

Reproduction Mode in *Hypericum* and its Consequences

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ABSTRACT

Several modes of reproduction were described in the genus *Hypericum*. Besides obligate sexuality, irregularities in meiosis, polyembryony and pseudogamous apomixis were noticed in various taxa of the genus. This review summarizes previous knowledge and also analyses evolutionary consequences of different breeding systems and possible employment of particular reproductive modes for breeding in the genus *Hypericum*.

Keywords: apomixis, breeding, evolution, *Guttiferae*, sexuality

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INTRODUCTION

The microevolution of plants refers to evolutionary changes at or below the species level. It is the occurrence of small-scale changes in allele frequencies in a population, over a few generations (Wilkins 2006). These changes are the result of several processes: mutation, natural selection, gene flow and genetic drift (Briggs and Walters 1997). So, microevolution results from genetic, physiologic and further processes in interaction with their biotic and abiotic environment. Plants adapt themselves to this environment and are able to survive. The reproductive syndrome of plants is an important facet of their adaptational response (Stuessy 2009). Many flowering plants can choose between no less than three fundamentally different modes of reproduction: 1. outcrossing sex (panmixis); 2. asexuality, 3. selfing sex (autogamy). However, particular plant species usually do not strictly keep one reproduction mode. In various proportion they combine all of the three fundamental modes to fine-tune their reproductive strategies to changing ecological circumstances (Richards 1990, 1996, 1997, 2003). This is aimed at evolutionarily stable strategies (Holsinger 1992; Lloyd 1992). How is the situation in the big genus *Hypericum* (Guttiferae)?

REPRODUCTION MODE AND EMBRYOLOGY

In the genus *Hypericum* all three fundamental reproduction modes have been described. Besides outcrossing sex, self compatibility is widespread in the genus (Myers 1963; Culwell 1970; Robson 1981; Moraes *et al.* 2009), but not universal (Salisbury 1963 – incompatibility system in *H. calycinum*, Robson 1977, 1981). Asexuality is not rare (see later). The embryology of various species of the genus has been examined by different authors, however, only minor

variation was recorded for different species of the genus *Hypericum* (Schnarf 1914; Palm 1922; Souèges 1925; Hoar and Haertl 1932; Souèges 1936; Swamy 1946; Govindappa 1956; Rao 1957; Myers 1964; Davis 1966; Bougnicourt 1970, 1971; Philipson 1974, 1977). Normally, the anther is bisporangiate with 2-celled pollen grains. The ovule is anatropous and tenuinucellar. The archesporial cell functions directly as the megaspore mother cell, and a linear tetrad develops into a *Polygonum*-type embryo sac (Figs. 1, 2). Embryogeny in *Hypericum* is Solanad (i. e. the terminal cell divides by a transverse wall during the second cell generation and the basal cell forms a several-celled suspensor) (Prakash and Lau 1976; Robson 1981; Mártonfi *et al.* 1996).

ABNORMALITIES IN REPRODUCTION AND APOMIXIS

Abnormalities were found occasionally. Hoar (1931) discovered structural hybridity in *H. punctatum* Lam. similar to that occurring in *Oenothera* L. Adams (Robson and Adams 1968) refers to a similar situation in *H. mitchellianum* Rydb., a species (or hybrid) closely allied to *H. punctatum*. In both taxa, rings of 16 chromosomes were observed during meioses in pollen (Fig. 3). In *H. brasiliense*, abnormal anthers from sterile flowers were observed, presenting enlarged, unorganized tapetal cells and a thick deposit of sporopollenin, so it is the another case of cytoplasmic male sterility (Moraes *et al.* 2009). Besides pollen meiosis, Hoar (1931) observed the the ring of chromosomes also in the megaspore mother cells (Fig. 4). Bougnicourt (1970, 1971) found polyembryony in *H. tetrapterum* Fr. where additional embryos are formed from a synergid by apogamy and twin embryos are produced as buds from suspensor. Robson (1981) mentioned another one of Bougnicourt's results, where pseudo-polyembryony resulting from the con-

