Sexual reproduction of knotweed (*Fallopia* sect. *Reynoutria*) in Slovenia

Sexuální rozmnožování křídlatek (*Fallopia* sect. *Reynoutria*) ve Slovinsku

Simona Strgulc Krajšek & Jasna Dolenc Koce*

*Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, SI-1000 Ljubljana, Slovenia, e-mail: jasna.dolenc.koce@bf.uni-lj.si, * corresponding author*

Strgulc Krajšek S. & Dolenc Koce J. (2015): Sexual reproduction of knotweed (*Fallopia* sect. *Reynoutria*) in Slovenia. – Preslia 87: 17–30.

Fallopia japonica and *Fallopia* ×*bohemica* are two very invasive taxa belonging to the group *Fallopia* sect. *Reynoutria*, which cause serious problems in Europe and North America. Both of these taxa and the less invasive *F. sachalinensis* occur in Slovenia. Their main mode of reproduction is vegetative, although some *Fallopia* plants in Slovenia produce large numbers of seeds. Morphological analyses of selected natural populations reveal that *F. japonica* plants with only male sterile flowers typically produce many seeds. Fewer seeds were produced by *F.* ×*bohemica* and *F. sachalinensis* plants, although they produce viable pollen. Seed germination and survival of seedlings after three years were moderate outdoors in a botanical garden. The nuclear genome size of maternal plants and their corresponding seedlings was determined using DNA image cytometry in order to detect the ploidy level and potential donors of pollen. Based on the C-values all of the maternal plants were of one of three ploidy levels, which correspond to the octoploid *F. japonica* var. *japonica*, hexaploid *F.* ×*bohemica* and tetraploid *F. sachalinensis*. The variability in the genome size of the seedlings is high, and the most frequent pollen donor is *F.* ×*bohemica*.

K e y w o r d s: C-value, *Fallopia*, genome size, germination, reproduction, *Reynoutria*

Introduction

Fallopia japonica (Houtt.) Ronse Decr. (*Polygonaceae*) and its close relatives in the genus *Fallopia* Adans. in the section *Reynoutria* (Houtt.) Ronse Decr. (a group often named *Fallopia japonica* s.l.) are noxious invasive plant species worldwide. Their vegetative reproduction is well studied and recognized as their main means of spread in invaded regions. Four taxa of *Fallopia* sect. *Reynoutria* occur in Europe: Japanese knotweed [*Fallopia japonica* (Houtt.) Ronse Decr., with two varieties *F. japonica* var. *japonica* (Houtt.) Ronse Decr. and *F. japonica* var. *compacta* (Hooker fil.) J. P. Bailey]; giant knotweed [*Fallopia sachalinensis* (F. Schmidt) Ronse Decr.]; and Bohemian knotweed [*Fallopia* ×*bohemica* (Chrtek & Chrtková) J. P. Bailey], a hybrid between *F. japonica* var. *japonica* and *F. sachalinensis* (Bailey et al. 2009).

In Slovenia, *Fallopia japonica* var. *japonica* is very common along rivers, railways, roads and in some other ruderal habitats. *Fallopia sachalinensis* is found less frequently, with only a few known scattered localities. For the hybrid *F.* ×*bohemica*, there is little data for central Slovenia (Vreš 2007), although a recent study indicates that this hybrid is more frequent than previously thought, especially in central and eastern parts of Slovenia (Strgulc Krajšek & Jogan 2011). There are no records of *F. japonica* var. *compacta* occurring in Slovenia.

All octoploid specimens of *F. japonica* var. *japonica* in Europe belong to one extremely large clone of male sterile (female) plants (Hollingsworth & Bailey 2000). In North America, male fertile plants occur and sexual reproduction in *F. japonica* var. *japonica* is reported. The seedlings can grow quickly and flower within a single growing season (Forman & Kesseli 2003). In North America, *F. japonica* plants are highly genetically diverse and seed dispersal significantly contributes to the spread of this species along with predominant clonal growth (Grimsby et al. 2007). Sexual reproduction in *F. japonica* var. *japonica* is also reported in Europe (Tiébré et al. 2007, Bailey et al. 2009, Suda et al. 2010), although in this case the donors of pollen were usually the fertile male hybrid *F.* ×*bohemica*, or *F. sachalinensis*, or *F. baldschuanica* (Regel) Holub, a species belonging to the section *Pleuropterus* (Bailey 2013).

There are many potential reasons for the successful growth and spread of some plant taxa in newly colonized habitats, such as a wide tolerance of environmental conditions, phenotypic plasticity, self-reproduction, effective dispersal, high growth rate and polyploidy (te Beest et al. 2012). Among other factors, polyploidy is proposed as an important determinant of plant invasiveness. Several polyploidy-related factors and their combinations can influence the success of species invasion. Polyploids can have higher survival rates and fitness in the earliest establishment phase (preadaptation) as they are genetically very diverse, which allows for their subsequent adaptation and they can restore sexual reproduction following hybridization or they can reproduce asexually (te Beest et al. 2012). The amount of nuclear DNA (i.e. the genome size) is inherently associated with ploidy and usually expressed as the C-value. The total amount of DNA increases with polyploidy, although this increase is not always proportional, due to genome downsizing (Leitch & Bennett 2004). Some analyses of genome size have revealed that invasive plants are more likely to be polyploid than their non-invasive congeners, or the same plant species in its native range. This is the case for *Fallopia* sect. *Reynoutria* (te Beest et al. 2012). In this section there is a large variation in intraspecies ploidy based on the chromosome number x = 11 (Mandák et al. 2003, Bailey et al. 2007, 2009). Native populations of *F. japonica* include tetraploid (4x), hexaploid (6x), octoploid (8x) and decaploid (10x) cytotypes, whereas only tetraploids (*F. japonica* var. *compacta*) and octoploids (*F. japonica* var. *japonica*) occur in Europe. Native populations of *F. sachalinensis* are mainly 4x. The tetraploid cytotype also prevails in invaded areas, although some 6x and 8x individuals have developed, due to subsequent genome duplication. Hybridization between octoploid *F. japonica* and tetraploid *F. sachalinensis* has led to hexaploid *F.* ×*bohemica* in its native Asia and Europe, where some 4x and 8x cytotypes are also recorded. The genetic variation in this taxon is very high compared to the parental species (Bailey et al. 2009, Bailey 2013).

