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CHROMOSOME NUMBERS AND POLYPLOIDY IN LIFE FORMS OF ASTERACEAE, POACEAE AND ROSACEAE IN POLISH FLORA

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The chromosome numbers and frequency of polyploids were compared in life forms of Asteraceae, Poaceae and Rosaceae. Both parameters were higher in Poaceae and Rosaceae than in Asteraceae. Among the life forms, long-lived plants including perennials and woody plants (shrubs and trees) generally had higher chromosome numbers and consequently polyploid frequencies than short-lived species (annuals and biennials). The families surveyed have different frequencies of life forms. Asteraceae and Rosaceae are both dicots, but the life forms in Asteraceae are more similar to Poaceae than to Rosaceae. To separate the influence of life form, in a series of tests we compared life forms from the same families. These results also showed that long-lived forms generally have more chromosomes than short-lived ones.

Key words: Chromosome numbers, polyploidy, life forms, plants, evolution, Asteraceae, Rosaceae, Poaceae, threshold method, x method.

INTRODUCTION

Polyploidization plays an important role in plant evolution. Estimates of the frequency of polyploids in angiosperms range from 30 to 80% (Bennett, 2004) when traditional methods are applied. However, it is known that the frequency of polyploids and chromosome numbers differ between floras, geographical regions (e.g., Peruzzi et al., 2011; Peruzzi et al., 2012) and phylogenetic groups (Bedini et al., 2012a; Bedini et al., 2012c).

The simplest methods used to estimate polyploid frequency involve comparing the chromosome numbers of cytotypes to given chromosome numbers, for example the x value for a genus or multiples of the lowest haploid number in a genus (Wood et al., 2009). The so-called threshold methods simply use an arbitrarily chosen n number. They are relatively uncomplicated and are particularly applicable to large datasets for which not all x values are known. The most popular threshold values are n≥11 (Goldblatt, 1980) and n≥14 (Grant, 1981).

Indirect information about polyploidy may be obtained by comparing chromosome numbers in the groups examined. This does not give estimates of polyploid frequency but several of the calculated statistical values may give a broader view of the distribution of chromosome numbers, which may help identify polyploidization events and to describe them using mathematical functions, as was recently done for the Italian flora (Bedini et al., 2012b).

Methods based on molecular data may give more precise results but are much more time-consuming and expensive. However, they established that even such small genomes as those of *Arabidopsis thaliana* (n=5) may be of polyploid origin (Vision et al., 2000; Bowers et al., 2003). In recent years, many similar studies have gradually changed our understanding of the role of polyploids in plant evolution and have shown that almost all angiosperms had at least one episode of polyploidization in their evolutionary history (Soltis, 2005; Soltis et al., 2009). In light of such results it may seem pointless to study the frequency of polyploids in given groups of angiosperms. However, if we regard the results obtained by chromosome number methods as indicating the frequency of recently formed polyploids rather than the frequen-

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cy of plants that had at least one episode of polyploidization in their history, they become a useful tool in the search for relations between ploidy and different factors or features of plants (Góralski et al., 2014).

Studies of polyploidy very often focus on the features of polyploids that might explain their evolutionary success by genetic and phenotypic superiority (e.g., Ronfort, 1999; Otto and Whitton, 2000; Soltis and Soltis, 2000; Levin, 2002; Wang et al., 2006; Otto, 2007; te Beest et al., 2012) and features more directly related to competition and invasiveness (Lowry and Lester, 2006; Pandit, 2006; Hull-Sanders et al., 2009; Treier et al., 2009; Mráz et al., 2011a,b; Pandit et al., 2011; te Beest et al., 2012; Góralski et al., 2014). For decades there has also been an ongoing search for the relationship between chromosome number and life form, which may also be related to evolutionary success. In the second quarter of the 20th century, Stebbins (1938) compared the x values of dicots in temperate and boreal zones and found that the average x value was 9 for herbaceous plants (perennials, annuals and biennials) but 14.2 in woody plants, and that therefore the frequency of polyploids was lower in woody than in herbaceous plants, in which, according to Stebbins, the tendency toward polyploidy was manifested chiefly in perennial herbs.

