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ORIGINAL PAPER

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# Genome and species relationships in genus *Avena* based on RAPD and AFLP molecular markers

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**Abstract** Species and genome relationships among 11 diploid (A and C genomes), five tetraploid (AB and AC genomes) and two hexaploid (ACD genome) *Avena* taxa were investigated using amplified fragment length polymorphisms (AFLPs) and random amplified polymorphic DNA (RAPD) markers. The two primer pairs used for the AFLP reactions produced a total of 354 polymorphic bands, while 187 reproducible bands were generated using ten RAPD primers. Genetic similarities amongst the entries were estimated using the Jaccard and Dice algorithms, and cluster analyses were performed using UPGMA and neighbor joining methods. Principle coordinate analysis was also applied. The highest cophenetic correlation coefficient was obtained for the Jaccard algorithm and UPGMA clustering method ($r$=0.99 for AFLP and $r$=0.94 for RAPD). No major clustering differences were present between phenograms produced with AFLPs and RAPDs. Furthermore, data produced with AFLPs and RAPDs were highly correlated ($r$=0.92), indicating the reliability of our results. All A genome diploid taxa are clustered together according to their karyotype. The AB genome tetraploids were found to form a subcluster within the $A_s$ genome diploids (AFLPs), indicating their near-autoploid origin. The AC genome tetraploids are clustered to the ACD genome hexaploids. Finally, the C genome diploids form an outer branch, indicating the major genomic divergence between the A and C genomes in *Avena*.

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## Introduction

The genus *Avena* comprises diploid (2$n$=2x=14), tetraploid (2$n$=4$x$=28) and hexaploid (2$n$=6$x$=42) species. Diploid species have either the A or the C genome, tetraploids have either the AB or the AC genome and the hexaploids have the ACD genome designation. No diploid species have ever been identified with the B or D genome. Genome differentiation was initially based on cytological studies of interspecific hybrids and descriptions of species karyotypes (Rajhathy and Thomas 1974). At the diploid level, hybridization among the A and C genome species rarely produces interspecific hybrids, indicating major genomic differences among these species. The two major genomic diploid groups, AA and CC, are further subdivided according to their karyotypes (structural differences), and subscripts are introduced to discriminate them. The A genome diploid species consist of five different karyotypes, $A_s$, $A_l$, $A_d$, $A_p$ and $A_c$, which have been assigned to *Avena strigosa*, *A. longiglumis*, *A. damascena*, *A. prostrata* and *A. canariensis*, respectively. The C genome diploids are represented by two karyotypes, $C_v$ and $C_p$, of *A. ventricosa* and *A. pilosa* (now known as *A. eriantha*), respectively. However, the genetically controlled regulatory mechanism that prevents homoeologous chromosome pairing that exists in the *Avena*, along with the substantial structural differences of chromosomes among the oat taxa, could lead to an underestimation of the actual relationships between different species.

The application of different molecular techniques has provided further information on *Avena* genome relation-

ships. Genomic in situ hybridizations have revealed the close relationships among the A and D genomes (Chen and Armstrong 1994; Jellen et al. 1994a), and the A and B genomes (Leggett and Markhand 1995; Katsiotis et al. 1997). Molecular probes differentiated the D genome, by subtraction (Linares et al. 1998), and the B genome (Irigoyen et al. 2001) from the A genome. Finally, molecular markers, such as restriction fragment length polymorphism (RFLP) (Alichio et al. 1995; Nocelli et al. 1999), randomly amplified polymorphic DNA (RAPD) (Nocelli et al. 1999) and microsatellites (Li et al. 2000), along with isozymes (Sanchez de la Hoz and Fominaya 1989), have been used to identify relationships among *Avena* species, mainly within single ploidy levels.

In the investigation reported here, two methods of generating molecular markers—A FLPs and RAPDs—were used to study the genetic relationships among eight *Avena* A genome diploid taxa, three C genome diploid taxa, three AB and two AC genome tetraploid taxa and two ACD genome hexaploid taxa and to cross-check the validity of the species relationships obtained.