Even though only the female clone of *F. japonica* is present in Europe and it has spread mainly by vegetative reproduction hybridization between it and other *Fallopia* taxa is common. This has led to a large range of ploidy levels in the F1 generation, which has resulted in aneuploid and euploid progeny and polyploidy (Saad et al. 2011). Cytological studies has revealed hybrid taxa in the British Isles (Bailey & Stace 1992, Bailey et al. 2009), the Czech Republic (Mandák et al. 2003, Suda et al. 2010) and Belgium (Tiébré et al. 2007, Saad et al. 2011). Most commonly, *F. japonica* crosses with *F. sachalinensis* and *F.* ×*bohemica*, which has led to an extensive series of hybrids with chromosome numbers ranging from 2n = 44 to 67 and 2n = 66 to 110, respectively (Tiébré et al.

2007), and even 2n = 132 when backcrossing with octoploid *F.* ×*bohemica* occurs (Saad et al. 2011). Among the taxa present in certain areas, other combinations have also been reported, such as hybrids of *F. japonica* with *F. baldschuanica* (Bailey et al. 2007, Saad et al. 2011). This interspecies hybridization very likely increases the invasion potential of *Fallopia* taxa in recently colonized habitats and should not be underestimated (Bailey et al. 2007, Tiébré et al. 2007).

The aim of the present study was to determine whether *F. japonica* s.l. reproduces sexually in Slovenia. Genome size was measured using DNA image cytometry and the obtained C-value was used to estimate the ploidy level of the maternal plants, as well as that of the seedlings grown from seed produced by maternal plants and so detect the potential donors of pollen.

Materials and methods

Plant material

Field observations and the collection of plant material were carried out from 2009 to 2011. The plant material was collected from 29 locations in Slovenia (Fig. 1). A detailed description of these sampling sites is given in Appendix 1.

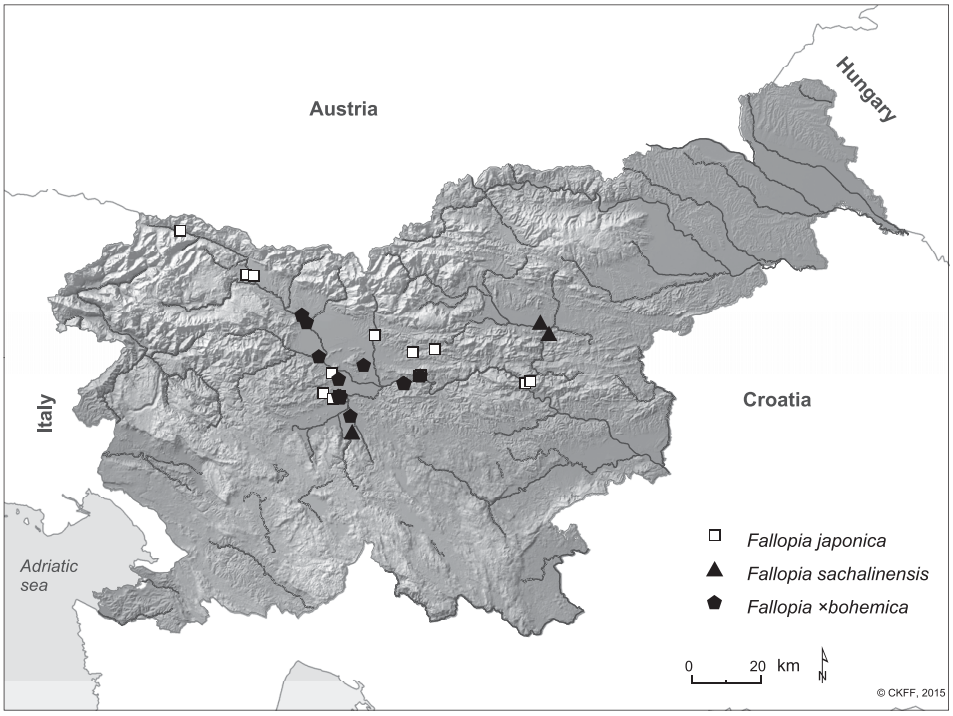

Fig. 1. – The sites in Slovenia at which the *Fallopia* plants were sampled.

In the late autumn of 2009, seeds were collected for germination tests from 22 specimens of *Fallopia* (13 *F. japonica*, 1 *F. sachalinensis*, 8 *F.* ×*bohemica*). As the seeds were collected late in the season it was impossible to collect fresh plant material (e.g. leaves, flowers) for herbarium voucher specimens or reliably determine the specimens based on their morphology. In the summers of 2010 and 2011, the following were collected: (i) fresh parts of rhizomes and shoots of all of the specimens from which seeds had previously been collected (i.e. the maternal plants); (ii) material for herbarium voucher specimens, which was dried and deposited in the University of Ljubljana Herbarium (Herbarium LJU); and (iii) flowers for the analyses of morphology and pollen viability, with parts of fresh inflorescences fixed and stored in 70% ethanol at 4 °C.

Field observations

During the sampling, the following data were collected for the *Fallopia* populations in the field: population size (small: up to 100 m^2; large: 100–1000 m^2; very large: more than 1000 m^2); number of *Fallopia* taxa in the population (monospecific and mixed population), and quantity of seed produced by the selected maternal plants (none; few: 1–20 seeds per inflorescence at a single node; many: more than 20 seeds per inflorescence at a single node).

Seed germination and seedling survival

Seeds were dried at room temperature (~20 °C) and then stored for at least 1 month in paper bags in a refrigerator at 8 °C, to simulate stratification prior to germination.