Similar observations have been reported for the flora of Pakistan (Baquar, 1976; Khatoon, 1991; Khatoon and Ali, 2006). The general view in the first two of those cited papers is that polyploidy is most common in perennials and less common in annuals, followed by shrubs, and finally least common in trees. Khatoon and Ali (2006) also found the lowest frequencies of polyploids in trees and higher ones in shrubs, although the life form they found most abundant in polyploids was annual plants, which prevailed over perennial herbs. Another sequence of polyploid percentages was given by Haskell (1952) for the British flora: lowest in annuals, higher in trees and shrubs, and highest in perennial herbs. Some other studies found no such differences between life forms: for example, Petit and Thompson (1999) for the flora of the Pyrenees, and Vamosi and Dickinson (2006) for Rosaceae.

Differences between the flora studied and the methods used by the various authors make it difficult to sort out their results and come to general conclusions. Although studies in this area have been done for at least 75 years, new research is still needed if we are to understand the connections between ploidy and life form in plants.

We studied representatives of three angiosperm families in the Polish flora: Asteraceae, Poaceae and Rosaceae. The first is the largest family of angiosperms, comprising over 1,600 genera and 23,000 species, and rich in life forms (Anderberg et al., 2007). Poaceae (grasses) is one of the largest and most widespread families and is very important in agriculture. Other interesting features of grasses are their wide range of x (2–18) and 2n (4 to more than 260) values achieved via such processes as chromosome doubling, aneuploid reduction and hybridization (Hilu, 2004). Rosaceae is also an economically important group of angiosperms that contains about 100 genera and 3,000 species representing perennial, tree and shrub life forms. Phenomena that influence evolution, such as hybridization, polyploidization and apomixis, are frequently observed in these families (Hummer and Janick, 2009). They are also the most data-rich families in our database (Góralski et al., 2009).

The Polish flora is relatively young because in the Pleistocene almost the entire area of the country was covered by a glacier (Szafer and Zarzycki, 1977). This makes it an interesting area for observing many evolutionary phenomena. In recent decades the chromosome numbers of ~40% of the Polish angiosperms have been examined (Gacek et al., 2011) and their 2n values have been collected in a database accessible online (Góralski et al., 2009).

MATERIALS AND METHODS

DATA AND DATA SOURCES

Chromosome numbers were obtained from the Chromosome Number Database (Góralski et al., 2009), which contains the chromosome numbers known for Polish angiosperms. The life forms of the examined species were found in different sources (Szafer et al., 1953; Rutkowski, 2004; USDA, 2007; Snowarski, 2012). The x values were estimated using data on somatic chromosome numbers and consulted with various syntheses and taxon-specific literature (previously described in Gacek et al., 2011).

GROUPS TESTED

For statistical analyses and comparisons at different levels we created 19 groups containing the data collected for three families: Asteraceae, Poaceae and Rosaceae. The first group contained all of the data (called "all tested"). The next three groups contained data for these three families separately ("Asteraceae," "Poaceae," "Rosaceae"). Five groups contained chromosome numbers for six life forms represented by at least ten cytotypes with self-explanatory names – "perennial," "annual," "biennial," "shrubs" and "annual or biennial" (containing species that can be annual or biennial). In addition, two groups containing two similar life forms were created – "annual and biennial" and "trees and

TABLE 1. Comparisons and statistical significance of tests. Signs indicate the statistical significance of the difference (- P>0.01, + P≤0.01) 1st – chromosome number (Mann-Whitney test), 2nd – polyploid frequency at n≥11 threshold (χ^2 test), 3rd – polyploid frequency at n≥14 threshold (χ^2 test), 4th – polyploid frequency estimated by x value (χ^2 test)