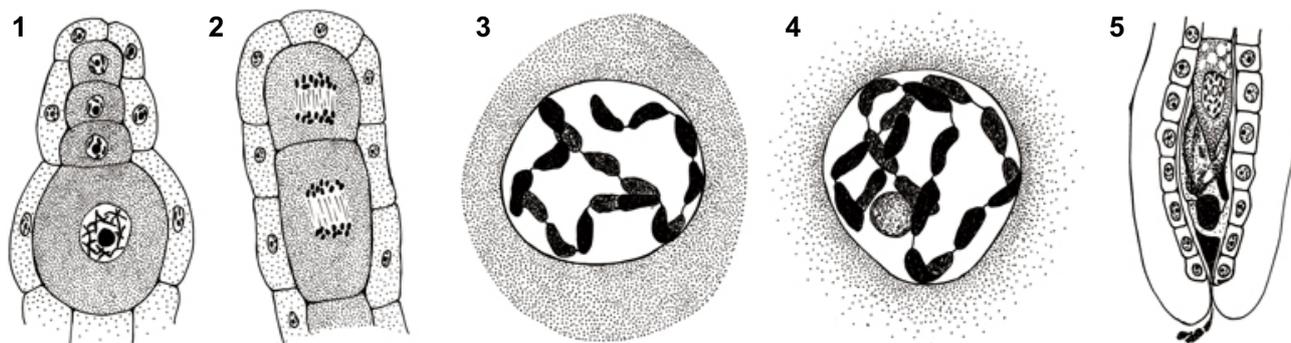


Fig. 1 *Hypericum setosum* (orig. *H. virginianum*): chain of four megaspores with upper three small and abortive – normal development (according to Hoar et Haertl 1932). **Fig. 2** *H. setosum* (orig. *H. virginianum*): megaspore mother cell undergoing second or homeotypic division – normal development (according to Hoar et Haertl 1932). **Fig. 3** *Hypericum punctatum*: pollen mother cell with nucleus enlarged, nucleolus disappeared, and chain of 16 chromosomes (according to Hoar 1931). **Fig. 4** *H. punctatum*: megaspore mother cell at diakinesis showing chain of 16 chromosomes; nucleolus still present (according to Hoar 1931). **Fig. 5** *Hypericum perforatum*: degenerative megaspore mother cell and initial cell of aposporic embryo sac (according to Noack 1939).

crecence of two or more ovules was observed in *H. maculatum* Crantz and *H. tetrapterum* Fr. as well as in *H. perforatum* L. (Mártonfi et al. 1996). Some of these results point out the presence of sporophytic apomixis in *Hypericum* sect. *Hypericum*. The presence of gametophytic apomixis is, however, much important, both facultative (see Table 1) and obligatory (in one case - *H. scabrum* from *Hypericum* sect. *Hirtella*, Matzk et al. 2003) gametophytic apomixis was detected. The results of the studies in the genus *Hypericum* presented up to now show that this apomixis is of *Hieracium*-type (Noack 1939, 1941; Matzk et al. 2003; Barcaccia et al. 2006, 2007). First results were published by Noack (1939, 1941) for *H. perforatum*. These results suggested that a normal reduced embryo sac occurred only in 3% of ovules and in the majority of the ovules (97%) aposporous embryo sacs (Fig. 5) with 32 chromosomes were present. Although the egg cell is capable of parthenogenetic

development, the polar nuclei must be fertilized (pseudogamy). Detailed study of reproductive diversity in *H. perforatum* was published by Matzk et al. (2001). They employed a new method FCSS (flow cytometric seed screen, Matzk et al. 2000), which allows to define, on the basis of DNA content in embryo and endosperm, the mode of seed formation. They identified 11 distinct routes of reproduction (Table 2). Several further authors studied degree of apomixis in *H. perforatum*: Mayo and Langridge (2003) studied reproduction using RFLP and AFLP. Between two Australian populations, plants displayed 14 polymorphisms from a total of 22 scorable RFLP markers when genomic DNA was probed with M13 bacteriophage. However, individuals within each population exhibited identical RFLP fingerprints. 49% of the progeny of four crosses made between the two populations exhibited fingerprint and ploidy level identical to the maternal parent and probably origi-

Table 1 List of apomictic taxa found in the genus *Hypericum* (according to Matzk et al. 2003 and others, completed). Asterisk denote obligate apomixis, other taxa are facultatively apomictic. Number in parentheses indicates source: 1 – Noack 1939; 2 – Lihová et al. 2000; 3 – Matzk et al. 2001; 4 – Mártonfi 2001; 5 – Matzk et al. 2003; 6 – Mártonfi 2008; 7 – Moraes et al. 2009.

