Preslia 95: 241–266, 2023
doi: 10.23855/preslia.2023.241

Preslia

Cytogeography of invasive knotweeds (*Fallopia* sect. *Reynoutria*) in central Europe: rare aneuploids and evidence for a climatically determined distribution

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Abstract: *Fallopia* sect. *Reynoutria* members (knotweeds) represent one of the most invasive alien plants in Europe. However, several aspects of their biology, including cytological variation are poorly understood. Specifically, some taxa have multiple ploidy levels and the frequency of cytotypes in seeds and seedlings does not correspond to that recorded in adult plants. In this study, flow cytometry was used to determine the relative genome size of 1,106 established plants of knotweeds (960 populations) collected in the Western Carpathians and adjacent area in Pannonia, and the results were combined with previous data (53 individuals from 43 populations) from the Krivánska Malá Fatra Mts. In total, 1,159 individuals from 1,003 populations (825 individuals from Slovakia, 173 from Hungary, 70 from Poland, 63 from Czechia and 28 from Austria) were evaluated. *Fallopia* sect. *Reynoutria* is represented mainly by the hexaploid ($2n = 6x \sim 66$) cytotype of *F.* ×*bohemica* (809 individuals) and octoploid ($2n = 8x \sim 88$) cytotype of *F. japonica* var. *japonica* (297 individuals) in the area studied. To a limited extent, the tetraploid ($2n = 4x \sim 44$) and hexaploid ($2n = 6x \sim 66$) cytotypes of *F. sachalinensis* (43 and 9 individuals, respectively) were also recorded. In addition, for the first time, adult aneuploid knotweed plants with $2n = 65$ and $2n = 107$ chromosomes (both in *F.* ×*bohemica*) were recorded in continental Europe and the world, respectively. In contrast, the occurrence of the tetraploid ($2n = 4x \sim 44$) cytotype of *F. japonica* previously reported in the area studied was not confirmed and it is likely this information is incorrect. This study revealed evidence of a climatically determined distribution of invasive knotweeds in the area studied. The occurrence of *F. japonica* var. *japonica* and *F. sachalinensis* decreased markedly along a north-west–south-east gradient, whereas the frequency of *F.* ×*bohemica* occurrence increased (from 15.7% in the Polish part of the Western Carpathians up to 98.5% in the Hungarian part of Pannonia).

Keywords: alien plants, chromosome numbers, distribution, flow cytometry, Pannonia, *Reynoutria*, Western Carpathians

Introduction

Fallopia sect. *Reynoutria* (Houtt.) Ronse Decr. (*Fallopia* Adanson; knotweed; *Polygonaceae*) includes six species native to East Asia (Desjardins 2015): *F. ciliinervis* (Nakai) K. Hammer, *F. compacta* (Hook.) G. H. Loos et P. Keil, *F. forbesii* (Hance) Yonekura et

Received: 22 Nov 2022; Revised: 16 Jan 2023; Accepted: 12 Feb 2023; Published: 5 Jun 2023

H. Ohashi, *F. japonica* (Houtt.) Ronse Decr., *F. multiflora* (Thunb.) Haraldson and *F. sachalinensis* (F. Schmidt) Ronse Decr. In the invaded European range, this section is represented by four naturalized alien species, including *F. compacta*, *F. japonica* var. *japonica*, *F. multiflora* and *F. sachalinensis*, and two formally described hybrids, including *F.* ×*bohemica* (Chrtek et Chrtková) J. P. Bailey (= *F. japonica* var. *japonica* × *F. sachalinensis*) and *F.* ×*moravica* Hodálová et Mereďa (= *F. compacta* × *F. sachalinensis*) (e.g. Bailey 1989, Bímová et al. 2001, Bailey et al. 2007, Hodálová et al. 2022). Three of them, namely *F.* ×*bohemica*, *F. japonica* var. *japonica* and *F. sachalinensis*, are among the most successful invasive plants in Europe (Beringen et al. 2019). *Fallopia japonica* is recognized as one of the worst invasive species in Europe (Nentwig et al. 2017) and the International Union for Conservation of Nature (IUCN) ranks it as one of the 100 most invasive organisms in the world (Lowe et al. 2000). Several works have confirmed that its (still overlooked) hybrid, *F.* ×*bohemica*, exhibits even greater invasiveness (Bímová et al. 2003, Alberternst & Böhmer 2011, Moravcová et al. 2011, Bailey 2013, Parepa et al. 2014, Gillies et al. 2016) and the newly described *F.* ×*moravica* (Hodálová et al. 2022) is likely to be similarly invasive.

Despite the fact that much attention is given to *F.* sect. *Reynoutria* taxa from the point of their invasiveness (Lavoie 2017), several aspects of their biology are still poorly understood, including the taxonomy of particular taxa (especially in their East-Asian native range; Kim & Park 2000, Bailey 2003, 2013, Pashley 2003, Bailey et al. 2007, Balogh 2008, Desjardins 2015, Beringen et al. 2019), their identification (Mereďa et al. 2019a), distribution (see Vuković et al. 2019, Stalažs 2022) and the frequency and importance of their inter- and intrasectional hybridization (Bailey & Stace 1992, Bailey 2013, Desjardins 2015).

One of the still insufficiently known aspects of knotweed biology is their cytological variation and cytotype distribution even though these features may be key determinants of knotweed invasiveness. In *F.* sect. *Reynoutria* seeds and seedlings, there are a large number of aneuploid cytotypes, which for some reason (most likely related to climate) are not able to develop into established plants in nature (Bailey 1989, 1999, 2013, Tiébré et al. 2007b, Bailey et al. 2009, Krebs 2009, Gammon et al. 2010, Saad et al. 2011, Strgulc Krajšek & Dolenc Koce 2015). In plants of *F.* sect. *Reynoutria* established in Europe, euploid cytotypes are mainly recorded, namely diploid ($2n = 2x = 22$), tetraploid ($2n = 4x = 44$), hexaploid ($2n = 6x = 66$) and octoploid ($2n = 8x = 88$; Bailey & Stace 1992, Pashley 2003, Suda et al. 2010, Park et al. 2018), whereas two or more cytotypes occur within most taxa. The cytotype frequency in *F.* sect. *Reynoutria* taxa differs not only between native and invaded ranges but also among countries on particular continents (Asia, Europe, North America, Australasia; Table 1, Supplementary Tables S1 and S2).

In Europe, only one ploidy level is reported for *F.* ×*moravica* and *F. multiflora* ($2n = 4x = 44$ and $2n = 2x = 22$, respectively). *Fallopia* ×*bohemica* is mostly hexa- and rarely octo- or decaploid ($2n = 10x = 110$). *Fallopia sachalinensis* is mostly tetra- and rarely hexa- or octoploid (Bailey 1989, Bailey & Stace 1992, Mandák et al. 2003, Bailey & Wisskirchen 2006, Bailey et al. 2007, Tiébré et al. 2007a, Suda et al. 2010, Duistermaat et al. 2012, Desjardins 2015, Bzdega et al. 2016, Holm et al. 2018; Table 1, Supplementary Tables S1 and S2). The cytological variation in other *F.* sect. *Reynoutria* taxa on the European continent is unclear. Apart from the doubtful tetraploid numbers reported for *F. japonica* from Slovakia (see below), *F. japonica* var. *japonica* was long considered to

Table 1. Chromosome numbers (2n) and ploidy levels (2x–12x) recorded for adult individuals (incl. artificial hybrids, excluding seeds and seedlings) of *Fallopia* sect. *Reynoutria* taxa in their native and invaded ranges. *Fallopia japonica* s.l. – cytological data for the country refer only to *F. japonica* incl. *F. compacta*; [] – chromosome number for the country is reported only for artificial hybrids or adult individuals grown from seeds collected in the field. Questionable (erroneous) chromosome counts (noted in Supplementary Table S1 in red) are not provided. Results referring to this study are in bold. For details, see Supplementary Table S1. [†]It is not clear whether the individuals with 2n = 44–67 reported by Tiébré et al. (2007a) and those with 2n = 44–66 reported by Saad et al. (2011) are identical.

Taxon	2n	Native range	Invaded range		
		East Asia	Europe	North America	Australasia
F. ×*bohemica*	44 and 4x	–	–	Canada, USA	–
	44–66, 44-67[†]	–	Belgium	–	–
	65	–	United Kingdom, **Czech Republic (this study)**	–	–
	66 and 6x	Japan	United Kingdom, Norway, Germany, Switzerland, Belgium, Netherlands, Poland, Czech Republic, Austria, Slovakia, Hungary, Slovenia, Croatia, Romania, Bulgaria	Canada, USA	Australia, New Zealand
	77 and 7x	–	–	USA	–
	80	–	Continental Europe	–	–
	88 and 8x	–	United Kingdom, France, Germany, Belgium, Poland?, Czech Republic	USA	–
	99 and 9x	–	–	USA	–
	100	–	[United Kingdom]	–	–
	107	–	**Hungary (this study)**	–	–
	108	–	[United Kingdom]	–	–
	110 and 10x	–	[United Kingdom]	USA	–
F. ciliinervis	unknown	–	–	–	–
F. compacta	44 and 4x	Japan	United Kingdom, Ireland, Germany, Belgium, Czech Republic	–	New Zealand
	88 and 8x	–	Netherlands?	–	–
F. forbesii	66 and 6x	Korea	–	–	–
	88 and 8x	Korea	–	–	–