The germination was recorded under laboratory conditions for 19 samples of *Fallopia*. One hundred seeds were generally used per sample, which were divided into four replicates of 25 seeds placed in Petri dishes (diameter, 9 cm) on wet filter paper. Distilled water was used for watering. Only one replicate (25 seeds in total) was prepared when specimens produced very little seed. The seeds were germinated in the light at room temperature, and distilled water was added daily, as necessary. The number of seeds that germinated were counted every third day. When the roots were ~1 cm long, the seedlings were removed from the Petri dishes and fixed for the genome size measurements, as described below.

In addition, the germination was recorded in open-air conditions, in the botanical garden. Fifty seeds of 11 *F. japonica* and 8 of *F.* ×*bohemica* maternal plants, and 10 from one *F. sachalinensis* maternal plant, were sown in soil in containers in April 2010 and left outside in the cultivation section of the Botanic Gardens of the University of Ljubljana. The soil was watered occasionally and seedlings of other plants were left to grow in containers next to young *Fallopia* plants. The number of *Fallopia* seedlings growing in the containers was counted twice in the first year and then once every year.

Flower morphology and pollen viability

The flower morphology was examined under a stereomicroscope (Stemi SV 11, Carl Zeiss, Germany) to determine the type of flower: male sterile (female) flower had pistil and poorly developed stamens, male flower had stamens and no or poorly developed pistil, hermaphrodite flower had developed pistil and stamens.

Five anthers from five different flowers of one specimen were used as the sample. The flowers were initially stored in 70% ethanol and then placed on microscope slides and the anthers were mechanically isolated with tweezers. The other parts of the flowers were removed. The anthers were broken open using a dissection needle and a drop of anilin blue in lactophenol used to colour the viable pollen grains, as described by Jacquemart & Thompson (1995). This method has been used previously in analyses of the genus *Fallopia* (Tiébré et al. 2007).

The slides were then covered with a cover slip and observed under a microscope (Axioscope MOT, Carl Zeiss, Germany) at 400× magnification. The viability of the pollen was estimated using the following criteria: no pollen; all of the pollen grains were sterile; 1–10% viable pollen grains; 11–50% viable pollen grains; 51–99% viable pollen grains; all of the pollen grains (100%) were viable.

Nuclear genome size

The nuclear genome size was measured for the maternal plants and the young seedlings grown from the seeds that were collected from the maternal plants.

Fresh shoot tips were collected from the maternal plants in the field. The young leaves were removed and the shoot tips were cut into smaller pieces to ensure the penetration of the fixative deep into the tissue and a high quality fixation. The shoot tips were fixed in 4% phosphate-buffered neutral formaldehyde at room temperature and post-fixed in 3:1 (v:v) methanol:acetic acid at 4 °C, as previously described (Bačič et al. 2007). The root tips of young *Fallopia* seedlings were fixed using the same procedure.

Pisum sativum cv. 'Kleine Rheinlaenderin' was used as the calibration standard. The *Pisum* seeds were germinated and the root tips of the seedlings were processed together with the *Fallopia* specimens in all of the experimental procedures (i.e. fixation, DNA staining, genome size measurement).

Following hydrolysis in 5 N HCl (30 min at 20 °C), the nuclear DNA was stained with Feulgen reagent (pararosanilin chloride; BDH, UK). The stained root tips were washed in several changes of SO_2-water, and then in distilled water. Squash preparations of dissected root meristems were prepared in 45% acetic acid. The amounts of nuclear DNA (i.e. the C-value) were measured densitometrically using interphase-peak DNA image cytometry (Vilhar et al. 2001). The image analysis was carried out under a light microscope (Axioscope MOT; Carl Zeiss, Germany) using a 63× oil immersion objective, a CCD camera (Sony DXC-950P), a frame grabber (Matrox Meteor), and the image analysis software package KS 400, version 3.0 (Carl Zeiss, Germany). The integrated optical density was measured for ~200 interphase nuclei per slide. For each specimen of a maternal plant, ~7 slides (replicates) were prepared.

Pisum sativum cv. 'Kleine Rheinlaenderin' used as the calibration standard has a 2C-value of 8.84 pg DNA (Greilhuber & Ebert 1994), which was used to convert the arbitrary units of the integrated optical density into pg DNA. The amount of DNA in the unreplicated (G1 phase) nuclei was expressed as the 2C-value.

To test differences among taxa statistical analyses ANOVA and t-test were carried out using Prism 3.02 (GraphPad) software.

Results

Samples of *Fallopia* section *Reynoutria* were collected at 29 sites in the central and western parts of Slovenia (Fig. 1). *Fallopia japonica* grew at 18 of these localities, *F. sachalinensis* at three and hybrid *F.* ×*bohemica* at 13. Monospecific populations of *Fallopia* occurred at most of these localities and mixed populations at only six localities, which were always a combination of *F. japonica* and *F.* ×*bohemica*. The size of the populations of *Fallopia* examined was variable; monospecific populations of *F. japonica* and *F. sachalinensis* were mainly small and covered less than 1000 m^2 and those of *F.* ×*bohemica* were large and mainly covered more than 1000 m^2. Very large populations were also recorded for all mixed populations.

The morphological analysis of flowers revealed that all the *F. japonica* plants examined were male sterile (female) with visible but small and undeveloped stamens and anthers that contained no pollen. The same was recorded in one female population of *F. sachalinensis* while the other two populations had hermaphrodite flowers. On the other hand *F.* ×*bohemica* plants developed mainly male flowers, with only one sample (F45, Appendix 1) having mostly hermaphrodite and just a few male flowers. Based on flower morphology, no pollen was detected in female flowers of *F. japonica* and *F. sachalinensis* and pollen viability was only tested for *F.* ×*bohemica* and the hermaphrodite samples of *F. sachalinensis*. In 89% of the *F.* ×*bohemica* samples less than half of the pollen was viable while in *F. sachalinensis* samples all of the pollen was viable.

The number of seeds on most of the *F. japonica* plants ranged from few (less than 20 seeds per inflorescence at a single node) to many (usually more than 50 seeds per inflorescence at a single node) and in only one sample (F23) were there no seeds. In this case the reason for the lack of seeds could be the absence of a pollen source in the vicinity. There were only a few seeds per inflorescence recorded for all *F.* ×*bohemica* and *F. sachalinensis* plants, which were developed by a small number of hermaphrodite flowers among the prevailing male flowers, which were not probably previously detected because of the rarity of this type of flower.