## Materials and methods

### Plant material and DNA isolation

Eleven diploid taxa, five tetraploid and two hexaploid taxa belonging to the genus *Avena* were included in the present study. Their genomic designation, accession number and the source from which they were obtained are listed in Table 1. Each taxon is represented with only one accession since interspecific polymorphism was found to exceed intraspecific polymorphism in previous studies in oats (Nocelli et al. 1999) and other genera (Kafkas and Perl-Treves 2001). Total genomic DNA was extracted from 2-week-old leaves. A bulk of three plants per accession was used and DNA was extracted using the DNeasy Plant Mini kit (QIAGEN, Valencia, Calif.) according to manufacturer's protocol.

### RAPD analysis

Twenty-five different 10-mer primers were tested, ten of which were subsequently selected for further analysis based on the number of reproducible polymorphic bands (Table 2). Each RAPD reaction was carried out in a total volume of 25$\mu$l containing PCR buffer (20 m*M* Tris-HCl pH 8.4, 50 m*M* KCl), 50 ng of genomic DNA, 0.625 $\mu M$ primer, 150 $\mu M$ of each dNTP (Promega, Madison,

**Table 1** Origin of *Avena* species studied, accession numbers, ploidy levels and genome designations

| | Species | Ploidy level | Genome | Accession number | Source |
|---|---|---|---|---|---|
| 1 | *Avena clauda* | 2*n*=2*x*=14 | $C_p$ | Cc2201 | IGER[b] |
| 2 | *A. eriantha* | 2*n*=2*x*=14 | $C_p$ | Cc2087 | IGER |
| 3 | *A. ventricosa* | 2*n*=2*x*=14 | $C_v$ | Cc 7064 | IGER |
| 4 | *A. hirtula* | 2*n*=2*x*=14 | $A_s$ | Cc 7046 | IGER |
| 5 | *A. matritensis* | 2*n*=2*x*=14 | $A_s$[a] | Cav 658411 | USDA[c] |
| 6 | *A. brevis* | 2*n*=2*x*=14 | $A_s$ | PI 258543 | USDA |
| 7 | *A. strigosa* | 2*n*=2*x*=14 | $A_s$ | Cc2080 | JIC[d] |
| 8 | *A. lusitanica* | 2*n*=2*x*=14 | $A_s$[a] | Cav 6317 | USDA |
| 9 | *A. damascena* | 2*n*=2*x*=14 | $A_d$ | Cc 7045 | IGER |
| 10 | *A. prostrata* | 2*n*=2*x*=14 | $A_p$ | Cc 7060 | IGER |
| 11 | *A. longiglumis* | 2*n*=2*x*=14 | $A_l$ | CIav 9089 | USDA |
| 12 | *A. abyssinica* | 2*n*=4*x*=28 | AB | Cc1161 | IGER |
| 13 | *A. barbata* | 2*n*=4*x*=28 | AB | Cc2048 | IGER |
| 14 | *A. vaviloviana* | 2*n*=4*x*=28 | AB | PI 412767 | USDA |
| 15 | *A. maroccana* | 2*n*=4*x*=28 | AC | Cc 7070 | IGER |
| 16 | *A. murphyi* | 2*n*=4*x*=28 | AC | Cc 7120 | IGER |
| 17 | *A. sativa* | 2*n*=6*x*=42 | ACD | Sun II | USDA |
| 18 | *A. sterilis* | 2*n*=6*x*=42 | ACD | Cc 4704 | IGER |

[a] Tentative genome assignment—see Discussion
[b] Institute of Grassland and Environmental Research, Aberystwyth, UK
[c] United States Department of Agriculture, Aberdeen, Idaho USA
[d] John Innes Center, Norwich UK

