

The mentor effect increases the rate of selfing in couch grasses

Efekt mentora zvyšuje míru autogamie u pýrů

Romana Urfusová^{1,2}, Václav Mahelka², František Krahulec², Ondřej Veškrna³
& Tomáš Urfus^{1*}

¹Department of Botany, Charles University, Benátská 2, CZ-128 00 Prague, Czech Republic, e-mail: tomas.urfus@natur.cuni.cz; ²Institute of Botany of the Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic; ³Research Center SELTON, Ltd., Stupice 24, CZ-250 84 Sibřina, Czech Republic; *corresponding author

Urfusová R., Mahelka V., Krahulec F., Veškrna O. & Urfus T. (2021) The mentor effect increases the rate of selfing in couchgrasses. – Preslia 93: 377–397.

Some self-incompatible species are thought to be capable of selfing via facilitation by foreign pollen (induced selfing or the mentor effect), which might be a phenomenon of underestimated importance. In addition, higher percentages of selfing are linked to polyploidy. Grasses are a very important plant group, but the degree to which they are capable of selfing (including via the mentor effect) has scarcely been investigated. We selected *Elymus hispidus*, *E. repens* and their hybrid (*Poaceae* tribus *Triticeae*) as model taxa in order to test their ability to reproduce by selfing, to assess the significance of induced selfing, and to examine the assumed link between selfing and ploidy level (particular cytotype). Because absolute genome size of the model species of *Elymus* differs significantly, we used flow cytometry as a marker for identifying selfing as opposed to outcrossing based on comparing parental plants with their progeny. The ability for selfing and induced selfing was determined in two complementary crossing experiments supplemented by an open-pollination control treatment. The results confirm that both species and their hybrid are capable of selfing and that the percentage of selfing increased significantly in the presence of foreign pollen. This is especially marked in *Elymus hispidus* (the realized seed set in autonomous selfing was 6.2% compared to 22.7% in induced selfing). Octoploids selfed more often than hexaploids and heptaploids, and heptaploids produced significantly fewer seeds after selfing than hexaploids and octoploids. The pronounced selfing ability in *E. hispidus* (observed in both experiments) may be linked to introgression. Despite probably being a very important reproductive feature, the significance of induced selfing (i.e. the mentor effect) is definitely underestimated.

Keywords: absolute genome size, *Elymus*, mentor effect, induced selfing, pollination experiment

Introduction

There are two predominant sexual reproductive pathways in plants: selfing (self-pollination, ~ autogamy) and outcrossing (cross-pollination, outbreeding, ~ allogamy; Darwin 1861, Richards 1996, Briggs & Walters 2016). A significant percentage of flowering plants (40–60%; East 1940, Igic et al. 2008) display some degree of self-incompatibility. Nevertheless, self-incompatibility is not necessarily followed by complete outcrossing (Igic & Kohn 2006). Analogously, self-compatibility is far from complete selfing (autogamy). The majority of angiosperms combine different percentages of selfing with outcrossing and obligate outcrossers, and especially obligate selfers, are probably rare (Igic & Kohn 2006, Oakley et al. 2007).

The percentage of selfing is related to life history traits and ecology; for example, annual species tend to be mainly selfers because their life is very short, so they need to produce seeds quickly and in larger amounts. One obvious disadvantage of selfing is a higher probability of inbreeding depression, leading to decreased individual fitness (Olmstead 1989, Charlesworth 2006). On the other hand, the main advantage of selfing is reproduction not dependent on other individuals, the services of pollinators or some external abiotic factors such as wind or water; the consequences of outcrossing are the opposite (Olmstead 1989). Even though a major percentage of flowering plants display some self-incompatibility, 40–60% of angiosperms are capable of self-pollination (Hörandl 2010, Fujii et al. 2016). In principle, there are three ways of avoiding self-pollination: heteromorphic self-incompatibility, dichogamy and homomorphic self-incompatibility (Barrett & Cruzan 1994).

In addition to highly specific spatial isolation (dioecism), heteromorphic self-incompatibility (SI) could be realized at the level of direct morphological differentiation, heterostyly (e.g. distyly in *Primula* or tristily in *Lythrum*; Darwin 1877, Barrett & Cruzan 1994). Reproductive elements of the same flower are frequently separated temporally (different timing of maturity), i.e. dichogamy (proterandry or proterogyny). The second way to achieve SI is called homomorphic, a genetically determined pollen recognition system.

Homomorphic mechanisms act at the gametophytic (GSI) or sporophytic (SSI) level. Generally, there are two ways in which GSI is realized in angiosperms: S-RNase-type GSI and *Papaver*-type GSI, and one way in which SSI is realized: *Brassicaceae*-type SSI (Hiscock & McInnis 2003). S-RNase-type of GSI is widely present in many families of angiosperms (reported from *Solanaceae*, *Rosaceae* and *Scrophulariaceae*; Newbigin et al. 1993). The mechanism is based on S-RNase cross-identification of pollen tubes and style tissue (Hiscock & McInnis 2003).

Papaver-type of GSI, which occurs in *Papaver rhoeas* seems to be restricted to *Papaveraceae* (Franklin-Tong & Franklin 1992). The incompatibility is mediated by a Ca^{2+} -based signalling system induced by exposure of pollen to a stigmatic S-protein of the same haplotype (e.g. Kao & Tsukamoto 2004, McClure & Franklin-Tong 2006, Yang et al. 2008). Sporophytic SI, *Brassicaceae*-type, is determined by the S phenotype of pollen (one multiallelic S locus), which is determined sporophytically by the diploid genotype of the paternal plant and inhibition of the growth of pollen tubes occurs on surface of stigma (Nasrallah & Nasrallah 1993) and is reported for instance in the families *Brassicaceae*, *Asteraceae*, *Betulaceae* and *Convolvulaceae* (Brennan et al. 2011).

The degree of selfing is usually variable, meaning that selfing and outcrossing are combined (e.g. Stebbins 1957). Selfing is realized via a number of mechanisms (cleistogamy – *Violaceae*, Oakley et al. 2007; geitonogamy – *Orchidaceae*, Johnson & Nilsson 1999; facilitated selfing – *Roridula*, Anderson et al. 2003) and at different floral stages (prior – *Violaceae*, competing – *Brassicaceae*, and delayed self-pollination – *Asteraceae*; Lloyd & Schoen 1992). Nevertheless, only a few cleistogamous species are thought to be obligatorily selfing (e.g. *Epipactis*, Pedersen & Ehlers 2000; *Bulbophyllum dischidii-folium* or *Dendrobium chryseum*, Uphof 1938; *Epipogium roseum*, Zhou et al. 2012).

The mentor effect (induced selfing) is a specific type of selfing in which a self-incompatible plant accepts its own pollen rather than that of a different species in the mixture on its stigma surface (Richards 1997, Krahulcová et al. 1999, Tas & van Dijk 1999, Mráz

2003). The mentor effect is reported both in sporophytic SI plant groups (*Asteraceae* – e.g. Desrochers & Rieseberg 1998, Krahulcová et al. 1999, Tas & van Dijk 1999, Mráz 2003) and in gametophytic SI (*Lotus*; Miri & Bubar 1966, Richards 1997).

The mentor effect has rarely been proved experimentally [*Asteraceae* – *Pilosella* (Krahulcová et al. 1999), *Hieracium* (Mráz 2003), *Helianthus* (Desrochers & Rieseberg 1998), *Salicaceae* – *Populus* (Vanden-Broeck et al. 2012) or in *Rosaceae* – *Potentilla* (Dobeš et al. 2018)]. Despite being potentially important, the mentor effect has not yet been studied in the *Poaceae*.

Grasses (*Poaceae*) are a reproductively highly specific group that is primarily anemogamous. The flowers of grasses are efficiently adapted to wind pollination; their stigmas are feathery to improve pollen capture and stamens are versatile (Connor 1979). There are exceptions, for example, entomogamous species of the genus *Pariana* (tribus *Olyreae*; Connor 1979) and *Paspalum dilatatum* (*Paspaleae*; Adams et al. 1981).

Self-incompatibility is reported in at least 16 genera belonging to five out of the 13 subfamilies of the *Poaceae* (Yang et al. 2008). Selfing is widespread in the *Poaceae*. Connor (1979) lists 45 self-compatible genera of grasses that combine selfing and outcrossing (simultaneous presence of cleistogamous and chasmogamous florets; Connor 1979). Generally, the majority of grass genera include both selfing and outcrossing species, which is probably related to perennial and annual life strategies (Baumann et al. 2000). Self-incompatible species are documented in the tribus *Poeae*, *Aveneae*, *Triticeae*, *Andropogoneae*, *Chlorideae*, *Danthoniae* and *Oryzae* (Connor 1979, Li et al. 1997, Baumann et al. 2000, Yang et al. 2008). Outcrossing could also be a result of dioecy, as is in the case of some species of *Poa* and *Festuca* (Connor 1979).

Self-incompatibility in grasses is highly specific. Despite being functionally gametophytic (S, Z loci represented by a polyallelic series), SI of the grass-type in effect acts like sporophytic SI (tri-nucleate pollen at the time of dispersal, the short-lived pollen with a high respiratory rate and dry stigma, rejection of pollen on stigma surface, maintenance of SI in polyploids, related pollen SI protein SCR/SP11 as in *Brassica*; Yang et al. 2008). The precise genetic components of grass-type SI are still unknown (Yang et al. 2008, Klaas et al. 2011).

