Use of flow cytometry in research on apomictic plants

Využití průtokové cytometrie ve výzkumu apomiktických rostlin

Anna K r a h u l c o v á[1] & Olga R o t r e k l o v á[2]

[1]*Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 43 Průhonice, Czech Republic; e-mail: anna.krahulcova@ibot.cas.cz;* [2]*Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic; e-mail: orotrekl@sci.muni.cz*

Krahulcová A. & Rotreková O. (2010): Use of flow cytometry in research on apomictic plants. – Preslia 82: 23–39.

This paper reviews recent use of flow cytometry in studies on apomictic plant taxa. The most of apomictic angiosperms are polyploid, often differing in ploidy level from their sexual counterparts within the agamic complex. Flow cytometry is widely used for screening the ploidy levels of mature plants and their seed generated both in the field and in experiments. Routine ploidy screening often accompanied by molecular markers distinguishing individual genotypes are used to reveal novel insights into the biosystematics and population biology of apomictic taxa. Apomixis (asexual seed formation) is mostly facultative, operating together with other less frequent reproductive pathways within the same individual. The diversity in modes of reproduction in apomicts is commonly reflected in the ploidy structure of their progeny in mixed-cytotype populations. Thus, flow cytometry facilitates the detection and quantification of particular progeny classes generated by different reproductive pathways. The specific embryo/endosperm ploidy ratios, typical of the different reproductive pathways, result from modifications of double fertilization in sexual/apomictic angiosperms. Thus, the reproductive origin of seed can be identified, including autonomous or pseudogamous apomixis, haploid parthenogenesis and sexual reproduction, involving either reduced or unreduced gametes. Collectively, flow cytometry has been used to address the following research topics: (i) assessing the variation in ploidy levels and genome sizes in agamic complexes, (ii) detection and quantification of different reproductive modes in facultative apomicts, (iii) elucidation of processes in populations with coexisting sexual and apomictic biotypes, (iv) evolution of agamic complexes, and (v) genetic basis of apomixis. The last topic is of paramount importance to crop breeding: the search for candidate gene(s) responsible for apomixis is the main objective of many research programmes. A list of the angiosperm taxa that could provide model systems for such research is provided.

K e y w o r d s: agamic complexes, cytotype, evolution, flow cytometry, genetic control of apomixis, genome size, hybridization, mixed populations, parthenogenesis, polyploidy, progeny screening, residual sexuality

Introduction

Flow cytometry (FCM) is advancing research in various fields of biological sciences, including evolutionary and population biology, and biosystematics (recently reviewed by Kron et al. 2007). This method of analysis is fast, accurate, non-destructive and can be used to process large numbers of samples (individuals) in a day. A recent monograph (Doležel et al. 2007), which summarizes principles and applications of FCM in plant sciences, includes two chapters related to apomixis research (Matzk 2007, Suda et al. 2007b). Apomixis is an alternative pathway to sexual reproduction and is defined in flowering plants as the asexual (uniparental) formation of seed, i.e. without meiosis and fertilization (Koltunow &

Grossniklaus 2003, Bicknell & Koltunow 2004). Replicating the maternal genome in the progeny, apomixis is reflected by specific variation in morphology, karyology, ecology and distribution (Czapik 1994). Although apomixis is not as common as sexual reproduction in angiosperms, it is recorded in at least 220 genera (Matzk 2007), predominantly in the families *Asteraceae*, *Rosaceae* and *Poaceae* (Bicknell & Koltunow 2004). Nearly all cases refer to maternal apomixis whereas paternal apomixis is a unique phenomenon that is recorded in the gymnosperm *Cupressus dupreziana*; the diploid (unreduced) pollen in this species can give rise directly to paternal-like progeny (Pichot et al. 2001, 2008).

As demonstrated here, a number of challenging questions about apomictic plants can be resolved using FCM. However, conventional chromosome counts are also essential reference standards in FCM analyses. When interpreting flow cytometric histograms it is important to be aware of some pitfalls resulting from patterns in variation in the amounts of nuclear DNA in plants (Suda et al. 2006, 2007b). For instance, some closely related taxa with the same number of chromosomes can have very different genome sizes and taxa with different chromosome numbers can have the same amount of nuclear DNA. Furthermore, the DNA content of polyploid complexes in plants often does not increase proportionally with ploidy level (Leitch & Bennett 2004). Additional challenges in the interpretation of FCM results are cited in the papers of Doležel et al. (2007) and Kron et al. (2007). Image densitometry is sometimes used as an alternative to FCM for measuring DNA content (Vilhar et al. 2001, Doležel et al. 2007). However, this method is less suitable for ploidy screening of large samples of seeds because embryo and endosperm tissue need to be separated prior to analysis (Hörandl et al. 2008).

This review attempts an overview of the results of the application of flow cytometry in studies on apomixis and summarizes progress in studies on biosystematics, ecology and evolution of apomictic plants. We describe promising research directions, list relevant publications showing benefits of FCM and introduce apomictic taxa that serve as model systems.

Structure of agamic complexes: variation in ploidy level and genome size

One of the research areas that has greatly benefited from flow cytometry is plant biosystematics. Flow cytometry is the method of choice for investigating closely related taxa characterized by specific nuclear DNA contents. This specifity is caused either by polyploidy, or by differences in the monoploid genome size (Cx-value according to Greilhuber et al. 2005), which often varies among taxa in a genus or subgenus. Such studies usually determine the structure of the complex and the phylogenetic relationships among the taxa involved, irrespective of their mode of reproduction (i.e. sexual or apomictic). As apomictic plants are largely polyploid (with only a few exceptions; see Asker & Jerling 1992, Savidan 2007), the agamic (apomictic) complexes are particularly suitable for FCM analysis, which simplifies the detection of differences in DNA ploidy levels (terminology follows Suda et al. 2006). The resulting pattern of variation is evaluated at different geographical scales, from population, across regional and continental levels up to the entire distribution area (Table 1). Data on genetic diversity often accompany cytogeographic studies of polyploid agamic complexes (e.g. Paun et al. 2006, Kao 2008).

Table 1 summarizes papers that used flow cytometry to determine the ploidy and/or genome size variation in agamic complexes in different geographic regions and at differ-

ent spatial scales. It is not possible to draw any general conclusions about the distribution of apomictic/sexual cytotypes from this data, however, it does indicate that the distribution pattern may be quite complex even at the regional scale (e.g. Mráz et al. 2008).

The variation in nuclear DNA content detected using FCM may be due to polyploidy, aneuploidy (*Poa pratensis*; Speckmann & van Dijk 1972), different genome sizes of the parental taxa of the hybrid genome (*Hieracium* subgen. *Pilosella*; Suda et al. 2007a) or the presence of B chromosomes (*Boechera holboellii* complex; Sharbel et al. 2005). In the latter case, it is likely there is an association between supernumerary elements (B chromosomes) and apomictic reproduction.

Facultative apomixis: determination and quantification of reproductive pathways and expression of apomixis

Versatility of reproduction

Facultatively apomictic plants, either open-pollinated in mixed-cytotype populations or experimentally crossed, are able to form heteroploid aberrant progeny by different reproductive pathways. In addition to true apomictic mother-replicating progeny (2n + 0 progeny), autonomously derived progeny can be generated by haploid parthenogenesis (n + 0, polyhaploid progeny) and/or several types of hybrids can be formed. The hybrids that result from the fusion of reduced or unreduced gametes are designated n + n, n + 2n, 2n + n or 2n + 2n, the female gamete being represented by the first symbol (Harlan & de Wet 1975). Flow cytometry provides a rapid and reliable tool for screening discrete categories of progeny as embryos, seedlings and/or mature plants have the specific ploidy level.

Experimental production of inter-cytotype crosses followed by ploidy screening of hundreds of their progeny at the seedling stage resulted in the detection and quantification of five reproductive pathways in *Hieracium* subgen. *Pilosella* (Krahulcová et al. 2004). Similarly, various reproductive modes are reported in the in vitro regenerants of *Hypericum perforatum* 'Topas' (Brutovská et al. 1998). In this study, four different regenerant cytotypes of *Hypericum* were cultivated together and allowed to pollinate each other. The ploidy categories of their seedling progeny were then screened using a combination of conventional chromosome counts and flow cytometry. While the diploid regenerants produced diploid progeny, large variation was recorded in seedling progeny of the polyploid regenerants, suggesting facultative sexuality. Using FCM analysis of seeds (see below) revealed a dominance of sexual reproduction in diploid regenerants of this *H. perforatum* cultivar, whereas the mode of reproduction in the regenerants with higher ploidy levels was very variable (Koperdáková et al. 2004). An analysis of the progeny of crosses between plants of *Rosa* sect. *Caninae* indicates they produce unreduced male and female gametes (Nybom et al. 2006). A combination of FCM and molecular markers (RAPD and microsatelite analyses) was used to infer the origin and genomic configuration in seedling progeny; a small fraction of the progeny developed apomictically (Nybom et al. 2006).