Section of the genus (according to Robson 1977)	Taxa
3. Ascyreia Choisy	<i>H. acmosepalum</i> N. Robson (5); <i>H. beanii</i> N. Robson (5); <i>H. bellum</i> Li subsp. <i>latisepalum</i> (5); <i>H. dyeri</i> Rehder (5); <i>H. forrestii</i> N. Robson (5); <i>H. hookerianum</i> Wight & Arn. (5); <i>H. kouytchense</i> H. Lév. (5); <i>H. oblongifolium</i> Choisy (5); <i>H. patulum</i> Thunb. (5); <i>H. pseudohenryi</i> N. Robson (5)
9. Hypericum	<i>H. × carinthiacum</i> A. Fröhl. (= <i>H. × desetangii</i> nothosubsp. <i>carinthiacum</i> A. Fröhl.) N. Robson (2); <i>H. × carpaticum</i> Mártonfi (4); <i>H. × desetangii</i> Lamotte (5); <i>H. dubium</i> Leers (= <i>H. maculatum</i> subsp. <i>obtusiusculum</i> (Tourlet) Hayek) (5, 6); <i>H. kamtschaticum</i> Ledeb. (5); <i>H. perforatum</i> L. (1, 3, 5); <i>H. yezoense</i> Maxim. (5)
17. Hirtella Stef.	<i>H. scabrum</i> * L. (5)
30. Spachium (R. Keller) N. Robson	<i>H. brasiliense</i> Choisy (7)

* - asterisk denote obligate apomixis, other taxa are facultatively apomictic.

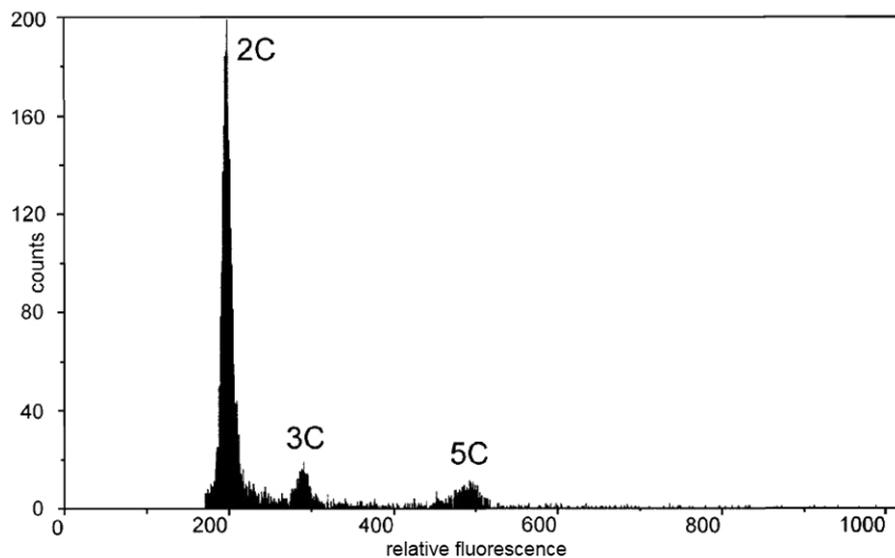
Table 2 Divergent reproductive pathways of *Hypericum perforatum* reconstructed by flow cytometric seed screen from 50 bulked seeds of several individuals per accession (according to Matzk et al. 2001, modified).

Route No.	C-values ^a of		Mode of reproduction ^a	% of accessions
	Embryo:	endosperm		
1	2 :	3	obligate: sexual (reduced double fertilized; 2x mother plants)	1.8
2	4 :	10	obligate: apomictic (unreduced pseudogamous; 4x mother plants)	1.8
3	4 :	8 + 10	obligate: apomictic (unreduced pseudogamous + autonomous; 4x mother plants)	0.9
4	4 :	6 + 10	facultative: apomictic/sexual (4x mother plants)	23.0
5	4 + 6 :	6? + 10	facultative: apomictic/sexual?/unreduced double fertilized (4x mother plants)	23.9
6	4 + 6 :	6? + 10 + 15	facultative: apomictic/sexual?/unreduced double fertilized (4x + 6x mother plants)	6.2
7	2 + 4 :	6 + 10	facultative: apomictic/sexual/reduced parthenogenetic (4x mother plants)	17.7
8	2 + 4 + 6 :	6 + 10	facultative: apomictic/sexual/reduced parthenogenetic/unreduced double fertilized (4x mother plants)	15.0
9	2 + 4 + 6 :	6 + 10 + 15	facultative: apomictic/sexual/reduced parthenogenetic/unreduced double fertilized (4x+6x mother plants)	7.1
10	4 + 5 + 6 :	6? + 9 + 10	facultative: apomictic/sexual?/unreduced double fertilized (pollen 1x and 2x; mother mixoploid?)	0.9
11	2 + 4 + 5 + 6 :	6 + 9 + 10	facultative: apomictic/sexual/reduced parthenogenetic/unreduced double fertilized (pollen 1x and 2x; mother mixoploid?)	1.8

^a in most cases endosperm peaks can be discriminated from embryo peaks by their height; only in few cases the small 6C endosperm peak is superimposed by a high 6C embryo peak and, therefore, the sexual path remains unproved (?) unless an additionally 2c embryo peak proved reduced embryo sacs. The C-values correspond with ploidy.

