromatin condensation in maize diploid nuclei. – New Phytol. 126: 31–35.

Bullock D. & Rayburn A. (1991): Genome size variation in southwestern US Indian maize populations may be a function of effective growing season. – Maydica 36: 247–250.

Bureš P., Wang Y. F., Horová L. & Suda J. (2004): Genome size variation in Central European species of *Cirsium* (*Compositae*) and their natural hybrids. – Ann. Bot. 94: 353–363.

Castro-Jimenez Y., Newton R. J., Price H. J. & Halliwell R. S. (1989): Drought stress responses of *Microseris* species differing in nuclear DNA content. – Amer. J. Bot. 76: 789–795.

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

Cavalier-Smith T. (1985): Cell volume and the evolution of eukaryotic genome size. – In: Cavalier-Smith T. (ed.), The evolution of genome size, p. 105–184, John Wiley & Sons, Chichester.

Cavallini A. & Natali L. (1990): Nuclear-DNA variability within *Pisum sativum* (*Leguminosae*): cytophotometric analyses. – Plant Syst. Evol. 173: 179–185.

Cavallini A. & Natali L. (1991): Intraspecific variation of nuclear DNA content in plant species. – Caryologia 44: 93–107.

Cavallini A. & Natali L. (1994): Chromosome endoreduplication variability in cultivars of *Pisum sativum* L. and its relationship to basic genome size. – Cytobios 79: 181–188.

Cavallini A., Natali L., Cionini G. & Gennai D. (1993): Nuclear DNA variability within *Pisum sativum* (*Leguminosae*) – nucleotypic effects on plant growth. – Heredity 70: 561–565.

Cavallini A., Natali L., Giordani T., Durante M. & Cionini P. G. (1996): Nuclear DNA changes within *Helianthus annuus* L.: variations in the amount and methylation of repetitive DNA within homozygous progenies. – Theor. Appl. Genet. 92: 285–291.

Cavallini A., Zolfino C., Cionini G., Cremonini R., Natali L., Sassoli O. & Cionini P. G. (1986): Nuclear DNA changes within *Helianthus annuus* L.: cytophotometric, karyological and biochemical analyses. – Theor. Appl. Genet. 73: 20–26.

Cavallini A., Zolfino C., Natali L., Cionini G. & Cionini P. G. (1989): Nuclear DNA changes within *Helianthus annuus* L.: origin and control mechanism. – Theor. Appl. Genet. 77: 12–16.

Costich D. E., Meagher T. R. & Yurkow E. J. (1991): A rapid means of sex identification in *Silene latifolia* by use of flow cytometry. – Plant Mol. Biol. Reporter 9: 359–370.

Devos K. M., Brown J. K. M. & Bennetzen J. L. (2002): Genome size reduction through illegitimate recombination counteracts genome expansion in *Arabidopsis*. – Genome Res. 12: 1075–1079.

Doležel J. & Bartoš J. (2005): Plant DNA flow cytometry and estimation of nuclear genome size. – Ann. Bot. 95: 99–110.

Doležel J. & Göhde W. (1995): Sex determination in dioecious plants *Melandrium album* and *M. rubrum* using high resolution flow cytometry. – Cytometry 19: 103–106.

Doležel J., Greilhuber J. & Suda J. (2007): Flow cytometry with plants: an overview. – In: Doležel J., Greilhuber J. & Suda J. (eds), Flow cytometry with plant cells, p. 41–65, Willey-VCH, Weinheim.

Durrant A. & Jones T. W. A. (1971): Reversion of induced changes in amount of nuclear DNA in *Linum*. – Heredity 27: 431–439.

Edwards G. A. & Endrizzi J. L. (1975): Cell size, nuclear size and DNA content relationships in *Gossypium*. – Can. J. Genet. Cytol. 17: 181–186.