The expressivity of apomictic/sexual reproduction can be, at least in some species, modified by environmental factors and/or by particular pollen donors (Asker & Jerling 1992, Bengtsson & Ceplitis 2000). The occurrence of aberrant (i.e. mother non-replicating) progeny in repeated runs of experiments carried out under different conditions was quantified using FCM, which revealed such interference in *Brachiaria decumbens* (Naumova et al. 1999) and *Hieracium* subgen. *Pilosella* (Krahulcová et al. 2004).

Table 1. – Selected FCM studies on apomictic taxa, which record the variation in ploidy/genome size and its correlation with phylogenetic relationships among taxa and/or include the geographic distribution of individual cytotypes. The karyological data is based on FCM and chromosome counts in some cases.

Taxon (genus, species, or complex)	Family	Scope of FCM application	Geographical scale	Reference
Arnica cordifolia	*Asteraceae*	ploidy variation related to habitat differentiation	local- and broad-scale distributions of cytotypes (Central and W United States)	Kao 2008
Hieracium alpinum	Asteraceae	ploidy variation and genome size, cytogeography	continental level (Europe)	Mráz et al. 2009
Hieracium bauhini group	*Asteraceae*	variation in ploidy and reproductive mode, cytogeography	regional level (Central Europe)	Rotreklová 2004
Hieracium subgen. *Hieracium*	Asteraceae	variation in ploidy, genome size and reproductive mode	continental level (Europe)	Chrtek et al. 2009
Hieracium nigrescens group	*Asteraceae*	ploidy variation	regional level (Central Europe)	Chrtek et al. 2007
Hieracium subgen. *Pilosella*	*Asteraceae*	ploidy variation and genome size	regional level (Central and SE Europe)	Suda et al. 2007a
		ploidy variation and genome size	population level (Germany)	Bräutigam & Bräutigam 1996
		variation in ploidy and reproductive mode	regional level (Šumava Mts, Czech Republic)	Krahulec et al. 2008
		variation in ploidy and reproductive mode	continental level (Europe)	Rotreklová et al. 2005
		variation in genome size in homoploid hybrids	secondary distribution area (New Zealand)	Morgan-Richards et al. 2004
Hieracium pilosella	*Asteraceae*	ploidy variation, cytogeography	regional level (Czech Republic, Slovakia and NE Hungary)	Mráz et al. 2008
Taraxacum	*Asteraceae*	variation in genome size (representatives of sections)	distribution area	Záveský et al. 2005
Boechera (*Arabis*) *holboellii* complex	*Brassicaceae*	ploidy variation	distribution area (North America)	Sharbel & Mitchel-Olds 2001
		ploidy variation, reproductive mode	distribution area (North America)	Schranz et al. 2005
Poa	*Poaceae*	ploidy variation, reproductive mode	distribution area (NPGS collection)	Kelley et al. 2009
Poa pratensis	*Poaceae*	variation in DNA content, reproductive mode	distribution area (USDA collection)	Wieners et al. 2006
Tripsacum dactyloides	*Poaceae*	ploidy and genome size variation	continental level (North America)	Bantin et al. 2001

Ranunculus carpaticola	*Ranunculaceae*	ploidy variation	regional level (Central Slovakia)	Paun et al. 2006
Crataegus, Mespilus	*Rosaceae*	ploidy variation	continental level (North America)	Talent & Dickinson 2005
Crataegus	*Rosaceae*	reproductive mode, endosperm ploidy balance	continental level (North America)	Talent & Dickinson 2007a
Sorbus	*Rosaceae*	genome size, check on homogeneity of ploidy	population level	Lepší et al. 2008, 2009

Advance in the detection of reproductive pathways: Flow Cytometric Seed Screen

The sexual versus apomictic origin of the seed is reflected in the specific ploidy level (nuclear DNA content) of the resulting embryo and endosperm (Table 2). FCM was used to determine the ploidy level and so determine the reproductive origin of the seeds of *Tripsacum* (Grimanelli et al. 1997). Endosperm and embryo were extracted from immature seeds and their ploidy level determined separately. Apomictic as well as sexual seeds were found. In both cases, the endosperm originated via the fusion of polar nuclei with either a reduced or unreduced sperm nucleus (pseudogamy).

The development of Flow Cytometric Seed Screen (FCSS) by Matzk et al. (2000) revolutionalized the routine screening of embryonic stages as a means of determining the reproductive pathways in angiosperms. This method exploits double fertilization and provides an easy and convenient way of determining the different modes of reproduction. Unlike the time consuming conventional embryological procedures, FCSS is based on the determination of the embryo/endosperm ploidy level ratio in mature seed, which corresponds to a particular reproductive pathway (for details see Matzk et al. 2000, Matzk 2007). FCSS not only reliably discriminates between sexually versus apomictically produced seed, but between different mechanisms of apomictic reproduction (autonomous vs pseudogamous endosperm development, true apomixis vs haploid parthenogenesis) and gamete characteristics (whether reduced or unreduced) (Hörandl et al. 2008, Table 2, Fig. 1). Compared to the FCM screening of seedlings, which only discriminates between different ploidy categories, the FCSS method discriminates between embryos produced apomictically or sexually, irrespective of their ploidy level. A potential problem is seed that contain a small non-maternal embryo. In such cases, the embryo peak may not be visible in the flow histogram because of the paucity of embryo nuclei, while the peak associated with the maternal seed coat may be visible. The interpretation of FCSS histograms may also be complicated by the occurrence of a peak corresponding to the G_2 phase of endosperm nuclei or by endopolyploidy. Furthermore, additional ploidy variation may occur in the endosperm of pseudogamous plants, when either one or both pollen nuclei are involved in the formation of endosperm.

If a sufficient number of seeds are processed, the quantification of particular progeny classes (corresponding to different reproductive pathways) is possible using FCSS based on the analysis of a single-seed (see e.g. *Hypericum perforatum*; Pank et al. 2003). It is possible to substitute for this rather laborious and time-consuming procedure a slightly modified protocol that simultaneously analyses several seeds. For example, FCM acquisitions using pairs of seeds were successfully performed on *Hieracium bauhini* (Fig. 1;

Krahulcová et al. 2009). Here, seeds of the pentaploid apomictic cytotype co-occurring in a mixed-ploidy, sexual-apomictic natural population were analyzed, and the following reproductive pathways identified: true apomixis with autonomous endosperm development, haploid parthenogenesis, and sexual reproduction via both reduced and unreduced female gametes (Fig. 1). To further simplify the screening and quantification of the different ploidy categories in embryos in large batches of seed, pooled seed samples can be used, as documented by Krahulcová & Suda (2006) who simultaneously analyzed 10 seeds. The accuracy of this modified FCSS method was confirmed by experimental analyses of seed mixtures with different frequencies of particular cytotypes of fully apomictic species of *Hieracium* subgen. *Hieracium*. Generally, the efficiency of pooled-seed analyses depends on the size of the seed and the embryo. Further studies aimed at determining the sensitivity and the resolution threshold of pooled-seed FCM acquisitions are needed.