Table 3 Expression of apomixis in *Hypericum perforatum* (according to Barcaccia *et al.* 2007, completed).

Degree of apomixis	Source of sexuality	Method of analysis	References
97%	B _{II} and B _{III} hybrids	Triploid frequency in interploidy crosses	Noack 1939
20-90%	(Poly)haploids, B _{II} and B _{III} hybrids	Flow cytometric seed screen of single seeds	Matzk <i>et al.</i> 2001
54-67%	(Poly)haploids, B _{II} and B _{III} hybrids	Flow cytometric seed screen of bulked and single seeds	Pank <i>et al.</i> 2003
94%	B _{III} hybrids and one aneuploid	Molecular fingerprints and chromosome counts	Mayo and Langridge 2003
23-82%	(Poly)haploids, B _{II} and B _{III} hybrids	Flow cytometric seed screen of bulked and single seeds	Barcaccia <i>et al.</i> 2006

**Fig. 6** *Hypericum dubium*: flow cytometric seed screen. 2C peak represents both sexual and apomictic embryo, 3C peak represents endosperm from sexual reproduction, 5C peak represents endosperm from apomictic pseudogamous reproduction. 2C peak corresponds to tetraploid level, $2n=32$.

nated apomictically. Seven seedlings with recombinant RFLP or AFLP fingerprints were found from a total of 121 progeny. Pank *et al.* (2003) studied, similarly to Matzk (2001) by FCSS method, a reproductive diversity in 92 accessions (656 plants) of the species in comparison with 66 plants of cv. 'Topaz' as a control. Among the plants from 92 accessions, 16 were obligate sexuals, 9 were obligate apomicts and all remaining ones were facultative apomicts. Among the controls, there were 6 obligate and 60 facultative apomicts. Special study was devoted to reproductive pathways of somaclonal families of *H. perforatum* in 2004 (Koperdákóvá *et al.* 2004) by FCSS. The prevalent mode of reproduction of diploid plants was sexual reproduction and seed samples of plants with higher ploidy levels showed an extensive variation in the mode of reproduction: B_{II} and B_{III} hybrid formation and/or aposporous pseudogamy including parthenogenetic development of a reduced embryo sac were observed. Barcaccia *et al.* (2006) used molecular markers to determine levels of genetic variation within and relationships among ecotypes of *H. perforatum*. All ecotypes were polyclonal, being not dominated by a single genotype, and characterised by different levels of differentiation among multilocus genotypes. FCSS indicated that all genotypes were facultatively apomictic, with varying degrees of apomixis and sexuality. Seeds set by haploid parthenogenesis and/or by fertilisation of aposporic egg cells were detected in most populations. Excellent overview of apomixis in *H. perforatum* is given by Barcaccia *et al.* (2007). They summarized results, mainly on the basis of Matzk *et al.* (2001) that with rare exceptions, seeds of tetraploids can demonstrate four distinct (embryo : endosperm) ploidy ratios: reduced, double fertilized (sexually produced) balanced B_{II} or selfed plants (4C embryo : 6C endosperm); unreduced, pseudogamous (apomictically produced) maternal types (4C : 10C); reduced, parthenogenetic amphihaploid types (2C : 6C); or unreduced, double-fertilized (poly)triploid B_{III} plants (6C : 10C). Various degree of apomixis (Table 3) such demonstrate the extreme plasticity of the reproductive system in *H. perforatum* (Barcaccia *et al.* 2007).

