Sexuality, apomixis, ploidy levels, and genomic relationships among four Paspalum species of the subgenus Anachyris (Poaceae)

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Abstract

The subgenus Anachyris of the genus Paspalum comprises six species, all native to the New World. Cytology, embryology, fertility, and hybridization experiments were examined in four of the six species and different chromosome races. Meiotic chromosome behavior supported autoploid origin for tetraploid P. procurrens and tetraploid P. usterii, and also corroborates autoploidy for P. malacophyllum. Meiosis of the three species was compared with the meiotic chromosome behavior of a tetraploid P. simplex strain acquired by artificial autopolyploidization. Moreover, chromosome associations observed in tetraploid interspecific hybrids of P. simplex/procurrens and P. simplex/usterii, in triploid hybrids of P. simplex × malacophyllum, and in diploid hybrids of P. simplex × malacophyllum, indicate that the four species share the same basic genome, and that the polyploid races are autoploids. Facultative apomixis of the aposporous type is reported for the first time in tetraploid strains of P. procurrens and P. usterii. It is also confirmed for tetraploid P. malacophyllum, a species whose diploid representative is here reported to reproduce sexually, though embryological analyses demonstrated some potential for apospory. Our results suggest that the whole subgenus Anachyris of Paspalum is basically an agamic complex. At least three species: P. simplex, P. procurrens, and P. malacophyllum involve sexual self-incompatible diploid races as well as apomictic polyploid (mainly tetraploid) representatives.

Keywords: Paspalum; Anachyris; Autoploidy; Interspecific hybrids

Introduction

The subgenus Anachyris of the genus Paspalum includes six species according to the taxonomic revision of Morrone et al. (2000). The subgenus is distributed from Mexico to Brazil, Paraguay, Bolivia, Argentina and Uruguay. The main distinctive morphological characteristics are: the boat-shaped, concave-convex spikelets; the second glume typically missing, though occasionally and incompletely developed in two species; and the upper lemma with prominent longitudinal nerves on the abaxial surface.

In the genus Paspalum, most species are characterized by the existence of different conspecific cytotypes. Usually, the diploid strains reproduce by sexuality and
cross-fertilization, due to self-incompatibility. The conspecific polyploid strains, usually tetraploids, reproduce by means of aposporous apomixis (Quarin, 1992). Among the species of the subgenus Anachyris, P. simplex Morong is a good example of this genetic system: the sexual cross-pollinating diploid cytotype has a limited distribution area in central-northern Argentina, while the apomictic tetraploid cytotype has a broader distribution including areas of Bolivia, Paraguay, Brazil, Argentina and Uruguay. Two uncommon triploid plants have been collected. One of these was sexual and the other was apomictic. In addition, hexaploid apomictic plants have occasionally been found accompanying tetraploid populations (Urbani et al., 2002). Sexual self-incompatible diploid accessions of P. procurrens Quarin are known to be geographically congruent and cytologically related to diploid P. simplex (Espinoza and Quarin, 1998). Until now, polyploid accessions have not been reported for P. procurrens.

P. malacophyllum Trin. is the most widely distributed species and shows the greatest morphological variation among the species (Morrone et al., 2000) of Anachyris. The literature contains conflicting reports concerning the mode of reproduction of this species, though all studies indicate that the species is tetraploid. It was first reported to be an aposporous apomict by Brown and Emery (1958). Later, Bennett and Bashaw (1966) indicated that the species was sexual and autotetraploid. Based on cytotoxicological studies of 16 accessions collected in different locations of Argentina and Brazil, Burson and Hussey (1998) considered P. malacophyllum to be an autotetraploid, with the mode of reproduction being facultative apomixis. All the analyzed accessions showed aposporous embryo sac development, though some meiotically derived embryo sacs were observed in all of them to different degrees of development. Recently, rare diploid accessions of P. malacophyllum were found in southern Brazil (Pozzobon, M., personal communication).

Limited information exists concerning ploidy level and mode of reproduction of the remaining three species of the subgenus Anachyris. Paspalum usterii Hackel and P. volcanensis Zuloaga, Morrone & Denham (sub Paspalum aff. malacophyllum) were reported to be tetraploid (Hunziker et al., 1998). There is no information concerning the reproductive mode of these two species and of the ploidy level and reproduction of P. costellatum Swallen.

Taking advantage of the existence of sexual diploid genotypes in P. simplex and P. procurrens, Espinoza and Quarin (1998) hybridized these species. The high level of cross-fertilization (66%), the regular meiotic chromosome pairing in the F1 hybrids, and the proportion of spikelets that set seeds in the hybrids (62%) suggested a very close evolutionary relationship between these species.

Because all known tetraploid strains of the subgenus Anachyris are aposporic or highly aposporic, it would be difficult to study the genomic relationship among its species with interspecific crosses. The acquisition of sexual tetraploid plants of P. simplex from colchicine treatment of diploid plants (Cáceres et al., 1999) bypassed the difficulty of hybridization. The induced tetraploid P. simplex reproduces sexually and could be emasculated and pollinated with pollen of the other apomictic species of the subgenus.

The aims of this investigation were: (1) to report the occurrence of tetraploidy in P. procurrens; (2) to determine the meiotic chromosome behavior and the mode of reproduction of tetraploid accessions of P. usterii and P. procurrens, and of a diploid and two newly acquired tetraploid germplasm accessions of P. malacophyllum; (3) to make interspecific crosses between a sexual, colchicine-induced tetraploid plant of P. simplex and three tetraploid species of the subgenus Anachyris: P. malacophyllum, P. procurrens, and P. usterii in order to determine their genomic relationships and the feasibility of introgression of genes among these species via hybridization programs; (4) to hybridize diploid P. simplex and the recently acquired diploid germplasm of P. malacophyllum to assess the degree of homology of their genomes and the level of fertility of their hybrids.

Material and methods

Plant materials

The sexual diploid accession of P. simplex Q4109 was originally collected at Los Gatos ranch, Santiago del Estero, Argentina. The individual plant no. 59 was used as female parent in crosses with diploid P. malacophyllum.

Cuttings of an original colchicine-induced tetraploid plant of P. simplex were introduced to Argentina from Italy. This sexually reproducing tetraploid plant was experimentally produced at the Institute of Plant Genetics, CNR Research Division of Perugia (Cáceres et al., 1999). The plant was indeed a mixoploid with diploid and tetraploid sectors, but predominantly tetraploid. It was identified as 11B1a, multiplied vegetatively, and grown in pots in a greenhouse at IBONE, Corrientes. Most crosses were carried out using plant 11B1a as the female parent in interspecific crosses. The exception was the P. simplex × usterii cross for which the plant named C1-2 of P. simplex was used as the female parent. Plant C1-2 is a sexual tetraploid that originated from self-pollination of plant 11B1a. 

