

Variation in DNA-ploidy Levels of *Reynoutria* Taxa in the Czech Republic

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The genus *Reynoutria* is represented by four taxa in the Czech Republic: *Reynoutria japonica* var. *japonica*, *R. japonica* var. *compacta*, *R. sachalinensis* and *R. ×bohemica*. By using flow cytometry, cytological variability within the genus is described based on 257 *Reynoutria* samples. The varieties of *R. japonica* are cytologically uniform, var. *japonica* is exclusively octoploid ($2n = 8x = 88$) and var. *compacta* occurs only as a tetraploid ($2n = 4x = 44$), but *R. sachalinensis* and *R. ×bohemica* exhibit some variation in chromosome numbers. *Reynoutria sachalinensis* is predominantly tetraploid ($2n = 4x = 44$), but also occurs occasionally as hexaploid and octoploid cytotypes. The most common ploidy level in *R. ×bohemica* is hexaploid ($2n = 6x = 66$), but tetraploid and octoploid clones were also found. The four taxa occurring in the Czech Republic are described briefly and the possible origins of the cytotypes discussed.

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Key words: Clonality, *Fallopia*, flow cytometry, hybridization, invasion, Polygonaceae, polyploidy, *Reynoutria japonica* var. *japonica*, *R. japonica* var. *compacta*, *R. sachalinensis*, *R. ×bohemica*.

INTRODUCTION

The spread of an alien species and its ecological impact is sometimes positively correlated with the presence of polyploidy (Barrett, 1982; Thompson, 1991). Polyploidy in general allows the plant to diversify its genome due to genome reorganization (Soltis and Soltis, 1999) conferring adaptive plasticity (Roose and Gotlieb, 1976; Thompson and Lumaret, 1992) to hide deleterious alleles through an increase in frequency of heterozygous loci (Parsons, 1959), and subsequently to reduce the incidence of inbreeding. Polyploidy is frequently linked to vegetative reproduction (Gibby, 1981), which allows new taxa to buffer the disadvantages of small population size (Levin, 1975). Vegetative reproduction can help a plant of one sex to await the arrival of an individual of the opposite sex (Baker, 1986). Relatively high numbers of invasive plants possess diaspores adapted to asexual reproduction (Vogt Andersen, 1995), and some highly invasive plants, such as *Reynoutria japonica* var. *japonica*, are restricted exclusively to this method of reproduction in their adventive range (Hollingsworth *et al.*, 1998).

Hybridization of related invasive taxa in the territory of their secondary distribution is a relatively common event (Vilà *et al.*, 2000). Compared with their parents, the hybrids can possess various levels of fitness (Rieseberg, 1995). Examples of hybridization generating new taxa more highly invasive than their parents have been known (Vilà *et al.*, 2000). Fast evolution of new taxa by coincidence of

hybridization and polyploidization and subsequent spread of newly evolved species have been documented in *Senecio* (Abbott, 1992; Lowe and Abbott, 1996), *Tragopogon* (Roose and Gotlieb, 1976; Novak *et al.*, 1991), *Spartina* (Marchant, 1967, 1968; Ayres and Strong, 2001) and *Carpobrotus* (Vilà and D'Antonio, 1998).

In members of the genus *Reynoutria* the combination of hybridization, the presence of various ploidy levels and pronounced clonality are probably the main determinants of their successful spread in the countryside. The basic scheme occurring within the genus has been described for the British Isles (Bailey and Stace, 1992; Bailey, 1994; Bailey *et al.*, 1995; Hollingsworth *et al.*, 1998; Hollingsworth and Bailey, 2000*a, b*), but little information is available for other parts of Europe. To understand better the determinants of invasiveness within the genus, it is useful to know the degree of cytological variation within the Czech Republic. Hence, the main aim of this paper is to describe the number of cytotypes and discuss their possible origins.