Eilam T., Anikster Y., Millet E., Manisterski J., Sagi-Assif O. & Feldman M. (2007): Genome size and genome evolution in diploid *Triticeae* species. – Genome 50: 1029–1037.

Evans G. M. (1968): Nuclear changes in flax. – Heredity 23: 25–38.

Evans G. M., Durrant A. & Rees H. (1966): Associated nuclear changes in the induction of flax genothrophs. – Nature 212: 697–699.

Evans G. M. & Rees H. (1971): Mitotic cycles in Dicotyledons and Monocotyledons. – Nature 233: 350–351.

Furuta Y., Nishikawa K. & Makino T. (1975): Intraspecific variation of nuclear DNA content in *Aegilops squarrosa* L. – Jap. J. Genet. 50: 257–263.

Galbraith D. W., Harkins K. R., Maddox J. M., Ayres N. M., Sharma D. P. & Firoozabady E. (1983): Rapid flow cytometric analysis of the cell cycle in intact plant tissues. – Science 220: 1049–1051.

Graham M. J., Nickell C. D. & Rayburn A. L. (1994): Relationship between genome size and maturity group in soybean. – Theor. Appl. Genet. 88: 429–432.

Greenlee J. K., Rai K. S. & Floyd A. D. (1984): Intraspecific variation in nuclear DNA content in *Collinsia verna* Nutt. (*Scrophulariaceae*). – Heredity 52: 235–242.

Gregory T. R. (2001): Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. – Biol. Rev. 76: 65–101.

Greilhuber J. (1986): Severely distorted Feulgen DNA amounts in *Pinus* (*Coniferophytina*) after nonadditive fixations as a result of meristematic self-tanning with vacuole contents. – Can. J. Genet. Cytol. 28: 409–415.