Taxon	2n	Native range	Invaded range		
		East Asia	Europe	North America	Australasia
F. japonica	44 and 4x	Russia (Vladivostok), Japan, Korea (*F. japonica* s.l.)	–	USA	–
	~45	Japan	–	–	–
	52	China?	–	–	–
	55 and 5x	–	–	USA	–
	66 and 6x	Japan, Korea (*F. japonica* s.l.)	Netherlands, Poland?	USA	–
	~80	–	Germany	–	–
	88 and 8x	Japan, Korea (*F. japonica* s.l.), China	United Kingdom, Ireland, Norway, France, Germany, Switzerland, Belgium, Netherlands, Poland, Czech Republic, Austria, Slovakia, Hungary, Slovenia, Croatia, Romania	USA	Australia, New Zealand
	99 and 9x	–	–	USA	–
	110 and 10x	China	–	–	–
F. ×*moravica*	44 and 4x	Japan?	United Kingdom, Czech Republic	–	New Zealand
F. multiflora	22 and 2x	Japan	?	–	–
F. sachalinensis	44 and 4x	Japan, Russia (Sakhalin)	United Kingdom, Norway, Belgium, Germany, Poland, Czech Republic, Austria, Slovakia, Slovenia	Canada, USA	–
	66 and 6x	Russia, Japan?	Poland, Czech Republic, **Slovakia (this study)**	–	–
	88 and 8x	–	Netherlands, Czech Republic	–	–
	102	Korea	–	–	–
	132 and 12x	Korea	–	–	–

be exclusively octoploid in Europe. However, hexaploid individuals morphologically similar to this taxon have recently been reported from the Netherlands (Duistermaat et al. 2012) and Poland (Bzdega et al. 2016). Similarly, *F. compacta* has thus far been considered to be an exclusively tetraploid species in Europe; however, Duistermaat et al. (2012) report octoploid level for one morphologically similar individual from the Netherlands. Occasionally (and not always reliably identified), hexaploid hybrid individuals between *F. compacta* × *F. japonica* var. *japonica* are also reported from several European countries (Bailey 1989, 2003, Bailey & Stace 1992, Duistermaat et al. 2012). Other cytotypes and taxa confirmed to date for the native East-Asian and invaded North-American knotweed ranges, which could also be found in Europe, are listed in Table 1.

Within Europe, the cytological variation in *F.* sect. *Reynoutria* taxa is best explored in the United Kingdom (Bailey & Conolly 1985, Bailey 1989, Bailey et al. 1996, Pashley 2003 and others) and the Czech Republic (Mandák et al. 2003, Hodálová et al. 2022, Mereďa et al. 2022 and others) with data from more than 300 cytological samples for each country. Cytological data from dozens (25 to 184) of invasive knotweeds have been obtained from Belgium (mainly in Tiébré et al. 2007a), Germany (mainly in Krebs et al. 2010), Poland (Wcisło 1977, Bzdega et al. 2016, Mereďa et al. 2022), Norway (Holm et al. 2018), Slovakia (Mereďa et al. 2019a, 2022) and Slovenia (Strgulc Krajšek & Dolenc Koce 2015). In other European countries, cytological data are lacking, or at most only a few reports are available (for details see Supplementary Table S1).

Regarding the cytological variation in invasive knotweeds, the central-European regions of the Western Carpathians and Pannonia (Poland, Czech Republic, Austria, Slovakia, Hungary) are relatively unexplored compared with the rest of the continent (Table 2). More detailed cytological data from this area were obtained from Slovakia (112 records; Marhold et al. 2007, Mereďa et al. 2019a, 2022) and the Czech Republic (34 records; Mandák et al. 2003). In addition, only sporadic chromosome counts are published for the Polish part of the Western Carpathians (three records; Wcisło 1977).

Three *F.* sect. *Reynoutria* taxa are reported from the Western Carpathians and Pannonia, namely *F. japonica* var. *japonica* (octoploid), *F. sachalinensis* (tetraploid) and their hybrid *F.* ×*bohemica* (hexaploid) (Mandák et al. 2003, 2004, Balogh 2008, Fischer et al. 2008, Zając et al. 2011, Šípošová 2016, Mandák & Pyšek 2019; Table 2, Supplementary Table S1). Within *F. japonica*, in addition to octoploid individuals (which are common in *F. japonica* var. *japonica* throughout Europe), the occurrence of tetraploid plants with 2n = 44 is repeatedly reported from the Western Carpathians (Ľubochňa village and Bratislava city; Feráková & Schwarzová 1974, Májovský et al. 1974, 1987). Nevertheless, these counts are questioned by Mandák et al. (2003) and Marhold et al. (2007).

The main aim of the present study was to explore the cytological (chromosome number, ploidy level and relative genome size) variation in *F.* sect. *Reynoutria* taxa in the Western Carpathians and adjacent part of Pannonia. The following questions were addressed: (i) Which *F.* sect. *Reynoutria* taxa/cytotypes occur in the area studied? (ii) What is the spatial distribution and frequency of these taxa/cytotypes in the area studied? (iii) Is relative genome size of *F.* sect. *Reynoutria* taxon-specific?

Table 2. Previously published chromosome counts and ploidy levels (Cytology) of taxa of *Fallopia* sect. *Reynoutria* for the area studied. N – number of analysed individuals; [] – erroneous chromosome numbers (for details, see Discussion). †Mereďa et al. (2022) analysed the same individuals as in this study; however, instead of relative genome size, only absolute genome size values were evaluated.

Taxon	Cytology	N	Country	Reference
F. ×bohemica	2n = [44]	1	Slovakia	Váchová & Feráková (1986: 60)
	2n = 6x ~ 66	13	Czech Republic	Mandák et al. (2003: Suppl.)
	2n = 66	1	Slovakia	Mráz (2006: 118)
	2n = 6x ~ 66	1	Slovakia	Urfus & Vít (2007: 259)
	2n = 6x ~ 66	23	Slovakia	Mereďa et al. (2019a: 218–220)
	2n = 6x ~ 66	30	Slovakia	Mereďa et al. (2022)†
F. japonica var. *japonica*	2n = 88	1	Poland	Wcisło (1977: 162–163)
	2n = [44]	1	Slovakia	Feráková & Schwarzová (1974: 42)
	2n = [44]	1	Slovakia	Májovský et al. (1974: 15)
	2n = [44]	1	Slovakia	Májovský et al. (1987: 107)
	2n = 8x ~ 88	17	Czech Republic	Mandák et al. (2003: Suppl.)
	2n = 8x ~ 88	18	Slovakia	Mereďa et al. (2019a: 218–220)
	2n = 8x ~ 88	21	Slovakia	Mereďa et al. (2022)†
F. sachalinensis	2n = 44	2	Poland	Wcisło (1977: 163)
	2n = 44	1	Slovakia	Májovský & Váchová (1986: 65)
	2n = 4x ~ 44	4	Czech Republic	Mandák et al. (2003: Suppl.)
	2n = 44	1	Slovakia	Feráková (2007: 258)
	2n = 4x ~ 44	2	Slovakia	Mereďa et al. (2019a: 218–220)
	2n = 4x ~ 44	3	Slovakia	Mereďa et al. (2022)†

Materials and methods

Plant material

Established (adult) plants of *F.* sect. *Reynoutria* were collected in 2016–2022 throughout the Western Carpathians and adjacent part of Pannonia (Fig. 1, Supplementary Table S3). Plants were determined based on morphological traits reported by Fojcik & Tokarska-Guzik (2000), Bailey & Wisskirchen (2006), Mandák & Pyšek (2019), Mereďa et al. (2019a) and Hodálová et al. (2022). Material from one to 10 individuals per locality was obtained, depending on the size and morphological variation of individuals. When two or more taxa occurred at one locality, the individuals were divided into separate populations based on their identity. In the current study, an individual (as well as a stand) is defined as a compact colony of knotweed shoots in one locality with at least a 3 m distance from other such colonies. In the analyses, each individual (stand) was typically represented by one shoot.

Details on the origin of the material included in this study are given in Supplementary Table S3. Voucher specimens are deposited in the herbarium SAV.

Chromosome counts

Chromosome numbers were counted in root tip meristems of 12 plants (*F. japonica* var. *japonica*, one individual; *F. sachalinensis*, five individuals from five populations; *F. ×bohemica*, six individuals from five populations; Supplementary Table S3). Six plants (*F. sachalinensis* no. 239S-2, 860-1 and 972, *F. ×bohemica* no. 70B and 861, *F. japonica* var. *japonica* no. 70J-1) were selected randomly for chromosome counting,