MATERIALS AND METHODS

Study species

Representatives of the genus *Reynoutria* Houtt. (syn. *Fallopia* Adans.) (Polygonaceae) are herbaceous perennials with robust erect stems, an extensive system of thick rhizomes, deeply three-parted styles with fimbriate stigmas, and a functionally dioecious breeding system. There are two distinct opinions on the classification at the generic level. Some authors treat the group as the distinct genus

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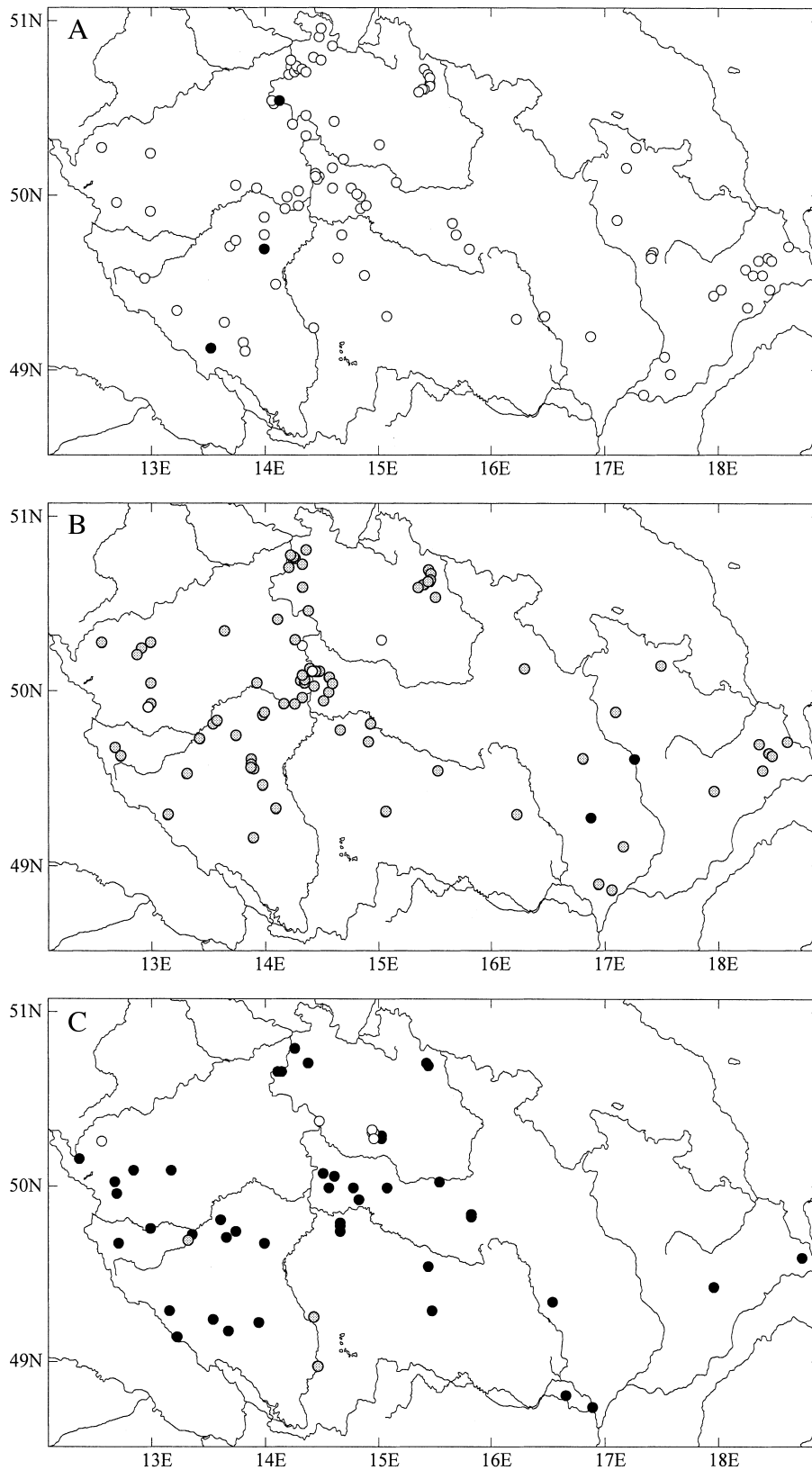


FIG. 1. Distribution of the 257 *Reynoutria* clones used in this study. A, *Reynoutria japonica* var. *japonica* (white circles) and *R. japonica* var. *compacta* (black circles). B, *R. ×bohemica*; C, *R. sachalinensis*. Black circles indicate tetraploids ($2n = 44$), grey circles hexaploids ($2n = 66$), and open circles octoploids ($2n = 88$). For localities see Supplementary data: (<http://www.aob.oupjournals.org>).