However, further papers show that apomixis is not an

exceptional trait of the taxon *H. perforatum* and possibly of its closest relatives. Lihová *et al.* (2000) studied reproduction of *H. × carinthiacum* A. Fröhl. nothosubsp. *carinthiacum* under the name *H. × desetangsii* nothosubsp. *carinthiacum* (A. Fröhl.) N. Robson (for nomenclature see Mártonfi 2008) = *H. perforatum* × *H. maculatum* subsp. *maculatum* in detail. The results suggest that facultative apomixis with pseudogamy is possible in hybrids, probably due to inheritance of this trait from maternal plant *H. perforatum*. Various ploidy levels from triploid to heptaploid ones as well as aneuploids and mixoploids were found among the progeny. Mixoploid status of hybrid plants is documented also by Schulte *et al.* (1999). Similarly facultative apomixis was described for the species *H. carpaticum* Mártonfi (Mártonfi 2001), which was, however, later reevaluated as a hybrid taxon *H. × carpaticum* (= *H. dubium* Leers × *H. maculatum* Crantz) (Mártonfi 2008). Facultative apomixis of *H. × carpaticum* was thus explained as inherited from *H. dubium* (= *H. maculatum* subsp. *obtusiusculum* (Tourlet) Hayek). In 2003 Matzk *et al.* (2003) confirmed that besides *H. perforatum*, tetraploid *H. dubium* (probable autotetraploid derived from *H. maculatum*) is facultative apomict, too (see also Mártonfi 2008, Fig. 6). Simultaneously Matzk *et al.* (2003) brings a set of further facultatively apomictic taxa (from the sections *Hypericum* sect. *Ascyreia* Choisy and *Hypericum* sect. *Hypericum*; see Table 1) and one obligatory apomict (from the section *Hypericum* sect. *Hirtella* Stef.). Recent result for *H. brasiliense* (Moraes *et al.* 2009) shows that the presence of facultative apomixis in the genus *Hypericum* is extended also to the section *Hypericum* sect. *Spachium* (R. Keller) N. Robson.

IMPACT ON EVOLUTIONARY STUDIES AND BREEDING

The above survey of results concerning reproduction in the genus *Hypericum* suggests that evolutionary processes like hybridization and polyploidization together with apomictic reproduction play more important role in the evolution of the genus *Hypericum* than supposed initially. Recent data