The germination was determined for the seeds collected from the maternal *Fallopia* plants, both in the laboratory (grown in Petri dishes) and the botanical garden (planted in soil in containers) (Table 1). The highest percentage germination rate was recorded for *F. japonica* in the laboratory (between 49% and 100%). The average survival of seedlings was up to 20% after one year and only a few seedlings survived three years (seven from the original 11 samples of *F. japonica*, three from the original eight samples of *F.* ×*bohemica*, none for *F. sachalinensis*). ANOVAs and t-tests revealed that the germination of seeds collected from maternal plants of *F. japonica* was significantly higher than that collected from *F.* ×*bohemica* ($P = 0.0002$ for laboratory; $P = 0.0042$ for botanical garden). Comparison of germination conditions revealed that germination was significantly higher in the laboratory than the botanical garden ($P = 0.0007$ for *F. japonica;* $P = 0.0397$ for *F.* ×*bohemica*). Statistical analysis of *F. sachalinensis* was not performed because the numbers were too low.

The nuclear genome size was measured for 25 maternal plants for which the 2C-values ranged from 4.30 to 9.86 pg DNA. The 2C-values formed three distinct groups, which correspond to the three *Fallopia* taxa revealed by the morphological analysis (Fig. 2). The 12 maternal plants that belonged to *F. japonica* had the largest genome size, with

Table 1. – Seed germination and seedling survival recorded for the *Fallopia* samples. ND – no data available. Mean values and ranges are presented.

Species	Sample code	Seed germination (%)		Viable seedlings in botanical garden (%)	
		Laboratory	Botanical garden	After 1 year	After 3 years
F. japonica	F03	91	70	28	2
	F04	79	62	18	0
	F08	99	42	28	8
	F10	56	10	8	0
	F11	64	54	20	4
	F13	81	10	12	2
	F14	100	46	14	0
	F17	62	24	10	4
	F18	97	78	57	12
	F21	99	54	20	0
	F22a	49	24	12	2
Mean (min–max)		79.7 (49–100)	43.1 (10–78)	20.6 (8–57)	3.1 (0–12)
F. ×bohemica	F02	28	10	0	0
	F05	56	30	6	2
	F07	7	2	0	0
	F09	29	6	10	2
	F12	6	0	0	0
	F19	56	14	0	0
	F20	54	16	8	0
	F25	ND	29	8	6
Mean (min–max)		33.7 (6–56)	13.4 (0–29)	4.0 (0–10)	1.3 (0–6)
F. sachalinensis	F16	68	40	20	0

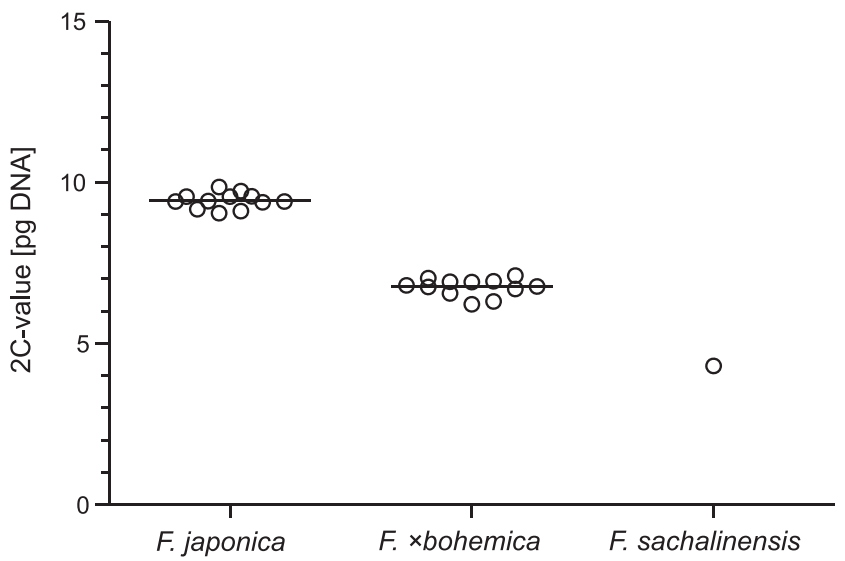

Fig. 2. – Genome size of the maternal *Fallopia* plants. For each specimen, the nuclear DNA amounts are expressed as 2C-values based on measuring ca. seven microscopic slides (means, circles). The mean value for each species is indicated by the lines (*F. japonica*, n = 12; *F. ×bohemica*, n = 12; *F. sachalinensis*, n = 1).

a mean 2C-value of 9.43±0.07 pg DNA (range, 9.04 to 9.86 pg DNA; coefficient of variation CV = 2.59%). Another 12 samples of maternal plants belonged to the hybrid *F.* ×*bohemica*, with a mean 2C-value of 6.75±0.07 pg DNA (range, 6.22 to 7.11 pg DNA; CV = 4.02%). Only one specimen of *F. sachalinensis* was analysed, and this had the lowest 2C-value, 4.30 pg DNA.

The genome sizes of the seedlings that germinated from the seed collected from the *Fallopia* plants were more variable than those collected from maternal plants (Fig. 3). The greatest variability in the genome size was recorded in the seedlings from *F. japonica*, with 2C-values ranging from 5.33 pg to 13.99 pg DNA (mean 2C-value, 8.74±0.12 pg DNA; CV = 16.75%; n = 152). The 2C-values of the *F.* ×*bohemica* seedlings ranged from 5.92 pg to 10.62 pg DNA (mean 2C-value, 7.28±0.15 pg DNA; CV = 16.08%; n = 64). The lowest variability in genome size was recorded for the *F. sachalinensis* seedlings, which ranged from 4.30 pg to 6.54 pg DNA (mean 2C-value, 6.00±0.12 pg DNA; CV = 8.70%; n = 16) (Fig. 3).