In addition to rare cases of obligatory selfing, such as in *Trichloris crinita* (Kozub et al. 2017), selfing in grasses exists mostly in mixed breeding systems, either as predominant with occasional outcrossing, such as in *Oryza sativa* (Nayar 1967), *Triticum aestivum*, *Hordeum vulgare* (Frankel & Galun 1977) and *Leersia oryzoides* (with variable percentage of outcrossing; Darwin 1877, Fogg 1928), or minimal, such as in the ‘seed rescue’ plan in *Zea mays* (Dresselhaus et al. 2011). Analogously to the rest of the angiosperms, annual species of grasses are also known to be extensively selfing (Smith 1944).

Even non-self-incompatible species are known among members of three grass subfamilies (*Bambusoideae*, *Oryzoidae* and *Centostecoideae*), self-incompatibility appears to be prevalent in grasses (Baumann et al. 2000). Probably because emasculation is difficult in grasses, reproductive modes are still understudied in grasses (Hanson & Carnahan 1956). Nevertheless, because they include crucial crops, several model species have been under intensive investigation, especially members of the *Triticeae* (Haudry et al. 2008, Escobar et al. 2010).

The tribus *Triticeae*, a key model group of grasses, includes species reproducing both via self-pollination and cross-pollination (Frankel & Galun 1977, Dewey 1984, Escobar

et al. 2010), spanning from obligate outcrossing due to strict self-incompatibility (*Secale cereale* or *Hordeum bulbosum*; Yang et al. 2008) to high incidence of selfing (*Hordeum vulgare* and *Triticum monococcum*; Escobar et al. 2010), which is dependent on each species' life strategy (see below, East 1940, Baumann et al. 2000).

Within the *Triticeae*, reproductive systems are mainly studied in crops (*Triticum aestivum*, *Hordeum vulgare*, *Secale cereale* – Kruse 1973, Lu & von Bothmer 1991, Petersen 1991, Escobar et al. 2010). Ancestral *Triticeae* were probably outcrossing species and the transition between selfing and outcrossing took place in the direction outcrossing to selfing, with annuals being predominantly selfers (Escobar et al. 2010).

To quantify the degree of selfing, we selected individuals of species of the central-European genus *Elymus* (*E. hispidus* and *E. repens*), which belong to the tribus *Triticeae*. The genus *Elymus* s.l. generally consists of both self-compatible species (e.g. diploid to octoploid *Elymus farctus*; Melderis 1980, Dewey 1984) and strict outcrossers (e.g. tetraploid *Elymus genyri*; Dewey 1984, Assadi 1996). These scarce reports may indicate that higher ploidal cytotypes tend to be capable of selfing (Dewey 1984).

Elymus hispidus (Opiz) Melderis [syn.: *Elytrigia intermedia* (Host) Nevski, *Agropyron intermedium* (Host) P. Beauv., *Thinopyrum intermedium* (Host) Barkworth et D. R. Dewey] is an allohexaploid species ($2n = 6x = 42$). *Elymus hispidus* is typical for steppe habitats (Melderis 1980). It has repeatedly been reported to be capable of hybridization with *Triticum aestivum* (wheat) and has extensively been used in wheat improvement (Verushkine & Shechurdine 1933, Han et al. 2004, Fedak & Han 2005, Salina et al. 2015). *Elymus hispidus* has been reported as a probable outcrosser (Dewey 1984).

Elymus repens (L.) Gould [syn.: *Elytrigia repens* (L.) Nevski, *Agropyron repens* (L.) P. Beauv., *Triticum repens* L.] mostly occurs as an allohexaploid cytotype ($2n = 6x = 42$). *Elymus repens* is known as a troublesome weed and commonly grows in cereal crops. Some breeding experiments with wheat as the male or female partner have been conducted, but with few results (Armstrong 1936, Tsitsin & Lubimova 1959, Franke et al. 1992). As a wild relative of wheat, *E. repens* has at least once been used in wheat improvement (Zeng et al. 2013). *Elymus repens* is also reported as a probable outcrosser (East 1940, Dewey 1984). In contrast to the aforesaid suggestions, Smith (1944) indicates an ability of selfing for *E. repens* and *E. hispidus*.

Elymus repens and *E. hispidus* hybridize frequently (incl. backcrossing; Mahelka et al. 2005, 2007, Urfusová et al. 2021), resulting in *Elymus ×mucronatus* (Opiz) Conert [syn.: *Agropyron mucronatum* Opiz, *Elytrigia mucronata* (Opiz) Prokudin]. This hybridization is confirmed at the $6x$ level ($2n = 6x = 42$). Both species and their interspecific hybrid include also rare higher ploidal cytotypes (octoploids, heptaploids, nonaploids; Mahelka et al. 2005, 2007, Paštová et al. 2019, Urfusová et al. 2021).

A previous investigation (Urfusová et al. 2021) revealed extensive homoploid hybridization between hexaploid *Elymus repens* and *E. hispidus* in central Europe (based on absolute genome sizes and morphometrics), which appears to be unidirectional towards *E. hispidus*. Hybridization is reported as a way of breaking SI (Rieseberg 1997, Nasrallah et al. 2007), which is why we hypothesize that, out of the above-mentioned taxa, *E. hispidus* and its hybrids are more capable of selfing (compared to *E. repens*). Moreover, knowledge of the reproductive modes of the model taxa is essential because they are wild relatives of cultivated crops and there are two inconsistent opinions regarding their reproductive systems (Smith 1944, Dewey 1984). A possible association of reproductive

modes with hybridization and ploidy level (linkage to particular cytotype) has never been tested in the *Triticeae*. Finally, the significance of the mentor effect in grasses has never been investigated. Therefore, to start filling these gaps we decided to address the following questions: (i) To what extent are model *Elymus* taxa capable of selfing? (ii) Does the mentor effect (induced fertilization) increase the degree of selfing? and (iii) Is the degree of selfing linked to particular ploidy levels?

Material and methods

We used six hexaploid plants, cloned in 24 ramets (*E. hispidus*, *E. repens* and their hybrid, two individuals per species, each in four copies; $2n = 6x = 42$) from a previous study (Urfusová et al. 2021) and six plants/24 ramets of higher cytotypes from the Čertoryje nature reserve (Bílé Karpaty Mts, eastern Czechia), which consisted of three heptaploids (two *E. repens* individuals and one hybrid; $2n = 7x = 49$) and three octoploids (two *E. hispidus* individuals and one hybrid; $2n = 8x = 56$), see Electronic Appendix 1. The taxonomic identity of model plants (incl. higher ploidal cytotypes) is based on their genome size, chromosome counts and morphometrics (Urfusová et al. 2021). Thirty-seven characters were measured in the same way as described in Urfusová et al. (2021), incorporated in the original matrix and then statistically evaluated by principal component analysis (PCA), which additionally confirmed our determination (Electronic Appendix 2).

The identity of 48 ramets of 12 *Elymus* parental plants (used in the main pollination experiment) was confirmed by absolute genome size analysis and chromosome counts (Electronic Appendix 3).

Experimental design

The experiment consisted of three main treatments: 1, 2a and 2b (Table 1), for testing for three different modes of reproduction (autonomous selfing, induced selfing via pollen of a close relative, and induced selfing via pollen of a distant relative). Moreover, a fourth treatment a positive control was adopted to confirm the overall seed production/fertility of the species (open-pollination; Table 1). The control experiment was done only with hexaploid *Elymus* plants. A more complete set of control treatments would have exceeded our time and budget constraints.

The main pollination experiments were carried out in a greenhouse of an experimental garden at the Institute of Botany of the Czech Academy of Sciences in Průhonice during the growing seasons of 2018 and 2019. Altogether we cultivated 48 ramets of 12 *Elymus* individuals of different species and ploidy levels (Electronic Appendix 1). The reproductive mode experiments involved controlled pollination of different species/cytotypes (Table 2). To test the significance of the mentor effect (induced selfing), we pollinated a set of model plants with pollen of different cereals, including both diploid (*Triticum monococcum*, *Hordeum vulgare*, *Secale cereale*) and polyploid (*Triticum aestivum* and \times *Triticosecale*) cytotypes, in addition to different *Elymus* cytotypes (Electronic Appendix 1). Details of the experimental set-up are presented in Table 2.

Spikes were covered by pollination bags (solid flat bag made of pergamin used by *Triticum* breeders; Baumann, Saat-zuchtbedarf, Waldenburg, Germany) before anther and pistil maturity and then pollinated according to a particular treatment (Table 1).

Table 1. – Design of greenhouse pollination experiments and methods of working with *Elymus* plants.

Treatment	Test for	Manipulation
1	autonomous selfing	covering a single spike or two spikes of a plant with a pollination bag before anther maturity
2a	mentor effect caused by close relative taxa	covering one spike with a pollination bag before anther maturity, then pollinating with pollen from other <i>Elymus</i> sp.
b	mentor effect caused by distant relative taxa	covering one spike with a pollination bag before anther maturity, then pollination with pollen from cereals
control	seed production/fertility	open pollination

Table 2. – Results of crossing experiments. Values in bold indicate the incidence of selfing in each species studied. The first number (N) is the number of spikes pollinated, followed by the number of pollinated florets and the number of seeds that developed. The names of genera included in the experiments are abbreviated as follows: *E.* – *Elymus*, *T.* – *Triticum*, *H.* – *Hordeum*, *S.* – *Secale*.