P. procurrens, accession Q4094, is a newly acquired germplasm collected at El Salvador, Chuquisaca,
Bolivia, by C. Saravia-Toledo, and grown from seeds in Corrientes, Argentina, by routine cultural practices.

Two 4x accessions of *P. malacophyllum* were used for interspecific crosses with induced 4x *P. simplex*: accession TK2449, collected by Tim Killeen 2 km N of Concepción, Estancia El Recreo, Santa Cruz, Bolivia (16°12’S, 62°08’W), and grown from seed at Corrientes. It was previously reported to be tetraploid (Norrmann et al., 1994) and represents the typical morphology of the species, very similar to the drawings published with the original description by Trimius (1831). The second accession, V5095, was collected by José F. Valls, on the road between Itumbiara and Bom Jesus de Goiás, Brazil, and was reported to be tetraploid (Honfi et al., 1990). Then Pagliarini et al. (2001) studied the meiotic behavior of this same accession, under its Brazilian germplasm code BRA-003077, and found an average of 0.86 univalent, 10.34 bivalent, 0.48 trivalent and 4.05 quadrivalent chromosomes per cell. It is an atypical plant with short and extremely broad leaf blades (usually more than 4 cm wide). The plant was introduced as cuttings to Corrientes, Argentina, in the early 1990s and maintained in the field by routine cultural practices. Unlike the accession TK2449 that represents the morphological archetype of the species, the plant V5095 represents an extreme degree of variation regarding the shape and size of leaf blades. It might well be considered a different taxon due to these morphological dissimilarities and its decumbent growth habit.

*P. usterii* was collected by Ana Honfi, accession 1175, at Teyucuare, Misiones, Argentina, and cultivated from pieces of rhizomes in Corrientes, Argentina.

The diploid accession V14855 of *P. malacophyllum* was collected 7 km north of Correia Pinto, state of Santa Catarina, Brazil (27°31’48.3”S, 50°22’11.7”W). The growth habit and the leaf shape of this diploid plant resemble the tetraploid accession V5095, but the size of the whole plant and particularly the leaves is much smaller.

Vouchers of the studied material are deposited in the CTES herbarium at Corrientes, Argentina, with exception of *P. malacophyllum*, accessions V5095 and V14855, which are deposited in the CEN herbarium at Brasilia, Brazil.

**Interspecific hybridizations**

The female parent, a potted plant of 11B1a, was introduced into a special humidity chamber late in the evening. Only one inflorescence, ready to start blooming the next day, was left on the plant. Early in the morning, a programmed humidifier raised the relative humidity in the chamber to about 100%. At blooming time, soon after sunrise, the anthers remained indehiscent after anthesis due to the high humidity in the chamber. The hanging indehiscent anthers were eliminated with thin-pointed tweezers. Then, pollen of the desired male parent was harvested in glassine bags and dusted on the emasculated inflorescence. The plant was returned to the greenhouse with the inflorescence bagged in a glassine bag to prevent later undesired pollination. The whole procedure was repeated for 5–6 days until all the spikelets of the target inflorescence completed blooming and were pollinated. Approximately 30 days later, the inflorescence was harvested, the fertile spikelets were separated from the empty ones and sown in sterilized soil the following spring. Seedlings were separately grown in 200 cm³ pots and then transplanted to the field. The same procedure was followed when the tetraploid plant C1-2 was used as female parent.

To cross 2x accessions of *P. simplex* and *P. malacophyllum*, a self-incompatible single plant of 2x *P. simplex*, accession Q4109, was isolated and daily dusted with pollen of accession V14855 of diploid *P. malacophyllum* until the target inflorescence completed flowering.

**Hybrid identification and crossability**

Because the emasculation procedure might allow some minimal degree of self-pollination, and although the female parents produced few seed under self-pollination conditions, the progenies were controlled to ensure that each descendant was of hybrid origin. The recognition of the hybrids in the progeny of *P. simplex × procurrens* crosses was accomplished by observation of the growth habit: the female parent, *P. simplex*, has an erect habit while *P. procurrens* is stoloniferous, a dominant character. Thus, the occurrence of stoloniferous branches in the offspring was an indicator of hybridity. The hybrid origin among tetraploid individuals of the *P. simplex × malacophyllum* was estimated by morphological characteristics because there are clear differences between the parental species. Because the morphological characteristics of *P. simplex* and *P. usterii* are less contrasting than in the interspecific crosses mentioned above, the recognition of the hybrid origin of the progeny was conducted by molecular marker analysis: Specific bands generated by randomly amplified polymorphic DNA markers (RAPD) in the male parent were considered indicative of hybridization when the bands were observed in any individual plant of the progeny. The DNA extraction and RAPD procedures were as described in Acuña et al. (2004).

The whole progeny of the 2x *P. simplex* (female) × 2x *P. malacophyllum* (male) cross was assumed to be hybrids since the female progenitor was a self-incompatible plant, and because most morphological characteristics of the progeny were intermediate between parents.
The degree of crossability among the different species was calculated as the number of hybrids recovered for every hundred pollinated spikelets. In *Paspalum*, each spikelet bears a single perfect floret and the ovary supports a single ovule.

**Cytology**

Mitotic chromosome numbers were determined in root tips, collected from potted plants, pretreated in a saturated aqueous solution of *z*-bromenaphthalene for 2 h, hydrolyzed with 1N HCl for 10 min at 60 °C, stained with Feulgen’s reagent and squashed in a drop of 2% acetic orcein.

Inflorescences of parental plants and of selected hybrids were fixed in 3:1 solution of absolute ethanol–glacial acetic acid and stored in 70% ethanol at 5 °C. Pollen mother cells (PMC) for the study of meiotic chromosome behavior were stained with 2% aceto-carmine.

**Mode of reproduction**

The mode of reproduction of tetraploid *P. procurrens* and of two 4x accessions of *P. malacophyllum* was determined by the study of megasporogenesis and embryo sac development. Ovaries at different stages of development were fixed in FAA (10% ethanol, glacial acetic acid, and 37% formaldehyde in the ratio 18:1:1), dehydrated in a tertiary butyl alcohol series, and embedded in paraffin. Material was sectioned 12 μm thick, stained in safranin–fast green staining series, and observed under bright-field microscopy. The proportion of ovules carrying aposporous or meiotic embryo sacs was determined in ovaries fixed at the time of anthesis. Only mature ovaries, fixed at the time of anthesis, were sectioned, stained, and analyzed to assess the mode of reproduction of *P. usterii* and the diploid accession V14855 of *P. malacophyllum*.