Using FCSS has revealed the existence of apomixis in a number of taxa, including species in the genera *Allium*, *Arabis*, *Hieracium*, *Hypericum*, *Panicum*, *Paspalum*, *Pennisetum*, *Poa*, *Potentilla*, *Ranunculus*, *Taraxacum* and *Tripsacum* (Matzk et al. 2000). Soon after the details of this method were published FCSS was established in plant laboratories worldwide and the number of plant groups analyzed increased dramatically. Recently the reproductive pathways in, for example, *Paspalum simplex* (Cáceres et al. 2001, Sartor et al 2009), *Tripsacum dactyloides* (Bantin et al. 2001), *Arabis gunnisoniana* (Taskin et al. 2004), *Crataegus* spp. (Talent & Dickinson 2007c), *Hypericum dubium* (Mártonfi 2008) and *Ranunculus* (Hörandl et al. 2008, 2009) have been determined. In the grass *Tripsacum dactyloides*, there are an enormous diversity of reproductive pathways in the tetraploid cytotype, including sexual reproduction (with reduced and unreduced egg and sperm cells) and pseudogamous apomixis (with endosperm originating via reduced or unreduced sperm cells). Seeds with twin embryos of different origin (sexual and apomictic) have also been found (Bantin et al. 2001). Similarly, versatility in the mode of reproduction is repeatedly documented for *Hypericum perforatum*, including (i) apomixis with both autonomous and pseudogamous development of the endosperm, (ii) haploid parthenogenesis with pseudogamous endosperm development from both reduced and unreduced

Table 2. – C-values of unreplicated embryo and endosperm nuclei that originated either from reduced or unreduced gametes. The 2C-value corresponds to the DNA content of the unreplicated, non-reduced chromosome complement, irrespective of the ploidy level. Following reproductive origins of embryo and endosperm were identified based on the embryo/endosperm DNA content ratio: (1) sexual (blue); (2) apomixis with pseudogamous endosperm development (pink); (3) apomixis with autonomous endosperm development (grey); (4) haploid parthenogenesis with autonomous endosperm development (green); and (5) haploid parthenogenesis with pseudogamous endosperm development (yellow). C-values of endosperm and nuclei participating in endosperm development are in bold. Adopted from Matzk et al. (2000) and Matzk (2007).

	Embryo sacs		Reduced		Unreduced	
Sperm cells			egg cell 1C	**polar nuclei 1C + 1C**	egg cell 2C	**polar nuclei 2C + 2C**
reduced	1C	**1C**	2C	**3C**	3C	**5C**
unreduced	2C	**2C**	3C	**4C**	4C	**6C**
reduced	0	**1C**	1C	**3C**	2C	**5C**
unreduced	0	**2C**	1C	**4C**	2C	**6C**
–	0	**0**	1C	**2C**	2C	**4C**
			embryo	**endosperm**	embryo	**endosperm**

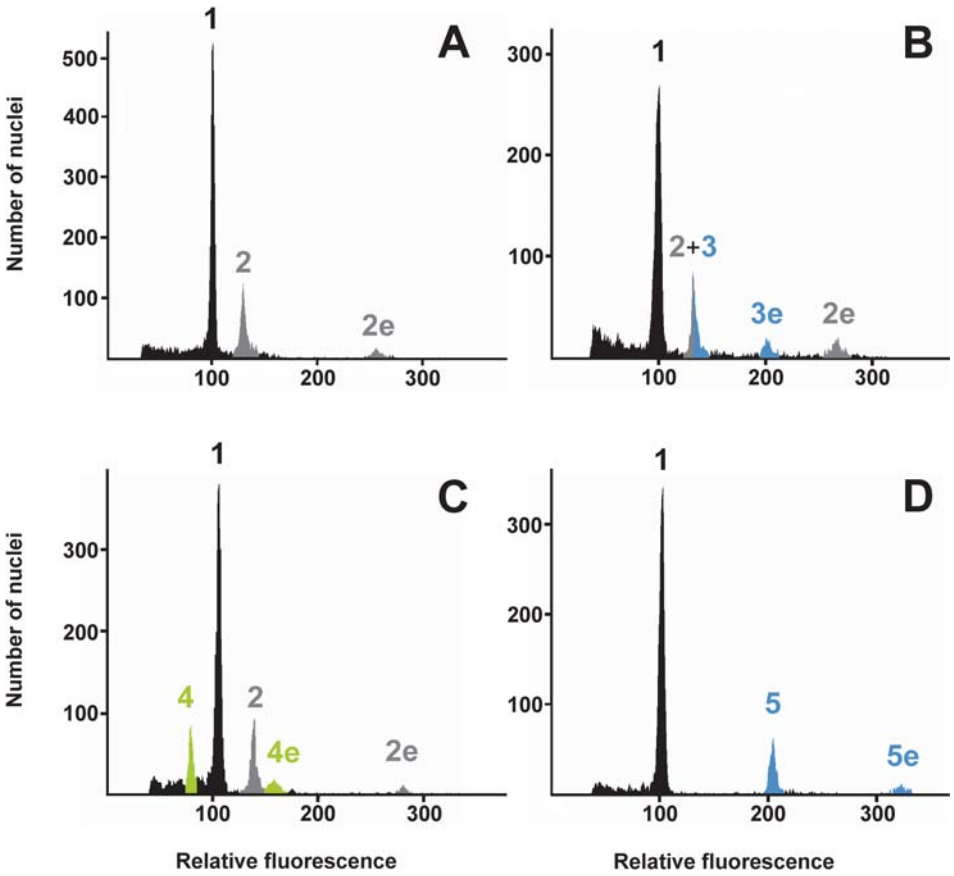

Fig. 1. – Flow cytometric screening of pairs of seeds of *Hieracium bauhini* collected from a mixed population consisting of the pentaploid facultative apomictic *Hieracium bauhini* and hexaploid sexual *H. pilosella*. Four different reproductive pathways were identified based on the different ploidy levels and embryo/endosperm ratios in these seeds: (1) apomictic with autonomous endosperm development (embryo/endosperm ploidy ratio is 5×/10×; grey peaks marked **2** and **2e** in panels **A**, **B**, **C**); (2) sexual via fusion of two reduced gametes (ploidy of the gametes is 2×–3×, embryo/endosperm ploidy ratio is 5×/7×; blue peaks marked **3** and **3e** in panel **B**); (3) parthenogenetic development of the reduced embryo sac with autonomous endosperm development (embryo/endosperm ploidy ratio is 3×/6×; green peaks marked **4** and **4e** in panel **C**); and (4) hybridization via fusion of one reduced pollen grain (3×) and one unreduced egg cell (5×) (embryo/endosperm ploidy ratio is 8×/13×; blue peaks marked **5** and **5e** in panel **D**). In all histograms, peak of the internal standard (the tetraploid *H. bauhini*) is marked **1** (black), embryo peaks are marked **2–5** and endosperm peaks are marked **2e–5e**.

central cells, and (iii) sexual reproduction involving the fusion of both reduced and unreduced gametes (Matzk et al. 2001, Pank et al. 2003, Koperdáková et al. 2004). FCSS identified maternal plants in *Panicum maximum*, which frequently produced either the polyhaploid (n + 0) progeny, or the high-polyploid progeny via 2n + n/n + 2n fertilization (Kaushal et al. 2009). Such genotypes are worthwile in breeding programmes. FCSS has resulted in the first verified record of apomictic reproduction in the family *Rubiaceae*: in cultivated *Coprosma waima* (Heenan et al. 2002) and a natural population of *Coprosma robusta* (Heenan et al. 2003).

Although apomixis mostly occurs in polyploid species (Asker & Jerling 1992), some diploid apomictic biotypes are rarely found in the field as for instance in the agamic complex of *Boechera* (Schranz et al. 2005, 2006, Sharbel et al. 2005). In addition, using embryological and genetical analyses combined with FCSS revealed the co-ocurrence of sexual and aposporic embryo sacs in diploid *Paspalum rufum* in a natural population in Argentina (Siena et al. 2008). FCSS was also successfully used to detect autogamy in *Ranunculus carpaticola,* as by analysing seed from an experimental hybridization of this otherwise self-incompatible taxon. Hörandl et al. (2008) revealed the presence of autogamous progeny induced by foreign pollen (mentor effect). Similarly, embryo cytotypes were screened in the progeny of seeds which were obtained from intercytotype crosses between the sexual and apomictic parent in the *Ranunculus auricomus* complex (Hörandl & Temsch 2009).

The facultativeness of apomixis

In *Poaceae*, a varying expression of apomixis is attributed to distinct species morphotypes in the field, which behave as either facultative or obligatory apomicts, respectively (*Eulaliopsis binata*; Yao et al. 2007). In the polyploid complex of *Poa pratensis*, a variety of reproductive modes was detected using the auxin test, which ranged from nearly obligate apomixis to complete sexuality (Matzk 1991). Combining FCM and RAPD-marker analyses revealed an unexpected high incidence of sexually produced progeny (both n + n and 2n + n) among the seedlings generated by self-fertilization of dodecaploid *Poa pratensis* 'Baron' (Huff & Bara 1993, Stephens et al. 2006). The same method was used to analyse the progeny of controlled crosses between the apomictic and sexual genotypes of *Poa pratensis* in order to trace the inheritance of parental genomes and quantify the progeny according to their mode of production (Barcaccia et al. 1997).