N/florets/seeds	Mother species						
	<i>E. hispidus</i> 6x	<i>E. hispidus</i> 8x	<i>E. repens</i> 6x	<i>E. repens</i> 7x	hybrid 6x	hybrid 7x	hybrid 8x
<i>E. hispidus</i> 6x	12/676/2	1/72/2	1/72/0	1/102/0	1/60/0	1/105/0	1/33/0
<i>E. hispidus</i> 8x	0/0/0	19/1483/11	0/0/0	0/0/0	2/235/0	0/0/0	1/132/0
<i>E. repens</i> 6x	1/72/0	2/123/5	18/1222/3	1/90/0	1/100/0	1/115/0	0/0/0
<i>E. repens</i> 7x	3/280/1	0/0/0	0/0/0	17/1547/0	1/80/0	0/0/0	0/0/0
hybrid 6x	3/212/0	4/325/18	3/246/0	1/138/0	15/1186/0	2/170/0	0/0/0
hybrid 7x	0/0/0	0/0/0	2/58/0	0/0/0	1/72/0	11/960/3	0/0/0
hybrid 8x	1/60/0	1/95/8	0/0/0	0/0/0	0/0/0	0/0/0	24/1928/7
<i>T. monococcum</i>	1/85/0	1/100/2	0/0/0	0/0/0	0/0/0	1/95/0	0/0/0
<i>T. aestivum</i>	18/1118/17	10/742/17	6/401/0	4/321/0	25/1641/0	8/664/10	7/491/7
<i>H. vulgare</i>	0/0/0	1/110/15	3/144/0	1/64/0	2/134/6	1/88/0	0/0/0
× <i>Triticosecale</i>	2/195/4	1/76/0	3/244/0	2/246/0	3/399/0	1/100/0	0/0/0
<i>S. cereale</i>	0/0/0	1/105/5	3/274/0	1/120/0	3/274/0	1/80/0	1/126/0
Control (open pollination)	39/814/9	0/0/0	11/786/9	0/0/0	5/208/0	0/0/0	0/0/0

Pollinations (induced selfing treatment) were done as follows: The upper part of the pollination bag was cut, when stigmas emerged from spikelets, foreign pollen was sprinkled on them. The pollination bag was then stapled. No embryo rescue or hormone treatment frequently used in *Triticeae* experiments (e.g. Lu & von Bothmer 1991, Petersen 2008) was applied. After pollination, the spikes stayed covered until seed maturity. We counted the spikelets on each spike. Subsequently, we counted florets per spikelet in five selected spikelets in the central part of the spike (the average number of florets was multiplied by the number of spikelets). Mature seeds were counted and then allowed to germinate.

Control open-pollination was checked in the experimental garden of the Faculty of Science at Charles University in Prague. A control set of 23 ramets of nine hexaploid *Elymus* individuals was left to open-pollinate with any of the *Elymus* plants cultivated in

experimental fields (Electronic Appendix 4). Plants were grown in pots in close proximity to flowering plants of different *Elymus* accessions, according to the study of Kozub et al. (2017), with the aim to maximize the probability of pollination. Open-pollination was employed to assess the overall fertility of hexaploid plants and to exclude completely sterile plants.

Seed germination

Seeds were freed from glumes, lemmas and palaeas and then germinated in Petri dishes on wet filter paper in a germination chamber (23°C/10°C; 12 h photoperiod). After germination, seedlings were transferred to a greenhouse (Charles University, Prague, Czechia).

Flow cytometry

Flow cytometry was used for the determination of absolute genome size (GS) of parental plants and progeny. In total, absolute genome size of 31 parental (average variation coefficient CV = 2.04) and 128 progeny (average CV = 2.43) were analysed. *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg; Doležel et al. 1998) served as the internal standard. We followed the protocol of Doležel et al. (2007) with Otto buffers, only slightly modified as described in Urfusová et al. (2021). Each sample was analysed using a Partec CyFlow SL flow cytometer (Partec GmbH, Münster, Germany) equipped with a green solid-state laser (Cobolt Samba, 532 nm, 100 mW). All samples were analysed twice on different days, and only analyses in which the variation between the two measurements did not exceed 3% were considered, provided that the coefficient of variance of individual samples was below 5%. Isolated stained nuclei were excited with a laser beam and the fluorescence intensity of 5000 particles was recorded. The resulting histograms were analysed using FloMax software (version 2.4d, Partec, Münster, Germany). Genome sizes of progeny and parental plants are presented as graphs produced in Microsoft Excel 2010.

The distribution of GS of progeny was compared with that of their parents (Fig. 1). The progeny GS values of the induced selfing experiment were classified into one of three categories (selfing, outcrossing, unresolved) and of two categories (selfing, unresolved) for the autonomous selfing experiment. A logical step to establish categories of progeny would be to derive a threshold for the induced selfing experiment from the autonomous variation of the GS. Regrettably, autonomous selfing resulted in very few seeds. That is why we defined the selfing category as the differences between absolute GS analyses of progeny using an arbitrary threshold of 10% (i.e. the interval of ± 0.05 times the maternal GS) in the autonomous selfing experiment. The outcrossing category was established as the interval of ± 0.05 times around hypothetical intermediate GS value between maternal and paternal plants. GS values that did not fit into the selfing or outcrossing category (or fell into both) were classified as unresolved (the category also includes other potential sources of GS variation, such as irregular meiosis).

Chromosome counting

Chromosomes were counted in 12 parental *Elymus* plants. Root tips of cultivated plants were collected and pretreated according to Kopecký et al. (2005). Mitotic metaphase

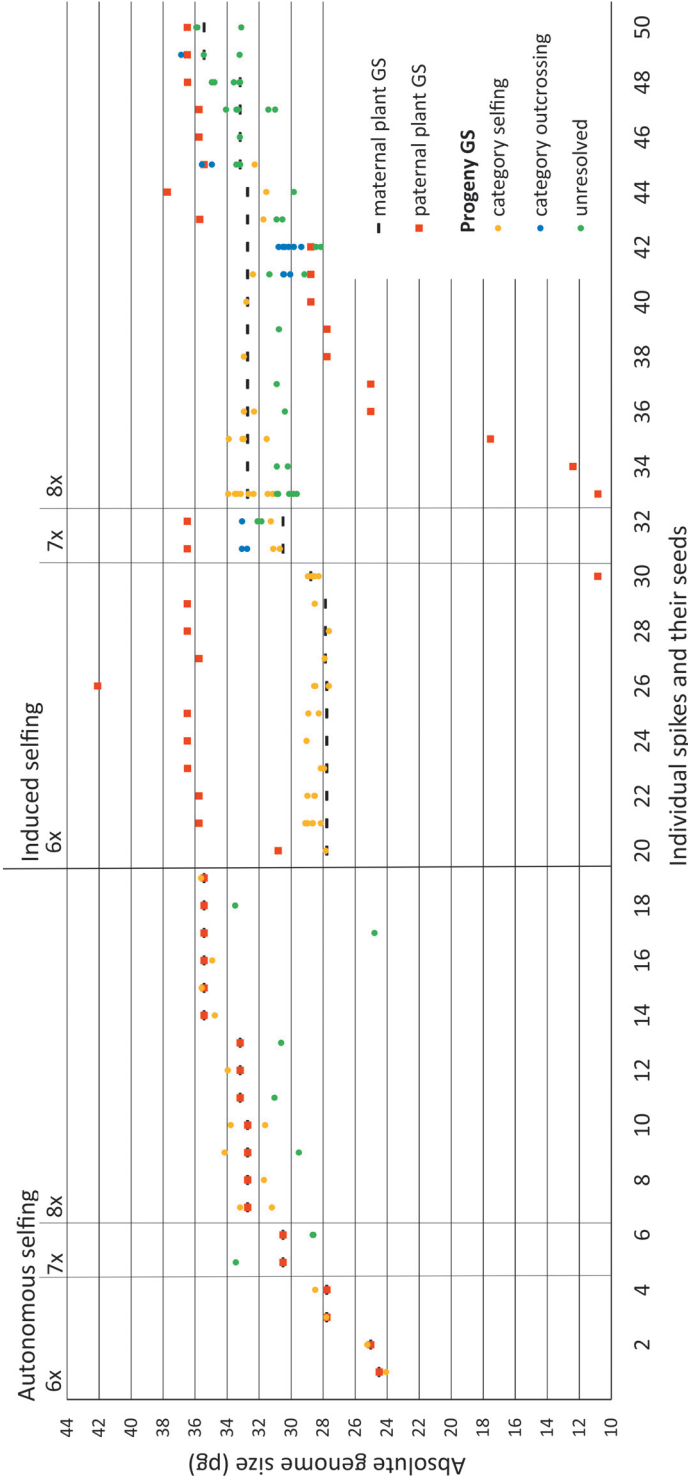


Fig. 1. – Absolute genome sizes of all the progeny and parental plants in the autonomous selfing and induced selfing experiments. The progeny of each pollinated spike is represented by a single column. The graph is sorted by the GS of maternal plants and divided according to the two major treatments.

spreads were prepared from root tips following the protocol of Masoudi-Nejad et al. (2002). Slides with chromosomal spreads were evaluated under a Zeiss PrimoStar HAL microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with phase contrast and an AxioCam ERc 5s Rev.2 camera (Carl Zeiss Microscopy GmbH, Jena, Germany). ZEN 2.3 imaging software (Carl Zeiss Microscopy GmbH, Jena, Germany) and Zoner Photo Studio 18 software were used for the processing of images. Ploidy level data and chromosome counts for crops were obtained from the breeding stations Selgen, a.s. (CZ) and Danko Hodowla Roślin Sp. z o.o. (PL).