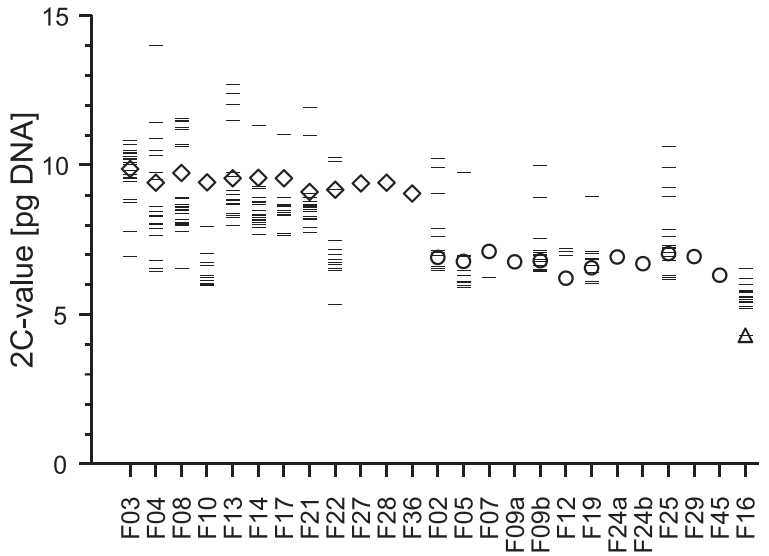

Fig. 3. – Genome sizes of the *Fallopia* seedlings. For each specimen, the nuclear DNA amount is expressed as 2C-value based on measurements for each root tip (line symbols). The mean value for the corresponding maternal plant is indicated by an open diamond for *F. japonica*, open circle for *F.* ×*bohemica* and open triangle for *F. sachalinensis*.

Discussion

All three invasive alien taxa of *Fallopia* sect. *Reynoutria* in Slovenia were analysed for flower morphology, seed germination and genome size. Whilst the first study on invasive *Fallopia* in Slovenia (Vreš 2007) indicates that *F. japonica* is the main taxon, both Strgulc Krajšek & Jogan (2011) and the current study indicate that equal amounts of the hybrid *F.* ×*bohemica* were also present. The reason for this is the earlier misidentification of the hybrid as *F. japonica*, which is by no means an uncommon situation. The successful

spread of *F.* ×*bohemica* in the last few years can be attributed to the superior competitive ability of the hybrids compared to the native flora (Parepa et al. 2014). In the present study, the more frequently found taxa *F. japonica* and *F.* ×*bohemica* were sampled at 18 and 15 localities, respectively, while *F. sachalinensis* was only found at three of the sites sampled. The habitats where these *Fallopia* specimens were collected are linked to human activities, such as roads, railways and ruderal sites (Appendix 1). This is especially relevant for *F. japonic*a and *F.* ×*bohemica*, which are more invasive. Similar distribution patterns of this group are reported in Europe (Mandák et al. 2003, Bailey et al. 2009, Pyšek et al. 2012). At the majority of the sites sampled there were only monospecific *Fallopia* populations, with a few mixed populations, always consisting of *F. japonica* and *F.* ×*bohemica*. All *F. japonica* plants were male sterile (female), as expected from the previous studies carried out in the rest of Europe (Hollingsworth & Bailey 2000, Tiébré et al. 2007). The *F.* ×*bohemica* plants mostly developed male flowers, with only few hermaphrodite flowers per plant. The pollen of these specimens was only partly viable (~ 50%), which is in agreement with the data for Belgium (Tiébré et al. 2007).

The field observations revealed that the *Fallopia* plants examined produced different quantities of seed. *Fallopia japonica* produced more seed than either *F. sachalinensis* or *F.* ×*bohemica*. The germination of the seed was tested, along with an analysis of the genome sizes of the young seedlings, in order to determine the sources of the pollen. The source of the pollen for the pollination of the *F. japonica* flowers could have been the hybrid (*F.* ×*bohemica*) or hermaphrodite plants of *F. sachalinensis*, the pollen of which was almost 100% viable, or it could have been representatives of other *Fallopia* species present in the flora of Slovenia (i.e. *F. baldschuanica*, *F. convolvulus*, *F. dumetorum*) (Strgulc Krajšek & Jogan 2011). The data on germination and survival revealed that a relatively high percentage of the seed from *F. japonica* and lower percentage of the seeds from *F.* ×*bohemica* and *F. sachalinensis* germinated. Some young seedlings successfully overwintered in the open air in the botanical garden and some were still alive three years after sowing. In one sample of *F. japonica* (F13) and one of *F.* ×*bohemica* (F09) more seeds germinated in the second year than in the first year. This might also have happened in the other samples but it was not detectable because seeds were not marked. The seedlings from the *F. japonica* maternal plants were more successful in terms of the survival test, compared to the seedlings from the *F.* ×*bohemica* and *F. sachalinensis* maternal plants. Though the survival was not high it is important to stress that after succesful germination and seedling establishment *Fallopia* plants quickly spread by vegetative reproduction; therefore, only one seedling is needed to initiate a new population. In the field in North America, seedlings survive and regrow the following spring if they achieved sufficient growth the previous year (Forman & Kesseli 2003). In the present study, the conditions in the botanical garden were similar to natural conditions, so we can assume that sexual reproduction is also one of the possible means by which *Fallopia* is spreading in Slovenia.

