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, genetic 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 proportions 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). Embryogenesis in *Hypericum* is Solanum (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 *Onoethera* 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. tetramerum* 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-
crescence 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>
<thead>
<tr>
<th>Section of the genus</th>
<th>Taxa</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Aseyrie Choisy</td>
<td><em>H. acmosepalum</em> N. Robson (5); <em>H. bearni</em> N. Robson (5); <em>H. bellum</em> Li subsp. <em>latisepalum</em> (5); <em>H. dyeri</em> Rehdler (5); <em>H. forrestii</em> N. Robson (5); <em>H. hookeranum</em> Wight &amp; Arn. (5); <em>H. koytchensa</em> H. Lév. (5); <em>H. oblongifolium</em> Choisy (5); <em>H. patdum</em> Thunb. (5); <em>H. pseudohenryi</em> N. Robson (5)</td>
<td></td>
</tr>
<tr>
<td>9. Hypericum</td>
<td><em>H. × carinthiacum</em> A. Fröh (+= <em>H. × desetangii</em> nothosubsp. <em>carinthiacum</em> A. Fröh) N. Robson (2); <em>H. × carpaticum</em> Mártonfi (4); <em>H. × desetangii</em> Lamotte (5); <em>H. dubium</em> Leers (+= <em>H. maculatum</em> subsp. <em>obtusiusculum</em> (Tourlet) Hayek) (5, 6); <em>H. kamtschaticum</em> Ledeb. (5); <em>H. perforatum</em> L. (1, 3, 5); <em>H. yezoense</em> Maxim. (5)</td>
<td></td>
</tr>
<tr>
<td>17. Hirtella Stef</td>
<td><em>H. scabrum</em> L. (5)</td>
<td></td>
</tr>
<tr>
<td>30. Spachium (R. Keller) N. Robson</td>
<td><em>H. brasiliense</em> Choisy (7)</td>
<td></td>
</tr>
</tbody>
</table>

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

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).

<table>
<thead>
<tr>
<th>Route</th>
<th>Embryo: endosperm</th>
<th>Mode of reproduction</th>
<th>% of accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>C-values <em>a</em> of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 : 3</td>
<td>obligate: sexual (reduced double fertilized; 2x mother plants)</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>2 : 10</td>
<td>obligate: apomictic (unreduced pseudogamous; 4x mother plants)</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>4 : 6 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (4x mother plants)</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>4 : 6 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (4x + 6x mother plants)</td>
<td>23.9</td>
</tr>
<tr>
<td>5</td>
<td>4 : 6 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (4x + 6x mother plants)</td>
<td>6.2</td>
</tr>
<tr>
<td>6</td>
<td>2 + 4 : 6 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (4x + 6x mother plants)</td>
<td>17.7</td>
</tr>
<tr>
<td>7</td>
<td>2 + 4 : 6 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (4x + 6x mother plants)</td>
<td>15.0</td>
</tr>
<tr>
<td>8</td>
<td>2 + 4 + 6 : 6 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (4x + 6x mother plants)</td>
<td>7.1</td>
</tr>
<tr>
<td>9</td>
<td>2 + 4 + 6 : 6 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (4x + 6x mother plants)</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>2 + 4 + 5 + 6 : 6 + 9 + 10</td>
<td>facultative: apomictic/sexual?/unreduced double fertilized (pollen 1x and 2x; mother mixoploid?)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* 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.

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**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, nucleus disappeared, and chain of 16 chromosomes (according to Hoar 1931). **Fig. 4** *H. punctatum*: megaspore mother cell at diakinesis showing chain of 16 chromosomes; nucleus 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).
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 \textit{H. perforatum} in 2004 (Koperdáková 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 aposporic 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 \textit{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 brings a set of further facultatively apomictic taxa (from the section \textit{Hypericum sect. Hirtella}) and one obligatory apomict \textit{H. brasiliense} (probable autotetraploid derived from \textit{H. maculatum}) is facultative apomict, too (see also Mártonfi 2001). Matzk et al. (2003) confirmed that besides \textit{H. perforatum}, tetraploid \textit{H. dubium} (probable autotetraploid derived from \textit{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 \textit{Hypericum sect. Ascyreia} \textit{Choisy} and \textit{Hypericum sect. Hirtella} \textit{Stef.}). Recent result for \textit{H. brasiiliense} (Moraes et al. 2009) shows that the presence of facultative apomixis in the genus \textit{Hypericum} is extended also to the section \textit{Hypericum sect. Spachium} (R. Keller) N. Robson.

**IMPACT ON EVOLUTIONARY STUDIES AND BREEDING**

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

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

**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.
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 1991 and Pank *et al.* 2003). 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 percentage 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

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**Fig. 7 Hypericum perforatum: A simplified breeding scheme with recurrent hybridisation.** (according to Matzk 1991 and Pank *et al.* 2003).
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 a novelty in plant development, but rather has evolved through the rearrangement of the sub-programs 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 have developed through a 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.

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