Greilhuber J. (1988): Self-tanning: a new and important source of stoichiometric error in cytophotometric determination of nuclear DNA content in plants. – Plant. Syst. Evol. 158: 87–96.
Greilhuber J. (1998): Intraspecific variation in genome size: a critical reassessment. – Ann. Bot. 82/Suppl. A: 27–35.
Greilhuber J. (2005): Intraspecific variation in genome size in angiosperms: identifying its existence. – Ann. Bot. 95: 91–98.
Greilhuber J. (2008): Cytochemistry and C-values: the less-well-known world of nuclear DNA amounts. – Ann. Bot. 101: 791–804.
Greilhuber J. & Ebert I. (1994): Genome size variation in *Pisum sativum*. – Genome 37: 646–655.
Greilhuber J. & Obermayer R. (1997): Genome size and maturity group in *Glycine max* (soybean). – Heredity 78: 547–551.
Greilhuber J. & Speta F. (1985): Geographical variation of genome size at low taxonomic levels in *Scilla bifolia* alliance (*Hyacinthaceae*). – Flora 176: 431–438.
Greilhuber J., Temsch E. M. & Loureiro J. C. M. (2007): Nuclear DNA content measurement. – In: Doležel J., Greilhuber J. & Suda J. (eds), Flow cytometry with plant cells, p. 67–101, Willey-VCH, Weinheim.
Grover C. E., Kim H. R., Wing R. A., Paterson A. H. & Wendel J. F. (2004): Incongruent patterns of local and global genome size evolution in cotton. – Gen. Res. 14: 1474–1482.
Gupta P. K. & Rees H. (1975): Tolerance of *Lolium* hybrids to quantitative variation in nuclear DNA. – Nature 257: 587–588.
Heller F. O. (1973): DNA-Bestimmungen an Keimwurzellen von *Vicia faba* L. mit Hilfe der Impulszytophotometrie. – Ber. Deutsch. Bot. Ges. 86: 437–441.
Hutchinson J., Rees H. & Seal A. G. (1979): An assay of the activity of supplementary DNA in *Lolium*. – Heredity 43: 411–421.
Jenkins G. (1985): Synaptonemal complex formation in hybrids of *Lolium temulentum* × *Lolium perenne* (L.). – Chromosoma 92: 81–88.
Jenkins G. & Rees H. (1983): Synaptonemal complex formation in a *Festuca* hybrid. – In: Brandham P. E. & Bennett M. D. (eds), Kew chromosome conference, Vol 2, p. 233–242, Allen & Unwin, London.
Joarder I. O., Alsaheal Y., Begum J. & Durrant A. (1975): Environments inducing changes in amount of DNA in flax. – Heredity 34: 247–253.
Jones R. N. & Rees H. (1982): B chromosomes. – Academic Press, New York & London.
Kalendar R., Tanskanen J., Immonen S., Nevo E. & Schulman A. H. (2000): Genome evolution of wild barley (*Hordeum spontaneum*) by *BARE-1* retrotransposon dynamics in response to sharp microclimatic divergence. – Proc. Natl. Acad. Sci. USA 97: 6603–6607.
Kankanpää J., Mannonen L. & Schulman A. H. (1996): The genome sizes of *Hordeum* species show considerable variation. – Genome 39: 730–735.
Kessler B. & Reches S. (1977): Structural and functional changes of chromosomal DNA during aging and phase change in plants. – Chromosomes Today 6: 237–246.
Knight C. A. & Ackerly D. D. (2002): Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. – Ecol. Lett. 5: 66–76.
Knight C. A., Molinari N. A. & Petrov D. A. (2005): The large genome constraint hypothesis: evolution, ecology and phenotype. – Ann. Bot. 95: 177–190.
König C., Ebert I. & Greilhuber J. (1987): A DNA cytophotometric and chromosome banding study in *Hedera helix* (*Araliaceae*), with reference to differential DNA replication associated with juvenile-adult phase change. – Genome 29: 498–503.
Kuwada Y. (1925): On the number of chromosomes in maize. – Bot. Mag. (Tokyo) 39: 227–234.
Lamb J. C., Meyer J. M., Corcoran B., Kato A., Han F. P. & Birchler J. A. (2007): Distinct chromosomal distributions of highly repetitive sequences in maize. – Chromosome Res. 15: 33–49.
Lang G. (1994): Quartäre Vegetationsgeschichte Europas. – Gustav Fischer, Jena.
Laurie D. A. & Bennett M. D. (1985): Nuclear DNA content in the genera *Zea* and *Sorghum*: intergeneric, interspecific and intraspecific variation. – Heredity 55: 307–313.
Leong-Škorničková J., Šída O., Jarolímová V., Sabu M., Fér T., Trávníček P. & Suda J. (2007): Chromosome numbers and genome size variation in Indian species of *Curcuma* (*Zingiberaceae*). – Ann. Bot. 100: 505–526.
Lohwasser U., Granda A. & Blattner F. R. (2004): Phylogenetic analysis of *Microseris* (*Asteraceae*), including a newly discovered Andean population from Peru. – Syst. Bot. 29: 774–780.
Longley A. E. (1927): Supernumerary chromosomes in *Zea mays*. – J. Agric. Res. 35: 769–784.
Longley A. E. (1938): Chromosomes of maize from North American Indians. – J. Agric. Res. 56: 177–195.

