CHROMOSOME NUMBERS OF POLISH FERNS

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The paper reports the results of karyological studies on seven fern species from the Polish Carpathians. Their chromosome numbers counted in root tip mitoses are reported for the first time from Poland. The specimens originated from the Tatra Mts and Pieniny range. Chromosome numbers were established for Asplenium viride (2n = 72), Athyrium filix-femina (2n = 80), Dryopteris filix-mas (2n = 164), Gymnocarpium dryopteris (2n = 160), Gymnocarpium robertianum (2n = 160), Phegopteris connectilis (2n = 90) and Phyllitis scolopendrium subsp. scolopendrium (2n = 72). The chromosome numbers from the Polish material match those reported from other countries. Information on the distribution and habitats of the investigated fern species is also provided.

Key words: Pteridophytes, ferns, chromosome numbers, southern Poland, Carpathians.

INTRODUCTION

Much scientific work on pteridophytes has been done over the last three centuries. One field of very intensive research is pteridophyte cytology, initiated by Manton’s (1950) classic work PROBLEMS OF CYTOLOGY AND EVOLUTION IN THE PTERIDOPHYTA. Probably no other group of plants has been the object of such an explosive increase in cytological research on such a worldwide scale, yielding valuable information for problems of phylogeny and classification. Cytological studies have resolved various taxonomic problems and have revealed details of evolutionary patterns and speciation processes (Lovis, 1977).

Much of the work of fern cytologists has concentrated on determining present-day basic chromosome numbers and their interpretation in terms of affinities. Unlike in flowering plants, the most important general characteristic of the pteridophytes is the high level of basic numbers. The basic chromosome numbers of homosporous pteridophytes are the highest known in vascular plants, averaging somewhere around x = 35–69. Some genera have much higher basic numbers, such as Ophioglossum with x = 120 and Equisetum with x = 108. For the most part, heterosporous pteridophytes tend to have low chromosome numbers like those in seed plants, for example Isoëtes with x = 11, Salvinia with x = 9 or Selaginella with x = 7, 8, 9 and 10 (Wagner and Wagner, 1980; Pichi Sermolli, 1987).

Among the processes that have played significant roles in pteridophyte differentiation and speciation, polyploidy seems to be the most important. Other mechanisms such as aneuploidy and apomixis have also contributed to differentiation, but are much less common. Cytological and cyrogenetic investigations have revealed that polyploidy is very frequent in Pteridophyta. At least half the fern species are thought to be polyploids or to consist of polyploid cytotypes. The majority of polyploid ferns appear to be allopolyploids, and the success of polyploidy seems closely associated with hybridization (Vida, 1976; Bir, 1988; Cubas, 1989).

With very few exceptions the European ferns have been studied cytologically somewhere in Eu-
rope, so the chromosome numbers of the typical European ferns are basically known. Chromosome counts assist in answering different questions: whether polyploidy is connected with the geographical distribution and ecology, how frequent polyploidy is, etc. Cytological studies should help settle which species are uniform in chromosome number and which show variation in ploidy, whether one or more species should be recognized in some problematic taxa, the parentage of species, and identification of species cytologically distinct from others.

In Poland, many karyological studies of flowering plants have been done, but very few on pteridophytes. Of 73 ferns and fern allies occurring in Poland (Piękos-Mirkowa, 1999), chromosome numbers have been established for only eight species and one hybrid so far. Among them is one representative of the quillwort *Isoëtes lacustris* L. (2n = 110) (Rychlewski and Jankun, 1972), the hybrid *D. expansa × d. dilatata* (2n = 123) (Piękos, 1974) and seven species of ferns: *Cystopteris fragilis* (L.) Bernh. (2n = 168) (Vida, 1974), *Asplenium ruta-muraria* L. (2n = 144), *Polypodium vulgare* L. (2n = 148), *Salvinia natans* (L.) All. (2n = 18) (Rychlewski and Jankun, 1972), *Dryopteris expansa* (C. Presl) Fraser-Jenkins et Jermy × *d. dilatata* (Hoffm.) A. Gray (2n = 164) and *D. carthusiana* (Vill.) H. P. Fuchs (2n = 164) (Piękos-Mirkowa, 1979).


### MATERIALS AND METHODS

Plant material was collected from natural habitats in the Tatra Mts. and Pieniny range in May and June 2000 (Table 1). Vouchers are deposited in the herbarium of one of the authors (DI).