Statistical analysis

The effect of selfing type, maternal and paternal plant ploidy level and species identity on seed production in the entire dataset (autonomous selfing and induced selfing experiment – treatments 1, 2a, 2b; Table 1) was tested using generalized linear mixed-effects models (GLMM, using R 3.6.2, functions `glmer`, `glht`, `anova`, `overdisp_fun`; R Core Team 2013, Bates et al. 2015; Tables S3, S4 and S5). We tested separately the effect of selfing type (autonomous vs. induced selfing), the effect of the ploidy level of the maternal and paternal plant, and the effect of the maternal and the paternal plant species on seed production (number of seeds/number of florets per spike) using GLMM (using R 3.6.2, functions `glmer`, `glht`, `overdisp_fun`; testing of one factor combined with random effect, binomial distribution). We divided our dataset according to selfing type (autonomous vs. induced selfing) and tested separately for the effect of the maternal and paternal plant species identity and the ploidy level of maternal and paternal plants in these two partial datasets. In addition, we tested the effect of diploid and polyploid pollen donors on induced selfing in the induced selfing partial dataset. Maternal plant identity was entered as a random effect in all analyses. After GLMM we performed post-hoc tests (general linear hypothesis, Tukey contrasts) to assess which groups differed significantly.

We tested overdispersion in the generalized linear mixed-effects models (GLMM, using R 3.6.2, function `overdisp_fun`; R Core Team 2013, Bates et al. 2015), where the ratio was lower than 1 and $P = 1$. Generalized mixed-effect model (GLMM) with binomial distribution was used to explain the effect of ploidy, maternal species, paternal species and type of selfing as fixed factors and maternal plant identity as a random factor on seed production. The same model was used to explain the percentage of germination. The statistical significance of terms was inferred using a likelihood ratio test (LRT), which compared a fully fitted model to the model from which the tested term was removed (Bretz et al. 2016).

Results

We tested whether the *Elymus* species under study were capable of autonomous selfing (self-fertilization) or induced selfing (via the mentor effect) and whether there was any correlation with ploidy level or hybrid origin. For the plants used in the crossing experiments, we checked 19,721 florets (keeping in mind that the number was partially derived) from 261 spikes and found that 51 spikes contained 143 seeds (Table 2, 3, 4, Fig. 1, Electronic Appendix 5). Control seed production in nine individuals/23 ramets yielded 50 seeds from 23 spikes and 1808 florets.

Absolute genome size of progeny plants

Absolute genome sizes of plants were determined in order to assess the origin of the progeny. The genome sizes of 128 seedlings originating from 143 seeds (a few died at the 2-leaved seedling stage, Table 4) were measured and compared with the genome sizes of both parental species (Fig. 1).

Autonomous selfing

The autonomous selfing treatment of both hexaploids and higher cytotypes yielded 26 seeds (23 germinated) from 7272 florets, and the GS of 11 fell within the $\pm 5\%$ interval of the selfing category (Table 2, Fig. 1). Whereas both hexaploid *Elymus* species had low percentages of autonomous selfing (*E. hispidus* 0.3%; *E. repens* 0.25%), hexaploid hybrid plants did not yield any progeny, despite a relatively high number of selfed spikes among the fifteen spikes and 1186 florets (Table 2).

In the cases of selfed (autonomous selfing) hexaploid *Elymus* plants, the GS of the progeny reached the values of the maternal plants: Two seedlings of *E. repens*, which developed from 18 spikes with 1222 florets, and two seedlings of *E. hispidus*, which developed on 12 spikes with 676 florets, fell within the arbitrary $\pm 5\%$ interval of the selfing category (Table 2, Fig. 3).

The progeny of heptaploid and octoploid cytotypes was markedly heterogeneous (see Table 2 and Fig. 3). Octoploid cytotypes were capable of autonomous selfing that resulted in 18 seeds from 43 spikes of three individuals and 3411 florets. Nevertheless, the GS of a significant part of their progeny markedly differed from that of their maternal plants (by up to 30%) and five of the 16 seedlings exceeded the arbitrary $\pm 5\%$ interval of selfing, thus they fall into the unresolved category (Fig. 1). In contrast to even ploidies, heptaploid *E. repens* did not produce any viable progeny from 17 spikes and 1547 florets. Heptaploid hybrid produced three seedlings from 11 spikes and 960 florets, and their GSs again reached very high values (up to 10% difference) compared to the GS of maternal plants; no seed from the three seedlings fell within the arbitrary $\pm 5\%$ interval of the selfing category (Fig. 1).

Induced selfing

Induced selfing of hexaploid *E. repens* did not result in any seedlings from 21 spikes and 1439 florets. By contrast, the response of hexaploid *E. hispidus* was markedly stronger and its progeny was categorized as selfed: 22 seeds and 22 seedlings from 29 spikes and 2022 florets. The genome size of the progeny corresponded to the selfing category, even in cases of hexaploid hybrid maternal plants: six seedlings from 39 spikes and 2995 florets.

Heptaploid *E. repens* did not produce any seed in the induced selfing experiment, which involved 11 spikes and 1081 florets (treatments 2a, 2b in Table 1), similar to that in the autonomous selfing treatment (Table 2, 3, 4). By contrast, heptaploid hybrid plants (from 16 spikes and 1417 florets) produced progeny that fell within all three arbitrary categories: three seedlings in the selfing category, three seedlings in the outcrossing category and two in the unresolved category, because of being in the overlap between selfing and outcrossing intervals of categories (categories defined above, Table 2–4, Fig. 1). Octoploids, both hybrid and *E. hispidus*, again, produced progeny of all three categories,

Table 3. – Numbers of spikes pollinated, seeds and florets in autonomous selfing, induced selfing and control open-pollination experiments. Induced selfing involved using pollen from *Elymus hispidus*, *E. repens*, hybrids, *Hordeum vulgare*, *Secale cereale*, *Triticum aestivum*, *T. monococcum* or \times *Triticosecale*.

Mother species	Mother ploidy	Type of reproduction	No. of seeds	No. of florets	Seed per floret [%]	Fertile/total nr. of spikes
<i>E. hispidus</i>	6x	induced selfing	22	2022	1.09	10/29 (34%)
<i>E. hispidus</i>	8x	induced selfing	72	1748	4.12	15/22 (68%)
<i>E. repens</i>	6x	induced selfing	0	1439	0	0/21 (0%)
<i>E. repens</i>	7x	induced selfing	0	1081	0	0/11 (0%)
hybrid	6x	induced selfing	6	2995	0.20	1/39 (3%)
hybrid	7x	induced selfing	10	1417	0.71	2/16 (13%)
hybrid	8x	induced selfing	7	782	0.90	2/10 (20%)
<i>E. hispidus</i>	6x	autonomous selfing	2	676	0.30	2/12 (17%)
<i>E. hispidus</i>	8x	autonomous selfing	11	1483	0.74	7/19 (37%)
<i>E. repens</i>	6x	autonomous selfing	3	1222	0.25	3/18 (17%)
<i>E. repens</i>	7x	autonomous selfing	0	1547	0	0/17 (0%)
hybrid	6x	autonomous selfing	0	1186	0	0/15 (0%)
hybrid	7x	autonomous selfing	3	960	0.31	2/11 (18%)
hybrid	8x	autonomous selfing	7	1928	0.36	7/24 (29%)
<i>E. hispidus</i>	6x	open pollination control	39	814	4.79	5/9 (56%)
<i>E. repens</i>	6x	open pollination control	11	786	1.40	7/9 (78%)
hybrid	6x	open pollination control	0	208	0	0/5 (0%)

Table 4. – Seed germination and seedling survival; N – total number of spikes with developed seed.

Mother species	Mother ploidy	Type of reproduction	N	No. of seeds	Germinated	Percentage of seed that germinated	Surviving	Percentage of seedlings that survived
<i>E. hispidus</i>	6x	induced selfing	10	22	22	100	21	95.5
<i>E. hispidus</i>	6x	autonomous selfing	2	2	2	100	2	100.0
<i>E. repens</i>	6x	autonomous selfing	7	3	3	100	2	66.7
hybrid	6x	induced selfing	1	6	6	100	6	100.0
hybrid	7x	induced selfing	3	10	8	80	8	100.0
hybrid	7x	autonomous selfing	1	3	3	100	3	100.0
<i>E. hispidus</i>	8x	induced selfing	2	72	66	92	63	95.5
<i>E. hispidus</i>	8x	autonomous selfing	2	11	10	91	10	100.0
hybrid	8x	induced selfing	2	7	7	100	6	85.7
hybrid	8x	autonomous selfing	7	7	6	86	6	100.0

21 seedlings in the selfing category, 12 seedlings in the outcrossing category and 36 seedlings in the unresolved category (16 of those in the unresolved category were again due to overlap), from a total of 32 spikes and 2530 florets (Table 2–4, Fig. 1). The selfing of the progeny of octoploids in the induced selfing experiment was mainly facilitated by pollen from cereals (15 out of 21 seedlings), whereas for outcrossing it was predominantly *Elymus* (11 of 12 seedlings).

Open pollination: control experiment

Hexaploid *Elymus repens* produced 11 seeds from of 786 florets and *E. hispidus* produced 39 seeds from 814 florets in the control experiment. The percentage of seed per floret was generally greater than in the experimental pollinations (see Table 3).

Elymus hispidus

Whereas *E. repens* and its hybrids did not yield any progeny in some of the treatments, the results for *E. hispidus* were positive in all treatments (including the control open-pollination experiment: 39 seeds from 814 florets; Table 2, 3, Fig. 1), so we selected their partial dataset for further statistical comparison.

To relate the results from the open-pollination control experiment (realized seed set 4.8% per floret for hexaploid *E. hispidus*) to those of both the autonomous and induced selfing treatments, the realized open pollination seed set was set at the maximum seed production (100%), and then expressed as a relative value. The recalculated realized seed set (i.e. expressed as a percentage of the control seed set) of hexaploid *E. hispidus* was 6.2% of the maximum realized seed set in the autonomous selfing treatment and 22.7% of the maximum realized seed set in the induced selfing treatment. The mentor effect resulted in a 3.7-fold greater percentage of selfing.