Reynoutria (Holub, 1971), others as a section of the genus *Fallopia* [i.e. *Fallopia* sect. *Reynoutria* (Houtt.) Ronse Decr.] (Ronse Decraene and Akeroyd, 1988; Bailey and Stace, 1992). In this paper, we follow the former approach represented by the taxonomy of Holub (1971).

All species present in the Czech Republic were introduced into Europe as garden ornamentals from eastern Asia in the 19th century (Conolly, 1977; Bailey and Conolly, 2000). In the Czech Republic, the genus is represented by *R. japonica* Houtt. var. *japonica*, *R. japonica* Houtt. var. *compacta* Moldenke, *R. sachalinensis* (F. Schmidt) Nakai and the hybrid between *R. sachalinensis* and *R. japonica*, namely *R. ×bohemica* Chrtek et Chrtková described from the Czech Republic by Chrtek and Chrtková (1983). All invade riparian and various human-made habitats and often spread into semi-natural vegetation (Brabec and Pyšek, 2000; Pyšek *et al.*, 2001). The spread of *Reynoutria* taxa in the Czech Republic is mainly vegetative through regeneration from rhizome and stem segments (Mandák and Pyšek, 1996; Bímová *et al.*, 2001, 2003) resulting from the unusual form of sexual reproduction where members of the genus *Reynoutria* have been reported to sexually reproduce only rarely within the adventive distribution area due to inefficient seedling establishment (Bailey *et al.*, 1995).

In *R. sachalinensis*, hermaphrodite and female tetraploid ($2n = 4x = 44$) clones have been recorded in Europe (Bailey and Stace, 1992). All European plants of *R. japonica* var. *japonica* recorded to date have been octoploid ($2n = 8x = 88$) and those of *R. japonica* var. *compacta* exclusively tetraploid ($2n = 4x = 44$) (Bailey and Stace, 1992). As yet, only female clones of *R. japonica* var. *japonica* have been recorded in the Czech Republic. However, despite the absence of pollen, the plants produce seeds because they are fertilized by pollen from *Fallopia aubertii* (L. Henry) Holub to give either *Fallopia ×conollyana* J. P. Bailey (Bailey, 2001) or *R. sachalinensis* (Mandák and Pyšek, 1996). In the latter case, the hybrid *Reynoutria ×bohemica* is produced ($2n = 6x = 66$) (Bailey and Stace, 1992).

Material collection

In total, 257 *Reynoutria* plants were collected from the wild in the Czech Republic between 1998 and 2001. Sampling locations were chosen such that they were representative of the whole of the Czech Republic and to obtain a sound basis for sampling the cytological variability [Fig. 1 and see Supplementary data (<http://www.aob.oupjournals.org>) for list of localities]. Samples were transplanted to the experimental garden of the Institute of Botany in Průhonice, Czech Republic. Rhizomes were planted in 12 l plastic pots filled with garden soil, and regenerated plants were used to ascertain chromosome numbers.

Chromosome counts

A modified method of Bailey and Stace (1992) was used to prepare slides for chromosome counting. Actively growing roots were pretreated in 0.002 M 8-hydroxyquinoline for 22–24 h at 4 °C, fixed overnight in 3 : 1 ethanol : acetic acid and stored at 4 °C in 70 % ethanol

until used. Root-tips were hydrolysed in 2N HCl for 10 min at 60 °C, rinsed in water and the meristematic tissue excised and squashed in a drop of lacto-propionic orcein (Dyer, 1963). Chromosomes were counted using a phase-contrast microscope.