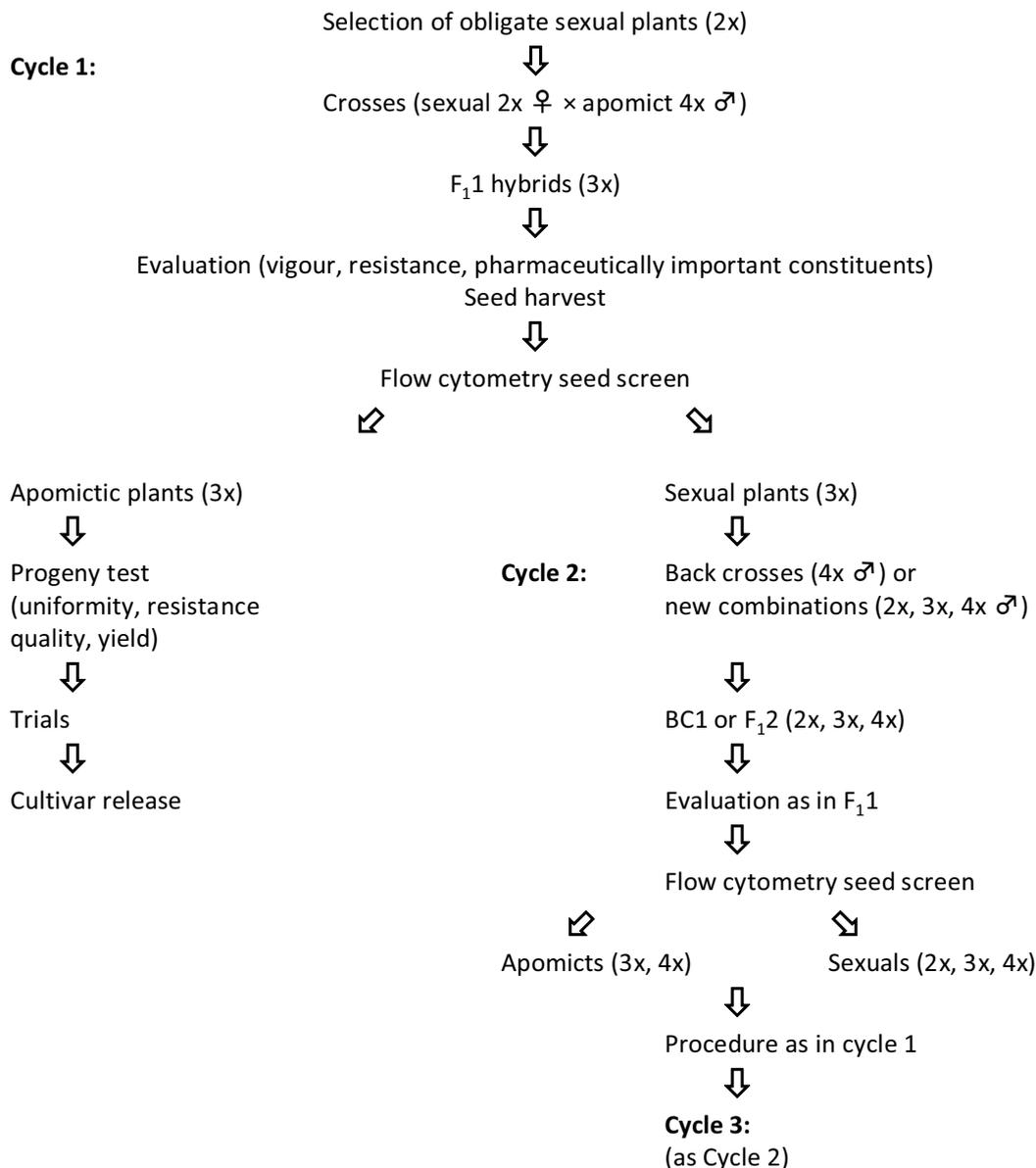


Fig. 7 *Hypericum perforatum*: A simplified breeding scheme with recurrent hybridisation. (according to Matzk 1991 and Pank *et al.* 2003).

on the occurrence of apomixis in the genus encourage more detailed research of all sections and species of the genus in order to obtain complex view of this genus. Detailed study of secondary metabolites, which may be useful for pharmacy and further human activities is needed, too. For this purposes, *H. perforatum* and *H. maculatum*, or eventually their hybrids are exploited the most often. As stated by Pank *et al.* (2003), plant breeders are interested now in combining desired characters from different genotypes into new commercial cultivars. However, in plants with apomixis standard breeding schemes are not practicable because apomixis excludes recombination and segregation. To overcome this barrier, Pank *et al.* (2003) proposed a breeding scheme (Fig. 7) based on crosses between obligate sexual and apomictic plants and on efficient screening method for the reproduction (Matzk *et al.* 2000). Employment of sporadically occurring obligate apomicts guarantee propagation of uniform progenies despite their heterozygosity, which is, however, fixed in particular lines (Richards 1997). Pank *et al.* (2003) states the use of facultative apomicts for breeding. As fixed heterotic effects is inherent in an apomictic genotype, the progeny which arose through sexual reproduction will have a reduced vitality and may not survive in competition with apomictic progeny under field growing conditions. Additionally, strong inbreeding effects reduce the vitality of recombinants in monoclonal apomictic cultivars. The per-

centage of sexual progenies in facultative apomicts can be reliably detected by single seed analyses with the FCSS.

Besides the study of evolution of the genus *Hypericum* itself and practical exploitation of plants from this genus in pharmaceutical industry, breeding systems in this genus are, owing to their wide plasticity, employable also directly for the study of mechanisms of apomixis. This is due to many factors (small genome size, high degree of molecular polymorphism between ecotypes, the versatile and dynamic mode of reproduction, the self-compatibility and easy crossability, etc. – see Matzk *et al.* 2001; Barcaccia *et al.* 2007), which make *H. perforatum* and further taxa of the genus model objects for biological studies.

FUTURE PERSPECTIVES

The genus *Hypericum* is widespread in all temperate parts of the world. It comprises about 450 species. Only about 15% of these species were tested for presence or absence of apomictic reproduction. Of the species tested, only one quarter was apomictic (cf. Table 1). These apomictic species belong to 4 out of 30 sections of the genus. The latest record on apomixis concerns the species *H. brasiliense* Choisy from the sect. *Spachium* (R. Keller) N. Robson (Moraes *et al.* 2009) suggests many surprises following from detailed studies of the genus. It is not only the presence or

absence of apomixis itself that represents an interesting character of particular species of the genus. It is necessary to understand heredity of apomixis in the genus *Hypericum* in forthcoming time. Employment of molecular analyses for genomic and transcriptome levels is necessary. Knowledge of apomixis in the genus *Hypericum* will substantially contribute to general comprehension of apomixis. Apomixis seems not to be true a novelty in plant development, but rather has evolved through the rearrangement of the subprograms that constitute a normal sexual pathway (Grimanelli *et al.* 2001). Knowledge obtained on molecular level and its application in plant breeding (not only in the genus *Hypericum*) are interesting from the point of view of their potential employment in private biotechnology sector and the seed industry. On the other hand, knowledge from different groups of angiosperms suggest that mechanisms of apomixis are very diverse. Therefore, understanding the regulation of apomixis will depend on a better understanding of the basic process of sexual plant reproduction (Grimanelli *et al.* 2001). In the genus as large as *Hypericum* this knowledge is inevitable for better understanding of evolutionary relationship in the genus, its relations to allied genera and other taxa of higher plants. Breeding systems represent basic piece of grit to the mosaic of understanding of evolutionary development of this genus.