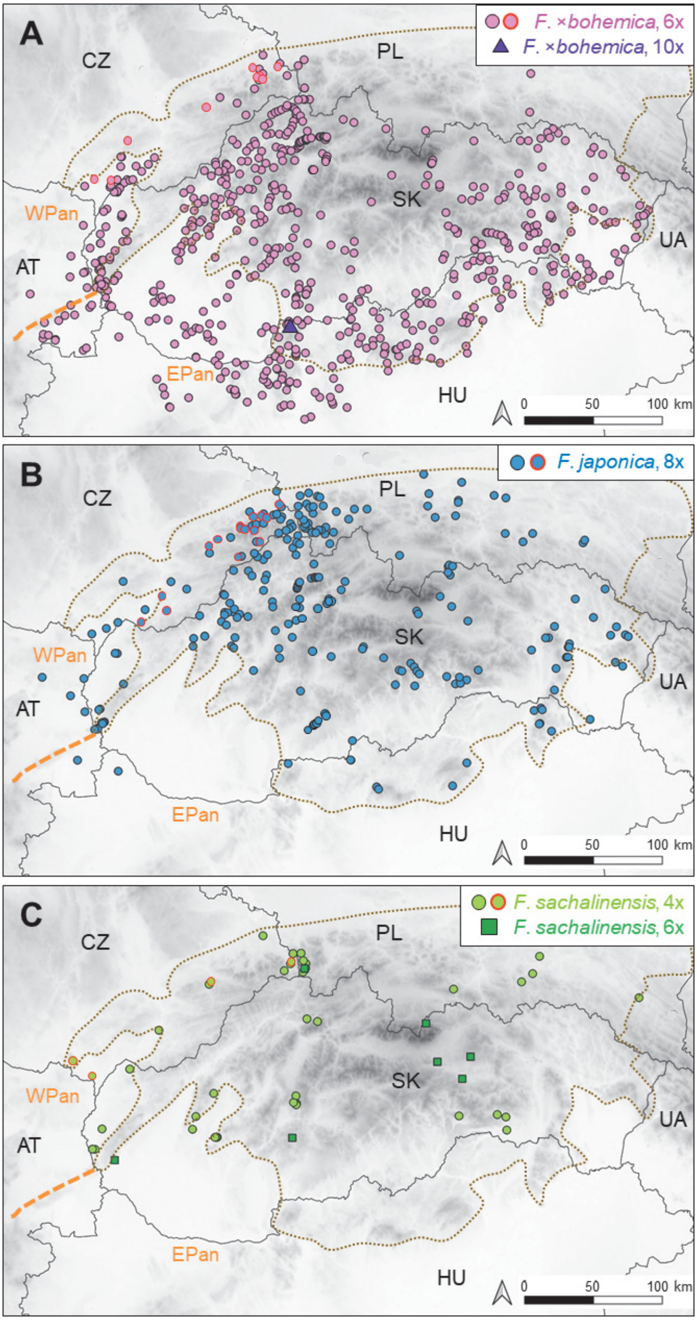

Fig. 1. Distribution of the 1,003 *Fallopia* sect. *Reynoutria* populations used in this study (black circles: 43 populations from Mereďa et al. 2019a) and 34 populations from Mandák et al. (2003; red circles). (A) *F.* ×*bohemica*, 2n = 6x ~ 66 (incl. 2n = 65) and 2n = 10x ~ 110 (namely, 2n = 107), (B) *F. japonica* var. *japonica*, 2n = 8x ~ 88, (C) *F. sachalinensis*, 2n = 4x ~ 44 and 2n = 6x ~ 66. Yellowish brown dotted line – borders of the Western Carpathians; orange dashed line – borders between the north-western (WPan) and north-eastern (EPan) parts of Pannonia.

and six individuals were selected, based on the extreme values of their relative genome size (*F. sachalinensis* no. 797 and 799, *F. ×bohemica* no. 326, 444B, 503-1 and 503-1).

Rhizomes were collected in the field and then either planted in a greenhouse at the Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences (PSBC SAS), Bratislava, or left for 5–14 days at a temperature of 22 °C in a plastic bag with sufficient moisture for rooting (Supplementary Fig. S1A).

The root tips were pretreated in a 0.002 M aqueous solution of 8-hydroxyquinoline at 4 °C for approximately 16 h (overnight), fixed in a 1:3 mixture of 98% acetic acid and 96% ethanol for 1–24 h, washed in distilled water, macerated in 1N HCl at 60 °C for 9 min and washed in distilled water again. Tip squashes were made using the cellophane square technique (Murín 1960). Permanent slides were stained with a 7% solution of Giemsa Stain – Modified Solution (Fluka Analytical) in Sörensen phosphate buffer, dried and examined in a drop of immersion oil using a Leica DM 1000 microscope equipped with an HDCE-X5 camera. ScopeImage 9.0 software was used for taking pictures of chromosomes.

Relative genome size

The relative genome sizes (hereafter RGS) of 1,106 individuals from 960 populations of *F.* sect. *Reynoutria* were newly analysed. These data were combined with flow cytometry (hereafter FCM) accessions of 53 individuals (from 43 populations) collected in the Krivánska Malá Fatra Mts (Slovakia) and reported in a previous study (Mereďa et al. 2019a). In total, information on the RGS and ploidy level of 1,159 *Fallopia* individuals from 1,003 populations (70 individuals from 66 populations from Poland, 63/59 from Czechia, 28/27 from Austria, 825/680 from Slovakia and 173/171 from Hungary) were included in the analyses (Table 3). FCM accessions published from the study area in Mereďa et al. (2022) were not included, as they are based on the same individuals as used in this study. However, instead of RGS, absolute genome size values were published for these individuals.

The RGS was estimated by FCM at the Institute of Botany PSBC SAS in Bratislava. The relationship between RGS and ploidy level was established using reference plants with known chromosome counts (see Results). In the absence of chromosome counts, all the data refer to the 'DNA ploidy level' in the sense of Suda et al. (2006). The symbol "~" indicates "approximately" and is used when the referenced ploidy level corresponds not only to the euploid chromosome number but also to the derived aneuploid number(s) of chromosomes.

Fresh leaves were preferably used in the analyses. If fresh leaves were no longer present on the plant at the end of the growing season (from October to December), a portion of the underground rhizome was obtained from the soil, washed, and stored in a plastic bag with sufficient moisture for 5–14 days in the dark at room temperature until young roots and shoots formed (Supplementary Fig. S1A). Young roots were subsequently used in FCM analyses instead of leaves. Young shoots were not used in FCM analyses as they released extremely mucilaginous compounds into the nuclear suspension due to which the RGS values of young shoots significantly differed from leaves and young roots (Supplementary Figs S1B and S1C). Fresh material (leaves or young roots) was favoured over silica-gel-dried samples because FCM using fresh material provides more accurate results than those obtained using desiccated tissues (Mereďa et al. 2019b).

Table 3. Number of individuals (stands) and populations of *Fallopia* taxa used in this study. Geographical regions: EPan – north-eastern part of Pannonia, WCarp – Western Carpathians, WPan – north-western part of Pannonia (Vienna Basin). For details, see Supplementary Table S3. Total figures are in bold. [†]Including data from Mereďa et al. (2019a). [‡]Number of individuals analysed in Mandák et al. (2003).

Taxon, cytotype	Number of individuals (stands)/populations analysed in the different countries and geographical regions													
	Poland	Czech Republic		Austria		Slovakia			Hungary		**Total**			
	WCarp	WCarp	WPan	WPan	EPan	WCarp[†]	WPan	EPan	WCarp	EPan	**WCarp**[†]	**WPan**	**EPan**	**All**
F. ×*bohemica*, 2n = 6x ~ 66 (incl. 2n = 65)	11/8	12/11 (+11[‡])	15/14 (+2[‡])	9/9	13/12	494/386	25/24	72/70	94/93	64/63	611/498 (+11[‡])	49/47 (+2[‡])	149/145	809/690 (+13[‡])
F. ×*bohemica*, 2n = 10x ~ 110 (2n = 107)	0	0	0	0	0	0	0	0	1/1	0	1/1	0	0	1/1
F. japonica var. *japonica*, 2n = 8x ~ 88	46/46	24/24 (+16[‡])	4/4 (+1[‡])	5/5	1/1	198/167	3/3	2/2	13/13	1/1	281/250 (+16[‡])	12/12 (+1[‡])	4/4	297/266 (+17[‡])
F. sachalinensis, 2n = 4x ~ 44	10/10	8/6 (+3[‡])	0 (+1[‡])	0	0	18/15	6/6	1/1	0	0	36/31 (+3[‡])	6/6 (+1[‡])	1/1	43/38 (+4[‡])
F. sachalinensis, 2n = 6x ~ 66	3/2	0	0	0	0	5/5	0	1/1	0	0	8/7	0	1/1	9/8
		44/41 (+30[‡])	19/18 (+4[‡])	14/14	14/13	715/573	34/33	76/74	108/107	65/64	**937/787 (+30**[‡]**)**	**67/65** (4[‡])	**155/151**	**1,159/1,003** (+34[‡])
Total	**70/66**	**63/59 (+34**[‡]**)**		**28/27**		**825/680**			**173/171**					

The sample preparation and FCM procedure followed DAPI staining given in Mereďa et al. (2019a). As an internal reference standard, *Bellis perennis* L. (2C = 3.38 pg; Schönswetter et al. 2007) was used in all analyses. The RGS (relative 2C value) of each sample was calculated as the mean of the G0/G1 peak of the fluorescence intensity of the sample divided by the mean of the G0/G1 peak of the fluorescence intensity of the standard. The RGS is given in arbitrary units (a.u.) relative to the standard used.

To ensure the accuracy of RGS estimations, each plant was analysed separately and only those histograms in which both (*Fallopia* and standard) G0/G1 peaks were symmetrical and similar in height with a coefficient of variation (CV) less than 3.5% were considered. Each plant was measured at least once; 26 individuals, however, were measured twice or thrice (on different days) to ensure high reliability of the results. In the case of repetitions, the average values of RGS were considered in subsequent analyses.

Results

Chromosome numbers

Two *Fallopia* individuals were found to be tetraploid (2n = 4x = 44), seven were hexaploid (2n = 6x = 66), one was octoploid (2n = 8x = 88) and aneuploid numbers of 2n = 65 and 2n = 107 were detected in two individuals (Table 4, Supplementary Table S3). Tetraploid counts of 2n = 44 were recorded for *F. sachalinensis* individuals no. 860-1 and 972 (Supplementary Fig. S2A) from Slovakia. Hexaploid counts of 2n = 66 were recorded for three *F. sachalinensis* individuals, including no. 239S-2 from Poland (Supplementary Fig. S2B) and nos. 797 and 799 from Slovakia, and for four *F. ×bohemica* individuals, including no. 70B (Supplementary Fig. S2C) and 861 from Slovakia and no. 503-1 (Supplementary Fig. S3A) and 503-2 (Supplementary Fig. S3B) from the Czech Republic. An octoploid count of 2n = 88 was recorded for *F. japonica* var. *japonica* individual no. 70J-1 from Slovakia (Supplementary Fig. S2D).