**Fertility**

In most species of *Paspalum*, including all species of the subgenus *Anachyris*, each spikelet contains a single perfect flower bearing a single ovule in its ovary. The degree of fertility was assessed as the proportion of spikelets that formed a mature caryopsis. Fertility was determined under self-pollination conditions, open-pollination, and by backcrossing. Self-pollination was achieved by bagging whole inflorescences with glassine bags to enforce self-pollination and prevent outcrossing. Open pollination was accomplished by harvesting mature inflorescences from a plant that flowered among other genotypes of the same species. For interspecific hybrids, open pollination fertility was analyzed in mature inflorescences of some selected plants of the hybrid population cultivated in the field without any control of pollination. Backcrossing was achieved by supplying parental pollen to some selected inflorescences of the hybrids.

**Results**

**The ploidy of *P. procurrens* and *P. usterii***

The accession Q4094 of *P. procurrens* was tetraploid with 2n = 40 chromosomes. Counts were performed on root tips of several plants obtained from seed of the original collection. The accession H1175 of *P. usterii* was established by cuttings of the original collection and had 2n = 40 chromosomes.

**Reproductive mode in male parental species**

Eighty-six mature ovules were observed in the diploid accession V14855 of *P. malacophyllum*. Most ovules (89.6%) showed a single embryo sac of the meiotic type (Table 1) with the egg apparatus (two synergids and the egg) toward the micropylar end, a large oblong and highly vacuolated central cell with two polar nuclei, and a mass of proliferated antipodal cells at the chalazal end. This is the typical feature of the embryo sac in sexually reproducing grass species. Occasionally, an aposporous embryo sac, bearing an egg cell and a central cell with two polar nuclei, was observed beside the typical meiotic sac and sharing the same ovule (4.6%), while 5.8% of the mature ovules failed to develop an embryo sac.

The reproductive mode was determined by the examination of megasporogenesis, megagametogenesis, and mature embryo sacs in tetraploid accessions of *P. malacophyllum* and *P. procurrens*. A conspicuously elongated megasporocyte was observed in young ovules of *P. malacophyllum* TK2449, before the inner integument completed development and before the micropyle was formed toward the base of the ovary. Meiosis took place during ovule rotation. The first cycle of meiosis and cytokinesis resulted in the formation of two dyad cells. The second division and cytokinesis took place in the chalazal member of the dyad and formed a final linear triad of megaspores with the simultaneous arrest of the second meiotic cycle in the micropylar dyad. The central megaspore degenerated immediately, followed by the abortion of the micropylar megaspore. The residue of this megaspore surrounded by a thick layer of callose remained as a dark and dense body in the micropylar end of the nucellus. By the time the ovule had finished rotation and the inner integument was complete (the outer integument was arrested in development and failed to cover the whole ovule), both
Table 1.  Embryo sac types in mature ovules of different Paspalum species and accessions

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>Number of ovules observed</th>
<th>Percentage of ovules with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>One meiotic sac</td>
</tr>
<tr>
<td>P. malacophyllum V14855</td>
<td>20</td>
<td>86</td>
<td>89.6</td>
</tr>
<tr>
<td>P. malacophyllum TK2449</td>
<td>40</td>
<td>67</td>
<td>3.0</td>
</tr>
<tr>
<td>P. malacophyllum V5095</td>
<td>40</td>
<td>76</td>
<td>17.1</td>
</tr>
<tr>
<td>P. procurrens Q4094</td>
<td>40</td>
<td>72</td>
<td>27.8</td>
</tr>
<tr>
<td>P. usterii H1175</td>
<td>40</td>
<td>79</td>
<td>35.4</td>
</tr>
</tbody>
</table>

Megaspores closest to the micropyle had already degenerated. The chalazal megaspore remained as the functional megaspore in 10 out of 17 ovules analyzed at this stage, while in the other seven ovules all three megaspores aborted. Active nucellar cells (initials of aposporous embryo sacs) with dark cytoplasm and a conspicuously enlarged nucleolus were observed close to the degenerated megaspores. Usually, when the chalazal megaspore remained functional it was also accompanied by one to several active nucellar cells. Thus, the majority of the ovules showed initials of aposporous embryo sacs at the end of megasporogenesis regardless of whether the chalazal megaspore remained functional or not. Aposporous embryo sacs developed from these initials.

At maturity, the aposporous sacs organized with an egg cell, one or two synergids (occasionally no synergids were observed), a large central cell with two (eventually three) polar nuclei, and usually an absence of antipodal cells. The number of aposporous sacs observed per mature ovule was variable, though usually only one or two became fully developed at maturity. When the chalazal megaspore escaped abortion it developed into a meiotic embryo sac, distinguishable by the proliferation of antipodal cells. At maturity, embryo sacs with few antipodal cells (3–6) were occasionally observed. Although these sacs might be aposporous sacs of the *Hieracium* type, we considered that all sacs bearing antipodal cells were meiotic embryo sacs. If this judgment is incorrect, the degree of sexuality would be somewhat overestimated. Proembryos were frequently observed in aposporous embryo sacs by the time of anthesis, prior to pollination. In a limited number of ovules, no female gametophyte developed. Megasporogenesis and embryo sac development of *P. malacophyllum* V5095 and *P. procurrens* Q4094 followed a similar pattern to the ones described above for *P. malacophyllum* TK2449. The main differences were that in *P. malacophyllum* V5095 usually female meiosis gave rise to four megaspores. Frequently, the four megaspores degenerated. A normal functional megaspore was observed in less than 10% of the ovules analyzed at the end of female meiosis. Development of proembryos by the time of anthesis, in aposporous embryo sacs, was observed less frequently in *P. malacophyllum* V5095 and in *P. procurrens* Q4094 than in *P. malacophyllum* TK2449.

In summary, four developmental classes of ovules were observed at maturity in all the tetraploid species or strains studied: (1) with one meiotic embryo sac; (2) with the meiotic embryo sac plus one or more aposporous sacs; (3) with one to several aposporous sacs; or (4) aborted ovules. The proportion of mature ovaries (fixed at anthesis) observed with these four classes of ovules in *P. malacophyllum* TK2449, *P. malacophyllum* V5095, *P. procurrens* Q4094 and *P. usterii* H1175 is shown in Table 1. It is considered that mature ovules bearing one or more aposporous sacs would lead to the formation of seed through parthenogenesis of the unreduced (2n) egg cell, and hence by mean of apomixis. On the other hand, those ovules carrying a meiotic embryo sac would need fertilization of the haploid (n) egg cell to form the embryo of a sexually formed seed. Those ovules carrying aposporous sacs together with the normal meiotic sac would give rise to seeds that may contain either a sexually formed embryo or a parthenogenic embryo, or both in the same seed. Although polyembryonic seeds are possible, germination of multiple seedlings from one seed was not observed in this material.