	Asteraceae	Poaceae	annual	(Asteraceae) biennial	annual or biennial	annual and biennial	perennial	perennial (Asteraceae & Poaceae)	Asteraceae – annual	Asteraceae – perennial	Poaceae – perennial	Rosaceae – perennial
Poaceae	--+-											
Rosaceae	-+++	-+++										
(Asteraceae) biennial			----						----			
annual or biennial			----	----								
perennial			----	---+	----	++-+						
(Rosaceae) shrubs			-+++	-+++	++++	-+++	-+++					----
(Rosaceae) trees and shrubs			-+++	-+++	++++	-+++	-+++					----
annual (Asteraceae & Poaceae)								----				
Asteraceae – annual and biennial										-+-+		
Asteraceae – perennial				---+					----		--+-	--++
Poaceae – annual									----		----	
Rosaceae – perennial											----	

shrubs." When a given life form was represented only by members of one family, the name of the family in the tables and charts is given in parentheses before the name of the life form and this life form is not displayed separately, when the life forms of a given family are specified. Two groups were created from life forms shared by two families: "perennial (Asteraceae & Poaceae)" and "annual (Asteraceae & Poaceae)". The next groups contained data on the chromosome numbers of life forms represented by a large enough number of cytotypes in each family: "Asteraceae – annual," "Asteraceae – perennial," "Poaceae – annual," "Poaceae – perennial" and "Rosaceae – perennial." As explained above, the groups "Asteraceae – biennial," "Rosaceae – shrubs" and "Rosaceae – trees & shrubs" are the same as "(Asteraceae) biennial", "(Rosaceae) shrubs" and "(Rosaceae) trees and shrubs," respectively, and are not specified separately in the tables and charts. The last two groups included two life forms with similar characteristics in two families ("Asteraceae – annual and biennial", "Rosaceae – trees and shrubs"). All comparisons and the statistical significance of the results are shown in Table 1.

STATISTICAL TOOLS AND PROCEDURES

All of the statistical tests and graphs employed the R environment for statistical computing (R Development Core Team, 2012). Two types of tests were applied for comparisons of groups: comparisons of chromosome numbers and comparisons of the frequency of polyploids. Several values were calculated for the chromosome numbers and are given in the tables: mean and SD, mode, median, minimum and maximum values. Differences in chromosome number between groups were tested using the nonparametric Mann-Whitney U (Wilcoxon) test (Wilcox.test function from stats library).

Additionally, the frequency of polyploids was estimated using two threshold methods, n≥11 (Goldblatt 1980) and n≥14 (Grant 1981), and the x value. The frequency of polyploids in the studied groups that had at least ten cytotypes was compared using Pearson's χ^2 test (chisq.test from stats library). This test was also used when the proportions of life forms were calculated.

Because multiple comparisons were used for all of the tests in our study (32 comparisons for every test) we took 0.01 as the significance level.

RESULTS

We tested 1,645 cytotypes belonging to three families (Asteraceae, Poaceae, Rosaceae). The chromosome numbers (2n) of the tested cytotypes ranged from 6 to 96. The mean for the cytotypes was slight-

TABLE 2. Chromosome number parameters calculated for the tested groups

Group	Cytotypes	Mean *x* (SD)	2n				
			mean (SD)	min.	max.	mode	median
all tested	577	8.49 (2.75)	31.27 (15.63)	6	96	28	28
Asteraceae	242	9.52 (3.04)	29.75 (14.74)	6	90	18	25.5
Poaceae	180	7.53 (1.50)	32.97 (19.22)	10	94	14	28
Rosaceae	155	7.99 (2.87)	31.67 (11.68)	14	96	28	28
annual	49	8.67 (2.33)	26.24 (11.63)	10	54	18	24
(Asteraceae) biennial	19	10.47 (4.5)	27.16 (14.27)	12	68	12	22
annual or biennial	10	7.7 (2.06)	22.5 (11.65)	6	42	14	19
annual and biennial	78	8.99 (3.07)	25.99 (12.24)	6	68	18	23
perennial	389	8.43 (2.49)	32.52 (17.35)	8	96	28	28
(Rosaceae) shrubs	94	7.26 (1.47)	29.21 (7.23)	14	68	28	28
(Rosaceae) trees and shrubs	99	7.66 (2.40)	29.43 (7.11)	14	68	28	28
annual (Asteraceae & Poaceae)	47	8.7 (2.38)	26.00 (11.34)	10	54	18	24
perennial (Asteraceae & Poaceae)	345	8.6 (2.59)	32.34 (17.53)	8	94	28	28
Asteraceae-annual	28	9.54 (2.63)	26.14 (10.23)	10	48	18	24
Asteraceae-perennial	187	9.49 (2.94)	30.99 (15.29)	8	90	18	27
Asteraceae-annual & biennial	55	9.62 (3.41)	25.51 (11.85)	6	68	18	20
Poaceae-annual	19	7.47 (1.17)	25.79 (13.1)	10	54	14	28
Poaceae-perennial	158	7.54 (1.55)	33.92 (19.79)	10	94	28	28
Rosaceae-perennial	44	7.07 (0.33)	33.93 (15.97)	14	96	28	31.5

