Sexual reproduction as a factor influencing population genetic structure in agamic complex of *Hieracium* subgenus *Pilosella*

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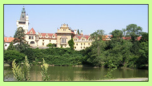


• Introduction:

Hieracium subgen. *Pilosella* is one of the most complex groups in flowering plants. Its structure is strongly influenced by hybridisation, polyploidisation, and apomixis. Apomixis represents clonal reproduction through seeds, no genetic variation is generated among progeny. However apomixis usually coexists with some level of sexuality. Gametes fusing during sexual reproduction can be reduced or unreduced, and inter-cytotype or inter-specific crosses are frequent. Parthenogenetic development of reduced gamete (haploid parthenogenesis) is also possible. The principal task of our study is significance of variability generated by sexual reproduction and haploid parthenogenesis among progeny of apomicts in subgen. *Pilosella*.

• Questions:

What kind of variability can be generated by sexual reproduction and haploid parthenogenesis among progeny of apomictic plants?
In what way does arising progeny impact subsequent development of population genetic structure?

• Model system:

Hexaploid (6x) apomictic accession of *H. bauhini* and tetraploid (4x) sexual accession of *H. pilosella* were chosen for the study. All plants were collected near Valov (NW Bohemia) where natural hybridisations between *H. bauhini* and *H. pilosella* occur.


H. bauhini (6x, apomictic)


H. pilosella (4x, sexual)

• Results 1): What kind of variability can be generated by sexual reproduction and haploid parthenogenesis?

Variability arising among progeny of apomictic *H. bauhini* was investigated in crossing experiments. *H. bauhini* and *H. pilosella* were used as seed parent and pollen donor respectively. Origin of each offspring (fusion of reduced / unreduced gametes, haploid parthenogenesis or apomixis) was assessed on the base of its ploidy level (Tab. 1) using flow cytometry. Proportion of haploid parthenogenesis was tested also in emasculated plants of *H. bauhini*.

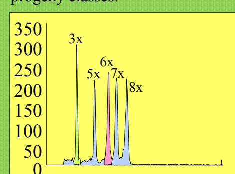
Tab. 1) Ploidy level of each progeny class, which can be theoretically obtained in crossing experiments:

		♀ - *H. bauhini*; 6x, apomict		
		3x	6x	6x
♂ - *H. pilosella*; 4x, sexual	2x	5x	8x	
	4x	7x	10x	
	0	3x		6x

Ploidy level of:
- gametes (reduced / unreduced)
- apomictic embryo sac
- sexually-derived progeny
- haploid parthenogenesis
- progeny from apomixis

Progeny classes obtained in crossing experiments:

Flow cytometry profile of progeny classes:



Percentage proportion of each progeny class:

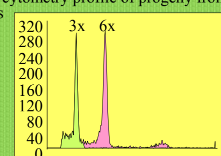
- 5x - 3.59%
- 7x - 0.13%
- 8x - 0.77%
- 10x - 0
- 3x - 1.28%
- 6x - 94.23%

780 seedlings were analysed. All progeny classes were obtained except decaploids (10x). Decaploidy seems to be too high ploidy to survive.

Proportion of haploid parthenogenesis

Crossing experiments - 1.28% of seedlings
Emasculated plants - 0.31% of seedlings

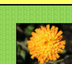
Flow cytometry profile of progeny from emasculated plants



Pollination seems to influence proportion of haploid parthenogenesis positively.

• Results 2): In what way does arising progeny impact subsequent development of population genetic structure?

Natural populations represent only rarely simple crossing experiments consisting of parental species and F1 hybrids. Arising hybrids can also give rise to variability and contribute to formation of complex structure of natural populations. Therefore it is necessary to study processes occurring on population level and compare results from experiments with situation in nature.



Model population: (Valov - NW Bohemia)

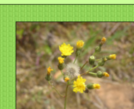
Properties of plants from model population:

plant type	ploidy	breeding system	number of plants
H. bauhini	6x	ap.	
	5x	ap.	
H. pilosella	4x	sex.	
Hybrid types	4x	sex.	4
	5x	sex.	9
	7x	ap.	1


5x hybrids from model population

H. bauhini (5x and 6x, apomictic), *H. pilosella* (4x, sexual), and hybrid types (4x, 5x, 7x) were detected among 71 samples collected at locality. 15 hybrid individuals were analysed for breeding system.
No triploids (3x) or octoploids (8x) were detected. Haven´t they established in population yet or is it not possible for them to arise there at all?

Processes in population - plans for future



What kind of variability arises only in the population?
- analysis of seeds and seedlings from model population

How does each progeny class behave in crosses?
- next crossing experiments are planned between progeny from experiments and parental species, and between hybrids from population and parental species

Schema of planned experiments:

plants from experiments / population:
1) 5x X *H. pilosella*
2) 5x X *H. bauhini*
3) 7x X *H. pilosella*
4) 7x X *H. bauhini*

plants from experiments only:
5) 8x X *H. pilosella*
6) 8x X *H. bauhini*
7) 3x X *H. pilosella*
8) 3x X *H. bauhini*

• Conclusions:

Although most of the progeny (94.23%) is produced asexually in apomictic *H. bauhini*, 4.31% of retained sexuality is sufficient to generate extensive variability. Three different classes of sexually derived progeny (5x, 7x, 8x) arose in crossing experiments. 1.28% of haploid parthenogenesis was detected in crossing experiments but only 0.31% in emasculated plants. Pollination seems to influence proportion of haploid parthenogenesis positively.

Nevertheless situation in natural populations is more complicated than it is in crossing experiments and challenge to next thorough investigation. No triploid (3x) or octoploid (8x) plants were detected in model population - Why? What kind of variability can arise just in population? How does each progeny class behave in crosses?

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