The reproductive mode of tetraploid *P. usterii* H1175 was assessed by the analysis of embryo sac types in mature ovules fixed at the time of anthesis. Nearly 42% of the ovules showed embryo sacs of the aposporous type (Table 1). In these ovules, usually one or two well-developed embryo sacs were present while some underdeveloped sacs were sporadically observed. Over 35% of the ovules had a single embryo sac characterized by an egg cell and two synergids toward the micropylar end, a large, highly vacuolated central cell with two nuclei (occasionally three), and three, rarely four, antipodal cells at the chalazal end. These antipodals showed mostly vacuolated and transparent cytoplasm with an inconspicuous or collapsed nucleus at the time of anthesis. We classified these sacs as meiotic embryo sacs because the development of antipodal cells, in the grass family, is a typical feature of embryo sacs that develop from the functional megaspore after meiosis.
Less frequently, some ovules (13.9%) showed a meiotic sac flanked by one or two aposporous sacs, and mature ovules lacking embryo sac were also observed (8.9%).

**Interspecific hybridization**

*Paspalum simplex 11B1a × procurrens Q4094*

From 542 spikelets of sexual-induced tetraploid *P. simplex*, which were emasculated and dusted with pollen of tetraploid *P. procurrens*, 92 caryopses were formed, and 74 seedlings were recovered after germination. Two seedlings died and 72 were transplanted to the field. Four adult plants showed the typical upright phenotype of the female parent and were considered to have originated from self-pollination of the female parent due to occasional failure in the emasculation process. The remaining 68 plants were classified as hybrids because all of them developed stoloniferous branches, which is a characteristic of the male parent, *P. procurrens*. Thus, the effective crossability between *P. simplex* and *P. procurrens* was 12.5% at the tetraploid level.

*Paspalum simplex 11B1a × malacophyllum TK2449*

One hundred and sixty-eight spikelets of a single *P. simplex* inflorescence were emasculated and pollinated with pollen of accession TK2449. Twenty-six spikelets formed caryopses and 22 seedlings were recovered after germination. The seedlings were transplanted to the field. Selected phenotypic characteristics were analyzed and compared with the parental traits. In all 22 progenies, most of the traits assessed were intermediate to those of the parents or closer to the parental plants, mainly in the size and shape of their leaf blades, the nine plants were easily classified as hybrids. All of them showed prominently broad leaf blades, a characteristic of the V5095 accession. The nine plants were cultivated in the field and showed very broad leaf blades ranging between 1.7 and 2.9 cm in width with a mean of 2.4 cm, while the mean leaf blade width for the 11B1a female parent was 0.9 cm, and 4.4 cm for the male parent V5095. Because the nine plants were classified as hybrids, the crossability was 3.78%.

*Paspalum simplex C1-2 × usterii H1175*

The crosses were conducted using the tetraploid plant C1-2 of *P. simplex* as female parent. Plant C1-2 in turn had been obtained by selfing the 11B1a plant. The pollinator was the tetraploid accession H1175 of *P. usterii*. A total of 2200 spikelets was emasculated and dusted with pollen of H1175. Only 29 spikelets set seed and 12 seedlings were recovered after germination in sterilized soil. However, three plants died in the field soon after transplanting, so the effective crossability was very low: 0.41%. In these crosses, the hybrid nature of the progeny was assessed through molecular markers because the morphological characteristics of the parents would not allow identifying the hybrid origin without any doubt. Genomic DNA from both parents was screened in order to identify specific bands from the

**Table 2. Morphological phenotypic characteristics of 4x Paspalum simplex (11B1a), 4x P. malacophyllum (TK-2449), and 20 individuals of their F₁ hybrids**

<table>
<thead>
<tr>
<th>Character</th>
<th>P. simplex 11B1a (female parent)</th>
<th>P. malacophyllum TK-2449 (pollen donor)</th>
<th>20 F₁ hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of leaf blades (cm)</td>
<td>20.4 (14–23)</td>
<td>39.6 (28–46)</td>
<td>33.6 (25–42)</td>
</tr>
<tr>
<td>Maximum width of the leaf blades (cm)</td>
<td>0.9 (0.6–1.3)</td>
<td>2.7 (2–3)</td>
<td>1.6 (1.3–2.5)</td>
</tr>
<tr>
<td>Distance from the place of maximum leaf blade width up to the base of the blade (cm)</td>
<td>6.0 (4.5–9)</td>
<td>19.4 (15–24)</td>
<td>12.7 (8–20)</td>
</tr>
<tr>
<td>Width of the leaf blade at one cm above its basal end (cm)</td>
<td>0.6 (0.4–0.8)</td>
<td>0.2 (0.15–0.25)</td>
<td>0.48 (0.2–0.9)</td>
</tr>
<tr>
<td>Number of racemes per inflorescence</td>
<td>13.5 (11–16)</td>
<td>35.6 (28–58)</td>
<td>32.5 (19–40)</td>
</tr>
<tr>
<td>Length of the central axis of the inflorescence (cm)</td>
<td>13.2 (9–17)</td>
<td>26.7 (19–45)</td>
<td>23.5 (14–29)</td>
</tr>
<tr>
<td>Length of the basal raceme (cm)</td>
<td>4.6 (3.5–6.2)</td>
<td>10.4 (7–14)</td>
<td>8.0 (6–11)</td>
</tr>
<tr>
<td>Length of the uppermost raceme of the inflorescence (cm)</td>
<td>2.6 (1.4–4.2)</td>
<td>2.2 (1–3.7)</td>
<td>2.7 (1.1–4.6)</td>
</tr>
</tbody>
</table>

Mean and range (between parentheses) of 10 records for each parent and five records for each hybrid.

*Measurements implicate the third leaf below the inflorescence.*
male parent *P. usterii* H1175. Forty arbitrary decamer oligonucleotides from two RAPD Primer Sets of the University of British Columbia were evaluated. A total of eight primers was selected because they generate several bands specific to the male parent. As a total, 65 bands were observed to be specific to *P. usterii* H1175. The nine progenies recovered from the *P. simplex* Q4109 each descendant was a hybrid. A minimum of 37 to a maximum of 47, indicating that derived from the pollen donor. Most bands specific to bands were observed to be specific to several bands specific to the male parent. As a total, 65 of eight primers was selected because they generate oligonucleotides from two RAPD Primer Sets of the University of British Columbia were evaluated. A total of 709 pollinated spikelets. Out of the sub-sample grown, it was not established due to the fact that only one sample varied between 1.2 and 2 cm. The degree of crossability offspring had leaves much wider than the female parent, diploid *P. malacophyllum* width in about the hybrid origin of these offspring. The leaf size, being intermediate between parents, left little doubt characteristics of the progeny concerning leaf shape and varyible nature of the female parent, all seedlings were recovered. In view of the self-incompatibility of the female parent, all seedlings were assumed to be of hybrid origin. The morphological characteristics of the progeny concerning leaf shape and size, being intermediate between parents, left little doubt about the hybrid origin of these offspring. The leaf width in *P. simplex* ranged from 0.5 to 1 cm, and in diploid *P. malacophyllum* from 2 to 2.7 cm, while all offspring had leaves much wider than the female parent, varying between 1.2 and 2 cm. The degree of crossability was not established due to the fact that only one sample of seed was germinated. Out of the sub-sample grown, it could be estimated to be around 30% considering the percentage of spikelets that set seed and the proportion of seed that produced hybrid plants.