Aneuploid chromosome numbers were recorded for two *F. ×bohemica* individuals: 2n = 65 in individual no. 326 from the Czech Republic (Fig. 2A) and 2n = 107 in individual no. 444B from Hungary (Fig. 2B).

Relative genome size

DAPI staining (after excluding analyses of inadequate quality) yielded high-resolution histograms with little background noise and low CVs (Supplementary Fig. S4). The mean CV of *Fallopia* samples was 2.09% and that of the standard was 2.53%. The between-day RGS variation of repeatedly analysed *Fallopia* individuals was very small and ranged from 0 to 2.0% (0.5% on average).

FCM runs of all 1,159 samples (from 1,003 populations) revealed five non-overlapping groups of fluorescence intensities (Fig. 3): (A) 43 samples (from 38 populations) with an RGS between 1.141 and 1.166 corresponded to tetraploid (2n = 4x ~ 44) *F. sachalinensis*, (B) nine samples (from eight populations) with an RGS between 1.720 and 1.751 corresponded to hexaploid (2n = 6x ~ 66) *F. sachalinensis*, (C) 809 samples (from 690 populations) with an RGS between 1.826 and 1.972 corresponded to hexaploid (2n = 6x ~ 66) *F. ×bohemica*, (D) 297 samples (from 266 populations) with an RGS between 2.545 and 2.629 corresponded to octoploid (2n = 8x ~ 88) *F. japonica* var. *japonica* and (E) one

Table 4. Chromosome counts of *Fallopia* taxa recorded in this study. Ind. no. – population and individual number. For details, see Supplementary Table S3.

Taxon	Chromosome count (photo)	Country	Ind. no.
F. ×bohemica	2n = 65 (Fig. 2A)	Czech Republic	326
	2n = 66 (Supplementary Fig. S2C)	Slovakia	70B
	2n = 66 (Supplementary Fig. S3A)	Czech Republic	503-1
	2n = 66 (Supplementary Fig. S3B)	Czech Republic	503-2
	2n = 66	Slovakia	861
	2n = 107 (Fig. 2B)	Hungary	444B
F. japonica var. *japonica*	2n = 88 (Supplementary Fig. S2D)	Slovakia	70J-1
F. sachalinensis	2n = 44	Slovakia	860-1
	2n = 44 (Supplementary Fig. S2A)	Slovakia	972
	2n = 66 (Supplementary Fig. S2B)	Poland	239S-2
	2n = 66	Slovakia	797
	2n = 66	Slovakia	799

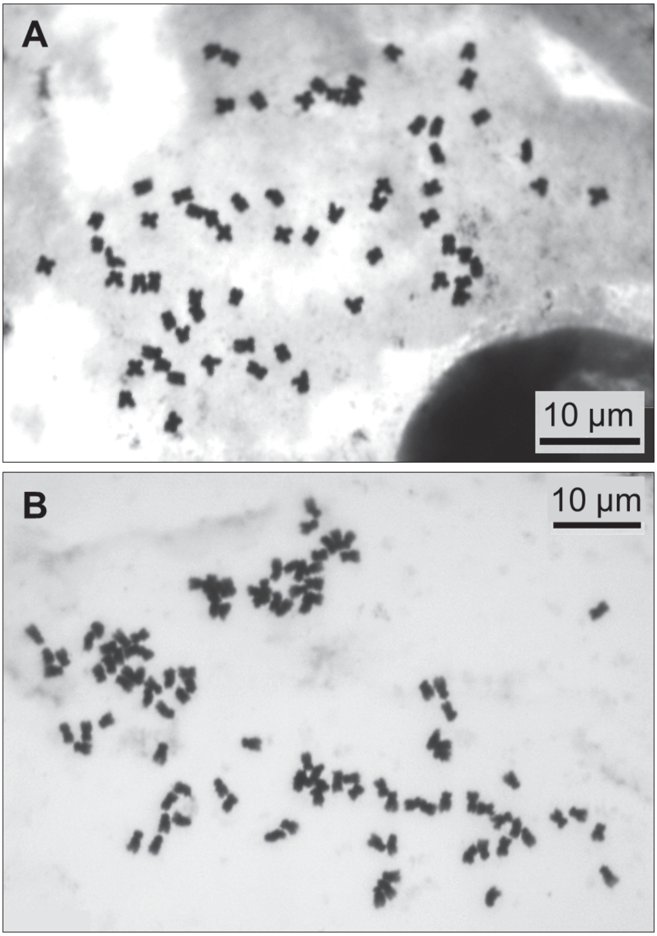

Fig. 2. Mitotic chromosomes of aneuploid plants of *Fallopia* sect. *Reynoutria*. (A) *F. ×bohemica* no. 326, 2n = 65, (B) *F. ×bohemica* no. 444B, 2n = 107. The population numbers correspond to those cited in Supplementary Table S3. Photographed by L. Mártonfiová.

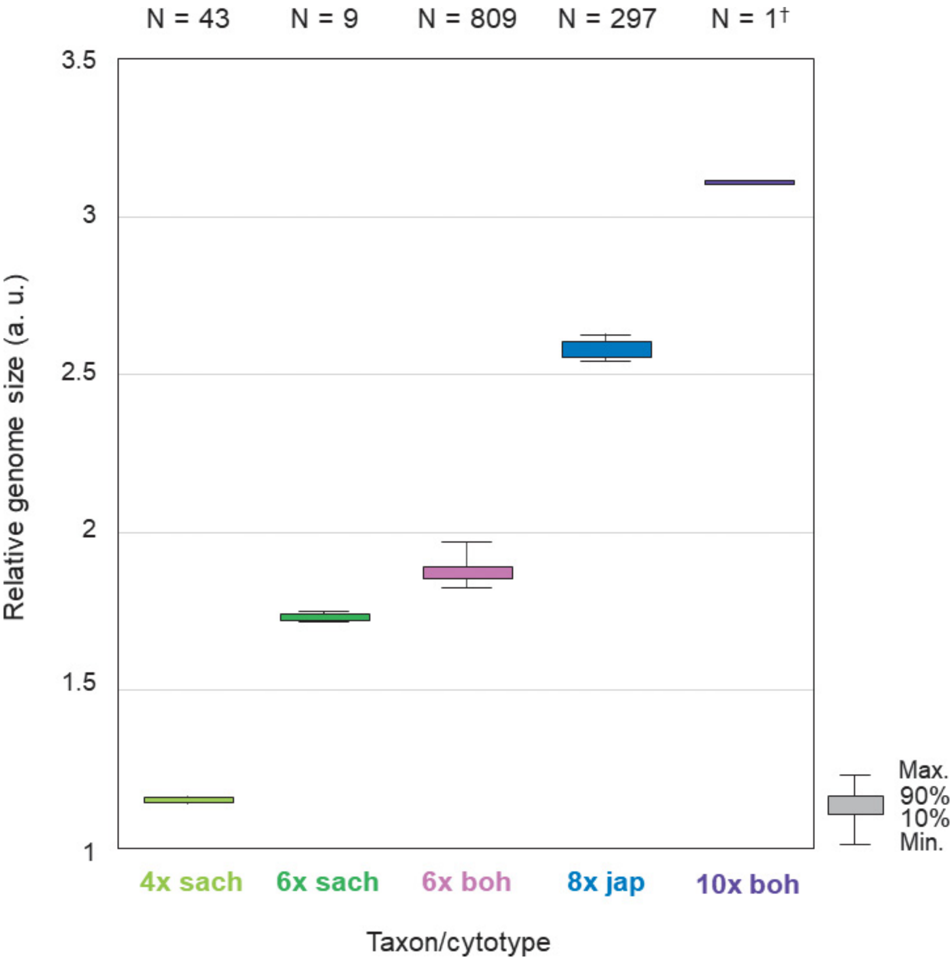

Fig. 3. Variation in the 2C relative genome sizes (RGS, in arbitrary units, a.u.; DAPI FCM, reference standard: *Bellis perennis*) of plants of *Fallopia* sect. *Reynoutria* from the area studied. Taxa/cytotypes are arranged according to their mean RGS. *N* – number of individuals analysed. 4x sach – *F. sachalinensis*, 2n = 4x ~ 44; 6x sach – *F. sachalinensis*, 2n = 6x ~ 66; 6x boh – *F.* ×*bohemica*, 2n = 6x ~ 66 (incl. 2n = 65); 8x jap – *F. japonica*, 2n = 8x ~ 88; 10x boh – *F.* ×*bohemica*, 2n = 10x ~ 110 (2n = 107; † one individual was measured on three different days).

sample (repeatedly measured on three different days) with an RGS between 3.101 and 3.112 corresponded to decaploid (2n = 10x ~ 110) *F.* ×*bohemica*. Table 5 presents RGS values for individual taxa and cytotypes. The numbers of taxa and cytotypes in particular countries and geographical regions within the area studied are presented in Table 3 and Fig. 4.

The RGS of 10 euploid individuals with counted chromosomes is marked in Supplementary Figs S5 and S6. The RGS of *F.* ×*bohemica* individual no. 326 with an aneuploid chromosome number of 2n = 65 was 1.828 a.u. and is within the variation of the RGS values for hexaploid *F.* ×*bohemica* individuals (Supplementary Fig. S5A). The *F.* ×*bohemica* plant no. 444B with an aneuploid chromosome number of 2n = 107 has an average RGS of 3.106 a.u. and represents the decaploid level (Fig. 3).