**Table 2** Primers used for the RAPD and AFLP analyses and the number of bands—both total and polymorphic—they produced

| RAPD-primers | Sequence (5′-3′) | Number of total bands produced | Number of polymorphic bands produced |
|---|---|---|---|
| RAPD-2 | CAC TTC CGC T | 20 | 20 |
| RAPD-5 | TCC ACC GGC T | 18 | 18 |
| RAPD-9 | GGC AGT TCT C | 20 | 18 |
| RAPD-11 | AAG CCC CCC A | 22 | 22 |
| RAPD-12 | ACG GCG ATG A | 17 | 17 |
| RAPD-18 | GAA ACG GGT G | 18 | 18 |
| OPA-2 | TGC CGA GCT G | 21 | 21 |
| OPAH-17 | CAG TGG GGA G | 17 | 16 |
| OPH-13 | GAC GCC ACA C | 17 | 17 |
| RI-16 | GTC GCC GTC A | 17 | 17 |
| AFLP-primer pairs | | | |
| ACA/CAC | *Eco*RI-ACA/*Mse*I-CAC | 228 | 222 |
| ACA/CAG | *Eco*RI-ACA/*Mse*I-CAG | 138 | 132 |

Wis.), 1 U *Taq* DNA polymerase (Invitrogen, Carlsbad, Calif.) and 2 m*M* $MgCl_2$. The amplification reactions were carried out in an OMN-E Hybaid thermal cycler with an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 1 min at 94°C, 1 min at 37°C, 1 min at 72°C and a final extension step of 10 min at 72°C. The PCR products were separated in a 1.2% agarose gel (Seakem-FMC, Rockland, Me.) and stained with ethidium bromide. Reproducibility of the banding patterns was checked by performing the reactions twice.

### AFLP analysis

AFLP analysis was carried out using the AFLP Plant Mapping kit (Applied Biosystems, Foster City, Calif.), as previously described by Katsiotis et al. (2003). Primer pair *Eco*RI+A/*Mse*I+C was used for the preselective amplification, and amongst the 64 selective amplification primer combinations tested, *Eco*RI+ACA/*Mse*I+CAC and *Eco*RI+ACA/*Mse*I+CAG were chosen based on their number of polymorphic and high intensity peaks (Table 2). The selective amplification products were separated in an automated Genetic Analyzer ABI Prism 310 (Applied Biosystems). Electrophoresis results were analyzed with GENESCAN analysis software, while selective amplification products were scored using the GENOTYPER software.

### Data analysis

The reproducible polymorphic amplified fragments were scored as present (1) or absent (0) for each marker and were assembled in a data matrix table. Genetic similarities (GS) between taxa were calculated using the Jaccard (1908) and Dice (1945) similarity coefficients with the SIMQUAL program. Cluster analyses were performed using the unweighted pair group method with arithmetic mean (UPGMA) and neighbor joining (NJ) methods. The correlation between the similarity and the cophenetic matrices for each similarity coefficient and clustering method was computed. Comparison between the data produced with RAPDs and with AFLPs was carried out using the Mantel test. Principal coordinate analysis was also applied for both methods. All of the above analyses were performed using NTSYS software (Rohlf 1998).

## Results

The ten RAPD primers used amplified a total of 187 reproducible bands, three of which were monomorphic, while the two AFLP primer pair combinations produced a total of 366 peaks, 12 of which were monomorphic. The genetic similarities calculated using the Jaccard and Dice coefficients gave comparable values. Both similarity matrices, when clustered either with UPGMA or NJ, produced similar topology with minor clustering differences among the entries. However, in all cases, taxa clustered according to their present genomic designation.