The nuclear genome size can also be used as a taxonomic marker when there is little intraspecies morphological variation and insufficient divergence among the different taxa (Suda et al. 2010). *Fallopia japonica* var. *japonica* is predominantly octoploid, while tetraploids are characteristic for *F. japonica* var. *compacta* and *F. sachalinensis* (Mandák et al. 2007, Bailey 2013). Nevertheless, some other cytotypes are also reported

in the Czech Republic (Mandák et al. 2003, Suda et al. 2010). The hybrid *F.* ×*bohemica* is much more variable, due to irregular meiosis and backcrossing, although the majority of these plants are hexaploid. In the present study, the genome size was measured using the shoot tips of the maternal plants and roots of seedling and DNA image cytometry. The data revealed that the maternal plants can be unambiguously distributed into three distinct groups that correspond to their morphological characterization. The greatest genome size was measured for *F. japonica*, medium for *F.* ×*bohemica* and smallest for *F. sachalinensis*. When expressed as 2C-values, genome size was similar and comparable to that previously measured in octoploid (8x) *F. japonica* var. *japonica,* hexaploid (6x) *F.* ×*bohemica* and tetraploid (4x) *F. sachalinensis* by Suda et al. (2010) using flow cytometry. The intraspecies variability was slightly higher in the present study than that recorded using flow cytometry by these authors, although it was still below 5%, which is the proposed variation (CV) limit in medical and plant DNA image cytometry (Vilhar et al. 2001). In another study conducted by Bailey & Stace (1992), much lower amounts of DNA and higher variability were reported, although they ascribe these underestimated values to the methodology used, so these values are not directly comparable with the present results.

The genome sizes of seedlings that developed after hybridization between known female and unknown male parents were more variable and did not correspond to that of any of the ploidy levels. This variability expressed as a coefficient of variation (CV) was >15% for the *F. japonica* and *F.* ×*bohemica* seedlings, and 8.7% for *F. sachalinensis*. The much lower variability recorded for *F. sachalinensis* could be related to the small number of seedlings tested. In *F. sachalinensis*, 16 seedlings from only a single maternal plant were analysed, whereas for the other two taxa 152 seedlings from 12 *F. japonica* and 64 seedlings from 12 *F.* ×*bohemica* maternal plants were used in the genome size analysis. High variability in seedling genome size is also reported by Saad et al. (2011) and the main reason for this could be the occurrence of aneuploid gametes and backcrossing in the *Fallopia* complex (Saad et al. 2011, Bailey 2013). The hybrid *F.* ×*bohemica* has very irregular meiosis, with a large number of univalents and multivalents (Bailey & Stace 1992). Fertilization with such pollen results in seedlings with a genome size that is smaller than that of the maternal plants. In the second generation hybrids, polyploidy can occur, and the seedlings have a larger genome size. Fertilization with unreduced gametes can also occur, which further complicates the picture (Saad et al. 2011). In the present study, the genome size data for the seedlings of the maternal plants of one species of *Fallopia* were tested using ANOVA. In *F. japonica*, the seedlings of two maternal plants (F10, F22) had significantly lower 2C-values. For these two plants, *F. sachalinensis* might have been the pollen donor, although this species is rare in Slovenia and was not found nearby. Another explanation might be fertilization by some of the other *Fallopia* taxa, e.g. *F. baldschuanica*, which mainly grows in gardens and is the main pollen source in the UK and in some parts of mainland Europe (Bailey et al. 2009, Saad et al. 2011). In Slovenia, *F. baldschuanica* is grown as an ornamental plant in gardens but only in the south-western part of the country is it present as an invasive species (Strgulc Krajšek & Jogan 2011). In the present study, no specimens of this species were found in the vicinity of the sites sampled, and here it is therefore less likely to have been the source of pollen. Seedlings of seven *F. japonica* plants had higher 2C-values and were most likely pollinated by *F.* ×*bohemica,* which is the most common backcrossing as is also reported in Belgium (Tiébré et al. 2007). The seedlings that germinated from the seeds that developed on the

F. ×*bohemica* plants had lower mean 2C-values than *F. japonica* seedlings, and the differences among them were not significant. Most of these seedlings had bigger genomes than their maternal plants suggesting that fertilization by pollen from *F.* ×*bohemica* occurred, while in those seedlings with a small genome the maternal plants were fertilized by pollen from *F. sachalinensis*. The seedlings that germinated from the seeds that developed on the *F. sachalinensis* plant had higher 2C-values than the maternal plant, which was probably fertilized by *F.* ×*bohemica* pollen.

More reliable conclusions about the pollen donor could be made if the chromosome numbers were available. Unfortunately, in the plant tissues examined, cell divisions were rarely detected, even though the measurement of the DNA amounts and chromosome numbers were obtained from the same slide, which is one of the important benefits of DNA image cytometry.

To conclude, in Slovenia sexual reproduction in the group *F. japonica* s.l. is confirmed by the presence of large numbers of seeds with relatively high percentages of germination and of seedlings that are able to survive outdoors. In the future, additional studies are needed to compare our findings with what occurs in nature where these seedlings germinate in well-established *Fallopia* stands. According to the genome size data, *F. japonica* is most frequently fertilized by *F.* ×*bohemica* pollen. The seeds produced by *F.* ×*bohemica* originated from backcrosses with the same taxon and hybridization with *F. sachalinensis*.

As *Fallopia* species can reproduce sexually, improved management techniques are needed to prevent their extensive colonization of new habitats. As well as the control of their vegetative reproduction, care needs to be taken to remove plants before they reach the flowering stage.

Acknowledgements

This study was partly financially supported by the Slovenian Research Agency, grant no. P1-0212. The authors thank Tamara Erhatič, Metka Škornik, Branka Trčak (Centre for Cartography of Fauna and Flora) and Dr. Aleš Kladnik for technical help, and Dr. Chris Berrie for editorial assistance.

Souhrn

Fallopia japonica a *F.* ×*bohemica*, invazní taxony ze skupiny *Fallopia* sect. *Reynoutria*, působí značné problémy v Evropě a v Severní Americe. Oba se vyskytují ve Slovinsku, stejně jako další, poněkud méně invazní druh *F. sachalinensis*. Rozmnožují se převážně vegetativně, i když některé rostliny ve Slovinsku vytvářejí velké množství semen. Morfologická analýza vybraných populací ukázala, že takto plodné jsou zejména rostliny *F. japonica* s květy obsahujícími sterilní samčí orgány; *F.* ×*bohemica* and *F. sachalinensis* vytvářejí sice méně semen, produkují však životaschopný pyl. Na pokusné zahradě jsme sledovali klíčivost semen a přežívání semenáčů po dobu tří let. Pomocí průtokové cytometrie jsme stanovili velikost jaderného genomu mateřských rostlin a jejich potomstva. Všechny mateřské rostliny byly buď oktoploidní *F. japonica* var. *japonica*, hexaploidní *F.* ×*bohemica* nebo tetraploidní *F. sachalinensis*. Variabilita velikosti genomu semenáčů byla značná a nejčastějším donorem pylu je *F.* ×*bohemica*.