Loureiro J., Rodriguez E., Doležel J. & Santos C. (2006): Flow cytometric and microscopic analysis of the effect of tannic acid on plant nuclei and estimation of DNA content. – Ann. Bot. 98: 515–527.
Loureiro J., Trávníček P., Rauchová J., Urfus T., Vít P., Štech M., Castro S. & Suda J. (2010): The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. – Preslia 82: 3–21.
Ma J., Devos K. M. & Bennetzen J. L. (2004): Analyses of LTR-retrotransposon structures reveal recent and rapid genomic DNA loss in rice. – Genome Res. 14: 860–869.
McClintock B. (1929): Chromosome morphology in *Zea mays*. – Science 69: 629.
McClintock B.(1950): The origin and behavior of mutable loci in maize. – Proc. Natl. Acad. Sci. USA 36: 344–355.
McClintock B. (1951): Chromosome organization and genetic expression. – Cold Spring Harbor Symp. Quant. Biol. 16: 13–47.
McClintock B. (1953): Induction of instability at selected loci in maize. – Genetics 38: 579–599.
Meagher T. R. & Costich D. E. (1994): Sexual dimorphism in nuclear DNA content and floral morphology in populations of *Silene latifolia* (*Caryophyllaceae*). – Amer. J. Bot. 81: 1198–1204.
Meagher T. R. & Costich D. E. (1996): Nuclear DNA content and floral evolution in *Silene latifolia*. – Proc. R. Soc. London, ser. B, Biol. Sci. 263: 1455–1460.
Meagher T. R. & Costich D. E. (2004): 'Junk' DNA and long-term phenotypic evolution in *Silene* section *Elisanthe* (*Caryophyllaceae*). – Proc. R. Soc. London, ser. B, Biol. Sci. 271: S493–S497.
Meagher T. R. & Costich D. E. (2008): 'Junk' DNA and phenotypic evolution in *Silene* section *Siphonomorpha*. – Genet. Res. 90: 111–118.
Meagher T. R., Gillies A. C. M. & Costich D. E. (2005): Genome size, quantitative genetics and the genomic basis for flower size evolution in *Silene latifolia*. – Ann. Bot. 95: 247–254.
Meagher T. R. & Vassiliadis C. (2005): Phenotypic impacts of repetitive DNA in flowering plants. – New. Phytol. 168: 71–80.
Messing J., Bharti A. K., Karlowski W. M., Grundlach H., Kim H. R., Yu Y., Wei F., Fuks G., Soderlund C. A., Mayer K. F. X. & Wing R. A. (2004): Sequence composition and genome organization of maize. – Proc. Natl. Acad. Sci. USA 101: 14349–14354.
Michaelson M. J., Price H. J., Ellison J. R. & Johnston J. S. (1991a): Comparison of plant DNA contents determined by Feulgen microspectrophotometry and laser flow cytometry. – Amer. J. Bot. 78: 183–188.
Michaelson M. J., Price H. J., Johnston J. S. & Ellison J. R. (1991b): Variation of nuclear DNA content in *Helianthus annuus* (*Asteraceae*). – Amer. J. Bot. 78: 1238–1243.
Nagl W. (1979): Differential DNA replication in plants: a critical review. – Z. Pflanzenphysiol. 95: 283–314.
Nagl W., Jeanjour M., Kling H., Kuhner S., Michels I., Müller T. & Stein B. (1983): Genome and chromatin organization in higher plants. – Biol. Zentralbl. 102: 129–148.
Natali L., Cavallini A., Cionini G., Sassoli O., Cionini P. G. & Durante M. (1993): Nuclear DNA changes within *Helianthus annuus* L.: changes within single progenies and their relationship with plant development. – Theor. Appl. Genet. 85: 506–512.
Nevo E., Beiles A. & Zohary D. (1986): Genetic resources of wild barley in the Near East: structure, evolution and application in breeding. – Biol. J. Linn. Soc. 27: 355–380.
Noirot M., Barre P., Duperray C., Hamon S. & De Kochko A. (2005): Investigation on the causes of stoichiometric error in genome size estimation using heat experiments: consequences on data interpretation. – Ann. Bot. 95: 111–118.
Noirot M., Barre P., Duperray C., Louarn J. & Hamon S. (2003): Effects of caffeine and chlorogenic acid on propidium iodide accessibility to DNA: consequences on genome size evaluation in coffee tree. – Ann. Bot. 92: 259–264.
Noirot M., Barre P., Louarn J., Duperray C. & Hamon S. (2000): Nucleus-cytosol interactions: a source of stoichiometric error in flow cytometric estimation of nuclear DNA content in plants. – Ann. Bot. 86: 309–316.
Ohri D. & Singh S. P. (1998): Genome size variation and plant systematics. – Ann. Bot. 82, Suppl. A: 75–83.
Peacock W. J., Dennis E. S., Rhoades M. M. & Pryor A. J. (1981): Highly repeated DNA sequence limited to knob heterochromatin in maize. – Proc. Natl. Acad. Sci. USA 78: 4490–4494.
Pecinka A., Suchánková P., Lysak M. A., Trávníček B. & Doležel J. (2006): Nuclear DNA content variation among Central European *Koeleria* taxa. – Ann. Bot. 98: 117–122.
Petrov D. A., Sangster T. A., Johnston J. S., Hartl D. L. & Shaw K. L. (2000): Evidence for DNA loss as a determinant of genome size. – Science 287: 1060–1062.
Pils G. (1981): Karyologie und Verbreitung von *Festuca pallens* Host in Österreich. – Linzer Biol. Beitr. 13: 231–241.