TABLE 3. Diploid and polyploid numbers and frequencies as estimated by n≥11 and n≥14 threshold methods and by x value; (%)

Group tested	Threshold *n*≥11		Threshold *n*≥14		By *x* value	
	diploids	polyploids	diploids	polyploids	diploids	polyploids
all tested	170 (29.46)	407 (70.54)	222 (38.47)	355 (61.53)	208 (36.05)	369 (63.95)
Asteraceae	87 (35.95)	155 (64.05)	129 (53.31)	113 (46.69)	117 (48.35)	125 (51.65)
Poaceae	59 (32.78)	121 (67.22)	69 (38.33)	111 (61.67)	66 (36.67)	114 (63.33)
Rosaceae	24 (15.48)	131 (84.52)	24 (15.48)	131 (84.52)	25 (16.13)	130 (83.87)
annual	23 (46.94)	26 (53.06)	25 (51.02)	24 (48.98)	27 (55.10)	22 (44.90)
(Asteraceae) biennial	9 (47.37)	10 (52.63)	10 (52.63)	9 (47.37)	14 (73.68)	5 (26.32)
annual or biennial	6 (60)	4 (40)	7 (70)	3 (30)	6 (60.00)	4 (40.00)
annual and biennial	38 (48.72)	40 (51.28)	42 (53.85)	36 (46.15)	47 (60.26)	31 (39.74)
perennial	118 (30.33)	271 (69.67)	166 (42.67)	223 (57.33)	145 (37.28)	244 (62.72)
(Rosaceae) shrubs	12 (12.77)	82 (87.23)	12 (12.77)	82 (87.23)	7 (7.45)	87 (92.55)
(Rosaceae) trees and shrubs	12 (12.12)	87 (87.88)	12 (12.12)	87 (87.88)	11 (11.11)	88 (88.89)
annual (Asteraceae & Poaceae)	22 (46.81)	25 (53.19)	24 (51.06)	23 (48.94)	26 (55.32)	21 (44.68)
perennial (Asteraceae & Poaceae)	108 (31.3)	237 (68.7)	156 (45.22)	189 (54.78)	136 (39.42)	209 (60.58)
Asteraceae-annual	13 (46.43)	15 (53.57)	15 (53.57)	13 (46.43)	17 (60.71)	11 (39.29)
Asteraceae-perennial	59 (31.55)	128 (68.45)	97 (51.87)	90 (48.13)	80 (42.78)	107 (57.22)
Asteraceae-annual & biennial	28 (50.91)	27 (49.09)	32 (58.18)	23 (41.82)	37 (67.27)	18 (32.73)
Poaceae-annual	9 (47.37)	10 (52.63)	9 (47.37)	10 (52.63)	9 (47.37)	10 (52.63)
Poaceae-perennial	49 (31.01)	109 (68.99)	59 (37.34)	99 (62.66)	56 (35.44)	102 (64.56)
Rosaceae-perennial	10 (22.73)	34 (77.27)	10 (22.73)	34 (77.27)	9 (20.45)	35 (79.55)