Total fertility (seed production)

The entire dataset (autonomous selfing and induced selfing experiment and treatments 1, 2a, 2b; Table 1) was analysed statistically as described above. Whereas up to this point the dataset consisted only of the values (genome size) of germinated seeds, in further analyses we considered the number of seeds produced per particular cross (number of seeds/number of florets expressed as a percentage for each pollinated spike). Nevertheless, the percentage of germination was generally very high: that of hexaploid plants was 100% and that of higher ploidies (7x, 8x) 91.5% on average (for more details see Table 4).

Elymus hispidus plants (octoploid and hexaploid) produced significantly more seeds [$\chi^2(2) = 17.474$, Df resid = 257, $P = 0.00016$; Electronic Appendix 6] than the other taxa, whereas hexaploid *E. hispidus* plants did not differ significantly from the rest of the plants in seed production (analysis of maternal plant species identity combined with its ploidy level, Electronic Appendix 6). Particularly, octoploid *E. hispidus* produced significantly more seeds than hexaploid *E. repens* and hexaploid hybrid plants [$\chi^2(2) = 14.884$, Df resid = 257, $P = 0.00059$; Electronic Appendix 6] and also hexaploid *E. hispidus* plants [$\chi^2(1) = 4.407$, Df resid = 79, $P = 0.03579$; Electronic Appendix 6].

Hexaploid hybrids produced significantly lower percentages of seeds than octoploid hybrids [separate analysis of hybrid plants, $\chi^2(2) = 6.0933$, Df resid = 108, $P = 0.04752$; Electronic Appendix 6].

If only ploidal groups are considered, octoploids (regardless of their taxonomic identity) produced significantly more seed than hexaploids [both treatments: $\chi^2(2) = 14.884$, Df resid = 257, $P = 0.00059$; induced selfing: $\chi^2(2) = 8.2165$, Df resid = 141, $P = 0.01644$; Electronic Appendix 6] and heptaploid plants [both treatments: $\chi^2(2) = 14.884$, Df resid = 257, $P = 0.00059$; autonomous selfing: $\chi^2(2) = 7.2812$, Df resid = 112, $P = 0.02624$; induced selfing: $\chi^2(2) = 8.2165$, Df resid = 141, $P = 0.01644$; Electronic Appendix 6].

Next, the ploidy levels and taxonomic categories were considered and their seed production compared within partial datasets. There was significant difference between hexaploid hybrids and hexaploid *E. hispidus* in seed production [hybrids produced a lower number of seeds, $\chi^2(2) = 9.7565$, Df resid = 126, $P = 0.00761$; Electronic Appendix 6], but there was no significant difference between 6x hybrids and 6x *E. repens* and also 6x *E. repens* and 6x *E. hispidus* in seed production [$\chi^2(2) = 9.7565$, Df resid = 126,

$P = 0.00761$; Electronic Appendix 6]. *Elymus hispidus* plants (both ploidies) produced significantly more seeds than *E. repens* plants (both ploidies) in the autonomous selfing dataset [$\chi^2(2) = 6.407$, Df resid = 112, $P = 0.04062$; Electronic Appendix 6] and more seeds than hybrid plants in the induced selfing dataset [$\chi^2(2) = 17.269$, Df resid = 141, $P = 0.00018$; Electronic Appendix 6].

In addition, the influence of paternal plant species and ploidy level were separately tested in the induced selfing experiment and no significant influence of diploid vs. polyploid paternal plant was found [$\chi^2(1) = 1.941$, Df resid = 142, $P = 0.1636$; Electronic Appendix 6], but there are significant differences in seed production in the induced selfing experiment between 6x and 2x and 8x and 6x paternal plants [$\chi^2(3) = 15.142$, Df resid = 140, $P = 0.0017$; Electronic Appendix 6]. An influence of paternal plant species on induced selfing was also recorded [$\chi^2(7) = 29.389$, Df resid = 136, $P = 0.00013$; Electronic Appendix 6].

Finally, the comparison of the results of the two experiments in the entire dataset revealed that induced selfing (mentor effect) produced significantly more seeds than autonomous selfing [$\chi^2(1) = 8.3748$, Df resid = 258, $P = 0.00381$; Electronic Appendix 6]. The same pattern as in the test of the entire dataset was also found in a partial dataset analysis of *E. hispidus* maternal plants [$\chi^2(1) = 0.0007$, Df resid = 79, $P = 0.00176$; Electronic Appendix 6].

In addition, we determined (by fitting generalized linear mixed-effects model) the characters that contributed most to seed production, using the entire dataset. There was no overdispersion in our models. Paternal plant species [$\chi^2(6) = 26.31$, Df resid = 250, $P = 0.00019$; Electronic Appendix 7] and maternal species [$\chi^2(2) = 12.247$, Df resid = 246, $P = 0.00219$; Electronic Appendix 7] were identified as the most significant factors, followed by the ploidy level of the maternal plants [$\chi^2(2) = 5.3194$, Df resid = 246, $P = 0.06997$; also confirming the significance of *E. hispidus* seed production indicated by GLMM with one factor tested; Electronic Appendix 6]. Furthermore, it supported the results of the two step GLMM analysis. The first step, analysis of successful vs. unsuccessful crosses confirmed the importance of the maternal plant species [$\chi^2(2) = 19.401$, Df resid = 246, $P = 6.127 \cdot 10^{-5}$; Electronic Appendix 8] followed by maternal plant ploidy level [$\chi^2(2) = 4.7052$, Df resid = 246, $P = 0.09512$; Electronic Appendix 8]. Subsequently, analysis of only successfully pollinated spikes confirmed the importance of paternal plant species [$\chi^2(6) = 16.055$, Df resid = 41, $P = 0.01346$; Electronic Appendix 8] and type of selfing [$\chi^2(1) = 7.6176$, Df resid = 36, $P = 0.00578$; Electronic Appendix 8]. The generalized linear mixed-effects model did not reveal any factors that significantly influenced the percentage of germination (Electronic Appendix 7).

Discussion

Despite being reported as outcrossers (Dewey 1984), the results presented indicate that *Elymus hispidus*, *E. repens* and their hybrids are capable of selfing. Although selfing remains a minor reproductive pathway in *E. repens*, in *E. hispidus* a significant percentage of the progeny may be produced via autogamy. Induction by foreign pollen (mentor effect) increased the percentage of selfing significantly, especially in hexaploid *E. hispidus*.

Fertility rate

Overall seed production by *Elymus hispidus* and *E. repens* was very low. The open-pollination control experiment of euploid plants resulted in a 3.1% increase in seed production per floret (compared to both selfing experiments: 0.5%). Nevertheless, our results are congruent with the pollination experiments of Smith (1944). In autonomous selfing experiment Smith (1944) reports 0.5% seeds per floret in *E. repens* (respectively 0.7% in *E. hispidus*) compared to our results of 0.2% in *E. repens* and 0.3% in *E. hispidus*. However, the design of Smith's (1944) and our experiments were different. Smith's study was on many individuals of each species whereas we used ramets of two genotypes per species.

Comparison of seed production in both selfing experiments with an open-pollination control may be affected by the different abiotic and biotic conditions in the greenhouse and experimental garden (different temperature fluctuations, light radiation, air flow, pathogens etc.) as well as between bagged and open-pollinated inflorescences. We are aware that temperature, light and humidity, time of the flowering season and age of a flower are relevant and generally accepted factors affecting the percentage of selfing (e.g. Lloyd & Schoen 1992). Finally, a factor, which may have influenced the results of the pollination experiments directly is the way they were pollinated (artificial vs. natural). Artificial pollination could positively affect seed production, especially by placing a sufficient amount of mature pollen grains directly on receptive stigmas (e.g. King et al. 2007).

In addition to the various limitations of the reproductive experiments, the low production of seed may also be linked to the clonality of both species of *Elymus* (Szabó 1979, 1981), as there is a trade-off between clonality and sexual reproduction (Herben et al. 2015, Van Drunen et al. 2015). The limited sexual reproduction of clonal plants is probably manifested by a lower pollen production (Vallejo-Marín et al. 2010).

In both selfing experiments, seed production was significantly greater in *E. hispidus* plants. The higher percentage of selfing in *Elymus hispidus* could be linked to ongoing introgressive hybridization (Urfusová et al. 2021), meaning that the hybrid origin may facilitate selfing (Snyder 1951, Rieseberg 1997, Petit et al. 1999, Nasrallah et al. 2007). Hybridization between *E. hispidus* and *E. repens* is asymmetrical towards the former species (Mahelka et al. 2007, Urfusová et al. 2021). By contrast, the majority of *E. repens* populations appear to be unaffected (Urfusová et al. 2021). Moreover, the greater percentage of self-compatibility in *E. hispidus* may be a consequence of its specific ecological preferences (Melderis 1980, Conert 1998) because steppe localities in central Europe are usually rare, small in area and isolated. As a result, its populations are smaller and consist of low numbers of genotypes, so a greater selfing ability could be advantageous under such conditions (Szczepaniak et al. 2009).

Evidence for a positive correlation between ploidy and the degree of autogamy is reported repeatedly (self-compatibility; Baker 1955, Thompson & Lumaret 1992, Otto & Whitton 2000), but our results do not support such link in the genus *Elymus*. *Elymus* plants of higher ploidy levels probably do not support the trend, because they are exceptional, abnormal cytotypes probably of hybrid origin and their natural abundance is very low (compared to the predominant hexaploids; Urfusová et al. 2021).