References

Bačič T., Jogan N. & Dolenc Koce J. (2007): *Luzula* sect. *Luzula* in the south-eastern Alps: karyology and genome size. – Taxon 56: 129–136.

Bailey J. P. (2013): The Japanese knotweed invasions viewed as a vast unintentional hybridisation experiment. – Heredity 110: 105–110.

Bailey J. P., Bímová K. & Mandák B. (2007): The potential role of polyploidy and hybridisation in the further evolution of the highly invasive *Fallopia* taxa in Europe. – Ecol. Res. 22: 920–928.

Bailey J. P., Bímová K. & Mandák B. (2009): Asexual spread versus sexual reproduction and evolution in Japanese knotweed s.l. sets the stage for the "Battle of the clones". – Biol. Inv. 11: 1189–1203.

Bailey J. P. & Stace C. A. (1992): Chromosome number, morphology, pairing, and DNA values of species and hybrids in the genus *Fallopia* (*Polygonaceae*). – Plant Syst. Evol. 180: 29–52.

Forman J. R. & Kesseli V. (2003): Sexual reproduction in the invasive species *Fallopia japonica* (*Polygonaceae*). – Am. J. Bot. 90: 586–592.

Greilhuber J. & Ebert I. (1994): Genome size variation in *Pisum sativum*. – Genome 37: 646–655.

Grimsby J. L., Tsirelson D., Gammon M. A. & Kesseli R. (2007): Genetic diversity and clonal vs. sexual reproduction in *Fallopia* spp. (*Polygonaceae*). – Am. J. Bot. 94: 957–964.

Hollingsworth M. L. & Bailey J. P. (2000): Evidence for massive clonal growth in the invasive *Fallopia japonica* (Japanese knotweed). – Bot. J. Linn. Soc. 133: 463–472.

Jacquemart A. L. & Thompson J. D. (1995): Floral and pollination biology of three sympatric *Vaccinium* (*Ericaceae*) species in the Upper Ardennes, Belgium. – Can. J. Bot. 74: 210–221.

Leitch I. J. & Bennett M. D. (2004): Genome downsizing in polyploid plants. – Biol. J. Linn. Soc. 82: 651–663.

Mandák B., Pyšek P., Lysák M., Suda J., Krahulcová K. & Bímová K. (2003): Variation in DNA-ploidy levels of *Reynoutria* taxa in the Czech Republic. – Ann. Bot. 92: 265–272.

Parepa M., Fischer M., Krebs C. & Bossford O. (2014): Hybridization increases invasive knotweed success. – Evol. Appl. 7: 413–420.

Pyšek P., Chytrý M., Pergl J., Sádlo J. & Wild J. (2012): Plant ivasions in the Czech Republic: current state, introduction dynamics, invasive species and invaded habitats. – Preslia 84: 575–629.

Saad L., Tiébré M. S., Hardy O. J., Mahy G. & Vanderhoeven S. (2011): Patterns of hybridization and hybrid survival in the invasive alien *Fallopia* complex (*Polygonaceae*). – Plant Ecol. Evol. 144: 12–18.

Strgulc Krajšek S. & Jogan N. (2011): The genus *Fallopia* Adans. in Slovenia (Rod *Fallopia* Adans. v Sloveniji). – Hladnikia 28: 17–40.

Suda J., Trávníček P., Mandák B. & Berchová-Bímová K. (2010): Genome size as a marker for identifying the invasive alien taxa in *Fallopia* section *Reynoutria*. – Preslia 82: 97–106.

te Beest M., Le Roux J. J., Richardson D. M., Brysting A. K., Suda J., Kubešová M. & Pyšek P. (2012): The more the better? The role of polyploidy in facilitating plant invasions. – Ann. Bot. 109: 19–45.

Tiébré M. S., Vanderhoeven S., Saad L. & Mahy G. (2007): Hybridization and sexual reproduction in the invasive alien *Fallopia* (*Polygonaceae*) complex in Belgium. – Ann. Bot. 99: 193–203.

Vilhar B., Greilhuber J., Dolenc Koce J., Temsch E. M. & Dermastia M. (2001): Plant genome size measurement with DNA image cytometry. – Ann. Bot. 87: 719–728.

Vreš B. (2007): *Fallopia* – knotweed (*Fallopia* – dresnik, slakovec). – In: Martinčič A., Wraber T., Jogan N., Podobnik A., Turk B. & Vreš B. (eds), Mala flora Slovenije: ključ za določanje praprotnic in semenk [Slovenian flora: determination key for *Pteridophyta* and *Spermatophyta*], Ed. 4, p. 211, Tehniška založba Slovenije, Ljubljana.

Received 15 October 2013
Revision received 19 September 2014
Accepted 27 October 2014

Appendix 1. – Sites sampled for the *Fallopia* specimens used in the present study.

