REPRODUCTIVE STRATEGY IN THE *RANUNCULUS AURICOMUS* COMPLEX (*RANUNCULACEAE*)

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ABSTRACT

A preliminary study of reproductive strategy was carried out on plants representing four cytotypes of the *Ranunculus auricomus* complex. Diploid plants reproduce sexually, triploids, tetraploids and hexaploids have an apomictic mode of reproduction (apospory combined with pseudogamy).

Some morphological characters of the plants connected with reproduction were analyzed at various phases and compared in two successive seasons. The following characters were estimated at the individual level: number of ovules and primary pollen grains per plant, pollen viability, pollen-ovule ratio, number of achenes per plant and their germinability, seed-ovule ratio. Most of data characteristic of reproductive strategy were calculated according to the formulae proposed by Urbańska (1989, 1990).

Apomictic cytotypes have both a significantly lower pollen viability and seed-ovule ratio (the lowest values in triploids) than sexual diploids.

KEY WORDS: *Angiospermae*, *Ranunculaceae*, *Ranunculus auricomus* complex, agamospermy, reproductive strategy.

INTRODUCTION

The *Ranunculus auricomus* complex in Europe comprises a polyploid series ranging from diploid ($2n=16$) to 9-ploid level ($2n=72$). Diploid species reproduce sexually, whereas polyploids are apomictic (Häfliger 1943, Rutishauser 1954a, b, Nogler 1971, Izmailow 1965, 1967).

The basic components of apomixis in the complex are as follows: 1) apomeiotic embryo sac formation; 2) parthenogenetic embryo development; 3) pseudogamous endosperm formation. The most regular endosperm development and successful formation of seeds are connected with pollination within the same ploidy level.

Exceptionally sexual components of reproduction may occur (i.e. meiotic functional embryo sac, double fertilization), resulting in the following possible modes of progeny formation: 1) fertilization of reduced egg cell results in B_{II} hybrid; 2) fertilization of unreduced egg cell results in B_{III} hybrid; 3) parthenogenetic development of reduced egg cell results in dihaploid or polyhaploid. Formation of karyologically new types in a progeny of apomictic biotypes was documented experimentally in our previous studies (Izmailow 1970, 1993). Polyploid biotypes of the *R. auricomus* complex show a high degree of apomixis (= % of maternal offspring). The degree of apomeiosis (98,8%) and degree of parthenogenesis (98,4%) were determined for some tetraploid and pentaploid individuals after controlled pollination (Izmailow 1970, 1993).

Presently a further study on reproductive biology in the complex has been undertaken; it refers to the preliminary quantitative assessment of female and male characters of some biotypes.

MATERIAL AND METHODS

The present comparative study of reproductive characters refers to four cytotypes of the *R. auricomus* complex. Their mode of reproduction was examined in our previous investigations. Diploid biotype ($2n=16$) represents sexual mode of reproduction whereas triploids ($2n=24$), tetraploids ($2n=32$) and hexaploids ($2n=48$) reproduce apomictically.

The studies were carried out on individuals of similar age in two successive seasons. The plants collected in natural habitats, were cultivated under the same environmental conditions in the experimental field.

Stainability of pollen grains was tested with acetocarmine; pollen viability was determined on medium consisting of 4% agar, 20% sucrose and 0,001% boric acid in distilled water. The achenes originated from open pollination. Their germination was determined in laboratory conditions on moist filter paper.

Reproductive offer, efficiency and reproductive success of the studied plants were calculated according to definitions and formulae of evaluation proposed by Urbańska (1989, 1990). In accordance with these patterns, the evaluation of male re-

Fig. 1. The mean percentage of well developed achenes (closed) and empty achenes (plain) in four cytotypes of the *R. auricomus* complex.

productive efficiency was based on results of the staining test. The data are related to the "mean individuals" of the particular cytotypes.

Definitions (acc. to Urbańska 1989)

Reproductive offer (FRO, MRO) – is defined as the amount of reproductive units occurring in female and/or male organs at early pre-mating stage (F = female; M = male).

Reproductive efficiency (FEFF, MEFF) – is defined as the ability to carry to term viable reproductive units by an individual (terminating points: pollen and seed dispersal). FEFF is expressed by the number of fertile seeds at dispersal relative to FRO. MEFF is expressed by the number of viable pollen grains at anthesis relative to MRO.

Reproductive success (FRS) – is determined by appearance of active offspring individuals. FRS relates to the germination percentage of the seeds produced by a given mother individual and is influenced by its FEFF. In the present study FRS is considered in female function only because most of the studied cytotypes reproduce apomictically.

RESULTS AND CONCLUSIONS

Achenes

The number of pistils per flower varies considerably in the studied cytotypes representing different microspecies. A lower variation was stated between individuals of the same microspecies and between flowers of the same plant.