### Table 1. List of localities and chromosome numbers of examined fern species

<table>
<thead>
<tr>
<th>Species</th>
<th>Place of origin</th>
<th>Habitat</th>
<th>N Lat.</th>
<th>E Long.</th>
<th>Altitude m.a.s.l.</th>
<th>2n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phegopteris connectilis</em> (Michx.) Watt</td>
<td>1. Tatra Mts.: Droga pod reglami</td>
<td>Carpathian beechwood</td>
<td>49°6'</td>
<td>19°56'</td>
<td>890</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>2. Tatra Mts.: Kuźnice</td>
<td>Dentario glandulosae-Fagetum</td>
<td></td>
<td></td>
<td>1060</td>
<td>90</td>
</tr>
<tr>
<td><em>Asplenium viride</em> Huds.</td>
<td>3. Tatra Mts.: Strążyska Valley</td>
<td>Dolomite rocks</td>
<td>49°13'</td>
<td>19°55'</td>
<td>1105</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>4. Tatra Mts.: Kościesia Valley</td>
<td>Calcareous rocks</td>
<td>49°15'</td>
<td>19°52'</td>
<td>–1000</td>
<td>72</td>
</tr>
<tr>
<td><em>Phyllitis scolopendrium</em> (L.) Newman subsp.</td>
<td>5. Pieniny Mts.: near Krościenko village</td>
<td>Phyllitido-Aceretum forest</td>
<td>49°45'</td>
<td>20°41'</td>
<td>–600</td>
<td>72</td>
</tr>
<tr>
<td><em>Athyrium filix-femina</em> (L.) Roth</td>
<td>6. Tatra Mts.: Droga pod reglami</td>
<td>Carpathian beechwood</td>
<td>49°16'</td>
<td>19°56'</td>
<td>890</td>
<td>80</td>
</tr>
<tr>
<td><em>Gymnocarpium dryopteris</em> (L.) Newman</td>
<td>7. Tatra Mts.: Kuźnice</td>
<td>Picea abies forest</td>
<td>49°15'</td>
<td>19°58'</td>
<td>1000</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>8. Tatra Mts.: Kościesia Valley</td>
<td>Picea abies forest</td>
<td>49°15'</td>
<td>19°52'</td>
<td>1000</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>10. Tatra Mts.: Carpathian beechwood</td>
<td>Dentario glandulosae-Fagetum</td>
<td></td>
<td></td>
<td>130</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>11. Tatra Mts.: Kuznice - Hala Gąsienicowa</td>
<td>Secondary spruce forest</td>
<td>49°15'</td>
<td>19°55'</td>
<td>1130</td>
<td>164</td>
</tr>
</tbody>
</table>

Sporophyte chromosome numbers were counted in root tip meristems using squash technique. Root tips were taken directly in the field and pretreated with 0.5% colchicine for 4 to 5 h, fixed in 96% ethanol:glacial acetic acid (3:1) and stored at -6°C for more than 24 h. Hydrolyzation was carried out with 1N HCl for about 40 min at 60°C. Root tips were stained with hematoxylin after Gomori (Melander and Wingstrand, 1953) at 60°C.
RESULTS AND DISCUSSION

The seven studied fern species (Tab. 1) occur widely in Europe and their chromosome numbers have been reported from different countries. Our results from Polish material proved to be in accordance with reports of other authors. The matching chromosome numbers found in plants from other parts of their areas of distribution indicate that the examined species are karyologically uniform.

**THELYPTERIDACEAE**

Phegopteris connectilis (Michx.) Watt

(2n = 3x = 90)

Three species belong to the genus Phegopteris: P. hexagonoptera (Michx.) Fée distributed in eastern North America, P. decursive-pinnata (H. C. Hall) Fée in East Asia, and P. connectilis (Michx.) Watt, a circumboreal species widely distributed in Eurasia and North America (Smith, 1990). The latter species is very common in Poland in both lowlands and mountains. In the Tatra Mts it reaches 1626 m a.s.l. (Pawłowski, 1956), occurring abundantly in a wide range of habitats and communities.

The base numbers in the family Thelypteridaceae include x = 27, 29–36. The genus Phegopteris has a single base number x = 30. Both diploids and tetraploids are known in many species of the family, and a few higher ploids are also known (Smith, 1990). Apomixis is found only in Phegopteris connectilis (Manton, 1950); this species is an apogamous triploid in most of its distribution area. A sexual diploid with n = 30 is known from Japan (Hirabayashi, 1969).