Fallopia japonica

F03-BLED; Slovenia: Gorenjska, Bled, Zagorica, Koritenska Road; N 46°21'58.2"; E 14°07'18.5"; shrubs; MTB: 9650/2. – F04-LESCE; Slovenia: Gorenjska, Lesce, beside Lesce to Bled road, near hippodrome; N 46°21' 49.3"; E 14°08'57.3"; ruderal place beside road; MTB: 9650/2. – F08-STANEŽIČE; Slovenia: Ljubljana, Stanežiče, beside main Medvode to Ljubljana road where it crosses the road to Stanežiče; N 46°06' 38.2"; E 14°26'44.1"; ruderal place beside road; MTB: 9852/4. – F10-KAMNIK; Slovenia: Gorenjska, Kamnik, Zgornje Perovo, beside petrol station, road branch to Zg. Perovo; N 46°12'34.0"; E 14°36'27.8"; ruderal place beside road; MTB: 9753/4. – F11-PODMILJ; Slovenia: Posavsko hribovje, Blagovica, beside Trojane to Blagovica road, near horticulture centre in Podmilju; N 46°10'25.9"; E 14°50'01.3"; meadow beside brook; MTB: 9855/1. – F13-ZIDANI MOST; Slovenia: Posavje, Zidani Most, beside Zidani Most to Radeče road, between the railway underpasses; N 46°05'03.1"; E 15°10'23.1"; ruderal place beside road; MTB: 9957/1. – F14-ŠIRJE; Slovenia: Posavje, Zidani Most, beside Zidani Most to Širje road, between road and river Savinja; N 46°05'27.0"; E 15°11'24.0"; ruderal place beside road; MTB: 9957/1. – F17-DOBROVA; Slovenia: Ljubjanska kotlina, Dobrova pri Ljubljani, by River Gradaščica, 100 m east of the bridge over Gradaščica; 46°03'29.3"; E 14°24'56.2"; meadow beside river; MTB: 9952/2. – F18-VRHOVCI; Slovenia: Ljubljana, Vrhovci, by bridge over Mali graben; N 46°02'33.9"; E 14°27'00.9"; brook bank; MTB: 9952/4. – F21-RIBČE; Slovenia: Zasavje, Ribče, by River Sava; N 46°06'11.7"; E 14°46'30.4"; meadow beside River Sava; MTB: 9854/4. – F22-KRESNICE; Slovenia: Zasavje, Kresnice, by River Sava; N 46°06'20.3"; E 14°46'44.6"; meadow beside River Sava; MTB: 9854/4. – F23-MARTULJEK; Slovenia: Gorenjska, Gozd-Martuljek, resting place between road and River Sava Dolinka; N 46°28'46.2"; E 13°52'10.5"; ruderal place beside road resting place; MTB: 9549/1. – F27-BF-MOST; Slovenia: Ljubljana, beside Večna pot, by Glinščica brook, beside bridge on eastern side of Biotechnical Faculty; N 46°02'58.3"; E 14°28'33.0"; bank of regulated brook; MTB: 9952/4. F28-BF-KOLESARSKA; Slovenia: Ljubljana, beside Večna pot, by cycle track near Glinščica brook, 100 m west of the bridge; N 46°02'59.8"; E 14°28'28.5"; bank of regulated brook; MTB: 9952/2. – F36-SP.LOKE; Slovenia: Posavsko hribovje, Krašnja, Spodnje Loke; N 46°09'54.0"; E 14°45'00.8"; hedge; MTB: 9854/2.

Fallopia ×bohemica

F02-KOKRICA; Slovenia: Gorenjska, Kranj, Naklo, crossroad on the Kranj to Naklo road to highway A2 (Kranj Zahod); N 46°15'45.3"; E 14°19'60.0"; ruderal place beside road; MTB: 9752/1. – F05-KRANJ; Slovenia: Gorenjska, Kranj, Savski otok, left riverbank of Sava; N 46°14'37.3"; E 14°21'01.0"; river bank; MTB: 9752/3. – F07-MEDVODE; Slovenia: Gorenjska, Medvode, Jeprca, unpaved parking place by Jeprca to Medvode road; N 46°09'15.6"; E 14°23'54.8"; ruderal place beside road resting place; MTB: 9852/1. – F09-ŠENTVID; Slovenia: Ljubljana, Šentvid, Poljane; N 46°05'44.6"; E 14°28'19.9"; ruderal place beside road; MTB: 9952/2. – F12-TRZIN; Slovenia: Ljubljanska kotlina, Trzin, by crossroad on the roads from Ljubljana to Mengeš and Domžale, by railway crossing; N 46°07'57.9"; E 14°33'59.5"; ruderal place between road and railway; MTB: 9853/3. – F19-SENOŽETI; Slovenia: Zasavje, Senožeti, by River Sava; N 46°05'09.7"; E 14°42'57.4"; meadow beside River Sava; MTB: 9954/1. – F20-SENOŽETI; Slovenia: Zasavje, Senožeti, by River Sava; N 46°05'05.6"; E 14°43'11.0"; meadow beside River Sava; MTB: 9954/1. – F22-KRESNICE; Slovenia: Zasavje, Kresnice, by River Sava; N 46°06'20.3"; E 14°46'44.6"; meadow beside River Sava; MTB: 9854/4. – F24-BF; Slovenia: Ljubljana, beside Večna pot, by potoku Glinščica brook, by fence of Biotechnical Faculty; N 46°03'02.7"; E 14°28'19.5"; bank of regulated brook; MTB: 9952/2. – F25-BF-GOZDARSTVO; Slovenia: Ljubljana, beside Rožna Dolina cesta XV, 100 m from crossroad with Večna pot, E of Forestry Department of Biotechnical Faculty; N 46°03'06.6"; E 14°28'44.6"; ruderal place beside road; MTB: 9952/3. – F29-ROŽNA-DOLINA; Slovenia: Ljubljana, Rožna dolina, ruderal place beside Rožna dolina cesta XVII; N 46°02'49.7"; E 14°28'40.4"; ruderal place; MTB: 9952/4. – F45-ČRNA VAS; Slovenia: Ljubljansko barje, Ljubljana, Črna vas, beside Ižanska cesta; N 45°59'58.1"; E 14°31'04.9"; ruderal place beside road MTB: 9953/1.

Fallopia sachalinensis

F16-IG; Slovenia: edge of Ljubljansko barje, Ig, Pungrt; N 45°57'24.6"; E 14°31'25.5"; meadow; MTB: 0053/1. – F50-CELJE-POLULE; Slovenia: Štajerska, Celje, Polule, around the bus station opposite the school; N 46°12'58.1", E 15°15'45.3", ruderal site beside road; MTB 9757/4. – F53-CELJE-MEDLOG; Štajerska, Celje, Medlog, beside connecting road Medlog to highway A1; N 46°14'45.2", E 15°13'46.0"; road bank; MTB 9757/3.