Percentage of germination

The evaluation of percentage of germination divided our maternal plants into two major groups: hexaploid plants (*E. hispidus*, *E. repens* and their homoploid hybrids) and aberrant cytotypes (heptaploids and octoploids). Whereas 100% germination was recorded for hexaploids, in higher polyploid cytotypes it was generally lower (80–100%, Table 4). The significant percentage of sterile/non-vigorous seed was probably a consequence of their hybrid origin and especially of an unbalanced composition of the subgenomes (Mason-Gamer 2008, Mahelka et al. 2011, Pařtová et al. 2019). Higher ploidy cytotypes of *Elymus* species are reported to be less fertile or even sterile (Mahelka et al. 2007), as has been reported for other plant groups (Richards 1997, Padmanaban et al. 2017).

Autonomous and induced selfing

Despite the low degree of autonomous selfing in hexaploid plants of *E. hispidus*, markedly increased seed production was recorded in the induced selfing experiments (3.7-fold, Table 3), which is also mirrored by the GLMM analysis of the entire dataset. Because pollen of related taxa is ubiquitous under natural conditions, especially that of anemogamous taxa, the true significance of induced selfing (i.e. the mentor effect) is likely to be underestimated and may have numerous consequences. Desrochers & Rieseberg (1998) suspect that the mentor effect increases the probability of the establishment of hybrid species. In addition, the mentor effect could induce selfing of otherwise sterile hybrids and enhance invasiveness (Vanden-Broeck et al. 2012). Koutecký et al. (2011) suggest that the mentor effect is an important mechanism preventing heteroploid hybridization. Therefore, the better ability of *Elymus hispidus* and the octoploid cytotype to reproduce via autonomous and induced selfing is probably linked to hybridization and isolation of their populations or clones (see above).

The majority of the selfing experiments (mainly on SI species) conducted so far indicate a merely theoretical value of autonomous selfing, which could be markedly greater because of the mentor effect even among entomogamous species under natural conditions (see *Hieracium* – Mráz & Paule 2006; *Pilosella* – Krahulcová et al. 1999; *Taraxacum* – Tas & van Dijk 1999; *Helianthus* – Desrochers & Rieseberg 1998; *Populus* – Vanden-Broeck et al. 2012; *Potentilla* – Dobeř et al. 2018; *Centaurea* – Koutecký et al. 2011).

Moreover, Shibaike et al. (2002) indicate the possible importance of phylogenetic relatedness in inducing selfing (higher rates of induced selfing in crosses of *Taraxacum* sect. *Mongolica* and *Ruderalia* than in crosses among species within the sect. *Mongolica*). Our data indicate a similar trend because the mentor effect induced by cereals in hexaploid *E. hispidus* is five-fold greater (1.5% seeds per florets) than when induced by other *Elymus* cytotypes and species (0.3% seeds per florets). Nevertheless, the tribus *Triticeae* is not a suitable model group for such an analysis, because its complex reticulate evolution does not enable us to determine phylogenetic relatedness (Escobar et al. 2011, Bernhardt et al. 2017). That is why we do not present this trend in the Results section.

The results of both our selfing experiments with higher polyploids can be interpreted in multiple ways because the genome size of their progeny is frequently variable compared to that of their parents (exceeding the arbitrary interval of $\pm 5\%$; see Fig. 1). Nevertheless, based on our results of comparing autonomous selfing and induced selfing, we hypothesize that despite being variable in GS, the majority of the seeds produced actually

arose via selfing and their variation was caused by unstable meiosis (Grandont et al. 2013, Kopecký et al. 2017). Generally, although the mechanisms of chromosome pairing are described in allopolyploids, they are not identical (reviewed in: Cifuentes et al. 2010, Yousafzai et al. 2010, Grandont et al. 2013, Svačina et al. 2020). Moreover, in a single case the genome size of the progeny of an octoploid maternal plant was markedly smaller (by up to more than one-third; see Fig. 1). The decrease in genome size could be explained by irregular meiosis enforced by selection leading to a standard hexaploid genomic constitution. An analogous way out of abnormally high polyploid composition is also documented in *Pilosella* (Krahulec et al. 2008, Krahulcová et al. 2009, 2011, Rosenbaumová & Krahulec 2015).

Conclusions

Despite being supposedly allogamous, *Elymus hispidus*, *E. repens* and their hybrids are capable of selfing (especially *E. hispidus*). Furthermore, the percentage of selfing increased significantly if it was induced by foreign pollen. The mentor effect is therefore an indisputable evolutionary force facilitating selfing in the *Elymus* taxa studied. Octoploids were significantly better at selfing than hexaploids, and heptaploids characteristically had a significantly lower ability to reproduce via selfing (both autonomous and induced). Whereas the progeny of hexaploids remain stable (based on genome size), that of higher polyploid cytotypes (heptaploids and octoploids) is characterized by marked variation in genome size, genomic downsizing and lower percentages of germination, probably a result of irregular meiosis.

See www.preslia.cz for Electronic Appendices 1–8

Acknowledgements

Jan Pinc and Petr Šmilauer helped us with the statistics. Reviewers carefully read the manuscript and suggested numerous valuable improvements. Frederick Rooks kindly improved the English of our submission and Tony Dixon edited the accepted manuscript. This study was supported by the Grant Agency of Charles University (project no. 1358218), the Czech Science Foundation (grants nos 17-06548S and 20-10019S), the Czech Academy of Sciences (long-term research development project RVO 67985939) and the Charles University Research Centre (programme no. 204069).

Souhrn

Některé autoinkompatibilní druhy jsou schopny samoopylení indukovaného cizím pylem (indukovaná autogamie, mentor efekt), jehož význam je pravděpodobně podhodnocen. Větší míra selfingu byla také opakovaně spojována s polyploidii. Přestože jsou líniovité (*Poaceae*) rostlinnou skupinou zásadního významu, nebyla u nich míra autogamie, včetně mentor efektu, dosud podrobně studována. V našem výzkumu jsme se zaměřili na druhy *Elymus hispidus*, *E. repens* a jejich hybridy (tribus *Triticeae*) a testovali jsme u nich schopnost autogamie, význam indukované autogamie a jejich vazbu na polyploidii. Reprodukční způsoby (alogamii, autogamii a mentor efekt) jsme testovali ve dvou opylovacích experimentech doplněných kontrolou (volné sprášení) a identitu rodičovských rostlin a jejich potomků jsme potvrdili prostřednictvím analýzy absolutní velikosti genomu. Výsledky experimentů potvrdily schopnost autogamie studovaných druhů i jejich hybridů. Míra autogamie se výrazně zvýšila při indukci cizím pylem (mentor efekt), což bylo patrné zejména u *Elymus hispidus* (produkce semen po korekci hodnotami z kontrolního volného sprášení u autonomní autogamie: 6.2 % vs. mentor efektu: 22.7 %). Oktoploidní rostliny tvořily potomky prostřednictvím autogamie více než hexaploidi

i heptaploidii, zatímco heptaploidii tvořili signifikantně méně semen autogamií než hexaploidii a oktoploidii. Schopnost vyšší míry autogamie *E. hispidus* (v obou experimentech) mohla být způsobena introgresivní hybridizací. Přestože je mentor efekt zjevně důležitý reprodukční způsob, je mu dosud překvapivě věnována jen malá pozornost.