Ploidy level estimation

All 257 clones were subjected to flow cytometric analysis of nuclear DNA content. A two-step procedure of sample preparation was employed (Otto, 1990). Approximately 2 cm² of fresh young leaf tissue of an analysed plant, together with approx. 1 cm² of leaf tissue of an internal standard with known chromosome number [*R. sachalinensis* $2n = 4x = 44$, *R. ×bohemica* $2n = 6x = 66$ or *R. japonica* $2n = 8x = 88$ (Supplementary data: <http://www.aob.oupjournals.org>)] were chopped with a new razor blade in a Petri dish containing 1 ml ice-cold Otto I buffer (0.1 M citric acid, 0.5 % Tween 20). The sample was filtered through a nylon mesh (42 µm) and centrifuged at 150g for 5 min. The supernatant was removed and the nuclei resuspended in 100 µl of ice-cold Otto I buffer. After 30 min incubation at room temperature, 1 ml of Otto II buffer (0.4 M Na₂HPO₄·12H₂O) with DAPI at a final concentration of 4 µg ml⁻¹ was added. Samples were stained for 30 min at room temperature. The relative fluorescence of isolated nuclei was analysed using a Partec PA II flow cytometer (Partec GmbH, Münster, Germany). The cytometer was adjusted so that the fluorescence of G₁ nuclei of tetraploid plants (internal standard) was localized on channel 200.

RESULTS

The most common taxon, *R. japonica* var. *japonica*, was represented by 99 samples, *R. ×bohemica* by 94 samples, *R. sachalinensis* by 61 samples and *R. japonica* var. *compacta* was found only in three localities [Fig. 1 and Supplementary data (<http://www.aob.oupjournals.org>)].

The analysis of DNA content of nuclei isolated from leaf tissue showed that most of the nuclei were in the G₀/G₁ phase of the cell cycle and thus formed a dominant peak in histograms of DNA content. This peak was localized at channel 205 for tetraploid plants (CV = 2.23), at channel 335 for hexaploid plants (CV = 1.71) and at channel 461 for octoploid plants (CV = 1.31). The peak ratios were 1 : 1.63 : 2.25 for *R. sachalinensis*, *R. ×bohemica* and *R. japonica*, respectively (Fig. 2).

Both varieties of *R. japonica* were cytologically uniform; all *R. j.* var. *japonica* plants were octoploid, and all those of *R. j.* var. *compacta* tetraploid (Fig. 3). *Reynoutria sachalinensis* was predominantly tetraploid. Their hybrid, *R. ×bohemica*, was hexaploid. In addition, ploidy levels deviating from the above pattern were found in *R. ×bohemica* and *R. sachalinensis* (Fig. 3). Their most likely origins are shown in Fig. 4.

The distribution of particular taxa and ploidy levels in the area studied is shown in Fig. 1. *Reynoutria* taxa occur more frequently in human-made and riparian habitats regardless of ploidy level of particular taxa in the Czech Republic

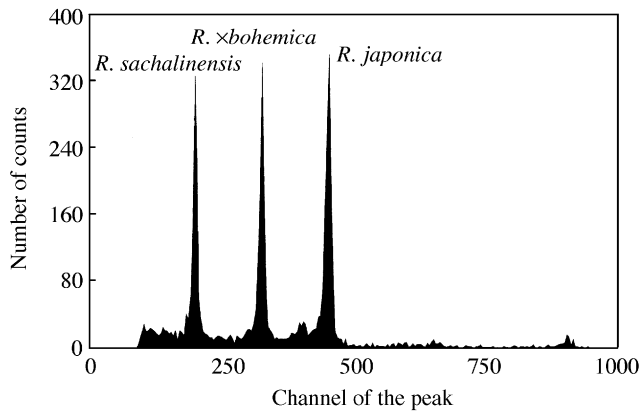


FIG. 2. Histogram of relative nuclear DNA content of particular *Reynoutria* species. The G_0/G_1 peak of tetraploid *R. sachalinensis* was on channel 205, that of hexaploid *R. xbohemica* on channel 335 and that of octoploid *R. japonica* var. *japonica* on channel 461, i.e. the peak ratios are 1 : 1.63 : 2.25 for *R. sachalinensis*, *R. xbohemica* and *R. japonica*, respectively.

(Fig. 5). The occurrence of octoploid *R. sachalinensis* and *R. xbohemica* is restricted to several localities that closely correspond to the presence of parks or old gardens. However, there is no general geographical pattern, and particular localities of different taxa and ploidy levels are often far from each other (Fig. 1).