REFERENCES

- Barcaccia G, Arzenton F, Sharbel TF, Varotto S, Parrini P, Lucchin M (2006) Genetic diversity and reproductive biology in ecotypes of the facultative apomict *Hypericum perforatum* L. *Heredity* **96**, 322-334
- Barcaccia G, Bäumllein H, Sharbel TF (2007) Apomixis in St. John's wort (*Hypericum perforatum* L.): an overview and glimpse towards the future. In: Hörandl E, Grossniklaus U, van Dijk PJ, Sharbel TF (Eds) *Apomixis. Evolution, Mechanisms and Perspectives. Regnum Vegetabile* (Vol 147), A. R. G. Gantner Verlag, Rugell, Liechtenstein, pp 259-280
- Bougnicourt M (1970) Polyembryonie chez l'*Hypericum tetrapterum* Fries (= *Hypericum acutum* Moench). *Bulletin de la Société Botanique de France, Paris* **116**, 495-504
- Bougnicourt M (1971) Embryogénie des Hypericacées. Développement de l'embryon chez l'*Hypericum tetrapterum* Fries. *Bulletin de la Société Botanique de France, Paris* **118**, 319-334
- Briggs D, Walters SM (1997) *Plant Variation and Evolution* (3rd Edn), Cambridge University Press, Cambridge, 513 pp
- Culwell DE (1970) A taxonomic study of the section *Hypericum* in the Eastern United States. PhD thesis, University of North Carolina, Chapel Hill, 185 pp
- Davis GL (1966) *The Systematic Embryology of Angiosperms*, John Wiley and Sons, New York, 528 pp
- Govindappa DA (1956) Morphological study of *Hypericum japonicum* Thunb. *Journal of Mysore University* **15**, 69-79
- Grimanelli D, Leblanc O, Perotti E, Grossniklaus U (2001) Developmental genetics of gametophytic apomixis. *Trends in Genetics* **17**, 597-604
- Hoar CS (1931) Meiosis in *Hypericum punctatum*. *Botanical Gazette* **92**, 396-406
- Hoar CS, Haertl EJ (1932) Meiosis in the genus *Hypericum*. *Botanical Gazette* **93**, 197-204
- Holsinger K (1992) Ecological models of plant mating systems and the evolutionary stability of mixed mating systems. In: Wyatt R (Ed) *Ecology and Evolution of Plant Reproduction*, Chapman and Hall, New York, pp 169-191
- Koperdákóvá J, Brutovská R, Čellárová E (2004) Reproduction pathway analysis of several *Hypericum perforatum* L. somaclonal families. *Hereditas* **140**, 34-41
- Lihová J, Mártonfi P, Mártonfiová L (2000) Experimental study on reproduction of *Hypericum* *desetangii* nothosubsp. *carinthiacum* (A. Fröhl.) N. Robson (Hypericaceae). *Caryologia* **53**, 127-132
- Lloyd DG (1992) Evolutionary stable strategies of reproduction in plants: who benefits and how? In: Wyatt R (Ed) *Ecology and Evolution of Plant Reproduction*, Chapman and Hall, pp 137-168
- Mártonfi P (2001) New species of the genus *Hypericum* sect. *Hypericum* (Guttiferae) from Slovakia. *Folia Geobotanica* **36**, 371-384
- Mártonfi P (2008) *Hypericum dubium* Leers – new data on taxonomy and biology. *Folia Geobotanica* **43**, 69-82
- Mártonfi P, Brutovská R, Čellárová E, Repčák M (1996) Apomixis and hybridity in *Hypericum perforatum*. *Folia Geobotanica et Phytotaxonomica* **31**, 389-396
- Matzk F (1991) New efforts to overcome apomixis in *Poa pratensis* L. *Euphytica* **55**, 65-72
- Matzk F, Hammer K, Schubert I (2003) Coevolution of apomixis and genome size within the genus *Hypericum*. *Sexual Plant Reproduction* **16**, 51-58
- Matzk F, Meister A, Schubert I (2000) An efficient screen for reproductive pathways using mature seeds of monocots and dicots. *Plant Journal* **21**, 97-108
- Matzk F, Meister A, Brutovská R, Schubert I (2001) Reconstruction of reproductive diversity in *Hypericum perforatum* L. opens novel strategies to manage apomixis. *Plant Journal* **26**, 275-282