Poggio L., Rosato M., Chiavarino A. M. & Naranjo C. A. (1998): Genome size and environmental correlations in maize (*Zea mays* ssp. *mays*, *Poaceae*). – Ann. Bot. 82, Suppl. A: 107–115.

Polito V. S. & Alliatta V. (1981): Growth of calluses derived from shoot apical meristems of adult and juvenile English ivy (*Hedera helix* L.). – Plant Sci. Lett. 22: 387–393.

Porter H. C. & Rayburn A. L. (1990): B-chromosome and C-band heterochromatin variations in Arizona maize populations adapted to different altitudes. – Genome 33: 659–662.

Price H. J., Bachmann K., Chambers K. L. & Riggs J. (1980): Detection of intraspecific variation in nuclear DNA content in *Microseris douglasii*. – Bot. Gaz. 141: 195–198.

Price H. J., Chambers K. L. & Bachmann K. (1981a): Geographic and ecological distribution of genomic DNA content variation in *Microseris douglasii* (*Asteraceae*). – Bot. Gaz. 142: 415–426.

Price H. J., Chambers K. L. & Bachmann K. (1981b): Genome size variation in diploid *Microseris bigelovii* (*Asteraceae*). – Bot. Gaz. 142: 156–159.

Price H. J., Chambers K. L., Bachmann K. & Riggs J. (1983): Inheritance of nuclear 2C DNA content variation in intraspecific and interspecific hybrids of *Microseris* (*Asteraceae*). – Amer. J. Bot. 70: 1133–1138.

Price H. J., Chambers K. L., Bachmann K. & Riggs J. (1986): Patterns of mean nuclear DNA content in *Microseris douglasii* (*Asteraceae*). – Bot. Gaz. 147: 496–507.

Price H. J., Hodnett G. & Johnston J. S. (2000): Sunflower (*Helianthus annuus*) leaves contain compounds that reduce nuclear propidium iodide fluorescence. – Ann. Bot. 86: 929–934.

Price H. J. & Johnston J. S. (1996): Influence of light on DNA content of *Helianthus annuus*. – Proc. Natl. Acad. Sci. USA 93: 11264–11267.

Price H. J., Morgan P. W. & Johnston J. S. (1998): Environmentally correlated variation in 2C nuclear DNA content measurements in *Helianthus annuus* L. – Ann. Bot. 82 (Suppl. A): 95–98.

Rayburn A. L. (1990): Genome size variation in southwestern Indian maize adapted to various altitudes. – Evol. Trends Plants 4: 53–57.

Rayburn A. L. & Auger J. A. (1990): Genome size variation in *Zea mays* subsp. *mays* adapted to different altitudes. – Theor. Appl. Genet. 79: 470–474.

Rayburn A. L., Biradar D. P., Bullock D. G. & McMurphy L. M. (1993): Nuclear DNA content in F_1 hybrids of maize. – Heredity 70: 294–300.