Flow cytometry greatly simplified the investigation of the versatility of reproductive systems in facultatively apomictic angiosperms. The most extreme diversity seems to occur in the species *Hypericum perforatum* (see the previous paragraph), where altogether 11 different modes of producing seed by diploid, tetraploid and hexaploid maternal plants (50 seeds of one mother plant were analysed together; Matzk et al. 2001) were identified. Aside from obligate sexual and obligate apomictic plants there are also different degrees of facultative apomixis, i.e. combinations of sexual reproduction, haploid parthenogenesis, true apomixis and pseudogamous apomixis. The facultative apomicts are capable of producing very diverse progeny by utilizing both sexual and asexual reproduction. Thus, in spite of the dominance of apomixis, which replicates the maternal genome, residual sexuality gives them an indispensable evolutionary potential.

Relationships and dynamics in populations with coexisting sexual and apomictic cytotypes

Due to non-destructiveness and rapid accummulation of data for a large numbers of individuals within a short period, flow cytometry facilitates plant population studies. Unlike conventional chromosome counting, which allowed the analysis of only few individuals, FCM can provide the data for whole populations. That also holds for those populations, in which sexual and apomictic cytotypes coexist, or two or more facultatively apomictic cytotypes

co-occur that differ in their degree of residual sexuality. Using FCM along with genetic markers for distinguishing the individual genotypes, the particular cytotypes/clones can be screened, and, analysing their progeny, the rate of apomixis can be evaluated. This has revealed that apomixis is an important factor promoting the widespread coexistence of different facultatively apomictic cytotypes of *Arnica cordifolia* in mixed populations (Kao 2007, 2008). In spite of the maternal genotype-fixing effect of apomixis, gene flow occurs between the sexual diploids and apomictic triploids and tetraploids in natural mixed populations of *Taraxacum* sect. *Ruderalia* (Verduijn et al. 2004a) and coexisting facultatively apomictic tetraploid biotypes of *Hyparrhenia diplandra* (Durand et al. 2000). Similarly, using multilocus AFLP genotyping surprisingly revealed that occassional sexuality occurs in predominantly apomictic populations of *Townsendia hookeri* (Thompson et al. 2008). Previously, the coexisting triploids and tetraploids in this population appeared to be male-sterile and were assumed to reproduce asexually.

Flow cytometry was also used to map the distribution of the diploid sexual and triploid apomictic biotypes of *Taraxacum officinale* s.l. in a mixed population in ecologically contrasting microhabitats (Verduijn et al. 2004b). Comparing the distribution, phenology and demography it was found that the sexual and apomictic plants preferred particular environments. It is important to note that, especially in some sections of the genus *Taraxacum*, the sexual and apomictic biotypes can simply be distinguished by their pollen. For instance, because they produce different sized pollen it is possible to study gene flow between the diploid sexual and triploid apomictic biotypes of the *Taraxacum* sect. *Ruderalia* coexisting in mixed populations (Menken et al. 1995).

FCM screening of ploidy level categories has been used to estimate the prospective rate of hybridization in mixed populations of *Hieracium* subgen. *Pilosella* in New Zealand. The recombined morpho-/cytotypes were detected among mature plants that were generated by hand-pollinating plants in the field (Houliston & Chapman 2004). The individual seed parent–progeny relationships in two mixed sexual/apomictic populations of this subgenus in Central Europe were traced by evaluating the ploidy of maternal plants and of their progenies. Apomicts, unlike coexisting sexual parents, produce seed that is extremely variable in terms of ploidy because of their diverse modes of reproduction. In this respect, apomictic maternal plants contribute more to population variation than maternal sexual plants (Krahulcová et al. 2009). Similarly, the flow cytometric screening of seeds has revealed that natural tetraploid populations of the facultatively apomictic *Hypericum perforatum* consist of facultatively apomictic ecotypes with varying degrees of apomixis and sexuality (Barcaccia et al. 2006). Among the reproductive pathways reconstructed, haploid parthenogenesis (n + 0 progeny) and fertilization of unreduced (aposporic) egg cells (2n + n hybrids), in addition to true pseudogamous apomixis (2n + 0 progeny) and to n + n hybridization, were detected in most populations.

It is relevant to note that many apomictic plants serve as pollen donors. Thus, their potential for increasing the genetic diversity of populations is dual: (i) via the above mentioned variation in reproductive pathways in facultative apomicts (i.e. the fate of female gametes) and (ii) via the sexual function of pollen (Whitton et al. 2008).

Evolutionary processes in agamic complexes

Hybridization

Flow cytometric screening confirmed the occurrence of hybridization in apomictic taxa of *Hieracium* subgen. *Pilosella,* not only in experimental crosses (Krahulcová et al. 2004) but also in the field in New Zealand (Houliston & Chapman 2004, Morgan-Richards et al. 2004). Here, it is likely that the effect of the residual sexuality of apomicts facilitated the success of highly invasive biotypes in their invaded distribution range (Morgan-Richards et al. 2004, Wilson et al. 2006). Actually, the DNA- and FCM-based population studies in New Zealand suggest that new tetraploid sexual lineages can be produced by crossing pentaploid facultatively apomictic parental genotypes (Chapman et al. 2003). A multidisciplinary approach combining molecular techniques with analysis of ploidy level and of the mode of reproduction, experimental hybridizations and evaluation of morphological variation can provide an integrated view of the structure of a hybrid agamic complex in the field. Such a study on *Hieracium* subgen. *Pilosella* in a Central European mountain range (Fehrer et al. 2005) revealed different levels of variability in the apomictic species (i.e. different extents of individual apomictic clones). Moreover, it was interspecific crosses between facultatively apomictic rather then sexual seed parents that resulted in the production of new hybrid species (Fehrer et al. 2005).

Gene flow between sexual and apomictic biotypes

Mártonfiová (2006) and Mártonfiová et al. (2007) have studied gene flow among the cytotypes of both the sexual and facultatively apomictic biotypes of *Taraxacum* sect. *Ruderalia*. Using FCSS to analyze the seed obtained from controlled crosses revealed the following trends: (i) The pollen of apomictic triploids has a reduced fertilization capacity if mixed with pollen of sexual diploids. Such interference probably also occurs in mixed diploid/triploid populations in the field. (ii) The pollen of tetraploid apomicts had higher hybridization potential than that of triploids when used to fertilize diploid sexual mothers. The tetraploid mothers formed hybrids via fertilization of an unreduced egg cell (2n + n hybrids) more frequently than triploid mothers. Consequently, the tetraploid apomicts, although less frequent in nature, seem to influence the gene exchange more effectively in mixed sexual/apomictic populations than the triploid apomicts (Mártonfiová 2006). Diploid (sexual seed parent) × tetraploid (apomictic pollen parent) crosses probably result in the production of triploid progenies in mixed-cytotype populations, which might increase population diversity (Mártonfiová et al. 2007). In *Crataegus*, where the diploid species is sexual, while the tri- and tetraploid species range from almost obligate to facultative apomixis, parallel inter-cytotype wide interspecific crosses have been made (Talent & Dickinson 2007b). The ploidy levels recorded for the embryos and the endosperm of the resulting hybrid progeny suggest that the gene flow from diploids to tetraploids might go via triploids. In *Ranunculus auricomus* complex, however, the heteroploid hybridization between sexuals and facultative apomicts is prevented by ploidy barriers; in addition, the mentor effects stimulate autogamy and thus limit interspecific crosses (Hörandl & Temsch 2009).

Manifestation of selection

The hybrids that occassionally arise in mixed sexual/apomictic populations are thought to be subjected to selection. This was demonstrated in *Taraxacum officinale* s.l. by comparing

the fitness of established and newly synthesized apomictic genotypes obtained from crosses and cultivated under different environmental conditions (de Kovel & de Jong 2000). As apomicts are triploid and sexuals are diploid in this group, FCM was used to determine the ploidy levels in the respective lineages. Hybridization between diploids and triploids is probably the source of new apomictic genotypes of *Taraxacum* in the field (de Kovel & de Jong 2000). A quantitative FCM analysis of the progeny structure, processed at successive ontogenetic stages (e.g. in the embryonic versus seedling stage), revealed the selection of specific progeny classes. In this way, it was possible to show selection against polyhaploid progeny and progeny from n + n hybridization during germination and early stages of seedling development in *Hieracium* subgen. *Pilosella* (Krahulec et al. 2006). Generally, this finding implies caution should be exercised when comparing the progeny structure generated by facultative apomicts based on information from different sources, which studied different developmental stages of the progeny, as the comparison might be biased.