DISCUSSION

Polyploidy is a common phenomenon in plants; it is estimated to occur in 47–70 % of angiosperm species (Ramsey and Schemske, 1998). Being of great importance for plant taxonomy, evolution and ecology, it is thus of interest to plant biologists. Formation of polyploid series of morphologically and ecologically different, but closely related, species allows us to understand the importance of polyploidy in nature using methods of comparative ecology.

Cytological variation of *R. japonica* var. *japonica* in its native regions is high and includes tetraploids, hexaploids, octoploids and decaploids (Table 1). This is in contrast to the situation in the adventive distribution area covered by the reported study where only octoploids were found. The frequency distribution of ploidy levels ascertained in *R. japonica* var. *japonica* does not differ from that reported from other European countries (Table 1), with the exception of Slovakia. However, the results of the reported study and of chromosome counts made by Bailey and Stace (1992) who only found octoploid plants in Europe, question the tetraploid chromosome numbers reported from Slovakia (Májovský *et al.*, 1987), which are probably due to misidentification of the taxa. *Reynoutria japonica* var. *japonica* is exclusively octoploid in Europe and does not possess any variation at either cytological or genotype level (Bailey and Stace, 1992; Hollingsworth and Bailey, 2000a). The present distribution probably resulted from a single introduction to Europe in the 19th century (Bailey and Conolly, 2000).

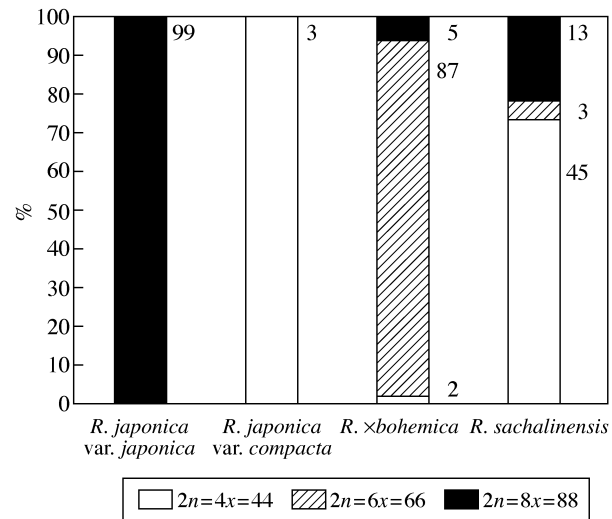


FIG. 3. Ploidy levels in particular *Reynoutria* taxa. Numbers indicate the number of samples analysed.

The dwarf alpine form *R. japonica* var. *compacta* has been repeatedly reported to be tetraploid (Table 1) which is in accordance with our study. Nevertheless, chromosome counts published to date are only from the adventive distribution area where *R. japonica* var. *compacta* is distinguished as a morphologically distinct type. This distinction certainly results from the bottleneck effect; in the native distribution area, there is a high morphological variability creating a continuum between *R. japonica* var. *japonica* and *R. japonica* var. *compacta* (Shiosaka and Shibata, 1993). For this reason, most authors working in the Far East do not recognize this variety (Lee, 1972; Kim and Park, 2000; but see Ohwi, 1965). On the other hand, Yonekura and Ohashi (1997) recognized, in addition to var. *japonica*, two more varieties of *R. japonica*, namely var. *hachidoensis* (Honda) Yonekura et H. Ohashi and var. *uzenesis* (Honda) Yonekura et H. Ohashi. Both are endemic to Japan and have never been introduced into Europe.