For the AFLP data, the cophenetic correlation coefficients (CCC) between the Jaccard similarity matrix and the derived UPGMA and NJ cophenetic matrices were 0.99 and 0.85, respectively, and the corresponding CCCs for the Dice similarity matrix were 0.98 and 0.83, respectively. For the RAPD data, the corresponding CCCs values were 0.94 and 0.76 using the Jaccard similarity matrix, and 0.88 and 0.76 using the Dice similarity matrix. Based on the CCC values, which can be used as a measure of the goodness-of-fit for cluster analysis, only the Jaccard/UPGMA phenograms are presented for both RAPD (Fig. 1A) and AFLP (Fig. 1B) data. The two phenograms show only minor clustering differences. The 18 taxa were clustered in three major groups: the C genome group, including *A. clauda*, *A. eriantha* and *A. ventricosa*; the AC/ACD genome group, including *A. sativa*, *A. sterilis*, *A. maroccana* and *A. murphyi*; and the A/AB genome group. In the latter group, the AB genome tetraploids (*A. abyssinica*, *A. vaviloviana* and *A. barbata*) form a subgroup and are clustered to the $A_s$ genome diploids (*A. brevis*, *A. strigosa*, *A. hirtula*), *A. matritensis*, *A. lusitanica*, *A. damascena* ($A_d$ genome), *A. longiglumis* ($A_l$ genome) and *A. prostrata* ($A_p$ genome). However, within this group clustering differences are evident between the RAPD- and AFLP-derived phenograms. In the RAPD phenogram, *A. damascena*, *A.*