Rayburn A. L., Biradar D. P., Bullock D. G., Nelson R. L., Gourmet C. & Wetzel J. B. (1997): Nuclear DNA content diversity in Chinese soybean introductions. – Ann. Bot. 80: 321–325.

Rayburn A. L., Price H. J., Smith J. D. & Gold J. R. (1985): C-band heterochromatin and DNA content in *Zea mays*. – Amer. J. Bot. 72: 1610–1617.

Rees H. & Jones G. H. (1967): Chromosome evolution in *Lolium*. – Heredity 22: 1–18.

Rosato M., Chiavarino A. M., Naranjo C. A., Camara Hernandez J. & Poggio L. (1998): Genome size and numerical polymorphism for the B chromosome in races of maize (*Zea mays* ssp. *mays*, *Poaceae*). – Amer. J. Bot. 85: 168–174.

SanMiguel P. & Bennetzen J. L. (1998): Evidence that a recent increase in maize genome size was caused by the massive amplification of intergene retrotransposons. – Ann. Bot. 82: 37–44.

SanMiguel P., Gaut B. S., Tikhonov A., Nakajima Y. & Bennetzen J. L. (1998): The paleontology of intergene retrotransposons of maize. – Nature Genet. 20: 43–45.

SanMiguel P., Tikhonov A., Jin Y. K., Motchoulskaia N., Zakharov D., Melake-Berhan A., Springer P. S., Edwards K. J., Lee M. & Avramova Z. (1996): Nested retrotransposons in the intergenic regions of the maize genome. – Science 274: 765–768.

Schäffner K-H. & Nagl W. (1979): Differential DNA replication involved in transition from juvenile to adult phase in *Hedera helix* (*Araliaceae*). – Plant Syst. Evol./Suppl. 2: 105–110.

Schönswetter P., Suda J., Popp M., Weiss-Schneeweiss H. & Brochmann C. (2007): Circumpolar phylogeography of *Juncus biglumis* (*Juncaceae*) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. – Molec. Phylogen. Evol. 42: 92–103.

Singh K. P., Raina S. N. & Singh A. K. (1996): Variation in chromosomal DNA associated with the evolution of *Arachis* species. – Genome 39: 890–897.

Slovák M., Vít P., Urfus T. & Suda J. (2009): Complex pattern of genome size variation in a polymorphic member of the *Asteraceae*. – J. Biogeogr. 36: 372–384.

Šmarda P. (2006): DNA ploidy levels and intraspecific DNA content variability in Romanian fescues (*Festuca* L., *Poaceae*), measured in fresh and herbarium material. – Folia Geobot. 41: 417–432.

Šmarda P. & Bureš P. (2006): Intraspecific DNA content variability in *Festuca pallens* on different geographical scales and ploidy levels. – Ann. Bot. 98: 665–678.

Šmarda P., Bureš P. & Horová L. (2007b): Random distribution pattern and non-adaptivity of genome size in a highly variable population of *Festuca pallens*. – Ann. Bot. 100: 141–150.

Šmarda P., Bureš P., Horová L., Foggi B. & Rossi G. (2008a): Genome size and GC content evolution of *Festuca*: ancestral expansion and subsequent reduction. – Ann. Bot. 101: 421–433.

Šmarda P., Bureš P., Horová L. & Rotreklová O. (2008b): Intrapopulation genome size dynamics in *Festuca pallens* and the early stages of genome size evolution in plants. – Ann. Bot. 102: 599–607.

Šmarda P., Müller J., Vrána J. & Kočí K. (2005): Ploidy level variability of some Central European fescues (*Festuca* subg. *Festuca*, *Poaceae*). – Biologia 60: 25–36.

Šmarda P., Šmerda J., Knoll A., Bureš P. & Danihelka J. (2007a): Revision of Central European taxa of *Festuca* ser. *Psammophilae* Pawlus: morphometrical, karyological and AFLP analysis. – Plant. Syst. Evol. 266: 197–232.