Evolutionary implications of variation in genome size in agamic complexes

Matzk et al. (2003) studied the trends in the coevolution of reproductive mode and variation in the genome size in *Hypericum*. They identified two independent agamic complexes at the section level. The phylogenetically younger *Hypericum* sect. *Hypericum* is characterized by an increased DNA content in apomicts, which is exclusively due to polyploidy. Apomicts of the evolutionary older *Hypericum* sect. *Ascyreia* have the highest DNA content of all species due to polyploidization and, in addition, a higher DNA content per chromosome. Thus, both polyploidization and increase in the monoploid genome size played a role in the evolution of apomixis in the latter section. The correlation between variation in genome size and species relationships has been studied in a taxonomically complicated group of *Hieracium* subgen. *Pilosella* (Suda et al. 2007a). The monoploid genome size (Cx-value according to Greilhuber et al. 2005) of those hybrid species, which inherited a part of the genome of *H. pilosella*, is influenced by the relatively low Cx-value of *H. pilosella*. Comparing the genome size of the hybrid species with that of their putative parents, the genome constitution of the respective polyploid hybrids can be inferred. Thus, the nuclear DNA content is a supportive marker for delimiting problematic taxa. Moreover, this character can be used to estimate the genome composition (proportion of the parental genomes) of hybrid cytotypes.

Current research on Hieracium subgen. Pilosella

The determination of the DNA ploidy level in mature plants and seedlings (Fehrer et al. 2007) by both the original and modified FCSS method (Krahulec et al. 2006, Krahulcová et al. 2009) are routinely used in investigations of the evolutionary processes in this agamic complex. The level of residual sexuality in the facultatively apomictic members of the complex is estimated by combining the results of emasculation experiments with the analysis of progeny from both field and experimental crosses. These results indicate that the degree of residual sexuality in facultative apomicts is influenced by the level of stabilization of the respective hybrid genotypes in the field (Fehrer et al. 2007, Krahulec et al. 2008, Krahulcová et al. 2009).

Genetic basis of apomixis in plants

The production of progeny with the maternal genome is a trait of major importance for agriculture and plant breeding. Fixation of hybrid vigour in crop plants via apomixis would have a positive effect on crop production. Therefore, the inheritance of apomixis and possible transfer of apomictic gene(s) to crops is the objective of many research programmes (e.g. Spillane et al. 2001, Koltunow & Grossniklaus 2003, Hörandl et al. 2007). Apomixis is a complex trait, which involves three elements: (i) absence of meiosis resulting in the formation of unreduced female gametes, (ii) absence of fertilization (parthenogenetic, i.e. the fertilization-independent development of embryo), and (iii) the developmental adaptations that ensure functional endosperm formation. Apomixis and sexuality are considered to be closely interrelated, sharing gene expression pathways and regulatory components (Koltunow & Grossniklaus 2003).

The following genera of angiosperms have recently been used as model organisms in research into the inheritance, expression and regulatory mechanisms of apomixis: *Arabidopsis*, *Boechera*, *Brachiaria*, *Erigeron*, *Hieracium* subgen. *Pilosella*, *Hypericum*, *Medicago*, *Paspalum*, *Pennisetum*, *Poa pratensis* agg., *Taraxacum*, and *Tripsacum* (for the references see Dresselhaus & Colombo 2001, Bicknell & Koltunow 2004, Catanach et al. 2006, Hörandl et al. 2007). Mutagenesis, FCM screening of progeny and genomic fingerprinting are often used to identify candidate genomic regions associated with apomixis. For example, Catanach et al. (2006) used these methods to identify two principal unlinked loci controlling apomixis in *Hieracium caespitosum*. Curtis & Grossniklaus (2007) studied the segregation in a mutant producing unreduced female gametophytes and the parent-of-origin effects acting as barriers to the introgression of apomixis in *Zea*. In this case, FCSS of F_2 families was used to evaluate the role of the parental genome balance in viable endosperm development. In addition to the model taxa cited above Cardone et al. (2006) suggest new species and genotypes suitable for studying the genetics of apomixis.

Experiments that use FCM screening of progeny commonly also use crosses between sexual (usually diploid) and apomictic (polyploid) parents and analysis of the segregating progenies with respect to ploidy, reproductive mode and inheritance of molecular markers. The FCM screening of progeny can even discriminate among particular aneuploid cytotypes ranging between the diploid and tetraploid level, which were obtained from controlled crosses (van Dijk et al. 2003, van Dijk & Bakx-Schotman 2004). A combination of FCM and molecular methods has been used to investigate the genetic basis of diplospory (a restitutional meiosis resulting in an unreduced embryo sac) in *Taraxacum* (Tas & van Dijk 1999, van Baarlen et al. 2002, van Dijk et al. 2003, van Dijk & Bakx-Schotman 2004). The results suggest the existence of a female-specific locus responsible for diplospory, whereas parthenogenesis (autonomous development of embryo, not requiring fertilization) is controlled by another independently segregating gene(s) (van Dijk & Bakx-Schotman 2004). An independent segregation of genes controlling the apomictic elements in *Taraxacum* was also confirmed by analysing the progenies from wide inter-sectional crosses (Záveský et al. 2007).

Flow Cytometric Seed Screen has also been used to study the segregation of progenies in *Poa pratensis*, which have originated from selfing and intercrosses between the sexual and facultatively apomictic parents (Matzk et al. 2005). The expression of apospory and parthenogenesis in F_1 plants remained constant over several years. The segregation of par-

ticular components of apomixis in this model system indicates that five major genes control asexual seed formation. The wide variation in the mode of reproduction in this species might be due to different expressions and interactions of these genes (Matzk et al. 2005). In addition, the regeneration of apomictic embryos in embryo tissue cultures of the polyploid apomictic clone of *Poa pratensis* 'Baron' is less frequent than expected (Stephens et al. 2006). Based on the results of crossing experiments and progeny screening Schranz et al. (2006) propose a complex genetic control of apomixis in *Boechera*. In contrast, a single dominant apospory-specific genomic region is thought to operate in *Pennisetum*; however, it is not transmitted at the same rate in the reciprocal backcrosses between sexual and apomictic types (Roche et al. 2001). Such segregation distortion indicates that apomictic reproduction has a rather complex genetic basis, which might be specific to different taxa of angiosperms. Thus, for the present, the introduction of apomixis into crop breeding programmes seems to be limited, due to the complexity of this trait.

Acknowledgements

This study was supported by the Czech Science Foundation (project no. 206/07/0059), the Ministry of Education, Youth and Sports of the Czech Republic (projects MSM 0021622416 and LC 06073), and by long-term institutional research plan AV0Z60050516 from the Academy of Sciences of the Czech Republic.