References

- Adams D. E., Perkins W. E. & Estes J. R. (1981) Pollination systems in *Paspalum dilatatum* Poir. (*Poaceae*): an example of insect pollination in a temperate grass. – *American Journal of Botany* 68: 389–394.
- Anderson B., Midgley J. J. & Stewart B. A. (2003) Facilitated selfing offers reproductive assurance: a mutualism between a hemipteran and carnivorous plant. – *American Journal of Botany* 90: 1009–1015.
- Armstrong J. M. (1936) Hybridization of *Triticum* and *Agropyron*: I. Crossing results and description of the first generation hybrids. – *Canadian Journal of Research* 14c: 190–202.
- Assadi M. (1996) A taxonomic revision of *Elymus* sect. *Caespitosae* and sect. *Elytrigia* (*Poaceae*, *Triticeae*) in Iran. – *Willdenowia* 26: 251–271.
- Baker H. G. (1955) Self-compatibility and establishment after “long-distance” dispersal. – *Evolution* 9: 347–349.
- Barrett S. C. H. & Cruzan M. B. (1994) Incompatibility in heterostylous plants. – In: Williams E. G., Clarke A. E. & Knox R. B. (eds), Genetic control of self-incompatibility and reproductive development in flowering plants, p. 189–219, *Advances in cellular and molecular biology of plants*, Vol. 2., Springer, Dordrecht.
- Bates D., Mächler M., Bolker B. & Walker S. (2015) Fitting linear mixed-effects models using lme4. – *Journal of Statistical Software* 67: 1–48.
- Baumann U., Juttner J., Bian X. & Langridge P. (2000) Self-incompatibility in the grasses. – *Annals of Botany* 85: 203–209.
- Bernhardt N., Brassac J., Kilian B. & Blattner F. R. (2017) Dated tribe-wide whole chloroplast genome phylogeny indicates recurrent hybridizations within *Triticeae*. – *BMC Evolutionary Biology* 17: 1–16.
- Brennan A. C., Tabah D. A., Harris S. A. & Hiscock S. J. (2011) Sporophytic self-incompatibility in *Senecio squalidus* (*Asteraceae*): S allele dominance interactions and modifiers of cross-compatibility and selfing rates. – *Heredity* 106: 113–123.
- Bretz F., Hothorn T. & Westfall P. (2016) Multiple comparisons using R. – Chapman and Hall/CRC, Boca Raton.
- Briggs D. & Walters M. S. (2016) Breeding systems. – In: Briggs D. & Walters M. S. (eds), *Plant variation and evolution*, p. 98–134, CRC Press.
- Charlesworth D. (2006) Evolution of plant breeding systems. – *Current Biology* 16: 726–735.
- Cifuentes M., Grandont L., Moore G., Chèvre A. M. & Jenczewski E. (2010) Genetic regulation of meiosis in polyploid species: new insights into an old question. – *New Phytologist* 186: 29–36.
- Conert H. J. (1998) *Tribus Triticeae*. – In: Conert H. J., Jäger E. J., Kadereit J. W., Schultze-Motel W., Wagenitz G. & Weber H. E. (eds), *Gustav Hegi Illustrierte Flora von Mitteleuropa*, p. 771–843, Parey, Berlin.
- Connor H. E. (1979) Breeding systems in the grasses: a survey. – *New Zealand Journal of Botany* 17: 547–574.
- Darwin C. (1861) *On the origin of species*. – D. Appleton and Company, New York.
- Darwin C. (1877) *The different forms of flowers on plants of the same species*. – Cambridge University Press, Cambridge.
- Desrochers A. M. & Rieseberg L. H. (1998) Mentor effects in wild species of *Helianthus* (*Asteraceae*). – *American Journal of Botany* 85: 770–775.
- Dewey D. R. (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial *Triticeae*. – In: Gustafson J. P. (ed.), *Gene manipulation in plant improvement*, p. 209–279, Columbia University Press, New York.
- Dobeš C., Scheffknecht S., Fenko Y., Prohaska D., Sykora C. & Hülber K. (2018) Asymmetric reproductive interference: the consequences of cross-pollination on reproductive success in sexual–apomictic populations of *Potentilla puberula* (*Rosaceae*). – *Ecology and Evolution* 8: 365–381.
- Doležel J., Greilhuber J., Lucretti S., Meister A., Lysák M. A., Nardi L. & Obermayer R. (1998) Plant genome size estimation by flow cytometry: inter-laboratory comparison. – *Annals of Botany* 82: 17–26.
- Doležel J., Greilhuber J. & Suda J. (2007) Estimation of nuclear DNA content in plants using flow cytometry. – *Nature Protocols* 2: 2233–2244.
- Dresselhaus T., Lausser A. & Márton M. L. (2011) Using maize as a model to study pollen tube growth and guidance, cross-incompatibility and sperm delivery in grasses. – *Annals of Botany* 108: 727–737.

- East E. M. (1940) The distribution of self-sterility in the flowering plants. – Proceedings of the American Philosophical Society 82: 449–518.
- Escobar J. S., Cenci A., Bolognini J., Haudry A., Laurent S., David J. & Glémin S. (2010) An integrative test of the dead-end hypothesis of selfing evolution in *Triticeae* (*Poaceae*). – Evolution 64: 2855–2872.
- Escobar J. S., Scornavacca C., Cenci A., Guilhaumon C., Santoni S., Douzery E. J. P., Ranwez V., Glémin S. & David J. (2011) Multigenic phylogeny and analysis of tree incongruences in *Triticeae* (*Poaceae*). – BMC Evolutionary Biology 11: 1–17.
- Fedak G. & Han F. (2005) Characterization of derivatives from wheat-*Thinopyrum* wide crosses. – Cytogenetic and Genome Research 109: 360–367.
- Fogg J. M. (1928) The clandestine of *Leersia oryzoides*. – Rhodora 30: 81–85.
- Franke R., Nestrowicz R., Senula A. & Staat B. (1992) Intergeneric hybrids between *Triticum aestivum* L. and wild *Triticeae*. – Hereditas 116: 225–231.
- Frankel R. & Galun E. (1977) Pollination mechanisms, reproduction and plant breeding. – Springer, Berlin & Heidelberg.
- Franklin-Tong V. E. & Franklin F. C. H. (1992) Gametophytic self-incompatibility in *Papaver rhoeas* L. – Sexual Plant Reproduction 5: 1–7.
- Fujii S., Kubo K. & Takayama S. (2016) Non-self- and self-recognition models in plant self-incompatibility. – Nature Plants 2: 16130.
- Grandont L., Jenczewski E. & Lloyd A. (2013) Meiosis and its deviations in polyploid plants. – Cytogenetic and Genome Research 140: 171–184.
- Han F., Liu B., Fedak G. & Liu Z. (2004) Genomic constitution and variation in five partial amphiploids of wheat-*Thinopyrum intermedium* as revealed by GISH, multicolor GISH and seed storage protein analysis. – Theoretical and Applied Genetics 109: 1070–1076.
- Hanson A. A. & Carnahan H. L. (1956) Breeding perennial forage grasses. – USDA Technical Bulletin 1145: 1–116.
- Haudry A., Cenci A., Guilhaumon C., Paux E., Poirier S., Santoni S., David J. & Glémin S. (2008) Mating system and recombination affect molecular evolution in four *Triticeae* species. – Genetics Research 90: 97–109.
- Herben T., Šerá B. & Klimešová J. (2015) Clonal growth and sexual reproduction: tradeoffs and environmental constraints. – Oikos 124: 469–476.
- Hiscock S. J. & McInnis S. M. (2003) The diversity of self-incompatibility systems in flowering plants. – Plant Biology 5: 23–32.
- Hörandl E. (2010) The evolution of self-fertility in apomictic plants. – Sexual Plant Reproduction 23: 73–86.
- Igic B. & Kohn J. R. (2006) The distribution of plant mating systems: study bias against obligately outcrossing species. – Evolution 60: 1098–1103.
- Igic B., Lande R. & Kohn J. R. (2008) Loss of self-incompatibility and its evolutionary consequences. – International Journal of Plant Sciences 169: 93–104.
- Johnson S. D. & Nilsson L. A. (1999) Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. – Ecology 80: 2607–2619.
- Kao T.-h. & Tsukamoto T. (2004) The molecular and genetic bases of S-RNase-based self-incompatibility. – The Plant Cell 16: S72–S83.
- King H., Rich T. C. G. & Cordrey L. (2007) Comparing hand pollination success with natural pollination of wild asparagus *Asparagus prostratus*, at colonies on The Gower, Pembrokeshire, west Wales, and in Cornwall, south-west England. – Conservation Evidence 4: 69–72.
- Klaas M., Yang B., Bosch M., Thorogood D., Manzanares C., Armstead I. P., Franklin F. C. H. & Barth S. (2011) Progress towards elucidating the mechanisms of self-incompatibility in the grasses: further insights from studies in *Lolium*. – Annals of Botany 108: 677–685.
- Kopecký D., Lukaszewski A. J. & Doležel J. (2005) Genomic constitution of *Festulolium* cultivars released in the Czech Republic. – Plant Breeding 124: 454–458.
- Kopecký D., Šimoníková D., Ghesquière M. & Doležel J. (2017) Stability of genome composition and recombination between homoeologous chromosomes in *Festulolium* (*Festuca* × *Lolium*) cultivars. – Cytogenetic and Genome Research 151: 106–114.
- Koutecký P., Baďurová T., Štech M., Košnar J. & Karásek J. (2011) Hybridization between diploid *Centaurea pseudophrygia* and tetraploid *C. jacea* (*Asteraceae*): the role of mixed pollination, unreduced gametes, and mentor effects. – Biological Journal of the Linnean Society 104: 93–106.
- Kozub P. C., Barboza K., Galdeano F., Quarin C. L., Cavagnaro J. B. & Cavagnaro P. F. (2017) Reproductive biology of the native forage grass *Trichloris crinita* (*Poaceae*, *Chloridoideae*). – Plant Biology 19: 444–453.