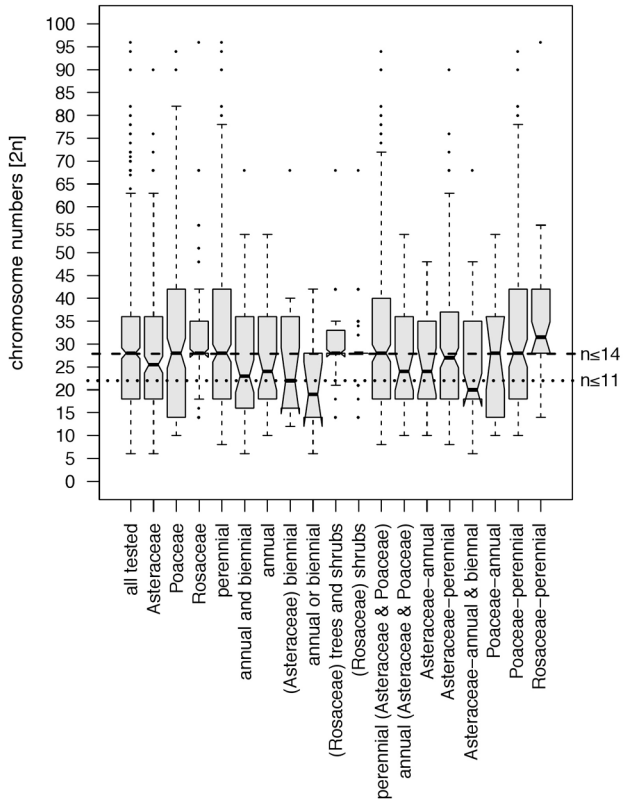

Fig. 1. Chromosome numbers in the tested groups. Horizontal bar – median; notched boxes – lower and upper quartiles; notches – +/-1.58 IQR divided by the square root of the number of data elements (IRQ – the difference between upper and lower quartiles); whiskers – the range of the data, excluding outliers; black dots – outliers (values that are farther than 1.5 IQR from the box).

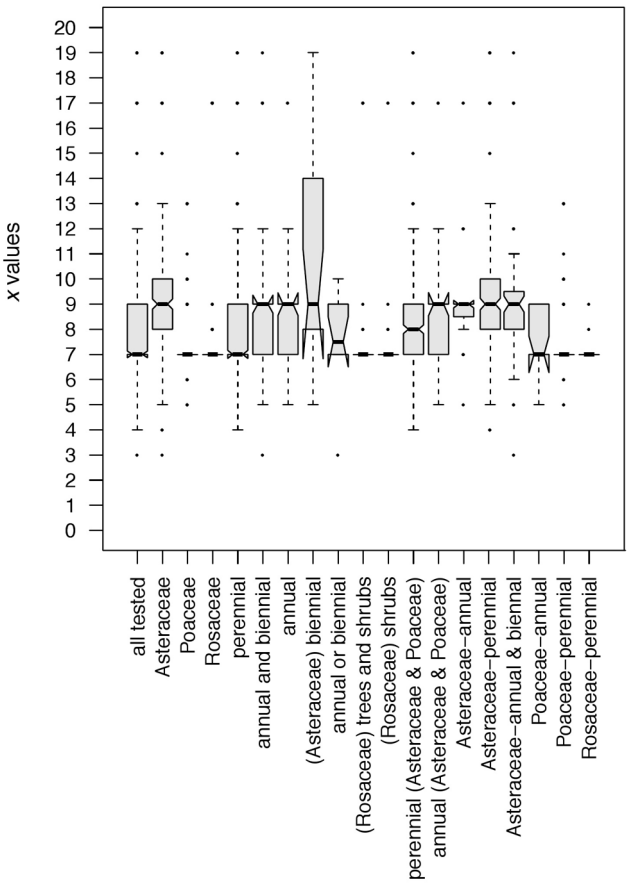

Fig. 2. Basic chromosome numbers (x values) for the tested groups. Horizontal bar – median; notched boxes – lower and upper quartiles; notches – +/-1.58 IQR divided by the square root of the number of data elements (IRQ – the difference between upper and lower quartiles); whiskers – the range of the data, excluding outliers; black dots – outliers (values that are farther than 1.5 IQR from the box).

ly higher than 31, and the median and mode equalled 28 (Tab. 2, Fig. 1). Depending on the method adopted, the frequency of polyploids was 70% (threshold n≥11), 62% (n≥14) and 64% (x method) (Tab. 3).

Among the tested families Asteraceae had the lowest mean (~30) and median (25.5). The medians for the other two families were the same (28) but the mode in Rosaceae (28) was twice that in Poaceae (14). The Mann-Whitney test did not indicate statistical significance (P>0.01).