Souhrn

Aplikace metody průtokové cytometrie v oborech zabývajících se biologií rostlin je spjatá s posledními dvěma desetiletími. Výhody této techniky (jednoduchost, rychlost, přesnost, nedestruktivnost) velmi pomohly i ke zdokonalení výzkumu apomixe. Tento způsob asexuálního rozmnožování semeny je sice mezi krytosemennými rostlinami menšinový, ale byl prokázán již u více než 200 rodů (převážně v čeledích *Asteraceae*, *Poaceae* a *Rosaceae*). Apomiktické rostliny jsou až na malé výjimky polyploidní a často se liší právě ploidií od příbuzných sexuálních taxonů nebo cytotypů, sdružených do jednoho polyploidního agamického komplexu. Protože pomocí průtokové cytometrie (FCM) lze měřit obsah jaderné DNA, je tato technika v botanice nejčastěji využívána k detekci ploidních úrovní. K analýzám lze použít části dospělých rostlin nebo jejich potomstva. Při biosystematických a populačních studiích je FCM často využívána spolu s molekulárními technikami detekujícími genotypy a s analýzami reprodukčního způsobu. V práci jsou citovány příklady polyploidních agamických komplexů studovaných těmito metodami. Důsledkem fakultativní apomixe je kombinace různých způsobů reprodukce v rámci jediné mateřské rostliny. Ve směsných populacích více cytotypů nebo při křížení rostlin s různou ploidií vznikají různé kategorie potomstva. Vedle obvykle dominujícího potomstva vzniklého apomixí a zachovávajícího mateřský genotyp se objevují i minoritní kategorie potomstva vzniklého buď sexuálně nebo haploidní parthenogenezí; takové potomstvo lze potom odlišit podle stupně ploidie. Průtoková cytometrie slouží k detekci a kvantifikaci jednotlivých reprodukčních způsobů, odvozených podle zastoupení různých kategorií potomstva. Při výzkumu apomixe je hojně využívána i odvozená metoda FCSS (Flow Cytometric Seed Screen), kterou lze analyzovat zralá semena a zjišťovat, jakým reprodukčním způsobem byla vytvořena. Tato technika využívá procesu dvojitého oplození u sexuálních krytosemenných rostlin a absence oplození při autonomním vývoji embrya apomiktů. Endosperm apomiktických rostlin se může vyvíjet buď autonomně nebo po oplození centrálního jádra zárodečného vaku (pseudogamie). Pomocí metody FCSS lze zjistit poměr ploidií, resp. obsahu DNA, mezi embryem a endospermem a z toho odvodit jednotlivé reprodukční způsoby. V současnosti se průtoková cytometrie uplatňuje zejména v těchto oblastech výzkumu: (i) variabilita ploidní úrovně a velikosti genomu v agamických komplexech, (ii) zbytková sexualita fakultativně apomiktických rostlin a diverzita reprodukčních způsobů, (iii) dynamika a struktura populací s koexistujícími sexuálními a apomiktickými biotypy, (iv) evoluční procesy v agamických komplexech a (v) genetická podstata apomixe. Hledání genů spojených se založením a regulací apomixe je náplní mnoha programů základního i aplikovaného výzkumu, při kterých se rovněž uplatňuje průtoková cytometrie. Identifikace těchto genů a jejich úspěšné přenesení do genomu kulturních plodin by měly zásadní význam pro šlechtění a zemědělskou produkci. Práce uvádí výčet taxonů, které při tomto výzkumu slouží jako modelové organismy.

References

Asker S. & Jerling L. (1992): Apomixis in plants. – CRC Press, Boca Raton.

Bantin J., Matzk F. & Dresselhaus T. (2001): *Tripsacum dactyloides* (*Poaceae*): a natural model system to study parthenogenesis. – Sexual Pl. Reprod. 14: 219–226.

Barcaccia G., Arzenton F., Sharbel T. F., Varotto S., Parrini P. & Lucchini M. (2006): Genetic diversity and reproductive biology in ecotypes of the facultative apomict *Hypericum perforatum* L. – Heredity 96: 322–334.

Barcaccia G., Mazzucato A., Belardinelli A., Pezzotti M., Lucretti S. & Falcinelli M. (1997): Inheritance of parental genomes in progenies of *Poa pratensis* L. from sexual and apomictic genotypes as assessed by RAPD markers and flow cytometry. – Theor. Appl. Genet. 95: 516–524.

Bengtsson O. & Ceplitis A. (2000): The balance between sexual and asexual reproduction in plants living in variable environments. – J. Evol. Biol. 13: 415–422.

Bicknell R. A. & Koltunow A. M. (2004): Understanding apomixis: recent advances and remaining conundrums. – Pl. Cell 16: 228–245.

Bräutigam S. & Bräutigam E. (1996): Determination of the ploidy level in the genus *Hieracium* subgenus *Pilosella* (Hill) S. F. Gray by flow cytometric DNA analysis. – Folia Geobot. Phytotax. 31: 315–321.

Brutovská R., Čellárová E. & Doležel J. (1998): Cytogenetic variability of in vitro regenerated *Hypericum perforatum* L. plants and in their progenies. – Pl. Sci. 133: 221–229.

Cáceres M. E., Matzk F., Busti A., Pupilli F. & Arcioni S. (2001): Apomixis and sexuality in *Paspalum simplex*: characterization of the mode of reproduction in segregating progenies by different methods. – Sexual Pl. Reprod. 14: 201–206.

Cardone S., Polci P., Selva J. P., Mecchia M., Pessino S., Hermann P., Cambi V., Voigt P., Spangeberg G. & Echenique V. (2006): Novel genotypes of the subtropical grass *Eragrostis curvula* for the study of apomixis (diplospory). – Euphytica 151: 263–272.

Catanach A. S., Erasmuson S. K., Podivinsky E., Jordan B. R. & Bicknell R. (2006): Deletion mapping of genetic regions associated with apomixis in *Hieracium*. – Proc. Natl. Acad. Sci. USA 103: 18650–18655.

Chapman H., Houliston G. J., Robson B. & Iline I. (2003): A case of reversal: the evolution and maintenance of sexuals from parthenogenetic clones in *Hieracium pilosella*. – Int. J. Pl. Sci. 164: 719–728.

Chrtek J. Jr., Tonková M., Mráz P., Marhold K., Plačková I., Krahulcová A. & Kirschner J. (2007): Morphological and allozyme diversity in the *Hieracium nigrescens* group (*Compositae*) in the Sudety Mountains and the Western Carpathians. – Bot. J. Linn. Soc. 153: 287–300.

Chrtek J., Zahradníček J., Krak K. & Fehrer J. (2009): Genome size in *Hieracium* subgenus *Hieracium* (*Asteraceae*) is strongly correlated with major phylogenetic groups. – Ann. Bot. 104: 161–178.

Curtis M. D. & Grossniklaus U. (2007): Amphimixis and apomixis: two sides of the same coin! – In: Hörandl E., Grossniklaus U. & Sharbel T. F. (eds), Apomixis: evolution, mechanisms and perspectives, p. 37–62, Regnum Veg. 147, A. R. G. Gantner Verlag, Rugell.

Czapik R. (1994): How to detect apomixis in *Angiospermae*. – Polish Bot. Stud. 8: 13–21.

de Kovel C. G. F. & de Jong G. (2000): Selection on apomictic lineages of *Taraxacum* at establishment in a mixed sexual-apomictic population. – J. Evol. Biol. 13: 561–568.

Doležel J., Greilhuber J. & Suda J. (eds) (2007): Flow cytometry with plant cells. – Wiley-VCH Verlag, Weinheim.

Dresselhaus T. & Colombo L. (2001): 2nd International Apomixis Conference (APO2001) at Como, Italy, 24–28 April 2001. – Sexual Pl. Reprod. 14: 245–251.

Durand J., Garnier L., Dajoz I., Mousset S. & Veuille M. (2000): Gene flow in a facultative apomictic *Poaceae*, the savanna grass *Hyparrhenia diplandra*. – Genetics 156: 823–831.

Fehrer J., Krahulcová A., Krahulec F., Chrtek J. Jr., Rosenbaumová R. & Bräutigam S. (2007): Evolutionary aspects in *Hieracium* subgenus *Pilosella*. – In: Hörandl E., Grossniklaus U. & Sharbel T. F. (eds), Apomixis: evolution, mechanisms and perspectives, p. 359–390, Regnum Veg. 147, A. R. G. Gantner Verlag, Rugell.

Fehrer J., Šimek R., Krahulcová A., Krahulec F., Chrtek J., Bräutigam E. & Bräutigam S. (2005): Evolution, hybridisation, and clonal distribution of apo- and amphimictic species of *Hieracium* subgen. *Pilosella* (*Asteraceae*, *Lactuceae*) in a Central European mountain range. – In: Bakker T., Chatrou L. W., Gravendeel B. & Pelser P. B. (eds), Plant species-level systematics: new perspectives on pattern and process, p. 175–201, Regnum Veg. 143, A. R. G. Gantner Verlag, Rugell.

Greilhuber J., Doležel J., Lysák M. A. & Bennett M. D. (2005): The origin, evolution and proposed stabilization of the terms "genome size" and "C-value" to describe nuclear DNA contents. – Ann. Bot. 95: 255–260.

Grimanelli D., Hernández M., Perotti E. & Savidan Y. (1997): Dosage effects in the endosperm of diplosporous apomictic *Tripsacum* (*Poaceae*). – Sexual Pl. Reprod. 10: 279–282.

Harlan J. R. & de Wet J. M. J. (1975): On Ö. Winge and a prayer: the origins of polyploidy. – Bot. Rev. 41: 361–390.

Heenan P. B., Dawson M. I. & Bicknell R. A. (2002): Evidence for apomictic seed formation in *Coprosma waima* (*Rubiaceae*). – N. Z. J. Bot. 40: 347–355.

Heenan P. B., Molloy B. P. & Bicknell R. A. (2003): Levels of apomictic and amphimictic seed formation in a natural population of *Coprosma robusta* (*Rubiaceae*) in Riccarton Bush, Christchurch, New Zealand. – N. Z. J. Bot. 41: 287–291.