Whereas *R. japonica* is cytologically uniform in Europe (Table 1), the situation with *R. sachalinensis* and *R. xbohemica* is much more complicated. Our study yielded new ploidy levels (Figs 3 and 4). *Reynoutria sachalinensis* is predominantly tetraploid, but we also found hexaploids and octoploids (Fig. 3). There are two possible methods by which this could arise: (1) by generative reproduction via unreduced gametes or (2) by somatic mutations (i.e. autopolyploidization leading to an octoploid cytotype). The former method is limited by rarely occurring sexual reproduction in *Reynoutria* taxa in the territory studied. Hence, occasional generative reproduction fixed by clonality over time could store ‘aberrant’ ploidy levels for a long time. Massive clonal growth could function in the same way in the case of somatic mutation without the necessity for generative reproduction. However, since the frequency of somatic mutations in *R. sachalinensis* is not known, the latter explanation of ‘aberrant’ ploidy levels only remains a speculation. An alternative explanation is the introduction

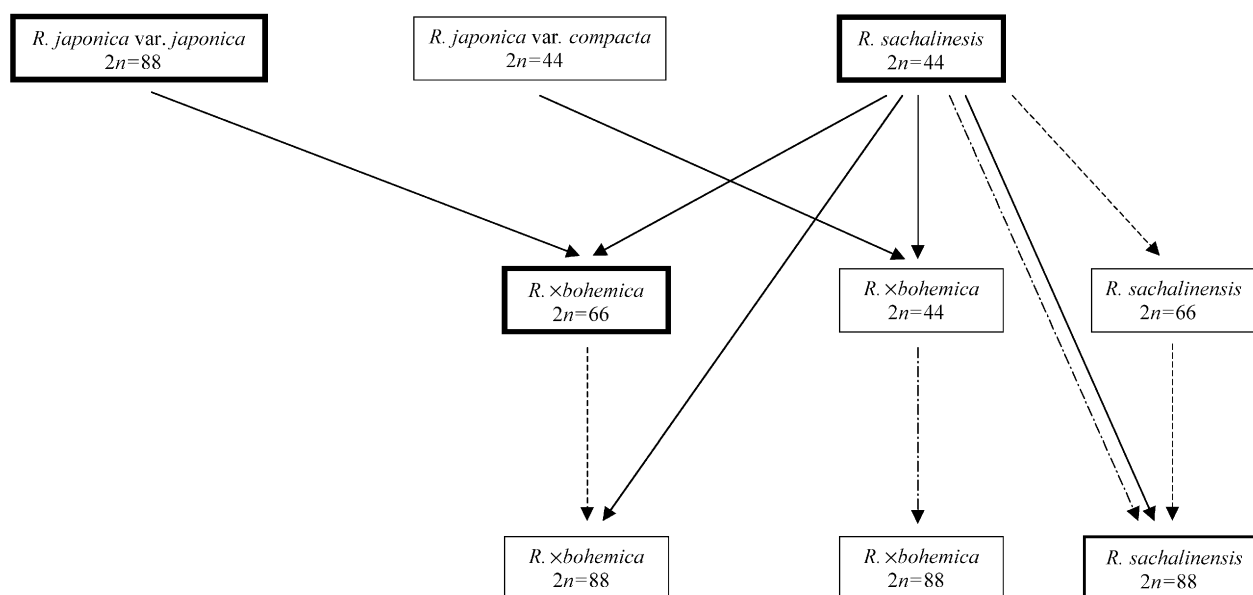


FIG. 4. Schema of hybridization and polyploidization within the genus *Reynoutria* in the Czech Republic. More frequent ploidy levels are indicated by wider frames: those with 6–20 localities have intermediate frames, those with more than 20 localities have wide frames. Solid lines indicate origin of particular taxa by hybridization, dashed lines indicate expected fusion of reduced and unreduced gametes, and dot-and-dashed lines indicate autopolyploidization.