The somatic triploid chromosome number 2n = 90 (Fig. 1), reported here for the first time from Poland, was found in all specimens investigated (Tab. 1). This agrees with previous counts of the meiotic number n = 90 or the mitotic number 2n = 90 from plants from Europe, Asia and America (Mitui, 1976; Mulligan and Cody, 1979; Khullar et al., 1983; Khullar et al., 1988; Matsumoto and Yano, 1989; Matsumoto and Nakaike, 1990. For other references see: Löve et al., 1977). Mulligan and Cody (1979) reported n = 120 in Canadian plants, while Matsumoto and Yano (1989) observed n = 60 in Japanese plants of this species.

**ASPLENIACEAE**

Asplenium viride Huds. (2n = 2x = 72)

The genus Asplenium is one of the largest fern genera in Europe, consisting of 30 species and 50 hybrids (Reichstein, 1981). This genus is notable for the large number of diploid species known – approximately half of the recorded taxa in Europe. Polyplody also occurs, but only tetraploid cytotypes have been recognized (Lovis, 1973). Both autopoloids and allopoloids appear to be common in the genus. Cytogenetic studies have clarified evolutionary relationships and taxonomic implications within Asplenium.

Asplenium viride is an Arctic-Alpine species. It occurs in southern Poland, having its center in the Sudety Mts and in the Carpathians. It is a monotypic species extending in the Tatra Mts from the base up to 2140 m a.s.l. (Pawłowski, 1956). It grows abundantly in limestone and dolomite rock crevices as a component of communities of the order Potentillata caulescentia.

Asplenium viride was the only representative of the genus Asplenium in Central Europe, a north-temperate species occurring in Macaronesia (except the Cape Verde Is.), Western, Central and Southern Europe, eastward to Crimea, North Africa from Morocco to Libya, the Caucasus, Turkey and north Iran. This montane fern is distributed in southern Poland, having its center of occurrence in the Carpathians and the Cracow-Częstochowa Jura. P. scolopendrium is locally frequent, but rare or absent elsewhere. It is encountered most frequently and abundantly in the Pieniny range. The species is confined to substrate rich in calcium carbonate. It grows on stony or rocky slopes exposed mainly to the north or northeast, within shaded and moist deciduous forests representing the Phylitido-Aceretum association or Dentario glandulosae-Fagetum lunarietosum subassociation.

Asplenium viride has not been studied from Polish material until now. Our count yielded a
Figs. 1–7. Metaphase plates from root tip meristems. Fig. 1. Phegopteris connectilis (2n = 90). Fig. 2. Asplenium viride (2n = 72). Fig. 3. Phyllitis scolopendrium subsp. scolopendrium (2n = 72). Fig. 4. Athyrium filix-femina (2n = 80). Fig. 5. Gymnocarpium dryopteris (2n = 160). Fig. 6. Gymnocarpium robertianum (2n = 160). Fig. 7. Dryopteris filix-mas (2n = 164). Bars = 10 µm.
diploid mitotic chromosome number of 2n = 72 (Tab. 1; Fig. 3). This count is in accordance with the results of many previous investigations from various European countries (Rivas Martínez et al., 1981; Jávůrková-J arolimová, 1992; Ivanova, 1997, 1998; refs. in: Löve et al., 1977; refs. in: Ivanova, 1997). Cody and Mulligan (1982) reported the meiotic number n = 72 for Canadian plants belonging to P. scolopendrium var. americanum.

WOODSIACEAE (ATHYRIACEAE)

Athyrium filix-femina (L.) Roth (2n = 2x = 80)

All three species of the genus Athyrium occurring in Europe are represented by sexual diploids (Schneller and Rasbach, 1984). Among them, A. filix-femina is one of the most common woodland ferns, with a circumboreal distribution pattern. It is widespread throughout Poland. It is very common and abundant in the Carpathians, extending up to 1620 m a.s.l. in the Tatra Mts, where it prefers rendzinas (especially brown and humus) derived from dolomites and limestones (Piękos-Mirkowa et al., 1999). This fern is most frequent in the forest belt in communities of the orders Fagetalia sylvaticae and Vaccinio-Piceetalia. It can also be found in the subalpine belt in tall herb vegetation of the order Adenostyletalia alliaei. The basic chromosome number of the genus Athyrium is x = 40 (Kato and Kramer, 1990). The diploid chromosome number 2n = 80 (Tab. 1; Fig. 4) is the first report of the cytology of A. filix-femina from Poland. It agrees with previous counts of n = 40 bivalents or 2n = 80 chromosomes (Mahabale and Kamble, 1981; Cody and Mulligan, 1982; Ivanova, 1997; Montgomery et al., 1997; refs. in: Löve et al., 1977; refs. in: Ivanova, 1997). Our result does not agree with the meiotic number n = 45 established by Horjales et al. (1990) in Spanish A. filix-femina, nor with n = 80 reported for var. dombei in South America by Kurita (1986).