- Krahulcová A., Chrtek J. & Krahulec F. (1999) Autogamy in *Hieracium* subgen. *Pilosella*. – *Folia Geobotanica* 34: 373–376.
- Krahulcová A., Krahulec F. & Rosenbaumová R. (2011) Expressivity of apomixis in $2n + n$ hybrids from an apomictic and a sexual parent: insights into variation detected in *Pilosella* (*Asteraceae: Lactuceae*). – *Sexual Plant Reproduction* 24: 63–74.
- 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 polyploid populations. – *Folia Geobotanica* 44: 281–306.
- 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.
- Kruse A. (1973) *Hordeum* × *Triticum* hybrids. – *Hereditas* 73: 157–161.
- Li X., Paech N., Nield J., Hayman D. & Langridge P. (1997) Self-incompatibility in the grasses: evolutionary relationship of the S gene from *Phalaris coerulea* to homologous sequences in other grasses. – *Plant Molecular Biology* 34: 223–232.
- Lloyd D. G. & Schoen D. J. (1992) Self- and cross-fertilization in plants. I. Functional dimensions. – *International Journal of Plant Sciences* 153: 358–369.
- Lu B. R. & von Bothmer R. (1991) Cytogenetic studies of the intergeneric hybrids between *Secale cereale* and *Elymus caninus*, *E. brevipes*, and *E. tsukushiensis* (*Triticeae: Poaceae*). – *Theoretical and Applied Genetics* 81: 524–532.
- Mahelka V., Fehrer J., Krahulec F. & Jarolímová V. (2007) Recent natural hybridization between two allopolyploid wheatgrass (*Elytrigia*, *Poaceae*): ecological and evolutionary implications. – *Annals of Botany* 100: 249–260.
- Mahelka V., Kopecký D. & Paštová L. (2011) On the genome constitution and evolution of intermediate wheatgrass (*Thinopyrum intermedium*: *Poaceae, Triticeae*). – *BMC Evolutionary Biology* 11: 127.
- Mahelka V., Suda J., Jarolímová V., Trávníček P. & Krahulec F. (2005) Genome size discriminates between closely related taxa *Elytrigia repens* and *E. intermedia* (*Poaceae: Triticeae*) and their hybrid. – *Folia Geobotanica* 40: 367–384.
- Mason-Gamer R. J. (2008) Allohexaploidy, introgression, and the complex phylogenetic history of *Elymus repens* (*Poaceae*). – *Molecular Phylogenetics and Evolution* 47: 598–611.
- Masoudi-Nejad A., Nasuda S., McIntosh R. A., Endo T. R. & Endo T. R. (2002) Transfer of rye chromosome segments to wheat by a gametocidal system. – *Chromosome Research* 10: 349–357.
- McClure B. A. & Franklin-Tong V. (2006) Gametophytic self-incompatibility: understanding the cellular mechanisms involved in “self” pollen tube inhibition. – *Planta* 224: 233–245.
- Melderis A. (1980) *Triticeae*. – In: Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H. & Walters S. M. (eds), *Flora Europaea* 5: 190–206, Cambridge University Press, New York.
- Miri R. K. & Bubar J. S. (1966) Self-incompatibility as an outcrossing mechanism in birdfoot trefoil (*Lotus corniculatus*). – *Canadian Journal of Plant Science* 46: 411–418.
- Mráz P. (2003) Mentor effects in the genus *Hieracium* s.str. (*Compositae, Lactuceae*). – *Folia Geobotanica* 38: 345–350.
- Mráz P. & Paule J. (2006) Experimental hybridization in the genus *Hieracium* s. str.: crosses between diploid taxa. – *Preslia* 78: 1–26.
- Nasrallah J. B., Liu P., Sherman-Broyles S., Schmidt R. & Nasrallah M. E. (2007) Epigenetic mechanisms for breakdown of self-incompatibility in interspecific hybrids. – *Genetics* 175: 1965–1973.
- Nasrallah J. B. & Nasrallah M. E. (1993) Pollen-stigma signaling in the sporophytic self-incompatibility response. – *The Plant Cell* 5: 1325–1335.
- Nayar N. M. (1967) Prevalence of self-incompatibility in *Oryza barthii* Cheval.: its bearing on the evolution of rice and related taxa. – *Genetica* 38: 521–527.
- Newbigin E., Anderson M. A. & Clarke A. E. (1993) Gametophytic self-incompatibility systems. – *The Plant Cell* 5: 1315–1324.
- Oakley C. G., Moriuchi K. S. & Winn A. A. (2007) The maintenance of outcrossing in predominantly selfing species: ideas and evidence from cleistogamous species. – *Annual Review of Ecology, Evolution, and Systematics* 38: 437–457.
- Olmstead R. G. (1989) The origin and function of self-incompatibility in flowering plants. – *Sexual Plant Reproduction* 2: 127–136.
- Otto S. P. & Whitton J. (2000) Polyploidy incidence and evolution. – *Annual Review of Genetics* 34: 401–437.

- Padmanaban S., Zhang P., Hare R. A., Sutherland M. W. & Martin A. (2017) Pentaploid wheat hybrids: applications, characterisation, and challenges. – *Frontiers in Plant Science* 8: 1–11.
- Paštová L., Belyayev A. & Mahelka V. (2019) Molecular cytogenetic characterisation of *Elytrigia × mucronata*, a natural hybrid of *E. intermedia* and *E. repens* (*Triticeae*, *Poaceae*). – *BMC Plant Biology* 19: 230.
- Pedersen H. A. E. & Ehlers B. K. (2000) Local evolution of obligate autogamy in *Epipactis helleborine* subsp. *neerlandica* (*Orchidaceae*). – *Plant Systematics and Evolution* 223: 173–183.
- Petersen G. (1991) Intergeneric hybridization between *Hordeum* and *Secale* (*Poaceae*). I. Crosses and development of hybrids. – *Nordic Journal of Botany* 11: 253–270.
- Petersen G. (2008) Meiosis of intergeneric hybrids between polyploid species of *Hordeum* and *Secale*. – *Hereditas* 116: 101–105.
- Petit C., Bretagnolle F. & Felber F. (1999) Evolutionary consequences of diploid-polyploid hybrid zones in wild species. – *Trends in Ecology and Evolution* 14: 306–311.
- R Core Team (2013) R: A language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, URL: <http://www.R-project.org>.
- Richards A. J. (1996) Breeding systems in flowering plants and the control of variability. – *Folia Geobotanica et Phytotaxonomica* 31: 283–293.
- Richards A. J. (1997) Plant breeding systems. – Chapman & Hall, London.
- Rieseberg L. H. (1997) Hybrid origins of plant species. – *Annual Review of Ecology and Systematics* 28: 359–389.
- Rosenbaumová R. & Krahulec F. (2015) Sexual reproduction as a source of ploidy level variation in the model agamic complex of *Pilosella bauhini* and *P. officinarum* (*Asteraceae: Lactuceae*). – *Plant Systematics and Evolution* 301: 279–290.
- Salina E. A., Adonina I. G., Badaeva E. D., Kroupin P. Y., Stasyuk A. I., Leonova I. N., Shishkina A. A., Divashuk M. G., Starikova E. V., Khuat T. M. L., Syukov V. V. & Karlov G. I. (2015) A *Thinopyrum intermedium* chromosome in bread wheat cultivars as a source of genes conferring resistance to fungal diseases. – *Euphytica* 204: 91–101.
- Shibaike H., Akiyama H., Uchiyama S., Kasai K. & Morita T. (2002) Hybridization between European and Asian dandelions (*Taraxacum* section *Ruderalia* and section *Mongolica*). – *Journal of Plant Research* 115: 321–328.
- Smith D. C. (1944) Pollination and seeds formation in grasses. – *Journal of Agricultural Research* 68: 79–95.
- Snyder L. A. (1951) Cytology of inter-strain hybrids and the probable origin of variability in *Elymus glaucus*. – *American Journal of Botany* 38: 195–202.
- Stebbins G. L. (1957) Self fertilization and population variability in the higher plants. – *The American Naturalist* 91: 337–354.
- Svačina R., Sourdille P., Kopecký D. & Bartoš J. (2020) Chromosome pairing in polyploid grasses. – *Frontiers in Plant Science* 11: 1–17.
- Szabó A. T. (1979) Wheatgrass variability (*Agropyron*, sect. *Elytrigia*) in a native collection from Transylvania. I. *Agropyron intermedium* complex. – *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 10: 89–99.
- Szabó A. T. (1981) Wheatgrass variability (*Agropyron*, sect. *Elytrigia*) in a native collection from Transylvania. II. *Agropyron repens* (L.) P. Beauv. – *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 11: 61–68.
- Szczepaniak M., Bieniek W., Boroń P., Szklarczyk M. & Mizianty M. (2009) A contribution to characterisation of genetic variation in some natural Polish populations of *Elymus repens* (L.) Gould and *Elymus hispidus* (Opiz) Melderis (*Poaceae*) as revealed by RAPD markers. – *Plant Biology* 11: 766–773.
- 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.
- Thompson J. D. & Lumaret R. (1992) The evolutionary dynamics of polyploid plants: origins, establishment and persistence. – *Trends in Ecology & Evolution* 7: 302–307.
- Tsitsin N. V. & Lubimova V. F. (1959) New species and forms of cereals derived from hybridization between wheat and couch grass. – *The American Naturalist* 93: 181–191.
- Uphof J. C. T. (1938) Cleistogamic flowers. – *Botanical Review* 4: 21–49.
- Urfusová R., Mahelka V., Krahulec F. & Urfus T. (2021) Evidence of widespread hybridization among couch grasses (*Elymus*, *Poaceae*). – *Journal of Systematics and Evolution* 59: 113–124.
- Vallejo-Marín M., Dorken M. E. & Barrett S. C. H. (2010) The ecological and evolutionary consequences of clonality for plant mating. – *Annual Review of Ecology, Evolution, and Systematics* 41: 193–213.
- Van Drunen W. E., van Kleunen M. & Dorken M. E. (2015) Consequences of clonality for sexual fitness: clonal expansion enhances fitness under spatially restricted dispersal. – *Proceedings of the National Academy of Sciences of the United States of America* 112: 8929–8936.

- Vanden-Broeck A., Cox K., Michiels B., Verschelde P. & Villar M. (2012) With a little help from my friends: hybrid fertility of exotic *Populus* × *canadensis* enhanced by related native *Populus nigra*. – *Biological Invasions* 14: 1683–1696.
- Verushkine S. & Shechurdine A. (1933) Hybrids between wheat and couch grass. – *Journal of Heredity* 24: 329–335.
- Yang B., Thorogood D., Armstead I. & Barth S. (2008) How far are we from unravelling self-incompatibility in grasses? – *New Phytologist* 178: 740–753.
- Yousafzai F. K., Al-Kaff N. & Moore G. (2010) The molecular features of chromosome pairing at meiosis: the polyploid challenge using wheat as a reference. – *Functional and Integrative Genomics* 10: 147–156.
- Zeng J., Cao W., Hucl P., Yang Y., Xue A., Chi D. & Fedak G. (2013) Molecular cytogenetic analysis of wheat – *Elymus repens* introgression lines with resistance to Fusarium head blight. – *Genome* 56: 75–82.
- Zhou X., Lin H., Fan X. L. & Gao J. Y. (2012) Autonomous self-pollination and insect visitation in a saprophytic orchid, *Epipogium roseum* (D. Don) Lindl. – *Australian Journal of Botany* 60: 154–159.

Received 4 November 2020
Revision received 19 July 2021
Accepted 12 October 2021