Paspalum simplex Q4109 × malacophyllum V14855

The crosses between these diploid accessions involved a single inflorescence of a self-incompatible plant of *P. simplex* as female parent. Two hundred and thirty-seven grains were formed from 579 pollinated spikelets. Only a sample of 50 filled spikelets was sown and 37 seedlings were recovered. In view of the self-incompatible nature of the female parent, all seedlings were assumed to be of hybrid origin. The morphological characteristics of the progeny concern the leaf shape and size, being intermediate between parents, left little doubt about the hybrid origin of these offspring. The leaf width in *P. simplex* ranged from 0.5 to 1 cm, and in diploid *P. malacophyllum* from 2 to 2.7 cm, while all offspring had leaves much wider than the female parent, varying between 1.2 and 2 cm. The degree of crossability was not established due to the fact that only one sample of seed was germinated. Out of the sub-sample grown, it could be estimated to be around 30% considering the percentage of spikelets that set seed and the proportion of seed that produced hybrid plants.

Chromosome numbers of the hybrids

Ploidy level of hybrids between 2x *P. simplex* and 2x *P. malacophyllum* was expected to be diploid. Therefore, a small sample of two individual plants was checked to substantiate this assumption, and both hybrids showed 2n = 20.

Chromosome counting was performed for a large fraction of the total number of plants classified as hybrids in those crosses involving the induced tetraploid materials of *P. simplex* as female parent. The counts were considered a necessary requisite because plant 11B1a is a mixoploid with some diploid tillers but with mainly tetraploid sectors, and because of the meiotic irregularities observed in the male parents. Due to the high number of *P. simplex* × procurrens hybrids, only a 10-plant sample was chosen randomly for chromosome counts in this cross.

The 10 *P. simplex* 11B1a × procurrens Q4094 sample hybrids, and all the 22 *P. simplex* 11B1a × malacophyllum TK2449 hybrids had 2n = 4x = 40 chromosomes, as expected if the emasculated inflorescences employed for crosses belonged to the tetraploid sector of the mixoploid (2x–4x) 11B1a plant.

The nine hybrids from *P. simplex* 11B1a × *malacophyllum* V5095 were triploid, 2n = 3x = 30, a result that could be expected only if the inflorescence used for this cross came from a rare diploid tiller of the mixoploid 11B1a plant. Since this plant had been obtained by treating seed of diploid Q4109 accession of *P. simplex* with colchicine, and because the triploid hybrids arose from a non-duplicated sector of the 11B1a plant, we judged the origin of these nine hybrids to be a cross between diploid *P. simplex* Q4109 and tetraploid *P. malacophyllum* V5095, to be identified subsequently as *P. simplex* Q4109 × *malacophyllum* V5095 triploid hybrids.

Chromosome number was established for the nine *P. simplex* × *usterii* hybrids that survived. Six hybrids had 2n = 40 chromosome as expected in a 4x × 4x cross. Two hybrids had 2n = 38 and one had 2n = 39 chromosomes. These aneuploids probably arose from gametes lacking one or two chromosome due to meiotic irregularities in the parental plants.

Meiotic chromosome behavior

Meiotic chromosome configurations were investigated in parental species and their hybrids, except for diploid *P. simplex* whose regular meiotic behavior has been previously reported (Espinoza and Quarin, 1997). Results are summarized in Table 3. The diploid accession of *P. malacophyllum* showed a fairly regular meiotic behavior with 10 chromosome bivalents in the majority of the observed PMC. Chromosomes were symmetrically distributed during anaphase of the first meiotic division.

As expected, most chromosomes of the induced autotetraploid *P. simplex* associated at meiosis forming mainly quadrivalents and bivalents. Despite the fact that the tetraploid plant 11B1a arose directly from colchicine treatment of a diploid seed (generation C0), nearly 40% of the chromosomes failed to form quadrivalent associations and paired as bivalents.

Chromosome pairing in all the natural tetraploid material, *P. procurrens* Q4094, *P. malacophyllum* TK2449 and V5095, *P. usterii* H1175, showed a high proportion of quadrivalent associations at diakinesis and metaphase I. Examples of meiotic chromosome pairing are illustrated in Fig. 1a (*P. procurrens* Q4094) and Fig. 1b (*P. malacophyllum* V5095).

Two individual hybrid plants of 2x *P. simplex* Q4109 × 2x *malacophyllum* V14855 had fairly regular meiotic behavior with mainly 10 chromosome bivalent at diakinesis and metaphase of the first division (Table 3), and showed a subsequent normal homologous
Table 3. Meiotic chromosome configurations observed at diakinesis and metaphase I in four *Paspalum* species and their interspecific hybrids

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>Scored plants</th>
<th>No. PMC</th>
<th>Mean chromosome associations per PMC and range per PMC (between parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. simplex</em> Q4109</td>
<td>20</td>
<td>Several</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td><em>P. malacophyllum</em> V14855</td>
<td>20</td>
<td>1</td>
<td>214</td>
<td>–</td>
</tr>
<tr>
<td><em>P. simplex</em> 11B1a Colchicine-induced 4x</td>
<td>40</td>
<td>1</td>
<td>68</td>
<td>0.55 (0–6)</td>
</tr>
<tr>
<td><em>P. malacophyllum</em> V5095</td>
<td>40</td>
<td>1</td>
<td>36</td>
<td>0.08 (0–2)</td>
</tr>
<tr>
<td><em>P. malacophyllum</em> TK2449</td>
<td>40</td>
<td>3</td>
<td>40</td>
<td>0.075 (0–2)</td>
</tr>
<tr>
<td><em>P. procurrens</em> Q4094</td>
<td>40</td>
<td>3</td>
<td>53</td>
<td>0.06 (0–2)</td>
</tr>
<tr>
<td><em>P. usterrii</em> HI1175</td>
<td>40</td>
<td>1</td>
<td>55</td>
<td>2.04 (0–8)</td>
</tr>
<tr>
<td><strong>Hybrids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. simplex</em> Q4109 × <em>malacophyllum</em> V14855</td>
<td>20</td>
<td>2</td>
<td>249</td>
<td>0.084 (0–4)</td>
</tr>
<tr>
<td><em>P. simplex</em> Q4109 × <em>malacophyllum</em> V5095</td>
<td>30</td>
<td>4</td>
<td>162</td>
<td>2.14 (0–7)</td>
</tr>
<tr>
<td><em>P. simplex</em> 11B1a × <em>malacophyllum</em> TK2449</td>
<td>40</td>
<td>3</td>
<td>113</td>
<td>1.34 (0–6)</td>
</tr>
<tr>
<td><em>P. procurrens</em> Q4094</td>
<td>40</td>
<td>3</td>
<td>73</td>
<td>1.75 (0–6)</td>
</tr>
<tr>
<td><em>P. usterrii</em> HI1175</td>
<td>40</td>
<td>2</td>
<td>104</td>
<td>0.97 (0–6)</td>
</tr>
</tbody>
</table>

*From Espinoza and Quarin (1997).* Several plants from the original Q4109 accession were analyzed. Seeds of diploid accession Q4109 were originally used by Cáceres et al. (1999) to induce sexual tetraploid plant 11B1a.