Table 5. 2C relative genome sizes (in arbitrary units, a.u.; DAPI FCM, reference standard: *Bellis perennis*) of adult plants of *Fallopia* sect. *Reynoutria* from the area studied (arranged according to their mean 2C RGS). N_I/N_P – number of individuals/populations analysed; var. – intraspecific variation (calculated as maximum×100/minimum). † Values refer to one individual, which was measured on three different days.

Taxon (DNA ploidy level and/or chromosome number)	N_I/N_P	2C relative genome size (a.u.)	
		mean ± S.D. (min.–)10%–90%(–max.)	var.
F. sachalinensis (2n = 4*x* ~ 44)	43/38	1.155 ± 0.006 (1.141–)1.147–1.163(–1.166)	2.2%
F. sachalinensis (2n = 6*x* ~ 66)	9/8	1.731 ± 0.009 (1.720–)1.722–1.743(–1.748)	1.6%
F. ×*bohemica* (2n = 6*x* = 65)	1/1	1.828	–
F. ×*bohemica* (2n = 6*x* ~ 66)	808/689	1.874 ± 0.014 (1.826–)1.858–1.890(–1.972)	8.0%
F. japonica var. *japonica* (2n = 8*x* ~ 88)	297/266	2.579 ± 0.018 (2.545–)2.556–2.604(–2.629)	3.3%
F. ×*bohemica* (2n = 10*x* = 107)	1/1†	3.106 ± 0.005 (3.101–)3.102–3.111(–3.112)	0.4%

The intraspecific RGS variation was relatively small in hexaploid *F. sachalinensis* (1.6%), tetraploid *F. sachalinensis* (2.2%) and octoploid *F. japonica* var. *japonica* (3.3%; Table 5). However, intraspecific RGS variation was much higher in hexaploid *F.* ×*bohemica* (8.0%), which was mainly caused by the extremely large values of two individuals from the same population, namely, no. 503-1 and 503-2 with RGS values of 1.949 and 1.972 a.u., respectively (Supplementary Fig. S6A). These outlying RGS values were also obtained in repeated analyses of the material. Surprisingly, chromosome counting showed that these plants were euploid with 2n = 66 (Supplementary Fig. S3A, B). If these two RGS values are not taken into account, then the intraspecific RGS variation of hexaploid *F.* ×*bohemica* was considerably smaller (5.3%).

Given that a chromosome number of 2n = 44 for *F. japonica* was reported (see Introduction) in Ľubochňa village and Železná studnička valley in Bratislava city (both Slovakia), all *F.* sect. *Reynoutria* stands occurring in these areas were analysed. Hundred-seventeen knotweed individuals from Ľubochňa village (altogether from 37 populations) were analysed; 23 of these samples (from eight populations) were octoploid *F. japonica* var. *japonica*, and 94 individuals (from 29 populations) were hexaploid *F.* ×*bohemica* (Supplementary Fig. S7). Sixteen knotweed individuals from Bratislava city, Železná studnička valley were analysed: 14 of these samples (14 populations) were octoploid *F. japonica* var. *japonica* and two individuals (two populations) were hexaploid *F.* ×*bohemica* (Supplementary Fig. S8).

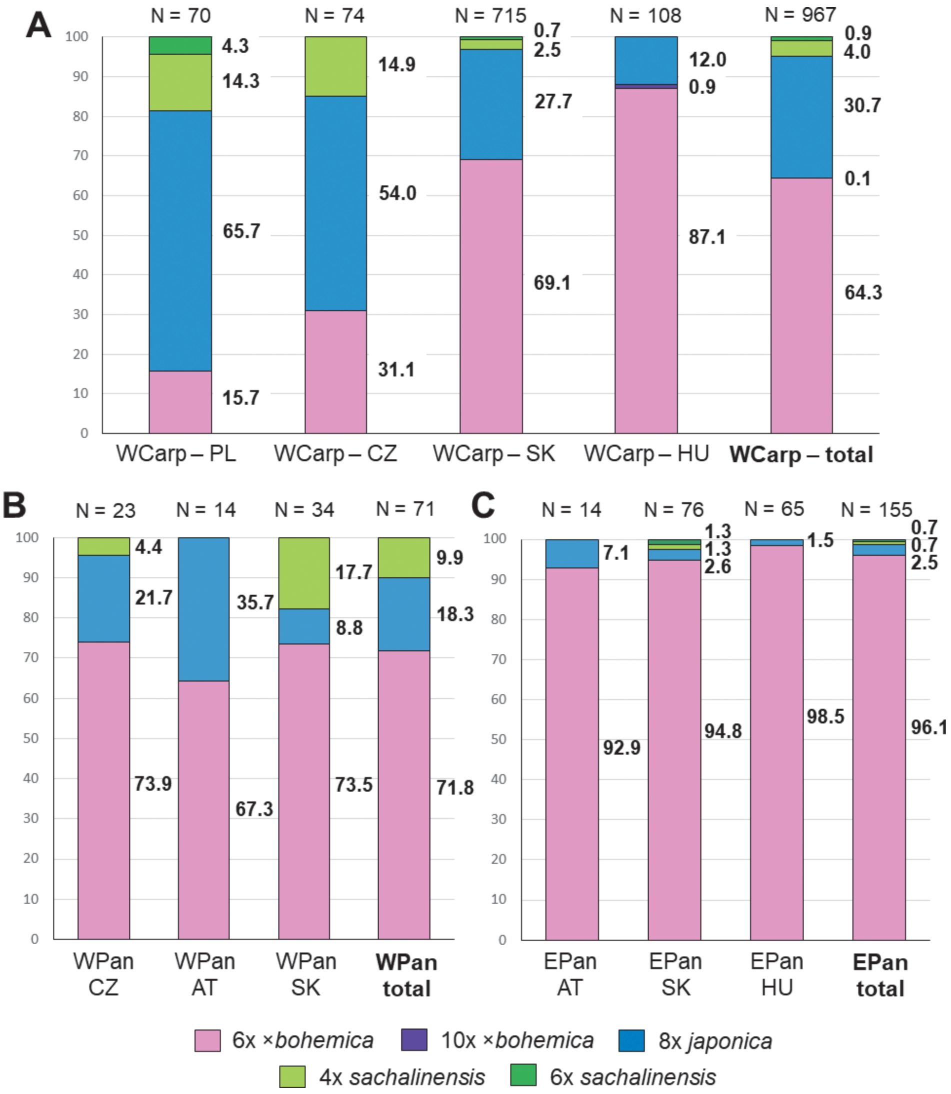

Fig. 4. Relative frequencies of the taxa and cytotypes of *Fallopia* sect. *Reynoutria* in selected regions of the area studied based on data presented in this study (1,159 individuals) and Mandák et al. (2003; 34 individuals) (see Fig. 1). (A) Western Carpathians (WCarp), (B) north-western part of Pannonia (WPan), (C) north-eastern part of Pannonia (EPan). N – number of individuals analysed for particular countries (AT – Austria, CZ – Czech Republic, HU – Hungary, PL – Poland, SK –Slovakia). Numbers on the graph indicate the percentage of individuals analysed.

Discussion

Diversity of Fallopia sect. Reynoutria taxa and cytotypes in the area studied

Data presented in this study (1,159 individuals from 1,003 populations, incl. 53 individuals and 43 populations from Mereďa et al. 2019a) represent the most detailed cytological screening of adult *F.* sect. *Reynoutria* plants in the world and are, in the number of individuals, comparable to the sum of all previous cytological data on invasive knotweeds from Europe (~1,200 individuals from 18 countries, see Supplementary Table S1). Within Europe, established plants of *F.* sect. *Reynoutria* have thus far been cytologically studied in detail only in Great Britain (more than 300 analysed individuals; mainly in Bailey & Conolly 1985, Bailey 1989, Pashley 2003) and the Czech Republic (more than 300 individuals; Mandák et al. 2003, Hodálová et al. 2022, Mereďa et al. 2022). Countries like Poland (184 individuals; Wcisło 1977, Bzdega et al. 2016, Mereďa et al. 2022), Germany (~130 individuals; mainly in Krebs et al. 2010), Norway (124 individuals; Holm et al. 2018), Belgium (~80 individuals; mainly in Tiébré et al. 2007a) and Slovenia (25 individuals; Strgulc Krajšek & Dolenc Koce 2015) are also fairly well explored. For other European countries, there are no cytological data for *F.* sect. *Reynoutria* taxa (e.g., from Spain, Denmark, Italy, Ukraine), or at most only a few reports (e.g. from Ireland, France, Switzerland, Netherlands; Table 1, Supplementary Tables S1 and S2).

In the area studied, three taxa and five cytotypes were revealed among established *F.* sect. *Reynoutria* plants: hexa- ($2n = 65$ and 66) and decaploid ($2n = 107$) *F.* ×*bohemica* (809 and one individuals, respectively), octoploid ($2n = 88$) *F. japonica* var. *japonica* (297 individuals) and tetra- ($2n = 44$) and hexaploid ($2n = 66$) *F. sachalinensis* (43 and nine individuals, respectively).

For the first time, (i) aneuploid chromosome number of $2n = 107$ was recorded in adult invasive knotweed, (ii) aneuploid chromosome number of $2n = 65$ in adult invasive knotweed in continental Europe (both counts in *F.* ×*bohemica*; for details see below) and (iii) hexaploid ($2n = 6x \sim 66$) cytotype of *F. sachalinensis* in the Western Carpathians and Pannonia (as well as in Slovakia). Our data is also the first cytological data for *F.* sect. *Reynoutria* from the Pannonian area in Austria and the Western Carpathian area in Hungary (in both countries, we revealed hexaploid *F.* ×*bohemica* and octoploid *F. japonica* var. *japonica*) and for *F.* ×*bohemica* from the western Carpathian area in Poland. This also significantly increased our knowledge of the cytological variation of invasive knotweeds in Slovakia (112 previous records, Marhold et al. 2007, Mereďa et al. 2019a, 2022, and 772 new records, this study) (Table 2, Supplementary Table S1).