Gymnocarpium dryopteris (L.) Newman (2n = 4x = 160)

The genus Gymnocarpium includes six species (Sarvela, 1978). Three of them belong to the European flora – two tetraploids and one diploid. One of the most common is G. dryopteris, with a wide circumboreal distribution. It occurs throughout Poland, reaching up to 1680 m a.s.l. (Pawłowski, 1956). In the Tatra Mts this fern can be found abundantly in various forest communities, both in beech woods of the order Fagetalia sylvaticae and in coniferous forest and Pinus mugo shrubs of the order Vaccinio-Piceetalia.

Gymnocarpium has a basic chromosome number of 40 (Kato and Kramer, 1990). No previous record of a G. dryopteris chromosome number is known from Poland. All specimens investigated showed a tetraploid mitotic number, 2n = 160 (Tab. 1; Fig. 5). The same number of 160 chromosomes or the meiotic number n = 80 were counted by the authors cited in Löve et al. (1977), and by Löve and Löve (1980), Cody and Mulligan (1982) and Pryer and Hauller (1993). Kato et al. (1992) indicated 2n = 80 for the same species in China, differing from the other mentioned authors.

Gymnocarpium robertianum (Hoffm.) Newman (2n = 4x = 160)

Gymnocarpium robertianum complex includes two species: G. jessoense (Koidz.) Koidz. and G. robertianum (Hoffm.) Newman s. str. (Sarvela et al., 1981). The latter has an amphiatlantic range. It occurs in Europe, in the Caucasus and in eastern North America. In Poland the species is distributed mainly in the mountains (Sudety Mts and West Carpathians) and in their forelands. It is much rarer and scattered in lowlands. In the Tatra Mts G. robertianum is a rather common species, mainly in the lower and upper montane forest belts, also extending up to the subalpine belt, reaching 1700 m a.s.l. (Pawłowski, 1956). It grows on substrate rich in calcium carbonate. The species is associated with scree and crevice vegetation of the class Thlaspietea rotundifoli and Asplenietea rupestria. It can also be found in stony and rocky forests, though avoiding places with dense shade.

Our count of 2n = 160 (Tab. 1; Fig. 6) is the first cytological record of G. robertianum from Poland. Previous reports from European and North American countries by Manton (1950), Vida (1963), Wagner (1963), Löve and Löve (1967) and Sarvela et al. (1981) have described it as a tetraploid sexual species with 80 bivalents or 160 chromosomes.

DRYOPTERIDACEAE

Dryopteris filix-mas (L.) Schott (2n = 4x = 164)

Dryopteris is largely a north-temperate genus including about 240 species and a great number of hybrids (Fraser-J enkins and Reichstein, 1984). Cy-
totaxonomically it is one of the most studied genera. Genome analysis of interspecific hybrids have shown how polyploid taxa originated and have shed light on the important role of hybridization and polyploidy in evolution and speciation within the genus Dryopteris (Gibby, 1979, 1985; Gibby and Walker, 1977).

In Poland, one of the most common and abundant Dryopteris species is D. filix-mas. It is widespread in both lowlands and mountains. In the Carpathian Mts it occurs in the forest belt and coniferous pine (subalpine) belt, extending up to 1770 m a.s.l in the Tatra Mts (Pie˛kos´-Mirkowa and Miechówka, 1992). It prefers deciduous forest communities and coniferous forests and Pinus mugo shrubs of the order Vaccino-Piceetalia. It can also be found in tall herb communities of the order Adonostyletalia. It occupies several soil types, but in the Tatra Mts it is most common on rendzinas derived from limestones, dolomites and marls (Pie˛kos´-Mirkowa et al., 1999).

High constancy of chromosome number is characteristic of the family Dryopteridaceae. Basic numbers of 40 and 41 have been established. The basic number in the genus Dryopteris is x = 41 (Kramer, 1990).

The established tetraploid chromosome number 2n = 164 (Tab. 1; Fig. 7) is reported for the first time from Poland material. It confirms determinations of n = 62 bivalents or 2n = 124 chromosomes by Schnell (1975), Windham (1983), Al-Bermani et al. (1993), Stepanov (1994), Jäger and Leonhards (1995), Ivanova (1997, 1998), and many other authors cited in Lê et al. (1977). Gibby (1985a) reported 2n = 164 for D. filix-mas from northern Pakistan. Our count is in disagreement with n = 41 reported by Mahabale and Kamble (1981) and by Singh (1974) for Indian plants.

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