**Fertility**

The selected diploid plants of *P. simplex* and *P. malacophyllum* failed to set seed when inflorescences were isolated from pollen of other genotypes, yet over 70% of the spikelets formed grains in 2× *P. simplex* and 2× *P. malacophyllum*. Occasionally, a quadrivalent had a close genomic homology between the two genomes of *P. simplex* and *P. malacophyllum* observed in some PMC of each of the hybrids (Table 3 and Fig. 1c). A mean of 7.86 quadrivalents (mean ± SD) at diakinesis and metaphase of the first meiotic division. The 40 chromosomes associated 3.08 per PMC with some cells showing up to eight quadrivalents. This regular chromosome distribution at anaphase. This regular chromosomal behavior was examined in four triploid hybrids of *P. simplex* × *malacophyllum* and metaphase I. Most chromosomes formed trivalents (Table 3 and Fig. 1d). A mean of 7.86 quadrivalents (mean ± SD) at diakinesis and metaphase of the first meiotic division. The 40 chromosomes associated 3.08 per PMC with some cells showing up to eight quadrivalents. This regular chromosome distribution at anaphase. This regular chromosomal behavior was examined in four triploid hybrids of *P. simplex* × *malacophyllum* and metaphase I. Most chromosomes formed trivalents (Table 3 and Fig. 1d). A mean of 7.86 quadrivalents (mean ± SD) at diakinesis and metaphase of the first meiotic division. The 40 chromosomes associated 3.08 per PMC with some cells showing up to eight quadrivalents. This regular chromosome distribution at anaphase. This regular chromosomal behavior was examined in four triploid hybrids of *P. simplex* × *malacophyllum* and metaphase I. Most chromosomes formed trivalents (Table 3 and Fig. 1d). A mean of 7.86 quadrivalents (mean ± SD) at diakinesis and metaphase of the first meiotic division. The 40 chromosomes associated 3.08 per PMC with some cells showing up to eight quadrivalents.
freely from other plants of the population (Table 4). These results confirmed that 2x races of *P. simplex* are sexual, self-sterile but cross-fertile, as reported earlier (Espinoza and Quarin, 1997). The incapacity of diploid *P. malacophyllum* for seed setting under self-pollination condition is an indication of self-sterility. Since we had only two genotypes of this diploid strain, and because they overlapped their flowering periods during a very short time, the low seed set under open pollination (2%) might not reveal the real outbreeding potential of these plants. These results, though incomplete, suggest that the diploid cytotype of *P. malacophyllum* is sexual and self-sterile due to a self-incompatibility genetic system, as is the rule for most diploid races of those *Paspalum* species involving sexual diploid and apomictic polyploid races (Quarin, 1992).

Our results indicate that the induction of autotetraploidy from the sexual self-sterile diploid *P. simplex* reported by Cáceres et al. (1999) had little effect on the self-incompatibility system. Less than 2% of the florets set seed when inflorescences of induced tetraploid plant 11B1a were selfed, while almost 45% produced grain under open pollination (Table 4). The three natural tetraploid species: *P. procurrens*, *P. malacophyllum* (accessions TK2449 and V5095), and *P. usterii* set seed under open pollination and also when selfed (Table 4). However, the degree of seed setting was very variable as characteristic of most apomictic tetraploid species of *Paspalum*.

Fertility was very erratic among the different interspecific hybrids obtained in this work. Some hybrids were sterile while others showed a wide array of fertility, from 0.02% in open-pollinated F1 #1 of *P. simplex* C1-2 × *usterii* H1175 to 36.6 in backcrossed F1 45 of *P. simplex* 11B1a × *procurrens* Q4094 (Table 4). The low degree of fertility in F1 hybrids of diploid *P. simplex* × diploid *P. malacophyllum* was unexpected since their meiotic chromosome behavior was fairly normal with mainly bivalent pairing. Seed production among the triploid hybrids *P. simplex* Q4109 × *malacophyllum* V5095 varied from sterility (0%) to 9.2%. The sterility or low fertility was attributable to meiotic irregularities caused by triploidy. The individual hybrid plant F1 #3, which had the highest fertility level (9.2%), was apomictic and most of its F2 descendants originated by 2n+n fertilization (data not shown). The fertility of tetraploid interspecific hybrids *P. simplex* 11B1a × *procurrens* Q4094 and *P. simplex* 11B1a × *malacophyllum* TK2449 in backcrossing experiments, though varying among individuals, showed fairly high levels corroborating the close phylogenetic relationship of the three species, and the feasibility of gene transferring among them when sexuality is available at the tetraploid level. The degree of fertility in tetraploid
interspecific hybrids *P. simplex* × *usterii* (Table 4) was below 1%, indicating a less significant phylogenetic relationship between *P. simplex* and *P. usterii* than was observed among *P. simplex*, *P. procurrens* and *P. malacophyllum*.

### Discussion

Cytological analysis of interspecific hybrids indicated that 2x and 4x cytotypes of *P. simplex*, *P. procurrens*, and *P. malacophyllum*, and a 4x cytotype of *P. usterii* have basically homologous genomes. The tetraploid races of all these species originated by autopolyploidy. Facultative apomixis of the aposporous type is reported for the first time in tetraploid strains of *P. procurrens* and *P. usterii*. It is also confirmed for tetraploid *P. malacophyllum*, a species whose diploid representative is here reported to reproduce sexually with some potential for apomixis.