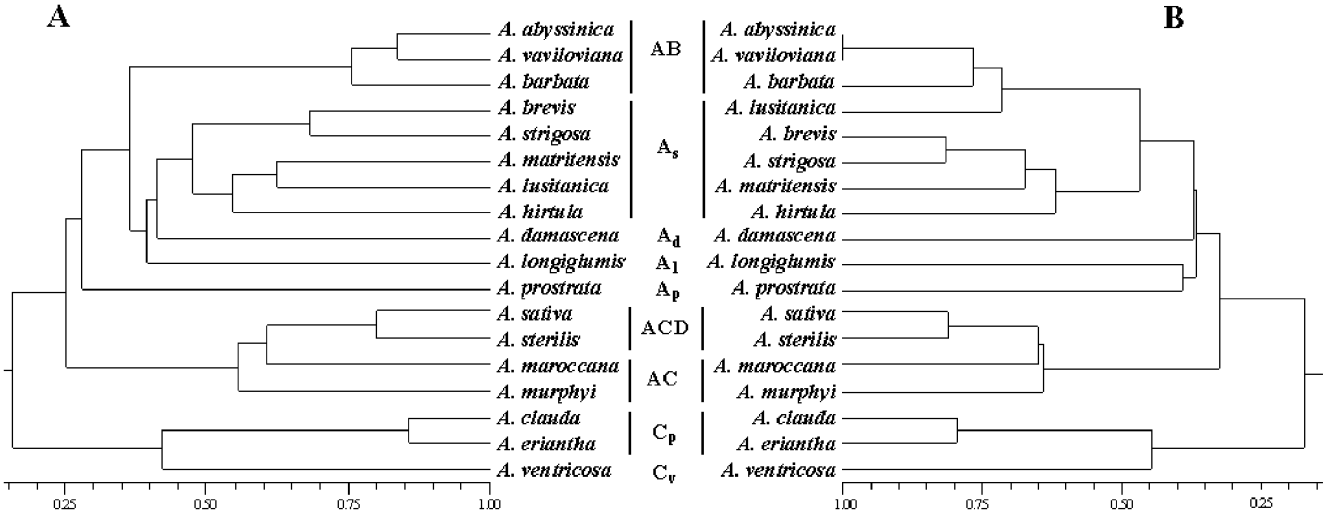


**Fig. 1** RAPD (**A**) and AFLP (**B**) cluster analyses for *Avena* species

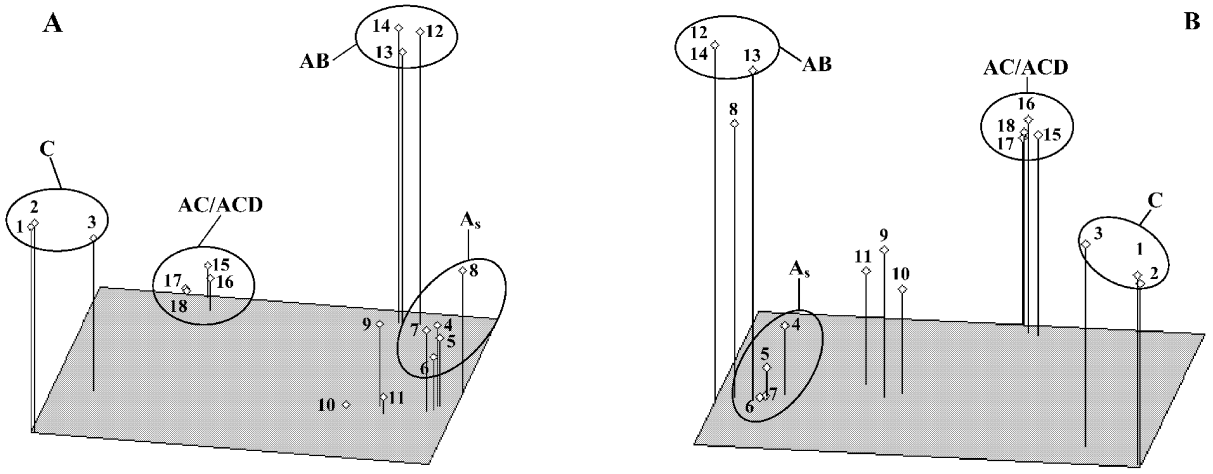


**Fig. 2** RAPD (**A**) and AFLP (**B**) principle coordinate analyses for *Avena* species. *Numbers* correspond to those designating specific species in Table 1

*longiglumis* and *A. prostrata* are clustered to the $A_s$ genome diploid taxa, while in the AFLP phenogram the $A_s$ taxa are first clustered to the AB genome tetraploid taxa.

The principal coordinate analysis for both RAPD (Fig. 2A) and AFLP (Fig. 2B) data revealed four groups: the CC diploid group, the AACCDD hexaploid/AACC tetraploid group, the AABB tetraploid and the AA diploid group. Within the latter group, subgrouping occurs depending on the karyotype; thus, the $A_sA_s$ diploids (*A. brevis*, *A. strigosa*, *A. hirtula*) along with *A. matritensis* and *A. lusitanica* are more closely related than *A. damascena* ($A_d$ genome), *A. longiglumis* ($A_l$ genome) and *A. prostrata* ($A_p$ genome). For the RAPD data, the first three principal coordinates explained almost half (46.64%) of the total variation, with PC-1, PC-2 and PC-3 accounting for 20.9%, 14.37% and 11.37% of the total variation, respectively. For the AFLP data, the amount of variation explained by the three first principal coordinates is slightly higher (49.49%) than in the RAPD data, with PC-1, PC-2 and PC-3 accounting for 21.98%, 15.04% and 12.47% of the total variation, respectively.

A comparison between the Jaccard genetic similarity matrices of the RAPD- and AFLP-derived data using the Mantel test revealed a high correlation value ($r$=0.92), indicating the reliability of our results.

## Discussion

Phylogenetic relationships and genome affinities in genus *Avena* were investigated using two molecular marker techniques, RAPDs and AFLPs, both of which have been widely used over the past few years in the assessment of intraspecific and interspecific genetic relationships (Wolfe and Liston 1998). Both methods proved efficient in detecting polymorphism in the genus *Avena*, with AFLPs producing more polymorphic bands per primer than RAPDs. The high correlation value ($r$=0.92) between the GS matrices of the RAPDs and AFLPs strengthens the reliability of the data generated and the dendrograms produced. In both methods the taxa were clustered according to their genomic designation. Furthermore, the A and C diploid taxa were clustered according to their karyotypes.