One of the significant results of this study is the detection of hexaploid ($2n = 6x \sim 66$) *F. sachalinensis* plants in the area studied. In Europe, this cytotype was only previously recorded in the Czech Republic (three individuals from Bohemia; Mandák et al. 2003) and Poland (26 individuals from southern Poland; Bzdega et al. 2016). Outside Europe, this cytotype is recorded only once in Russia (Bolkhovskikh et al. 1969; Table 1, Supplementary Table S1). Within the Western Carpathians, this cytotype was recorded in four regions (Fig. 1C), including (i) the south-western area in the Polish Western Carpathians (Istebna town), (ii) western Slovakia (Bratislava city), (iii) central Slovakia (Banská Štiavnica town) and (iv) north-eastern Slovakia (Spiš region), where *F. sachalinensis* is represented exclusively by this cytotype. In contrast, the tetraploid cytotype, which is more common in Europe, does not occur in north-eastern Slovakia.

The data presented also revealed that the occurrence of *F.* ×*bohemica* is greatly underreported in the area studied because this hybrid is often confused with the parental species (especially *F. japonica* var. *japonica*, see Beringen et al. 2019, Mereďa et al. 2019a). This confusion is discussed by Mereďa et al. (2019a) in regard to Slovakia and by Balogh (2008) and Balogh et al. (2008) for Hungary. This is especially so in the countries studied, for example, in Gerecse Hills in northern Hungary. Barina (2006) reports 22 localities for *F. japonica* and one site for *F. sachalinensis* (reported as *Reynoutria japonica* and *R. sachalinensis*, respectively) in this area, but not *F.* ×*bohemica*. In contrast, in this study the first two taxa were not recorded there, but 17 localities for *F.* ×*bohemica* were (Fig. 1, Supplementary Table S3). Despite the fact that taxa of *F.* sect. *Reynoutria* can now be reliably distinguished from each other based on their morphology (see, for example, identification manuals in Fojcik & Tokarska-Guzik 2000, Bailey & Wisskirchen 2006, Mereďa et al. 2019a), *F.* ×*bohemica* continues to be overlooked in several European countries (see Beringen et al. 2019, Vuković et al. 2019, Stalažs 2022). On the other hand, due to uncertainties encountered when differentiating between members of *F.* sect. *Reynoutria*, these invasive taxa are still not distinguished from each other when mapping their occurrence in many countries, which is also the case for several previous studies carried out in the area studied (e.g. Bartha et al. 2015, Gašparovičová et al. 2022).

The cytological variation in members of *F.* sect. *Reynoutria* is considerably less in the Western Carpathians and adjacent part of Pannonia than in the neighbouring Hercynian area in the Czech Republic (which is the only one of the neighbouring central-European regions where the cytology of invasive knotweeds has been examined in detail; Mandák et al. 2003, Hodálová et al. 2022, Mereďa et al. 2022). In contrast to the Hercynian area in the Czech Republic, the following taxa and cytotypes are absent in the area studied: tetraploid *F. compacta*, tetraploid *F.* ×*moravica*, octoploid *F.* ×*bohemica* and octoploid *F. sachalinensis*. The absence of the tetraploid *F.* ×*moravica*, which is relatively common in the neighbouring region of central Moravia (Hodálová et al. 2022) and the octoploid *F.* ×*bohemica*, which does occur in the Czech Republic (Mandák et al. 2003, Mereďa et al. 2022) and also in several other European countries (United Kingdom, France, Belgium, Netherlands, Germany, Poland; Bailey 1989, Bailey & Wisskirchen 2006, Tiébré et al. 2007a, Saad et al. 2011, Bzdega et al. 2016; Table 1), is particularly surprising.

In the area studied, intersectional hybrids between members of *F.* sect. *Reynoutria* and other sections of *Fallopia* (for exact RGS values of all central-European *Fallopia* taxa see Hodálová et al. 2022) were not detected. Such hybrids are occasionally reported from several European countries (Bailey 2003, 2013, Desjardins 2015). *Fallopia* ×*conollyana* Bailey, a hybrid between *F. baldschuanica* (Regel) Holub [member of *F.* sect. *Sarmentosae* (I. Grintz.) Holub] and *F.* ×*bohemica*, with 2n = 54, is the most frequently mentioned intersectional *Fallopia* hybrid in Europe. This hybrid is reported in many European countries [the United Kingdom, Ireland, Norway, France, Belgium, Netherlands, Germany, Czech Republic (only as seeds) and Austria; Bailey 1988, 2001, 2013, Stace & Crawley 2015, Hoste et al. 2017, Beringen et al. 2019, Keil & Fuchs 2019]. In addition, in 2000, *F.* ×*conollyana* was also found in the southern foothills of the Hungarian part of the Western Carpathians (at Vácrátot village; Bailey 2001). As both *F. baldschuanica* and *F.* ×*bohemica* are widely distributed in the Western Carpathians and adjacent part of Pannonia (*F. baldschuanica* is mainly cultivated in gardens) and often grow in close proximity, the occurrence of their hybrid at other sites in the area studied can be expected.

Distribution of Fallopia sect. Reynoutria taxa in the area studied

The distribution of invasive knotweed taxa differs between the Western Carpathians and adjacent part of Pannonia (see below), and within the Western Carpathians and Pannonia. These differences are depicted in Fig. 4.

In the Western Carpathians, in their northern and western parts (Poland, Czech Republic), *F.* sect. *Reynoutria* is most often represented by *F. japonica* var. *japonica* (65.7% and 54%, respectively) and *F. sachalinensis* is also present at a relatively high level in this area (18.6% and 14.9%, respectively; Fig. 4A). In the southern and eastern parts of this mountain range (Hungary), the presence of *F. japonica* var. *japonica* is much lower (12%) and *F. sachalinensis* is absent. In contrast, *F.* ×*bohemica* clearly dominates there (88%). In other words, in the northern and western parts of the Western Carpathians, the ratio of *F. japonica* var. *japonica*, *F. sachalinensis* and *F.* ×*bohemica* is approximately 3:1:1. In the southern and eastern areas, this ratio is approximately 1:0:7 (Fig. 4A).

In the north-western area of Pannonia (Vienna Basin), which has a more oceanic climate than the more north-eastern area of Pannonia (Bochníček et al. 2015: 119, Botti 2018), the average relative frequencies of *F.* sect. *Reynoutria* taxa (71.8% of *F.* ×*bohemica*, 18.3% of *F. japonica* var. *japonica*, 9.9% of *F. sachalinensis*, Fig. 4B) are similar to those recorded in the Western Carpathians (64.4%, 30.7%, 4.9%; Fig. 4A). In contrast, in north-eastern Pannonia, *F.* ×*bohemica* clearly dominates (96.1%) and the occurrence of *F. japonica* var. *japonica* and *F. sachalinensis* is almost zero (2.5% and 1.4%, respectively; Fig. 4C). The phytogeographical similarity in the distribution of invasive knotweeds in the north-western part of Pannonia and Western Carpathians is also reported for several species of native plants, including *Lycopodium annotinum* L. (Futák 1966), *Pinguicula vulgaris* L. (Krippel & Šípošová 1997) and *Viola palustris* L. (Mereďa et al. 2008). In these examples, Carpathian species occur in the north-western part of Pannonia (namely, in the Záhorská nížina Lowland) and are mostly absent in north-eastern Pannonia. This pattern mirrors the more humid and colder climate of the north-western Pannonia compared to the north-eastern Pannonia (Bochníček et al. 2015: 119, Botti 2018).

The absence of *F. sachalinensis* in the parts of Hungary studied (both Western Carpathians and north-eastern Pannonia) is consistent with previous reports that *F. sachalinensis* is generally restricted to botanical gardens in Hungary (Balogh 2008). The relatively abundant occurrence of *F. sachalinensis* in the northern and western parts of the area studied (Poland, Czech Republic) with a relatively cold and wet climate, and its absence in the drier and warmer southern and eastern parts of the area (Hungary) is not surprising given that this species has the most northerly located native distribution of the *F.* sect. *Reynoutria* taxa in southern Sakhalin and southern Kuril Islands in Russia and northern and central Japan; the only exception is the highly polyploid (dodecaploid, 12x) *F. sachalinensis* cytotype with 2n = 132 native in southern Korean islands (Bailey 2003, Pashley 2003, Park et al. 2018, Beringen et al. 2019).

In general, the data presented in our paper show a clear north-west–south-east gradient in the distribution of the relative frequencies of *F.* sect. *Reynoutria* taxa in the area studied (Fig. 4). Within this gradient, the relative abundances of *F. japonica* var. *japonica* and *F. sachalinensis* decrease towards the south and east, whereas that of *F.* ×*bohemica* increases. It is assumed that *F.* ×*bohemica* is better adapted to drier and warmer climate, which together with its faster spread (Mandák et al. 2004) and higher invasiveness compared to the parental species (Parepa et al. 2014) accounts for the dominance of *F.* ×*bohemica* in north-eastern Pannonia.