The frequency of polyploids was highest for Rosaceae for both thresholds (84.5%) and for the x method (84%). Asteraceae had the lowest share of polyploids for both n≥11 (64%) and n≥14 (46%) and for the x method (52%). Asteraceae had a higher median (9) and a much wider spread of x values than the other two families, for which the median was 7. The differences in polyploid frequency were significant (P≤0.01) between Rosaceae and the other families for all three methods, and between Asteraceae and Poaceae for the n≥14 threshold (Tab. 3).

Perennials, which shared the highest mode (28) and median (28) with shrubs and the group "trees and shrubs" had the highest mean (~32.5). Biennial plants were the group with the lowest mode (12) of chromosome number, but the lowest mean (22.5) and median (19) were for "annual or biennial" plants. This paper will not consider comparisons of groups containing many life forms versus the members of these groups, or comparisons of similar groups (e.g., biennial vs. "annual and biennial").

In the chromosome number comparisons of groups of life forms, 3 of 17 comparisons gave results at P≤0.01: "annual or biennial" versus shrubs, "annual or biennial" versus "trees and shrubs," and "annual and biennial" versus perennial.

For all three polyploid estimation methods the frequency of polyploids was lowest in the short-lived plants (30–55%), higher in perennials (57–70%) and highest in woody plants (87–93%). The differences in polyploid frequency were significant between shrubs and the other groups, and between "trees and shrubs" and the other groups, short-lived plants and perennials differed significantly in some cases for the n≥11 threshold and the x method but not for the n≥14 threshold.

We compared annuals with perennials in two of the three families, Asteraceae and Poaceae. Perennials had a higher mean (~32 vs. 26), median (28 vs. 24) and mode (28 vs. 18) but the differences were not significant.

Next we compared life forms within families. Three life forms (annual, biennial, perennial) had at least 10 cytotypes in Asteraceae. A common group (Asteraceae – annual and biennial) for short-lived plants that included annual, biennial and "annual or biennial" was also created. Perennials had the highest 2n mean (~30) and median (27), and "annual and biennial" plants the lowest (~25.5 mean, 20 median). The mode of chromosome numbers was 12 for biennials and 18 for the rest of the forms in Asteraceae. In Asteraceae the differences between "annual and biennial" and perennial were significant (P≤0.01). Perennials had the highest polyploid frequency under all estimation methods (57–68%); in all the short-lived groups it ranged from 26 to 54%. These differences were significant in some cases: for "annual and biennial" versus perennial for the n≥11 threshold and the x method, and for biennial versus perennial for the x method.

Poaceae were represented by two groups with ten or more cytotypes (annuals, perennials). Of these, perennials had higher mean and mode chromosome numbers but the differences were not significant by the Mann-Whitney test or tests for polyploid frequency.

Perennials and shrubs were two life forms having more than 10 cytotypes in Rosaceae, also common group ("trees and shrubs") was created for all woody plants: shrubs, trees and species that can be trees or shrubs.

The perennials had higher mean (~34 vs. ~29) and median (31.5 vs. 28) chromosome numbers than shrubs; the modes were the same (28). The polyploid frequencies tended to be higher in perennials than in shrubs and all woody plants but these differences were not significant.

The next series of comparisons was between groups of life form types from different families. The most common life form was perennial, so it was compared among all three families. Generally, all means, medians and modes for chromosome number were similar and the Mann-Whitney test did not show significant differences. The frequency of polyploids was highest in Rosaceae (77–80%); in the other two groups the frequencies were at least 9% lower; the χ^2 test indicated significant differences at P≤0.01 for Asteraceae versus Poaceae (n≥14 threshold) and for Rosaceae versus Asteraceae (n≥14 threshold and x method).

Annuals were shared between Asteraceae and Poaceae. In this case the mean chromosome numbers were similar (~26) but there were greater differences in the modes (Asteraceae 18, Poaceae 14) and medians (Asteraceae 24, Poaceae 28). For frequency of polyploids neither the Mann-Whitney test nor the χ^2 test showed significant differences between families.