- Mayo GM, Langridge P (2003) Modes of reproduction in Australian populations of *Hypericum perforatum* (St. John's wort) revealed by DNA fingerprinting and cytological methods. *Genome* **46**, 573-579
- Moraes ICR de, Pinto-Maglio CAF, Lombello RA (2009) Reproductive biology and cytology of *Hypericum brasiliense* Choisy (Hypericaceae). *Revista Brasileira de Botânica* **32**, 539-544
- Myers O (1963) Studies of reproduction and hybridization in five species of *Hypericum* native to eastern North America. PhD thesis, Cornell University, Ithaca, 233 pp
- Myers O (1964) Megasporeogenesis, megagametophyte development, and endosperm development in *Hypericum virginicum*. *American Journal of Botany* **51**, 664
- Noack KL (1939) Über *Hypericum*-Kreuzungen. VI. Fortpflanzungsverhältnisse und Bastarde von *Hypericum perforatum* L. *Zeitschrift für Induktive Abstammungs- und Vererbungslehre* **76**, 569-601
- Noack KL (1941) Geschlechtsverlust und Bastardierung beim Johanniskraut. *Forschungen und Fortschritte* **17**, 13-15
- Palm B (1922) Das Endosperm von *Hypericum*. *Svensk Botanisk Tidskrift Utgifven af Svenska Botaniska Foreningen* **16**, 60-68
- Pank F, Matzk F, Kästner U, Blüthner WD, Foltys de Gracia 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
- Philipson WR (1974) Ovular morphology and major classification of the dicotyledons. *Botanical Journal of the Linnean Society* **68**, 89-108
- Philipson WR (1977) Ovular morphology and the classification of the dicotyledons. *Plant Systematics and Evolution Supplement* **1**, 123-140
- Prakash N, Lau YY (1976) Morphology of *Ploiariium alternifolium* and the taxonomic position of *Ploiariium*. *Botaniska Notiser* **129**, 279-285
- Rao AN (1957) The embryology of *Hypericum patulum* Thunb. and *H. mysorense* Heyne. *Phytomorphology* **7**, 36-45
- Richards AJ (1990) The implications of reproductive versatility for the structure of grass populations. In: Chapman GP (Ed) *Reproductive Versatility in the Grasses*, Cambridge University Press, Cambridge, pp 131-153
- Richards AJ (1996) Genetic variability in obligate apomicts of the genus *Taraxacum*. In: Richards AJ, Kirschner J, Štěpánek J, Marhold K (Eds) *Apomixis and Taxonomy*, Opulus Press, Uppsala, pp 131-140
- Richards AJ (1997) *Plant Breeding Systems* (2nd Edn), Chapman and Hall, London, 529 pp
- Richards AJ (2003) Apomixis in flowering plants. *Philosophical Transactions of the Royal Society of London Series B* **358**, 1085-1093
- Robson NKB, Adams WP (1968) Chromosome numbers in *Hypericum* and related genera. *Brittonia* **20**, 95-106
- Robson NKB (1977) Studies in the genus *Hypericum* L. (Guttiferae). 1. Intra-generic classification. *Bulletin of the British Museum Natural History Botany* **5**, 291-355
- Robson NKB (1981) Studies in the genus *Hypericum* L. (Guttiferae) 2. Characters of the genus. *Bulletin of the British Museum Natural History Botany* **8**, 55-226
- Salisbury EJ (1963) Fertile seed production and self-incompatibility of *Hypericum calycinum* in England. *Watsonia* **5**, 368-376
- Schnarf K (1914) Beiträge zur Kenntniss der Samenentwicklung einer europäischer *Hypericum*-Arten. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-naturwissenschaftliche Klasse. Abteilung I* **123**, 159-188
- Schulte J, Schaffner W, Büter B, Berger Büter K (1999) Kreuzungsexperimente mit verschiedenen Arten der Gattung *Hypericum*. *Zeitschrift für Arznei- und Gewürzpflanzen* **4**, 126-133
- Souèges ECR (1925) Embryogénie des Hypericacées. Développement de l'embryon chez *H. perforatum*. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences Paris* **180**, 949-951