The mean numbers of pistils per flower were as follows: 54.0 in sexual diploids, 75.7 in triploids, 57.0 in tetraploids and 48.0 in hexaploids. A pistil contains one ovule, then the numbers mentioned above stand for the average number of ovules per flower. It appeared, however, that only a part of pistils form achenes containing a seed. The setting of well developed achenes is decreased by an irregular structure of some aposporous embryo sacs, degeneration of an egg cell, nucellus, embryo or/and endosperm. In flowers with a high number of pistils those at the top of the receptacle contain any mature embryo sac or it reaches maturity not before the flower sheds its perianth and then is unattractive for insect pollinators.

The mean percentage of well developed achenes was highest in diploids – 53.7%, in triploids it appeared to be 14.9%, in tetraploids – 37.2% and in hexaploids 35.2% (Fig. 1).

Pollen viability

Polyploid biotypes of the *R. auricomus* complex are pseudogamous apomicts, thus pollen viability is of importance for successful seed formation. Hybrid origin of the complex and polyploidy result in meiotic aberrations in microsporogenesis and are probably the main reason for decreased pollen viability (Rousi 1956, Jankun 1965).

The mature pollen grains of polyploids representing apparently various chromosome numbers reveal a wide scale of size differences; a significant part of them represents small and deformed grains and microsporocytes.

The average pollen stainability in the studied apomictic cytotypes was as follows: 2n=24 – 33.4%; 2n=32 – 54.2%; 2n=48 – 51.3%. Sexual diploid species (2n=16) produced 77.3% of viable pollen (Fig. 2).

A part of stained pollen grains proved incapable of germination on medium. The pollen germination test gave lower values than the former one: 2n=16 – 75.9%; 2n=24 – 28.0%; 2n=32 – 48.3%; 2n=48 – 46.6% (Fig. 2).

In spite of considerably decreased pollen viability in apomictic individuals, they have still at disposal a relatively good amount of viable mature pollen grains at anthesis. The pollen-ovule ratios (calculated from the number of stained pollen grains and ovules produced per plant) vary from 652 in triploids to 1684 in tetraploids. Such a high pollen-ovule ratio is typical for insect-pollinated plants (Kawano and Nagai 1975, Cruden 1977). Pollination can be strongly influenced by climate conditions, unfavourable for insect activity at flowering time in April.

Assessment of reproductive strategy

Pollen, ovule and seed production in the four cytotypes were analyzed and assessed according to the formulae proposed by Urbańska (1989, 1990). Table 1 summarizes the results of this quantitative assessment.

The sexual diploid cytotype (2n=16) is characterized by the highest value of FRS as compared with the other studied cytotypes. It is a result of regular development of meiotic em-

Fig. 2. Frequency histograms of stained and germinated pollen grains in various cytotypes of the *R. auricomus* complex. 1 – stained pollen grains, 2 – germinated pollen grains.

bryo sacs, regular meiosis in anthers, and of high percentage of viable pollen grains (high values of FEFF and MEFF).

Tetraploids ($2n=32$) and hexaploids ($2n=48$) appear to have very similar values of FEFF and MEFF as well as a similar reproductive success. In a sympatric population they seem to have an equal chance.

Triploid cytotype ($2n=24$) differs distinctly from tetraploids and hexaploids, showing the lowest values of FEFF, MEFF as well as of FRS. This cytotype, if produced spontaneously as polyploid in a natural population of tetraploids or hexaploids, may have a small success in production of seeds. On the other hand, in a population containing several triploid individuals a possibility of pollination within the same cytotype increases their chance for successful forming of achenes.

The seed-ovule ratio (the percentage of ovules developing into seeds) varied from 53,0% to 14,7% in sexual diploids and in apomictic triploids, respectively. The seed-ovule ratio in diploids remains within the limits characteristic of perennials (Wiens 1984), whereas the values obtained in tetraploids

(37,0%) and in hexaploids (35,0%) belong to the lowest ratios cited by this author, also in agamosperous plants.

All cytotypes are characterized by a high germinability of achenes: $2n=16$ – 95%; $2n=24$ – 88%; $2n=32$ – 90%; $2n=48$ – 91%. A contribution of germinated seedlings in natural populations is not yet well known and needs to be studied.

The low reproductive success of apomicts, as compared with sexual diploids, is supplemented by vegetative propagation, though in natural habitats it is not so intense as in experimental field.

LITERATURE CITED

- CRUDEN R.W., 1977. Pollen-ovule ratios: a conservative indicator of breeding system in flowering plants. *Evolution*, 31: 32-46.
 HÄFLIGER E., 1943. Zytologisch-embryologische Untersuchungen pseudogamer Ranunkeln der *Auricomus*-Gruppe. *Ber. der Schweiz. Bot. Ges.* 53: 318-382.