As expected, the diploid accession V14855 of *P. malacophyllum* has regular meiotic behavior and sexual reproduction, and is probably self-incompatible since this genotype failed to produce grain when grown in an isolated environment. However, it was not sterile because some seed was recovered at the end of its flowering phase when another genotype of the same species and ploidy level started flowering in the same greenhouse. This sexually reproducing diploid has some potential for apomictic reproduction because an aposporous embryo sac was sporadically observed beside the typical meiotic sac and

### Table 4. Seed fertility of four *Paspalum* species and their interspecific hybrids

<table>
<thead>
<tr>
<th>Parental species and hybrids</th>
<th>Percentage of spikelets that formed grain after self-pollination</th>
<th>Open-pollination</th>
<th>Backcrossed to male parent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. simplex</em> Q4109 (plant # 59) sexual 2x</td>
<td>0.0</td>
<td>70.2</td>
<td>–</td>
</tr>
<tr>
<td><em>P. malacophyllum</em> V14855 sexual 2x</td>
<td>0.0</td>
<td>2.1a</td>
<td>–</td>
</tr>
<tr>
<td><em>P. simplex</em> 11B1a sexual induced 4x</td>
<td>1.9</td>
<td>44.9</td>
<td>–</td>
</tr>
<tr>
<td><em>P. simplex</em> C1-2 sexual 4x</td>
<td>0.05</td>
<td>39.8</td>
<td>–</td>
</tr>
<tr>
<td><em>P. procurrens</em> Q4094 apomictic natural 4x</td>
<td>16.7</td>
<td>42.8</td>
<td>–</td>
</tr>
<tr>
<td><em>P. malacophyllum</em> TK2449 apomictic natural 4x</td>
<td>22.4</td>
<td>34.6</td>
<td>–</td>
</tr>
<tr>
<td><em>P. malacophyllum</em> V5095 apomictic natural 4x</td>
<td>7.2</td>
<td>13.3</td>
<td>–</td>
</tr>
<tr>
<td><em>P. usterii</em> H1175 apomictic natural 4x</td>
<td>5.88</td>
<td>5.8</td>
<td>–</td>
</tr>
<tr>
<td><em>P. simplex</em> Q4109 (2x) × <em>malacophyllum</em> V14855 (2x)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 02</td>
<td>0.5</td>
<td>1.2</td>
<td>–</td>
</tr>
<tr>
<td>F1 22</td>
<td>0.0</td>
<td>0.2</td>
<td>–</td>
</tr>
<tr>
<td><em>P. simplex</em> Q4109 (2x) × <em>malacophyllum</em> V5095 (4x)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 03</td>
<td>0.0</td>
<td>2.7</td>
<td>9.2</td>
</tr>
<tr>
<td>F1 06</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F1 07</td>
<td>0.0</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>F1 08</td>
<td>0.0</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>F1 09</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>P. simplex</em> 11B1a (4x) × <em>malacophyllum</em> TK2449 (4x)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 12</td>
<td>1.5</td>
<td>3.3</td>
<td>20.2</td>
</tr>
<tr>
<td>F1 16</td>
<td>0.4</td>
<td>3.7</td>
<td>17.5</td>
</tr>
<tr>
<td>F1 17</td>
<td>1.9</td>
<td>3.6</td>
<td>3.1</td>
</tr>
<tr>
<td><em>P. simplex</em> 11B1a (4x) × <em>procurrens</em> Q4094 (4x)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 22</td>
<td>4.9</td>
<td>4.3</td>
<td>8.2</td>
</tr>
<tr>
<td>F1 45</td>
<td>0.6</td>
<td>13.4</td>
<td>36.3</td>
</tr>
<tr>
<td>F1 47</td>
<td>1.7</td>
<td>26.6</td>
<td>33.2</td>
</tr>
<tr>
<td>F1 65</td>
<td>2.3</td>
<td>6.5</td>
<td>22.8</td>
</tr>
<tr>
<td>F1 66</td>
<td>0.1</td>
<td>4.6</td>
<td>4.1</td>
</tr>
<tr>
<td><em>P. simplex</em> C1-2 (4x) × <em>usterii</em> H1175 (4x)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 01</td>
<td>0.0</td>
<td>0.02</td>
<td>–</td>
</tr>
<tr>
<td>F1 02</td>
<td>0.12</td>
<td>0.36</td>
<td>–</td>
</tr>
<tr>
<td>F1 08</td>
<td>0.32</td>
<td>0.24</td>
<td>–</td>
</tr>
<tr>
<td>F1 11</td>
<td>0.0</td>
<td>0.9</td>
<td>–</td>
</tr>
</tbody>
</table>

*a* Only one different genotype, with limited flowering, was placed in the vicinity.
inside of the same ovule. Additional research is needed to
determine whether further parthenogenesis occurs in this
occasional aposporous sac to produce a descendant via
apomixis, or whether fertilization of the unreduced egg cell
with pollen of 4x plants would raise the ploidy level in the
progeny. In this condition, ploidy may also increase by two
steps through a triploid bridge. If this occurs, the relation-
ship between 2x and 4x levels might be a dynamic system of
one-way traffic carrying genetic variation from diploid
outbreeders to apomictic tetraploids. This would be an
effective dynamic system in a frame of autoploidy and
apomixis, but different from the two-way traffic increasing
and decreasing genome dosage in other plant species as
suggested by Bennett (2004).

A high proportion of chromosomes associated forming
quadrivalents at meiosis in tetraploid *P. procurrens*,
*P. usterii* and both accessions of tetraploid *P. malaco-
phyllum*. The number of quadrivalents observed in these
two accessions was somewhat lower than reported by
Bashaw et al. (1970) and considerably higher than
reported by Burson and Hussey (1998). Nonetheless,
these authors classified *P. malacophyllum* as autotetra-
ploid. Our results support this classification for this
species and also for *P. procurrens* and *P. usterii*. In the
genus *Paspalum* many apomictic tetraploid species have
sexual diploid counterparts; it has been suggested that
this should be a typical feature for most apomictic
polyploid species of the genus (Quarin, 1992). Our
research provides information supporting that this
scheme, with sexual diploid and apomictic autotetra-
ploid representatives, is the most common system
among species of *Paspalum*: the facultative apomictic
tetraploid accession of *P. procurrens* is added to the
sexual diploid representative reported previously (Quar-
in, 1993), and accession V14855 of *P. malacophyllum*
indicates that sexual diploid races exist for this species.
However, the sexual diploid representatives seem to be
much less frequent in the wild than are the tetraploid
races of *P. malacophyllum*.