DISCUSSION

Surprisingly, Rosaceae and Asteraceae (both dicots) seem to differ more from each other than they differ from Poaceae (monocots). The mean and median chromosome numbers were higher in Poaceae and Rosaceae than in Asteraceae. This order is similar to that given recently for the Italian flora (Bedini et al., 2012c); also worth noting is that for Asteraceae and Poaceae but not for Rosaceae the mean chromosome numbers for the Polish flora are higher than the means from that Italian study.

The order from polyploid-richest to polyploid-poorest in our study is Rosaceae→Poaceae→Asteraceae, but the differences between Asteraceae and Poaceae are weaker than between those two families and Rosaceae. Thus, for the three families that were examined, the phylogenetic similarities seem less responsible than other factors for the chromosome number distributions; life form apparently may be the factor chiefly responsible.

The examined life forms generally did not show statistically significant differences in chromosome number or polyploid frequency between the annual, biennial and "annual or biennial" groups, so a common group of short-lived plants was formed for them: "annual and biennial" (short-lived plants). For similar reasons the common group "trees and shrubs" (woody plants) was created.

When the life forms were compared for chromosome numbers, perennials appeared to have higher chromosome numbers than "trees and shrubs," followed by "annuals and biennials." Polyploid frequency gives a different series: "trees and shrubs" followed by perennials and then "annual and biennial" plants.

Our results are generally in line with those described for the British flora by Haskell (1952) and similar to earlier results from Stebbins (1938). The order that we arrived at, perennials→"trees and shrubs"→"annual and biennial" (with an occasional switch between perennials and woody plants), sug-

gests that the most obvious factor in increase of chromosome number is plant longevity. The differences were seen mainly between short-lived plants and those that live many years, such as perennials and woody plants. The differences between the latter two groups were not so marked in our study and both are regarded as long-lived forms.

Our results seem to support the idea that the differences between life forms are much more important determinants of differences in chromosome number than phylogenetic relations (though it should be mentioned that Asteraceae and Rosaceae are not very closely related). A closer look at the data shows that the situation is much more complicated. The important fact is that the distribution of life forms between families is not correlated with phylogenetic relations. The families that are more similar to each other (Asteraceae and Poaceae) in chromosome number and polyploid frequency are also similar in their life forms. Both groups share annuals, "annual or biennial" and perennials. Only the last category, perennials, is shared by Rosaceae, which is abundant in woody plants not present in the other families. Thus, a comparison of woody plants versus short-lived plants is almost entirely a comparison of part of Rosaceae versus part of Asteraceae and Poaceae (two Rosaceae cytotypes are annuals).

In this situation, it is not a simple exercise to distinguish the effects of life form and phylogeny on chromosome number or polyploidy. For example, it may be that Rosaceae has more chromosomes because it is abundant in long-lived plants, but it may also be that the ancestors of that family had a high number of chromosomes and that this is why perennials and woody plants in that family have high chromosome numbers. The first of those explanations may be supported by the higher chromosome numbers in perennials of Rosaceae generally than in the other families examined.

To separate the influence of life form from phylogenetic factors we performed the series of comparisons of life forms from the same families. The results indicated that the differences between the life forms within the tested families are comparatively weak, but there were some differences between short-lived and long-lived species. The weaker differences between life forms from the same family may suggest that taxonomy may yet be relevant here, but our results suggest that the relation between chromosome number and the phylogenetics of the studied families is not a straightforward one.

Figure 1 may shed some light on this problem. The distributions of the chromosome numbers of Asteraceae and Poaceae are wide, and although the medians are different, the ranges of values overlap significantly and therefore the statistical tests do not result in significant differences between these groups. By contrast, Rosaceae is represented by a smaller box and the part below the median is very compact, which indicates a high concentration of the same 2n values. Indeed, almost half of the chromosome numbers are at 28, the value of the mode and median. Shrubs are mainly responsible for the concentration of values at 2n=28, found in over 62% of the cytotypes (data not shown). The span of 2n values is wider in the group of all woody species in Rosaceae but still narrower than for the other groups. A similar concentration of chromosome numbers was also found more generally for the order Rosales in the Italian flora (Bedini et al., 2012c).