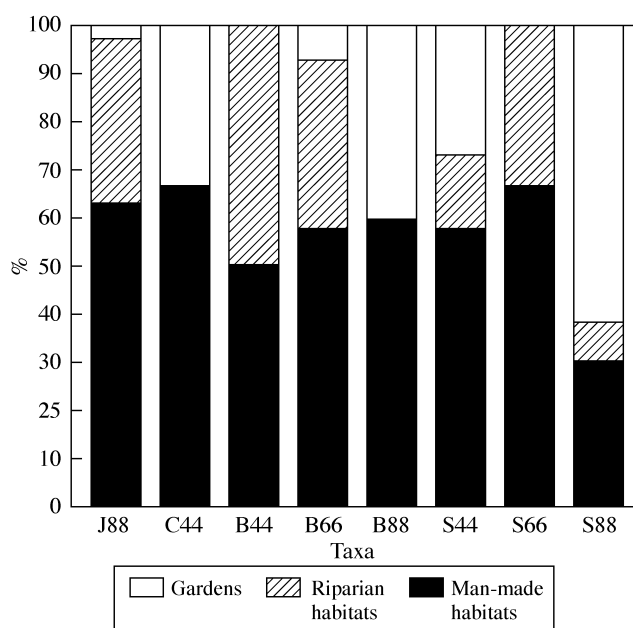


FIG. 5. Contribution of particular habitat types to the total number of localities for particular taxa and ploidy levels. J88, *Reynoutria japonica* var. *japonica* ($2n = 88$); C44, *R. japonica* var. *compacta* ($2n = 44$); B44, *R. xbohemica* ($2n = 44$); B66, *R. xbohemica* ($2n = 66$); B88, *R. xbohemica* ($2n = 88$); S44, *R. sachalinensis* ($2n = 44$); S66, *R. sachalinensis* ($2n = 66$); S88, *R. sachalinensis* ($2n = 88$).

of these ‘aberrant’ ploidy levels directly from the native distribution areas, Japan, Sakhalin or Ullung-do (an island between Japan and Korea). The ability of hexaploid and

octoploid *R. sachalinensis* to hybridize with other *Reynoutria* taxa has never been studied, i.e. there are no data concerning the fertility of pollen grains, seed viability or the ability of seedlings to survive. On the other hand, hybridization between and within particular *Reynoutria* taxa is common (Bailey and Stace, 1992). Hence, we can expect further hybridization with other *Reynoutria* taxa and the subsequent rise of new hybrid combinations with unknown properties.

Reynoutria xbohemica is a hybridogenous taxon that either originated in the adventive distribution area or was directly introduced from the native distribution area. Hybridization events of alien plants followed by the spread of the hybrid have been documented (Vilà *et al.*, 2000). Such events can lead to the rapid evolution of new taxa and expansion of their range. Eventually, such hybrid taxa may interfere with human objectives and become weedy or invasive (Abbott, 1992) as is the case with *R. xbohemica* (B. Mandák and P. Pyšek, unpubl. res.).

However, the pattern of cytological variation in *R. xbohemica* follows that found in *R. sachalinensis* but with different underlying mechanisms (Bailey *et al.*, 1995). *Reynoutria xbohemica* is predominantly hexaploid (Fig. 3). The clones with different ploidy levels, i.e. tetraploids and octoploids, have different origins. The tetraploid *R. xbohemica* is probably a hybrid between *R. sachalinensis* and *R. japonica* var. *compacta* (Fig. 4). Bailey and Stace (1992) pointed out that this hybrid is very difficult to distinguish on morphological grounds from hexaploid *R. xbohemica*, and chromosome numbers are often the only means of identifying these clones. The origin of octoploid *R. xbohemica* plants is inevitably more specula-

TABLE 1. Literature records of chromosome numbers found in *Reynoutria japonica* var. *japonica*, *R. japonica* var. *compacta*, *R. sachalinensis* and *R. ×bohemica* in both native and adventive territories