Hörandl E., Cosendai A.-C. & Temsch E. M. (2008): Understanding the geographic distributions of apomictic plants: a case for a pluralistic approach. – Pl. Ecol. Divers. 1: 309–320.

Hörandl E., Grossniklaus U., van Dijk P. J. & Sharbel T. F. (eds) (2007): Apomixis: evolution, mechanisms and perspectives. – Regnum Veg. 147, A. R. G. Gantner Verlag, Rugell.

Hörandl E. & Temsch E. M. (2009): Introgression of apomixis into sexual species is inhibited by menthor effects and ploidy barriers in the *Ranunculus auricomus* complex. – Ann. Bot. 104: 81–89.

Houliston G. J. & Chapman H. M. (2004): Reproductive strategy and population variability in the facultative apomict *Hieracium pilosella* (*Asteraceae*). – Amer. J. Bot. 91: 37–44.

Huff D. R. & Bara J. M. (1993): Determining genetic origins of aberrant progeny from facultative apomictic Kentucky bluegrass using a combination of flow cytometry and silver-stained RAPD markers. – Theor. Appl. Genet. 87: 201–208.

Kao R. H. (2007): Asexuality and the coexistence of cytotypes. – New Phytol. 175: 764–772.

Kao R. H. (2008): Origins and widespread distribution of co-existing polyploids in *Arnica cordifolia* (*Asteraceae*). – Ann. Bot. 101: 145–152.

Kaushal P., Agrawai A., Malaviya D. R., Siddiqui S. A. & Roy A. K. (2009): Ploidy manipulation in guinea grass (*Panicum maximum* Jacq., *Poaceae*) utilizing a Hybridization-supplemented Apomixis-components Partitioning Approach (HAPA). – Pl. Breeding 128: 295–303.

Kelley A. M., Johnson P. G., Waldron B. L. & Peel M. D. (2009): A survey of apomixis and ploidy levels among *Poa* L. (*Poaceae*) using flow cytometry. – Crop Sci. 49: 1395–1402.

Koltunow A. M. & Grossniklaus U. (2003): Apomixis: a developmental perspective. – Annu. Rev. Pl. Biol. 54: 547–574.

Koperdáková J., Brutovská R. & Čellárová E. (2004): Reproduction pathway analysis of several *Hypericum perforatum* L. somaclonal families. – Hereditas 140: 34–41.

Krahulcová A., Papoušková S. & Krahulec F. (2004): Reproduction mode in the allopolyploid facultatively apomictic hawkweed *Hieracium rubrum* (*Asteraceae*, *H.* subgen. *Pilosella*). – Hereditas 141: 19–30.

Krahulcová A., Rotreklová O., Krahulec F., Rosenbaumová R. & Plačková I. (2009): Enriching ploidy level diversity: the role of apomictic and sexual biotypes of *Hieracium* subgen. *Pilosella* (*Asteraceae*) that coexist in the polyploid populations. – Folia Geobot. 44: 281–306.

Krahulcová A. & Suda J. (2006): A modified method of flow cytometric seed screen simplifies the quantification of progeny classes with different ploidy levels. – Biol. Pl. 50: 457–460.

Krahulec F., Krahulcová A., Fehrer J., Bräutigam S. & Schuhwerk F. (2008): The structure of the agamic complex of *Hieracium* subgen. *Pilosella* in the Šumava Mts and its comparison with other regions in Central Europe. – Preslia 80: 1–26.

Krahulec F., Krahulcová A. & Papoušková S. (2006): Ploidy level selection during germination and early stage of seedling growth in the progeny of allohexaploid facultative apomict, *Hieracium rubrum* (*Asteraceae*). – Folia Geobot. 41: 407–416.

Kron P., Suda J. & Husband B. C. (2007): Applications of flow cytometry to evolutionary and population biology. – Annu. Rev. Ecol. Syst. 38: 847–876.

Leitch I. J. & Bennett M. D. (2004): Genome downsizing in polyploid plants. – Biol. J. Linn. Soc. 82: 651–663.

Lepší M., Vít P., Boublík K. & Suda J. (2008): *Sorbus milensis*, a new hybridogenous species from northwestern Bohemia. – Preslia 80: 229–244.

Lepší M., Vít P., Lepší P., Boublík K. & Kolář F. (2009): *Sorbus portae-bohemicae* and *Sorbus albensis*, two new endemic apomictic species recognized based on a revision of *Sorbus bohemica*. – Preslia 81: 63–89.

Mártonfi P. (2008): *Hypericum dubium* Leers: new data on taxonomy and biology. – Folia Geobot. 43: 69–82.

Mártonfiová L. (2006): Possible pathways of the gene flow in *Taraxacum* sect. *Ruderalia*. – Folia Geobot. 41: 183–201.

Mártonfiová L., Majeský L. & Mártonfi P. (2007): Polyploid progeny from crosses between diploid sexuals and tetraploid apomictic pollen donors in *Taraxacum* sect. *Ruderalia*. – Acta Biol. Cracov., ser. bot., 49: 47–54.

Matzk F. (1991): New effects to overcome apomixis in *Poa pratensis* L. – Euphytica 55: 65–72.

Matzk F. (2007): Reproduction mode screening. – In: Doležel J., Greilhuber J. & Suda J. (eds), Flow cytometry with plant cells, p. 131–152, Wiley-VCH Verlag, Weinheim.

Matzk F., Hammer K. & Schubert I. (2003): Coevolution of apomixis and genome size within the genus *Hypericum*. – Sexual Pl. Reprod. 16: 51–58.

Matzk F., Meister A., Brutovská R. & Schubert I. (2001): Reconstruction of reproductive diversity in *Hypericum perforatum* L. opens novel strategies to manage apomixis. – Pl. J. 26: 275–282.

Matzk F., Meister A. & Schubert I. (2000): An efficient screen for reproductive pathways using mature seeds of monocots and dicots. – Pl. J. 21: 97–108.

Matzk F., Prodanovic S., Bäumlein H. & Schubert I. (2005): The inheritance of apomixis in *Poa pratensis* confirms a five locus model with differences in gene expressivity and penetrance. – Pl. Cell 17: 13–24.

Menken S. B. J., Smit E. & den Nijs J. C. M. (1995): Genetical population structure in plants: gene flow between diploid sexual and triploid asexual dandelions (*Taraxacum* section *Ruderalia*). – Evolution 49: 1108–1118.

Morgan-Richards M., Trewick S. A., Chapman H. M. & Krahulcová A. (2004): Interspecific hybridization among *Hieracium* species in New Zealand: evidence from flow cytometry. – Heredity 93: 34–42.

Mráz P., Chrtek J. & Šingliarová B. (2009): Geographical parthenogenesis, genome size variation and pollen production in the arctic-alpine species *Hieracium alpinum.* – Bot. Helv. 119: 41–51.

Mráz P., Šingliarová B., Urfus T. & Krahulec F. (2008): Cytogeography of *Pilosella officinarum* (*Compositae*): altitudinal and longitudinal differences in ploidy level distribution in the Czech Republic and Slovakia and the general pattern in Europe. – Ann. Bot. 101: 59–71.

Naumova T. N., Hayward M. D. & Wagenvoort M. (1999): Apomixis and sexuality in diploid and tetraploid accessions of *Brachiaria decumbens.* – Sexual Pl. Reprod. 12: 43–52.

Nybom H., Esselink G. D., Werlemark G., Leus L. & Vosman B. (2006): Unique genomic configuration revealed by microsatellite DNA in polyploid dogroses, *Rosa* sect. *Caninae*. – J. Evol. Biol. 19: 635–648.

Pank F., Matzk F., Kästner U., Blüthner W. D., Foltys de Garcia E., Meister A., Ryschka U. & Schumann G. (2003): Reproductive diversity and strategies for breeding in St. John's wort (*Hypericum perforatum* L.). – Euphytica 134: 77–84.

Paun O., Greilhuber J., Temsch E. M. & Hörandl E. (2006): Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, *Ranunculaceae*). – Mol. Ecol. 15: 897–910.

Pichot Ch., El Maâtaoui M., Raddi S. & Raddi P. (2001): Surrogate mother for endangered *Cupressus*. – Nature 412: 39.

Pichot Ch., Liens B., Rivera Nava J. L., Bachelier J. B. & El Maâtaoui M. (2008): Cypress surrogate mother produces haploid progeny from alien pollen. – Genetics 178: 379–383.