Suda J., Krahulcová A., Trávníček P., Rosenbaumová R., Peckert T. & Krahulec F. (2007a): Genome size variation and species relationships in *Hieracium* sub-genus *Pilosella* (*Asteraceae*) as inferred by flow cytometry. – Ann. Bot. 100: 1323–1335.

Suda J., Weiss-Schneeweiss H., Tribsch A., Schneeweiss G. M., Trávníček P. & Schönswetter P. (2007b): Complex distribution patterns of di-, tetra-, and hexaploid cytotypes in the European high mountain plant *Senecio carniolicus* (*Asteraceae*). – Amer. J. Bot. 94: 1391–1401.

Sugiyama S., Yamaguchi K. & Yamada T. (2002): Intraspecific phenotypic variation associated with nuclear DNA content in *Lolium perenne* L. – Euphytica 128: 145–151.

Swift H. (1950): The constancy of desoxyribose nucleic acid in plant nuclei. – Proc. Natl. Acad. Sci. USA 36: 643–654.

Temsch E. M. & Greilhuber J. (2000): Genome size variation in *Arachis hypogaea* and *A. monticola* re-evaluated. – Genome 43: 449–451.

Temsch E. M., Greilhuber J., Hammett K. R. W. & Murray B. G. (2008): Genome size in *Dahlia* Cav. (*Asteraceae* – *Coreopsideae*). – Plant Syst. Evol. 276: 157–166.

Tischler G. (1921–1922): Allgemeine Pflanzenkaryologie. – In: Linsbauer K. (ed.), Handbuch der Pflanzenanatomie, Vol. 2/1, p. 521–683, Gebrüder Borntraeger, Berlin.

Tito C. M., Poggio L. & Naranjo C. A. (1991): Cytogenetic studies in the genus *Zea*. 3. DNA content and heterochromatin in species and hybrids. – Theor. Appl. Genet. 83: 58–64.

Turpeinen T., Kulmala J. & Nevo E. (1999): Genome size variation in *Hordeum spontaneum* populations. – Genome 42: 1094–1099.

Vagera J., Paulíková D. & Doležel J. (1994): The development of male and female regenerants by in vitro androgenesis in dioecious plant *Melandrium album*. – Ann. Bot. 73: 455–459.

Vicient C. M., Suoniemi A., Anamthawar-Jónsson K., Tanskanen J., Beharav A., Nevo E. & Schulman A. H. (1999): Retrotransposon *BARE-1* and its role in genome evolution in the genus *Hordeum*. – Plant Cell 11: 1769–1784.

Vinogradov A. E. (2003): Selfish DNA is maladaptive: evidence from the plant Red List. – Trends Genet. 19: 609–614.

Vitte C. & Bennetzen J. L. (2006): Analysis of retrotransposon structural diversity uncovers properties and propensities in angiosperm genome evolution. – Proc. Natl. Acad. Sci. USA 103: 17638–17643.

Vitte C. & Panaud O. (2003): Formation of solo-LTRs through unequal homologous recombination counterbalances amplifications of LTR retrotransposons in rice *Oryza sativa* L. – Mol. Biol. Evol. 20: 528–540.

Vitte C. & Panaud O. (2005): LTR retrotransposons and flowering plant genome size: emergence of the increase/decrease model. – Cytogenet. Gen. Res. 110: 91–107.

Walker D. J., Monino I. & Correal E. (2006): Genome size in *Bituminaria bituminosa* (L.) C. H. Stirton (*Fabaceae*) populations: separation of "true" differences from environmental effects on DNA determination. – Environm. Exp. Bot. 55: 258–265.

Zuccolo A., Sebastian A., Talag J., Yu Y., Kim H., Collura K., Kudrna D. & Wing R. A. (2007): Transposable element distribution, abundance and role in genome size variation in the genus *Oryza*. – BMC Evol. Biol. 7: 152.

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