Reference	Chromosome number (2n)	Locality
<i>Reynoutria japonica</i> Houtt. var. <i>japonica</i>		
Native distribution area		
Bailey (1999)	88	Korea
Bailey (2003)	44, 88	Japan
Bailey (1999), Hollingsworth and Bailey (2000b)	88, 110	China
Sugiura (1931), Doida (1960), Bailey (1999), Hollingsworth and Bailey (2000b)	44	Japan
Kim and Park (2000)	44, 66, 88	Korea
Lee (1972)	88	Korea
Adventive distribution area		
Bailey (2003)	88	Hungary, USA, Canada, Australia, New Zealand
Bailey and Stace (1992)	88	Great Britain
Graham and Wood (1965)	44, approx. 88	USA
Hollingsworth and Bailey (2000b)	88	Great Britain
Májovský <i>et al.</i> (1974), Murín (1974), Váchová and Feráková (1986), Májovský <i>et al.</i> (1987)	44	Slovakia
Mandák <i>et al.</i> , this study	88	Czech Republic
Wcisło (1977), Pogan and Wcisło (1983)	88	Poland
Zhukova [1967, in Bolkhovskikh <i>et al.</i> (1969)]	approx. 60	Russia
<i>Reynoutria japonica</i> Houtt. var. <i>compacta</i> Moldenke		
Native distribution area		
Bailey (2003)	44	Japan
Adventive distribution area		
Bailey and Conolly (1985), Bailey and Stace (1992), Hollingsworth and Bailey (2000b), Bailey (2003)	44	Great Britain
Bailey (2003), Mandák <i>et al.</i> , this study	44	Czech Republic
<i>Reynoutria sachalinensis</i> (F. Schmidt) Nakai		
Native distribution area		
Bailey (2003)	102, 103	Korea
Bailey and Stace (1992), Bailey (2003)	44	Japan
Kim and Park (2000)	132	Korea
Kim and Park (2000)	44	Japan
Lee (1972)	102	Korea
Sokolovskaya [1960, 1965 in Bolkhovskikh <i>et al.</i> (1969)]	44	Russia (Sakhalin)
Adventive distribution area		
Bailey (2003)	44, 66	USA
Bailey and Stace (1992)	44	Great Britain
Májovský and Váchová (1986), Májovský <i>et al.</i> (1987)	44	Slovakia
Mandák <i>et al.</i> , this study	44, 66, 88	Czech Republic
Menshikova [1964, in Bolkhovskikh <i>et al.</i> (1969)]	ca. 66	Russia
Wcisło (1977), Pogan and Wcisło (1983)	44	Poland
<i>Reynoutria × bohemica</i> Chrték et Chrtková		
Native distribution area		
Bailey (2003)	66	Japan
Adventive distribution area		
Bailey and Stace (1992), Bailey <i>et al.</i> (1996), Bailey (2003)	44, 66, 88	Great Britain
Bailey (2003)	88	France, Germany
Bailey (2003)	66	USA, Australia, New Zealand
Mandák <i>et al.</i> , this study	44, 66, 88	Czech Republic

tive (Bailey, 1999). They are probably the product of fusion of an unreduced (hexaploid plant of *R. ×bohemica*) and a reduced (tetraploid plant of *R. sachalinensis*) gamete. Another possibility is autopolyploidization of the tetraploid *R. ×bohemica* cytotype (Fig. 4).

Bailey and Stace (1992) showed that hexaploid *R. ×bohemica* has extremely irregular meiosis, with large numbers of univalents and multivalents up to quadrivalents.

This fact is reflected in low pollen fertility. In contrast, both tetraploid and octoploid *R. ×bohemica* have much more regular meiosis than the hexaploid. This agrees with our finding that the pollen grains of hexaploid *R. ×bohemica* showed lower germination than the octoploid clones (Koukolíková 2001). We can partly confirm this result that regularity of meiosis in *Reynoutria* hybrids is determined mainly by the ploidy level of these taxa.

The most common ploidy levels of particular *Reynoutria* taxa are scattered over the whole of the Czech Republic (Fig. 1). Most of the habitats occupied by *Reynoutria* taxa are in human settlements and the occurrence of the taxa is therefore associated with cultivation and subsequent accidental spreading by man, water or soil movements connected with building activities. Compared with the other *Reynoutria* taxa, the presence of octoploid *R. ×bohemica* and *R. sachalinensis* is more associated with gardens (Fig. 5). Hence, there is a possibility that these 'aberrant' ploidy levels were introduced directly from native distribution areas or evolved in gardens and then spread locally.

Although a direct genotype analysis is needed to confirm this conclusion, the high cytological variability of *R. ×bohemica* indicates that the evolution of new taxa is in progress within the complex studied. This fact, in combination with a high invasive potential within the group, is alarming. Selection of new, more invasive clones at the cytological level brings about the possibility that new cytotypes with a greater ability to spread and survive could emerge in central Europe.

SUPPLEMENTARY INFORMATION

Supplementary data contain a list of *Reynoutria* localities with ploidy levels and geographical coordinates in the Czech Republic.

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