Distribution of Fallopia sect. Reynoutria taxa in Europe

At a more global spatial scale on the European continent there is a similar north-west–south-east distribution gradient of invasive members of *F.* sect. *Reynoutria*. Bailey & Wisskirchen (2006) report that the ratio of *F. japonica* var. *japonica* relative to *F.* ×*bohemica* gradually decreases from Great Britain (where *F.* ×*bohemica* is outnumbered by *F. japonica* var. *japonica* by a factor of 100:1) through the Czech Republic to Hungary (where the hybrid is the major constituent). Within north-western Europe, *F. japonica* var. *japonica* prevails over *F.* ×*bohemica* in Norway (Holm et al. 2018), Belgium (Tiébré et al. 2007a) and the Netherlands (Beringen et al. 2019). The north-west–south-east gradient in the relative frequencies of *F. japonica* var. *japonica* and *F.* ×*bohemica* is also reported in Germany and Switzerland (Krebs et al. 2010) and is visible when data from the area studied (Western Carpathians and adjacent part of Pannonia) is compared with that from the Hercynian area of the Czech Republic (Mandák et al. 2003, 2004). In the Hercynian area in the Czech Republic, *F. japonica* var. *japonica* and *F.* ×*bohemica* occur in approximately the same percentages (36.8% vs. 35.4%, respectively) and a significant presence of *F. sachalinensis* is also recorded there (25.6%; Mandák et al. 2003). In the area studied, the relative frequencies of *F. japonica* var. *japonica*, *F.* ×*bohemica* and *F. sachalinensis* are 30.7%, 64.3% and 4.9% in the Western Carpathians, 18.3%, 71.8% and 9.9% in the north-western Pannonia, and 2.5%, 96.1% and 1.4% in the north-eastern Pannonia, respectively.

According to Bailey & Wisskirchen (2006), there are two different explanations for this distributional pattern: (i) the conditions in Pannonia are more conducive to the germination and establishment of *F.* ×*bohemica* seeds, or (ii) the hybrid rather than *F. japonica* var. *japonica* was initially introduced. Following Krebs et al. (2010), this gradient supports the suggestion that *F.* ×*bohemica* may be more prone to invade warmer areas located in sub-Mediterranean and Mediterranean regions than its parents and the niche of this hybrid is not only intermediate between its parents but includes other adaptations that enable it to extend its range. The data presented and the abovementioned observations of Bailey & Wisskirchen (2006) and Krebs et al. (2010) support the recent results of Jovanović et al. (2018) obtained by modelling the potential distribution of invasive knotweed species in south-eastern Europe. These authors report that the distribution of these taxa is climatically determined. As a consequence, the most suitable geographical areas for *F. japonica* var. *japonica* and *F. sachalinensis* are more northerly located than those of *F.* ×*bohemica*, and *F.* ×*bohemica* tolerates up to 60 mm less precipitation throughout the year than *F. japonica* var. *japonica* and *F. sachalinensis*.

Tetraploid cytotype of Fallopia japonica in Slovakia

In the past tetraploid (2n = 44) *F. japonica* plants were reported occurring at two Slovak localities: (i) Ľubochňa village in the Veľká Fatra Mts (Feráková & Schwarzová 1974 as *Reynoutria japonica*, and Májovský et al. 1974 as *Pleuropterus cuspidatus*) and (ii) Bratislava city, Železná studnička valley in the Malé Karpaty hills (Májovský et al. 1987 as *Reynoutria japonica*). In the past, two varieties of *F. japonica* were distinguished in

Europe: *F. japonica* var. *japonica* and *F. japonica* var. *compacta*. The first taxon, *F. japonica* var. *japonica*, in Europe, is exclusively octoploid and exceptionally hexaploid (see Introduction and Supplementary Table S1). The second taxon is currently recognized as a separate species, *F. compacta* (for more details see Hodálová et al. 2022). *Fallopia compacta* is predominantly (or exclusively) tetraploid in Europe (Supplementary Table S1) and has not been reported from the Western Carpathians or Slovakia (see Šípošová 2016). For these reasons, the 2n = 44 records for *F. japonica* from Slovakia were questioned by Mandák et al. (2003) and Marhold et al. (2007) and so far they have not been verified in the field. In the present study, all *F.* sect. *Reynoutria* stands occurring at the abovementioned localities were analysed, which revealed that none of them included tetraploids or individuals resembling *F. compacta*. In the localities where tetraploid *F. japonica* plants should occur, there were only octoploid (2n = 8x ~ 88) *F. japonica* var. *japonica* and hexaploid (2n = 6x ~ 66) *F.* ×*bohemica* (Supplementary Fig. S7 and Fig. S8). In addition, there are no herbarium specimens of these tetraploids in the SLO herbarium, where they should be stored. Thus, consistent with Mandák et al. (2003), the Slovakian data on tetraploid *F. japonica* is considered to be erroneous.

The utility of genome size for identifying knotweeds

The genus *Fallopia* is a taxonomically intricate polyploid complex of morphologically highly varied and often weakly differentiated taxa. Although these taxa are very dangerous invasive plants, their identification is often problematic due to their similar morphology (Kim & Park 2000, Mereďa et al. 2019a, Hodálová et al. 2022). In the last two decades, RGS has been shown to be a useful marker for identifying morphologically weakly differentiated plant complexes of different genera within both hetero- and homoploid taxa (for references see Mereďa et al. 2019b, Hodálová et al. 2020).

The utility of both absolute and relative genome size in the identification of *F.* sect. *Reynoutria* taxa and cytotypes is documented in detail by Suda et al. (2010) and is also used by several other authors (e.g. Tiébré et al. 2007a, Gammon et al. 2010, Krebs et al. 2010, Duistermaat et al. 2012, Bzdega et al. 2016, Mereďa et al. 2019a, 2022, Hodálová et al. 2022). However, in some of these studies, the basic information on the exact location and the number of individuals analysed, data on measured (absolute or relative) genome size values of idividual plants and cytotypes, the range of measured variation, etc. is often missing. The measurements presented here, based on five times more material than that reported by Suda et al. (2010) or in any other previous studies, confirmed the utility of RGS for identifying *F.* sect. *Reynoutria* taxa and cytotypes, as there is no overlap in the RGS values of the taxa and cytotypes studied (Fig. 3). This fact is especially important when identifying homoploid *Fallopia* taxa (e.g. tetraploid *F. compacta*, *F.* ×*moravica* and *F. sachalinensis*; hexaploid *F.* ×*bohemica* and *F. sachalinensis*; octoploid *F.* ×*bohemica*, *F. japonica* var. *japonica* and *F. sachalinensis*), which is also documented in recent papers (Hodálová et al. 2022, Mereďa et al. 2022).

Aneuploidy in Fallopia sect. Reynoutria

For the members of *F.* sect. *Reynoutria*, it is well-documented that there is a high percentage of aneuploid seeds and seedlings, which are rare in established plants in the wild (Bailey 1989, 1999, 2013, Tiébré et al. 2007b, Bailey et al. 2009, Krebs 2009, Saad et al.

2011, Strgulc Krajšek & Dolenc Koce 2015). For Europe, there are reports of chromosome numbers for more than 560 *F.* sect. *Reynoutria* adult plants and of this number only six (~1%) were aneuploid (with 2n = 44–67, 65, 80, ~80, 100 or 108). The remaining individuals were either euploid [~519 (93%) individuals, with 2n = 44, 66, 88 or 110] or the number of chromosomes could not be precisely determined [35 (~6%) individuals, with 2n = ~44, ~66, or ~88]; see Supplementary Table S1.

Currently, worldwide, aneuploid counts are reported for established plants of *F.* sect. *Reynoutria* mainly for hybrid *F.* ×*bohemica* (2n = 44–67, 65, 80, 100, 108, in plants from several European countries), but rarely for *F. japonica* (2n = ~45, 52 from East Asia) and *F. sachalinensis* (2n = 102 from Korea; Table 1, Supplementary Table S1).

In this research,12 individuals were selected for chromosome counting. Six of them, namely *F. sachalinensis* no. 239S-2, 860-1 and 972, *F.* ×*bohemica* no. 70B and 861, and *F. japonica* var. *japonica* no. 70J-1 (all with more or less average RGS values within the particular taxon) were selected randomly and six other individuals, namely *F. sachalinensis* no. 797 and 799, *F.* ×*bohemica* no. 326, 444B, 503-1 and 503-1, were selected based on their extreme RGS values (see Supplementary Figs S5 and S6). Aneuploidy was recorded in two plants of *F.* ×*bohemica*, both with high RGS values: no. 326 with 2n = 65 and no. 444B with 2n = 107 (Fig. 2).

The aneuploid individual, with 2n = 65, was recorded in the Czech Republic (Ostravice village). Currently, adult knotweed plants with this chromosome number have only been recorded once in the United Kingdom (also for *F.* ×*bohemica*; Pashley 2003; Table 1). A chromosome number of 2n = 107 has not yet been recorded for adult knotweed plants. The authors of previous studies (Bailey 1999, Pashley 2003, Bailey et al. 2007) assume that highly aneuploid individuals of *F.* ×*bohemica* at the decaploid level (2n = 10x ~110) arose when an unreduced gamete of hexaploid *F.* ×*bohemica* was pollinated by octoploid *F. japonica* var. *japonica*. It is assumed here that this is also the case for *F.* ×*bohemica* individual no. 444B, which has 107 chromosomes. This plant was growing in Hungary (Bernecebaráti village; Supplementary Fig. S9) approximately 20 m from the presumed maternal female octoploid *F. japonica* var. *japonica* (individual no. 444J). Only a few hundred metres from this aneuploid, hexaploid individuals of *F.* ×*bohemica* were recorded (one of them was analysed, individual no. 586), which probably served as the donor of unreduced pollen.

Both aneuploid individuals with 2n = 65 and 107 developed normally and flowered (both observed *F.* ×*bohemica* aneuploids were male-fertile). This finding confirms Bailey's previous observations that once established aneuploid plants of *F.* sect. *Reynoutria* (especially those of *F.* ×*bohemica*) do not experience difficulties surviving and forming normal plants (Bailey et al. 2007, 2009, see also Gammon et al. 2010).