TABLE 1. The values of reproductive offer, reproductive efficiency and reproductive success in four cytotypes of the *R. auricomus* complex.

Components of reproduction by seeds	2n = 16 sexual	2n = 24 apomictic	2n = 32 apomictic	2n = 48 apomictic
Reproductive offer				
F RO	302,4	393,6	456,0	336,0
M RO	978 745,6	769 574,0	1 417 460,0	957 133,1
Reproductive efficiency				
F EFF	0,53	0,15	0,37	0,35
M EFF	0,77	0,33	0,54	0,51
Reproductive success				
F RS	50,7	13,2	33,4	32,0

- IZMAIŁOW R., 1965. Macrosporogenesis in the apomictic species *Ranunculus cassubicus* L. Acta Biol. Cracov. Ser. Bot. 8: 183-195.
- IZMAIŁOW R., 1967. Observations in embryo and endosperm development in various chromosomic types of the apomictic species *Ranunculus cassubicus* L. Acta Biol. Cracov. Ser. Bot. 10: 99-111.
- IZMAIŁOW R., 1970. Cytogenetic studies in the apomictic species *Ranunculus cassubicus* L. Acta Biol. Cracov. Ser. Bot. 13: 37-50.
- IZMAIŁOW R., 1993. Biologia rozmnażania słowackich biotypów z kompleksu *Ranunculus auricomus-cassubicus*. In: Proces embryogenezy roślin in situ a in vitro. Zbornik referatov zo VI. konferencie rastlinnych embryologov Slovenska, Ceska a Polska. Nitra 1993. pp. 62-66.
- JANKUN A., 1965. Studies of meiosis in various chromosomic types of *Ranunculus cassubicus* L. Acta Biol. Cracov. Ser. Bot. 8: 171-181.
- KAWANO S. and NAGAI Y., 1975. The productive and reproductive biology of flowering plants. I. Life history strategies of three *Allium* species in Japan. Bot. Mag. Tokyo 88: 282-318.
- NOGLER G.A., 1971. Genetik der Aposporie bei *Ranunculus auricomus* s.l. W. Koch. I. Embryologie. Ber. Schweiz. Bot. Ges. 81: 139-179.
- ROUSI A., 1956. Cytotaxonomy and reproduction in the apomictic *Ranunculus auricomus* group. Ann. Bot. Soc. Vanamo. 29: 1-64.
- RUTISHAUSER A., 1954a. Die Entwicklungserregung des Endosperms bei pseudogamen Ranunculusarten. Mitt. Naturforsch. Ges. Schaffhausen 25: 1-45.
- RUTISHAUSER A., 1954b. Entwicklungserregung der Eizelle bei pseudogamen Arten der Gattung *Ranunculus*. Bull. Schweiz. Akad. Med. Wiss. 10: 491-512.
- URBAŃSKA K.M., 1989. Reproductive effort or reproductive offer? – A revised approach to reproductive strategies of flowering plants. Bot. Helv. 99: 49-63.
- URBAŃSKA K.M., 1990. Biology of asexually reproducing plants. In: Biological approaches and evolutionary trends in plants. Kawano S. (ed.). Academic Press, London. pp. 273-292.
- WIENS D., 1984. Ovule survivorship, broad size, life history, breeding systems, and reproductive success in plants. Oecologia (Berlin) 64: 47-53.

STRATEGIA ROZMNAŻANIA W KOMPLEKSIE *RANUNCULUS AURICOMUS* (*RANUNCULACEAE*)

STRESZCZENIE

Przeprowadzono wstępne badania dotyczące strategii rozmnażania roślin reprezentujących cztery cytotypy kompleksu *Ranunculus auricomus*. Diploidy z tego kompleksu rozmnażają się seksualnie; triploidy, tetraploidy i heksaploidy rozmnażają się apomiktycznie (aposporia połączona z pseudogamią).

W ciągu dwóch sezonów analizowano różne fazy rozmnażania wymienionych cytotypów. Następujące cechy zostały oszacowane dla indywidualnych okazów: liczba zalążków i pierwotnych ziarn pyłku wytwarzanych przez jednego osobnika, żywotność pyłku, stosunek ilości zalążków do ilości ziarn pyłku, liczba nasion produkowanych przez osobnika, zdolność kiełkowania nasion oraz stosunek ilości nasion do ilości zalążków tworzonych przez roślinę. Większość z tych cech oszacowano na podstawie wzorów proponowanych przez Urbańską (1989, 1990).

Apomiktyczne cytotypy w porównaniu z seksualnymi diploidami wykazują znacznie niższą żywotność pyłku oraz niższą wartość stosunku ilości nasion do ilości zalążków (najniższe wartości u triploidów).

SŁOWA KLUCZOWE: *Angiospermae*, *Ranunculaceae*, *Ranunculus auricomus* kompleks, agamosperma, strategia rozmnażania.