The C genome diploids form a distinct and isolated group in both dendrograms (Fig. 1) and three-dimensional plots (Fig. 2). *Avena eriantha* and *A. clauda* (both $C_p$ genome) are more closely related to each other than to *A. ventricosa* ($C_v$ genome). Interspecific hybrids of *A. eriantha-A. clauda* show regular meiosis forming seven bivalents (Rajhathy and Thomas 1974). Although *A. ventricosa* ($C_vC_v$) crosses readily with the former, their interspecific hybrids are completely sterile due to irregular meiosis, thereby reflecting the chromosomal structural rearrangements that differentiate the $C_p$ genome from the $C_v$ genome. The genetic divergence of the C genome diploids from the rest of the *Avena* species is clearly demonstrated in both dendrograms (Fig. 1) and three-dimensional plots (Fig. 2). Cytological data, such as C-banding patterns (Fominaya et al. 1988) and the failure of chromosome pairing in interspecific hybrids (*A. strigosa-A. eriantha*) reveal that the A and C genomes are totally isolated from one another (Nishiyama and Yabuno 1975; Leggett 1998). Their genomic divergence is also supported by RFLP analysis (Allichio et al. 1995) and sequence differences in their rDNA spacers (Jellen et al. 1994b). Furthermore, several C genome-specific repeated sequences have been isolated and characterized (Fabijanski et al. 1990; Gupta et al. 1992; Solano et al. 1992; Ananiev et al. 2002). Katsiotis et al. (2000) isolated two repetitive sequences that were present in all A, B and D genomes, and although they hybridized to *Arrhenatherum*, a closely related genus, they were absent from the *Avena* C genome. Finally, different retrotransposon

families have been reported between A and C genomes (Katsiotis et al. 1996).

Minor clustering differences within the A genome diploids are present between the two dendrograms (Fig. 1), although taxa grouped as expected on the basis of their karyotype. *Avena strigosa* and *A. brevis*, both carrying the $A_s$ genome, share morphological similarities and form interfertile $F_1$ hybrids with *A. hirtula* ($A_s$ genome) (Rajhathy and Thomas 1974). As for *A. matritensis*, there is currently no karyotypic classification available, although chromosome pairing of inter- and intraspecific hybrids (J.M. Leggett, unpublished data) indicates that it has the $A_s$ genome. This information together with the clustering of the two molecular marker types observed here leads us to propose an $A_s$ designation for its genome. Until recently, little information was available on the cytology of *A. lusitanica*. Baum (1977) refers to it as an $A_s$ diploid, while Thomas (1992) designates it as an A genome diploid. *Avena lusitanica* clusters within the $A_s$ group in the RAPD analysis although it is closer to the AABB tetraploids in the AFLP analysis. However, considering the overall positioning of this tetraploid group to the $A_s$ genome diploids in both forms of analyses, along with recent chromosome pairing studies (J.M. Leggett, unpublished data), it too should be considered as an $A_s$ genome diploid that may have played some evolutionary role in the formation of the AABB tetraploids. The other A genome diploids exhibit enough molecular diversity from the $A_sA_s$ diploids as well as from one another. *Avena damascena* ($A_dA_d$) seems to be more closely related to the $A_s$ genome species, and *A. prostrata* ($A_pA_p$) is the most distant. The meiotic behavior of hybrids between $A_s$ genome diploids and *A. damascena* (Leggett 1989), *A. longiglumis* (Rajhathy and Thomas 1974) and *A. prostrata* (Ladizinsky 1971), respectively, support our results. The low level of genetic similarity between *A. damascena* and *A. longiglumis/A. prostrata* (Fig. 1) reflects their reproductive isolation (Rajhathy and Baum 1972; Leggett 1984a).

Speciation of genus *Avena* at the diploid level relies mostly upon structural differentiation of the chromosomes (Rajhathy and Thomas 1974). If the assumption that structural rearrangements accumulated gradually is accepted, then the succession of the diploid species over time can be reconstructed. Therefore, based on the AFLP and the RAPD dendrograms (Fig. 1), *A. prostrata* and *A. longiglumis* have the most divergent A genomes and are considered to be the most ancient, while the $A_s$ genome is the most recently evolved. On the basis of the RAPD data reported here, the $A_p$ genome is even more ancient than the $A_l$ genome. These findings are in accordance with the sequence $A_p \rightarrow A_l \rightarrow A_d/A_c \rightarrow A_s$ (from the oldest to the youngest) reported by Rajhathy and Thomas (1974).