Although aneuploids have so far been reported with certainty for only approximately 1% of established plants of *F.* sect. *Reynoutria* in Europe and also in the current study, their percentage was low (~0.2% of all plants studied), it cannot be ruled out that *F.* sect. *Reynoutria* aneuploid plants (mainly *F.* ×*bohemica*) may be more frequent, due to limitation of FCM for detecting them. The detection of variation in one or two chromosomes in plant groups such as *F.* sect. *Reynoutria* with higher number of chromosomes (44 and more) by using FCM is poor. In addition, the graphs in Supplementary Figs S5 and S6, demonstrate that extreme RGS values in *F.* sect. *Reynoutria* may or may not indicate aneuploid chromosome numbers. This is especially the case for the two *F.* ×*bohemica* individuals, no. 503-1 and 503-2, with extremely high RGS values. The cells of these plants were repeatedly counted, but there were definitely not more than 66 chromosomes (Supplementary Fig. S3A, B).

Differences in the diversity of Fallopia sect. Reynoutria in its native and invaded ranges

Although the *F.* sect. *Reynoutria* is a small plant group with approximately six species and two hybrids recognized (Desjardins 2015, Hodálová et al. 2022), large differences in taxa and cytotype diversity among countries and continents exist (Table 1). The research carried out in the Western Carpathians and adjacent part of Pannonia confirms that the cytological variation in *F.* sect. *Reynoutria* and its taxonomic representation in the invaded European range is rather different from that recorded in the original East-Asian or invaded North-American ranges. In Europe, the taxa *F. ciliinervis*, *F. forbesii*, *F. japonica* var. *hachidyoensis* (Makino) Yonekura et Ohashi and *F. japonica* var. *uzenensis* (Honda) Yonekura et Ohashi, known from East Asia, have never been recorded (Kim & Park 2000, Pashley 2003, Bailey et al. 2007, Balogh 2008, Desjardins 2015, Park et al. 2018). On the European continent the following cytotypes are also absent: tetraploid cytotypes (2n = 44, ~44, 45) of *F. japonica* var. *japonica* (reported from Japan, Korea and USA; Pashley 2003, Gammon et al. 2010, Park et al. 2018), decaploid (2n = 110) individuals of *F. japonica* var. *japonica* (reported from China; Pashley 2003), 2n = 102 plants of *F. sachalinensis* (reported from Korea; Bailey 2003, Pashley 2003) and dodecaploid (2n = 132) individuals of *F. sachalinensis* (occurring in the Korean Islands of Ullung and Dok; Pashley 2003, Park et al. 2018). Similarly, penta- (2n = 5x = 55), nonaploid (2n = 9x = 99) cytotypes of *F. japonica*, and tetra-, hepta- (2n = 7x = 77) and nonaploid cytotypes of *F.* ×*bohemica*, reported from North America (Gammon et al. 2010) also do not occur in Europe. However, several cytotypes that are absent in East Asia and North America occur in Europe (e.g. octo- and decaploid *F.* ×*bohemica*, hexa- and octoploid *F. sachalinensis*; Table 1, Supplementary Table S1).

The absence of several of the taxa and cytotypes of *F.* sect. *Reynoutria* in Europe that are present in East Asia indicates that although this plant group is widely distributed on the European continent, only a small part of the variation of this section has thus far been introduced to Europe from its native range (see also Bailey & Wisskirchen 2006). On the other hand, the absence in East Asia of some *F.* sect. *Reynoutria* cytotypes and taxa that occur in Europe and North America indicates that either the cytology or taxonomy (or both) of *F.* sect. *Reynoutria* is insufficiently known in East Asia, or that the knotweed cytotypes at a selective advantage in the invaded ranges differ from those in its native range.

Supplementary materials

Fig. S1. Washed part of the rhizome of *Fallopia* ×*bohemica* individual, and variation in relative fluorescence intensities of hexaploid *Fallopia* ×*bohemica* and octoploid *F. japonica* var. *japonica* individuals.

Fig. S2. Mitotic chromosomes of *Fallopia* species.

Fig. S3. Mitotic chromosomes of *Fallopia* ×*bohemica* individuals with extremely large RGS values.

Fig. S4. Histograms of fluorescence intensity in simultaneous analyses of DAPI-stained nuclei isolated from several *Fallopia* sect. *Reynoutria* individuals.

Fig. S5. Variation in relative fluorescence intensities of tetraploid and hexaploid *Fallopia sachalinensis* individuals.

Fig. S6. Variation in relative fluorescence intensities of hexaploid *Fallopia ×bohemica* individuals and octoploid *F. japonica* var. *japonica* individuals.

Fig. S7. Location of analysed *Fallopia* populations in Ľubochňa village in Veľká Fatra Mts.

Fig. S8. Location of analysed *Fallopia* individuals in Železná studnička valley and in the surroundings in Bratislava city in Malé Karpaty hills.

Fig. S9. *Fallopia ×bohemica* individual no. 444B with aneuploid chromosome number of 2n = 107 from Bernecebaráti village in Hungary.

Table S1. Chromosome numbers and ploidy levels found in adult individuals of *Fallopia* sect. *Reynoutria*.

Table S2. Ploidy level frequencies found in adult *Fallopia* sect. *Reynoutria* taxa.

Table S3. The studied *Fallopia* populations.

Supplementary materials are available at www.preslia.cz

Acknowledgements

This study was financially supported by the Grant Agency VEGA, Bratislava, Slovakia (grants no. 2/0024/19 and 2/0161/21) and by the Slovak Research and Development Agency (grant no. APVV-19-0134). For information on the knotweed occurrence and help with providing material, we thank S. Botlová, D. R. Letz, Z. Koláriková, M. Kosorínová, H. Kothajová, J. Májeková, H. Šípošová and E. Uherčíková.

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Cytogeografie invazních křídlatek (*Fallopia* sect. *Reynoutria*) ve střední Evropě: vzácní aneuploidní jedinci a důkaz klimaticky podmíněného rozšíření taxonů

Zástupci sekce *Fallopia* sect. *Reynoutria* (křídlatky) patří k nejinvaznějším nepůvodním rostlinám v Evropě. Přesto zůstává několik aspektů jejich biologie, včetně cytologické variability, které jsou málo probádané. Významným fenoménem je skutečnost, že v semenech a semenáčcích křídlatek se setkáváme s častými aneuploidními chromozomovými počty, které u dospělých jedinců téměř absentují. V naší studii jsme zkoumali cytologickou variabilitu dospělých zástupců sekce *Fallopia* sect. *Reynoutria* v Západních Karpatech a přilehlých částech Panonie. U 12 jedinců jsme zkoumali počet chromozomů a u 1159 jedinců z 1003 populací (825 jedinců ze Slovenska, 173 z Maďarska, 70 z Polska, 63 z České republiky a 28 z Rakouska) jsme pomocí průtokové cytometrie zjišťovali relativní velikost genomu a ploidní úroveň. Zjistili jsme, že sekce *F.* sect. *Reynoutria* je na studovaném území reprezentována zejména hexaploidním (2n = 6x ~ 66) cytotypem *F.* ×*bohemica* (809 jedinců) a oktoploidním (2n = 8x ~ 88) cytotypem *F. japonica* var. *japonica* (297 jedinců). V malé míře se v území vyskytuje i tetraploidní (2n = 4x ~ 44) a dosud neuváděný hexaploidní (2n = 6x ~ 66) cytotyp *F. sachalinensis* (43, resp. 9 jedinců). Kromě toho jsme zjistili i přítomnost dvou aneuploidních jedinců s počty chromozomů 2n = 65 a 2n = 107 (oba u *F.* ×*bohemica*), přičemž uvedené počty nebyly dosud u dospělých křídlatek zjištěny v kontinentální Evropě ani jinde ve světě. Ve srovnání se sousedním českým hercynským regionem jsme na studovaném území nezaznamenali tetraploidní druh *F. compacta* (hodnocen dosud zpravidla na úrovni variety jako *F. japonica* var. *compacta*), tetraploidního křížence *F.* ×*moravica* (dosud zpravidla neodlišovaného od *F.* ×*bohemica*), oktoploidní cytotyp *F.* ×*bohemica*, ani oktoploidní cytotyp *F. sachalinensis*. Ve studovaném území jsme nezjistili ani přítomnost tetraploidního (2n = 4x ~ 44) cytotypu *F. japonica*, uváděného z Bratislavy a Ľubochne; tyto údaje považujeme za chybné. Podobně jako v některých jiných evropských oblastech, i v zastoupení křídlatek na studovaném území se projevuje určitá klimatická závislost. Frekvence výskytu *F. japonica* var. *japonica* a *F. sachalinensis* na studovaném území výrazně klesá ve směru od severozápadu k jihovýchodu, zatímco frekvence křížence *F.* ×*bohemica* podél tohoto gradientu naopak výrazně stoupá (z 15,7 % v polské části Západních Karpat na 98,5 % v maďarské části Panonie). Naše výsledky také odhalily, že zastoupení *F.* ×*bohemica* je ve studovaném území výrazně vyšší než ukazovala dosavadní data a že podhodnocování výskytu tohoto křížence je způsobeno jeho záměnou s rodičovskými taxony, zejména s druhem *F. japonica*.

How to cite: Mereďa P. Jr., Mártonfiová L., Skokanová K., Španiel S. & Hodálová I. (2023) Cytogeography of invasive knotweeds (*Fallopia* sect. *Reynoutria*) in central Europe: rare aneuploids and evidence for a climatically determined distribution. – Preslia 95: 241–266.

Preslia, a journal of the Czech Botanical Society

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