This concentration of chromosome numbers strongly influences the results of the tests for polyploid frequency. The threshold for the n≥14 method is 2n=28; this means that most of the cytotypes are qualified as polyploids for both threshold methods, resulting in a high representation of polyploids in woody plants and consequently in Rosaceae. This explains why "trees and shrubs" are more abundant in polyploids than the perennials in our comparisons. Such a strong influence of the exact 2n number on polyploid frequency as estimated by the threshold method suggests that this method is oversensitive in such situations. The result would have been dramatically different if the threshold had been only one or two chromosomes more. It is doubtful that such a small difference can really reveal the polyploid frequency.

The third method, based on x values, should give more reliable results. Trees are generally regarded as having a higher basic chromosome number than herbaceous plants (Stebbins, 1938; Levin and Wilson, 1976) but our dataset does not show that (Fig. 2). The median x values for short-lived plants were higher (7.5–9) than for woody plants (Rosaceae) and for the perennials of Rosaceae and Poaceae (7), and they had a much wider spread, mainly at the high values. The 2n values for the groups within Rosaceae were rather high, so consequently the polyploid frequency as estimated by the x method was also high. However, for short-lived plants (especially in Asteraceae), high x values and low 2n numbers resulted in comparatively low polyploid frequencies.

Although the differences in chromosome number distributions between Rosaceae and the other two families obscure the general gradation of chromosome number from perennials to woody plants to short-lived "annual and biennial" plants, this pattern is easily seen in Figure 1.

Perennials were also found to be richest in polyploids in the flora of Pakistan (Baquar, 1976; Khatoon, 1991), but other studies suggest that annual plants are the most polyploid-rich (Khatoon and Ali, 2006). Such a difference probably is connected with the different histories of the

floras of Pakistan and Poland; the latter is thought to have formed mainly during the Holocene after the last glaciation (Szafer and Zarzycki, 1977). Also, alien plants in the Polish flora generally have lower chromosome numbers and lower polyploid frequency than native plants (Góralski et al., 2014), and alien plants may be more common in annuals (and biennials) than in perennial or woody plants; that hypothesis needs to be confirmed in future studies.

The higher polyploid frequency in perennial herbs than in short-lived herbs may be explained by connecting polyploidization with hybridization – hybrids frequently are not fertile, but fertility may be reestablished by polyploidization, which is much more probable in long-lived perennials that can reproduce vegetatively than in herbs that live for only one or two years (Stebbins, 1938). Under such assumptions, "trees and shrubs" should have a high chromosome number, and indeed they seem to have more chromosomes than "annual and biennial" plants but fewer than perennials. Stebbins explained that polyploidy disturbs the size relationships of the cells so that wood fibers cannot be produced satisfactorily by cambium, and he also noted that trees are cytologically stable, but the relations between ploidy level and anatomical and histological features have not yet been thoroughly examined and understood.

The relationships between chromosome number or polyploidy and life forms are not yet clear. Our results add some valuable data to the discussion. Analyses and comparisons of more families and the life forms within them are needed. The differences in chromosome numbers between life forms may be correlated with other features such as the ability to colonize new areas or invasiveness, as recently found for the Polish flora (Góralski et al., 2014).

CONCLUSIONS

1. Chromosome numbers and polyploid frequencies were described for three families (Asteraceae, Poaceae, Rosaceae) in the Polish flora and for their life forms.
2. Asteraceae and Rosaceae are both dicots but Asteraceae is more similar in life forms to Poaceae than to Rosaceae.
3. Poaceae and Rosaceae generally have higher chromosome numbers and higher polyploid frequencies than Asteraceae.
4. The distribution of chromosome numbers is comparatively narrow in Rosaceae (in most cases 2n=28) and for its woody plants, especially shrubs. Wider distributions were found in Asteraceae and Poaceae and their life forms.
5. Long-lived plants (perennials, trees and shrubs) generally have higher chromosome numbers and higher polyploid frequencies than short-lived species (annuals and biennials). These results are similar to those for the British flora but differ from some results from Pakistan.
6. Threshold methods may be oversensitive in situations in which, as in Rosaceae, the most frequent 2n value is 28, the threshold for the n≥14 method.

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