We determined the mode of reproduction when those
accessions were used as male parents in the interspecific
crosses. Any given material was assumed to reproduce
sexually when the embryo sac developed from a
functional megaspore which originated by meiosis.
Accessions in which the embryo sac may develop from
cytologically unreduced nucellar cells (apospory) were
assumed to reproduce by means of apomixis. If
aposporo is the rule and meiotic embryo sacs fail to
develop, the accession would be classified as obligate
apomictic. In consequence, the diploid material of
*P. malacophyllum* is classified as a sexually reproducing
race with some potential for apomixis, while both
tetraploid accessions of this species are facultative
apomicts. Accession TK2449 showed the highest degree
of apomictic reproduction since only 3% of its mature
ovules had a single embryo sac of the meiotic type (with
proliferated antipodal cells), while in accession V5095,
17% of the ovules showed a single meiotic sac.
Variations in the degree of facultative apomixis had
been observed in several tetraploid accessions of
*P. malacophyllum* analyzed by Burson and Hussey
(1998) through embryological studies. However, the
degree of sexuality in these apomictic accessions needs
further confirmation. Aposporous embryo sacs bearing
antipodal cells (*Hieracium*-type) were observed in
tetraploid strains of *P. simplex*, a species of the same
subgenus. If *Hieracium*-type of apospory occurs in
*P. malacophyllum*, the degree of sexual reproduction
may be largely overestimated. Actually, Bashaw et al.
(1970), based on embryological studies, reported that a
40-chromosome accession of this species was sexual. The
apomictic mode of reproduction reported by Burson
and Hussey (1998) in 14 accessions, and in two
additional accessions in the present study, leaves little
doubt about the facultative apomictic reproduction
system in tetraploid *P. malacophyllum*, but progeny
tests would elucidate what is the real degree of sexuality.
Our embryological studies indicate that the tetraploid
strains of *P. procurrens* reproduce by facultative
apomixis of the aposporous type, while diploid races
of the same species were reported to be sexual out-
breeders due to the operation of a self-incompatibility
system (Quarin, 1993). *Paspalum usterii* is a tetraploid
species that reproduces by facultative apomixis. The
embryo sac types observed in mature ovules indicate
a higher degree of sexuality than that observed for
both the tetraploid accession of *P. malacophyllum*
and also for tetraploid *P. procurrens*. Nevertheless,
the low number of antipodal cells observed in the gameto-
phytes that were classified as meiotic embryo sacs
emphasizes some doubt in relation to the origin of these
sacs. Thus, further investigation and other experimental
approaches would be required to establish the actual
degree of sexual reproduction of this facultative
apomictic species.

Because sexual diploid strains usually exist for apomictic
tetraploid *Paspalum* species, it seems predictable to find
sexual diploid representatives for *P. usterii* in extensive
collections of the species.

The meiotic chromosome associations observed in the
parental species and in their interspecific hybrids suggest
on the one hand the autoploid origin of tetraploid races
and, on the other hand, the close genomic relationship
among species of the subgenus *Anachyris*. Hybrids
between *P. simplex* and *P. malacophyllum* at the diploid
level showed fairly regular meiotic chromosome pairing
as evidence of their homology. When the interspecific
hybrids were obtained at the tetraploid level, most
chromosomes associated forming bivalent and quad-
rrivalent associations as is the rule in the parental
species. Moreover, the triploid interspecific hybrids (2x
*P. simplex* × 4x *malacophyllum*, 2n = 3x = 30) showed
up to ten trivalent chromosome associations in some cells. The whole interpretation of meiotic chromosome pairing of interspecific hybrids between *P. simplex* and *P. malacophyllum* at different ploidy levels supports a common origin and almost complete homology among their genomes. A similar degree of homology was previously found among diploid races of *P. simplex* and *P. procurrens* (Espinoza and Quarin, 1998). The meiotic chromosome associations that we observed in tetraploid interspecific hybrids of *P. simplex* × *procurrens* confirm previous reports since most chromosomes associated as bivalents and quadrivalents with a maximum of seven quadrivalents per cell. However, the tetraploid interspecific hybrids *P. simplex* × *procurrens* showed a lower proportion of quadrivalents per cell (mean = 2.55) than those observed in their parents (means of 5.94 for *P. simplex* and 4.96 for *P. procurrens*). This would indicate that some changes have occurred in the speciation processes that affected their genomes, though basically they preserve their homology. The meiotic chromosome behavior of *P. simplex* × *usterii* tetraploid hybrids showed a high proportion of chromosomes associated as bivalents (mean = 9.02/cell) and quadrivalents (mean = 4.1/cell), with a maximum of 10 quadrivalents/cell. In view of the experimental autoploid origin of *P. simplex*, and the high proportion of quadrivalent associations in the hybrids, tetraploid *P. usterii* is to be classified as an autotetraploid with a basic genome homologous to the genome of *P. simplex*. The occasional chromosome associations higher than quadrivalent observed in *P. simplex* × *usterii* tetraploid hybrids suggest some chromosome rearrangements, probably translocations.

The present results, in addition to previous studies of 2x *P. simplex* × 2x *procurrens* hybrids, suggest that the whole subgenus *Anachyris* of *Paspalum* is basically an agamic complex for which six species are actually recognized: *P. simplex* (with sexual 2x, sexual and apomictic 3x, and apomictic 4x and 6x representatives), *P. malacophyllum* (sexual 2x and apomictic 4x), *P. procurrens* (sexual 2x and apomictic 4x), *P. usterii* (only apomictic 4x races known), *P. volcanensis* (4x), and *P. costellatum* for which no information on ploidy level or mode of reproduction is available. Our experimental research results indicate that the same basic genome is shared by at least four of the six species of the subgenus: *P. simplex*, *P. malacophyllum*, *P. procurrens*, and *P. usterii*. In these species the polyploid representatives originated by autoploidy and all reproduce by means of facultative apomixis with the exception of a rare triploid plant of *P. simplex* which reproduces sexually, though another 3x plant of the same species is apomictic (Urbani et al., 2002). The sexual tetraploid plants of *P. simplex* used in this work were obtained experimentally by colchicine treatment of diploid plants. Sexual tetraploid individuals have never been found in natural populations of any of these species. The results suggest that speciation took place at the diploid level, then polyploidization, apomixis, and eventually geographic barriers prevented hybridization and introgression. For example, experimental work demonstrated that *P. simplex* and *P. procurrens*, at the diploid level, hybridize and backcross very easily. However, the diploid races of these species retain geographical separation, although both belong to the same general phytogeographic Chaco region in northern Argentina (Espinoza and Quarin, 1998). The unique diploid representative of *P. malacophyllum*, as studied so far, was collected in the state of Santa Catarina, Brazil. Although our crosses between diploid races of *P. simplex* and *P. malacophyllum* produced barely fertile hybrids, introgression in the wild has been primarily prevented by a wide geographic barrier. Where these species distribution overlap as in areas of northern Paraguay or southwestern Brazil, introgression should be hardly accomplished due to the polyploid apomictic nature of these species. Interestingly enough, the general morphological characteristics of the diploid accession of *P. malacophyllum* resemble the unusual accession V5095 of *P. malacophyllum* which is tetraploid and apomictic and was originally collected in the state of Goiás, Brazil. The uncommon general morphology, the decumbent growth habit of this material, and the fact of their existence at both diploid and tetraploid levels, requires a special consideration of their taxonomic status. They clearly differ from the original description and illustration reported by Trinius (1831) when describing *P. malacophyllum*. Additional botanical collections and information regarding distribution and population characteristics of material with these unusual morphological attributes would help in the development of an improved taxonomic classification.

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References