Roche D., Chen Z., Hanna W. W. & Ozias-Akins P. (2001): Non-Mendelian transmission of an apospory-specific genomic region in a reciprocal cross between sexual pearl millet (*Pennisetum glaucum*) and an apomictic F1 (*P. glaucum* × *P. squamulatum*). – Sexual Pl. Reprod. 13: 217–223.

Rotreklová O. (2004): *Hieracium bauhini* group in Central Europe: chromosome numbers and breeding systems. – Preslia 76: 313–330.

Rotreklová O., Krahulcová A., Mráz P., Mrázová V., Mártonfiová L., Peckert T. & Šingliarová B. (2005): Chromosome numbers and breeding systems of some European species of *Hieracium* subgen. *Pilosella*. – Preslia 77: 177–195.

Sartor M. E., Quarin C. L. & Espinoza F. (2009): Mode of reproduction of colchicine-induced *Paspalum plicatulum* tetraploids. – Crop Sci. 49: 1270–1276.

Savidan Y. (2007): Apomixis in higher plants. – In: Hörandl E., Grossniklaus U. & Sharbel T. F. (eds), Apomixis: evolution, mechanisms and perspectives, p. 15–22, Regnum Veg. 147, A. R. G. Gantner Verlag, Rugell.

Schranz M. E., Dobeš Ch., Koch M. A. & Mitchell-Olds T. (2005): Sexual reproduction, hybridization, apomixis, and polyploidization in the genus *Boechera* (*Brassicaceae*). – Amer. J. Bot. 92: 1797–1810.

Schranz M. E., Kantama L., de Jong H. & Mitchell-Olds T. (2006): Asexual reproduction in a close relative of *Arabidopsis*: a genetic investigation of apomixis in *Boechera* (*Brassicaceae*). – New Phytol. 171: 425–438.

Sharbel T. F. & Mitchell-Olds T. (2001): Recurent polyploid origin and chloroplast phylogeography in the *Arabis holboellii* complex (*Brassicaceae*). – Heredity 87: 59–68.

Sharbel T. F., Mitchell-Olds T., Dobeš C., Kantama L. & de Jong H. (2005): Biogeographic distribution of polyploidy and B chromosomes in the apomictic *Boechera holboellii* complex. – Cytogenet. Genome Res. 109: 283–292.

Siena L. A., Sartot M. E., Espinoza F., Quarin C. L. & Ortiz J. P. A. (2008): Genetic and embryological evidences of apomixis at the diploid level in *Paspalum rufum* support recurrent auto-polyploidization in the species. – Sexual Pl. Reprod. 21: 205–215.

Speckmann G. J. & van Dijk P. E. (1972): Chromosome numbers and plant morphology in some ecotypes of *Poa pratensis* L. – Euphytica 21: 171–180.

Spillane C., Steimer A. & Grossniklaus U. (2001): Apomixis in agriculture: the quest for clonal seeds. – Sexual Pl. Reprod. 14: 179–187.

Stephens L. C., Fei S.-Z., Xiong Y. & Hodges C. F. (2006): Plants regenerated from embryo cultures of an apomictic clone of Kentucky bluegrass (*Poa pratensis* L. 'Baron') are not apomictic in origin. – Euphytica 147: 383–388.

Suda J., Krahulcová A., Trávníček P. & Krahulec F. (2006): Ploidy level versus DNA ploidy level: an appeal for consistent terminology. – Taxon 55: 447–450.

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., Kron P., Husband B. C. & Trávníček P. (2007b): Flow cytometry and ploidy: applications in plant systematics, ecology and evolutionary biology. – In: Doležel J., Greilhuber J. & Suda J. (eds), Flow cytometry with plant cells, p. 103–130, Wiley-VCH Verlag, Weinheim.

Talent N. & Dickinson T. A. (2005): Polyploidy in *Crataegus* and *Mespilus* (*Rosaceae*, *Maloideae*): evolutionary inferences from flow cytometry of nuclear DNA amounts. – Can. J. Bot. 83: 1268–1304.

Talent N. & Dickinson T. A. (2007a): Endosperm formation in aposporous *Crataegus* (*Rosaceae*, *Spiraeoideae*, tribe *Pyreae*): parallels to *Ranunculaceae* and *Poaceae*. – New Phytol. 173: 231–249.

Talent N. & Dickinson T. A. (2007b): The potential for ploidy level increases and decreases in *Crataegus* (*Rosaceae*, *Spiraeoideae*, tribe *Pyreae*). – Can. J. Bot. 85: 570–584.

Talent N. & Dickinson T. A. (2007c): Apomixis and hybridization in *Rosaceae* subtribe *Pyrinae* Dumort.: a new tool promises new insights. – In: Hörandl E., Grossniklaus U. & Sharbel T. F. (eds), Apomixis: evolution, mechanisms and perspectives, p. 301–316, Regnum Veg. 147, A. R. G. Gantner Verlag, Rugell.

Tas I. C. Q. & van Dijk P. J. (1999): Crosses between sexual and apomictic dandelions (*Taraxacum*). I. The inheritance of apomixis. – Heredity 83: 707–714.

Taskin K. M., Turgut K. & Scott R. J. (2004): Apomictic development in *Arabis gunnisoniana*. – Israel J. Pl. Sci. 52: 155–160.

Thompson S. L., Choe G., Ritland K. & Whitton J. (2008): Cryptic sex within male-sterile polyploid populations of the easter daisy, *Townsendia hookeri*. – Int. J. Pl. Sci. 169: 183–193.

van Baarlen P., de Jong J. H. & van Dijk P. J. (2002): Comparative cyto-embryological investigations of sexual and apomictic dandelions (*Taraxacum*) and their apomictic hybrids. – Sexual Pl. Reprod. 15: 31–38.

van Dijk P. J. & Bakx-Schotman J. M. T. (2004): Formation of unreduced megaspores (diplospory) in apomictic dandelions (*Taraxacum officinale* s.l.) is controlled by a sex-specific dominant locus. – Genetics 166: 483–492.

van Dijk P. J., van Baarlen P. & de Jong J. H. (2003): The occurrence of phenotypically complementary apomixis-recombinants in crosses between sexual and apomictic dandelions (*Taraxacum officinale*). – Sexual Pl. Reprod. 16: 71–76.

Verduijn M. H., van Dijk P. J. & van Damme J. M. M. (2004a): The role of tetraploids in the sexual-asexual cycle in dandelions (*Taraxacum*). – Heredity 93: 390–398.

Verduijn M. H., van Dijk P. J. & van Damme J. M. M. (2004b): Distribution, phenology and demography of sympatric sexual and asexual dandelions (*Taraxacum officinale* s.l.): geographic parthenogenesis on a small scale. – Biol. J. Linn. Soc. 82: 205–218.

Vilhar B., Greilhuber J., Koce J. D., Temsch E. M. & Dermastia M. (2001): Plant genome size measurement with DNA image cytometry. – Ann. Bot. 87: 719–728.

Whitton J., Sears Ch. J., Baack E. J. & Otto S. P. (2008): The dynamic nature of apomixis in the angiosperms. – Int. J. Pl. Sci. 169: 169–182.

Wieners R. R., Fei S.-Z. & Johnson R. C. (2006): Characterization of a USDA Kentucky bluegrass (*Poa pratensis* L.) core collection for reproductive mode and DNA content by flow cytometry. – Genet. Res. Crop Evol. 53: 1531–1541.

Wilson L. M., Fehrer J., Bräutigam S. & Grosskopf G. (2006): A new invasive hawkweed, *Hieracium glomeratum* (*Lactuceae*, *Asteraceae*), in the Pacific Northwest. – Can. J. Bot. 84: 133–142.

Yao J.-L., Zhou Y. & Hu C.-G. (2007): Apomixis in *Eulaliopsis binata*: characterization of reproductive mode and endosperm delelopment. – Sexual Pl. Reprod. 20: 151–158.

Záveský L., Jarolímová V. & Štěpánek J. (2005): Nuclear DNA content variation within the genus *Taraxacum* (*Asteraceae*). – Folia Geobot. 40: 91–104.

Záveský L., Jarolímová V. & Štěpánek J. (2007): Apomixis in *Taraxacum paludosum* (section *Palustria*, *Asteraceae*): recombinations of apomixis elements in inter-sectional crosses. – Pl. Syst. Evol. 265: 147–163.

Received 30 January 2008
Revision received 29 January 2009
Accepted 2 November 2009