The tetraploid species are also clearly differentiated into two groups—the AABB ($AAA'A'$) and AACC forms. All three AABB tetraploids form a subcluster within the A genome diploid taxa (Fig. 1). Although all three AABB tetraploids belong to the same biological species known as the barbata group and are interfertile, *A. abyssinica* is more closely related to *A. vaviloviana* than to *A. barbata*. The geographical distribution of *A. abyssinica* and *A. vaviloviana* is restricted, and these species are mainly found in Ethiopia, whilst *A. barbata* has a much wider distribution pattern. The two primer combinations used in the AFLP technique reported here did not produce any polymorphism between *A. abyssinica* and *A. vaviloviana*, a result that is in contrast to that obtained with the RAPD primers, which did produce polymorphic bands between the taxa. Cytological studies of the meiotic chromosome pairing of hybrids between AABB tetraploids and $A_sA_s$ autotetraploids revealed that the B genome chromosomes have large segments homologous with those of the $A_s$ genome (Sadasivaiah and Rajhathy 1968). The C-banding patterns of the B genome chromosomes are similar to those of the A genome chromosomes (Fominaya et al. 1988), and genomic in situ hybridization experiments using total *A. strigosa* genomic DNA as a probe uniformly labeled all 28 chromosomes of *A. barbata* (Leggett and Markhand 1995) and *A. vaviloviana* (Katsiotis et al. 1997), revealing the close relationship of the A and B genomes. Only minor genomic differences have been reported between the A and B genomes based on an $A_s$ genome-specific probe (Irigoyen et al. 2001). Thus, according to the results of the present study as well as those from previous studies (Oinuma 1952; Leggett and Markhand 1995; Katsiotis et al. 1997), the proposed genomic designation $AAA'A'$ for the barbata group tetraploids is justifiable. Chromosomal, morphological, biochemical and geographic evidence indicates a near-autoploid origin of the AABB tetraploids from the *A. hirtula/A. wiestii* population (Rajhathy and Thomas 1974). According to the AFLP results obtained in this study and chromosome pairing studies (J.M. Leggett, unpublished data) *A. lusitanica* ($A_s$ genome) is as strong a candidate to be the progenitor as the *A. hirtula/A. wiestii* taxa.

The AACC tetraploids (*A. murphyi* and *A. maroccana*) are grouped with the AACCDD hexaploids, and *A. maroccana* seems to be more closely related to the hexaploid species than *A. murphyi* (Figs. 1, 2). Karyotypically, *A. maroccana* and *A. murphyi* are quite distinct, and their hybrids are sterile on account of an irregular meiosis (Rajhathy and Thomas 1974). Their genomic constitution—AACC—has only recently been confirmed using genomic in situ hybridization (Leggett et al. 1994; Leggett and Markhand 1995). Until recently, accumulated karyotypic, meiotic and molecular evidence pointed to *A. maroccana* being the putative donor of the AC genome of the hexaploids (Rajhathy and Thomas 1974). However, further molecular and cytological data relating to the most recently discovered tetraploid *A. insularis* is required in the light of Ladizinsky's (1998) observations with regard to the chromosome affinities of *A. insularis* and *A. sativa*.

Hexaploids *A. sativa* and *A. sterilis* are interfertile and share the same chromosome structure. They represent the most recently formed polyploid species of the genus (Rajhathy and Thomas 1974). The D genome of the hexaploids is considered to have substantial similarities

with the A genome based on genomic in situ hybridization results (Chen and Armstrong 1994; Jellen et al. 1994a). Discrimination among chromosomes of these two genomes is possible using an A genome-specific probe (Linares et al. 1998). The fact that the hexaploids cluster with the AACC tetraploids (Figs. 1, 2) indicates that the D genome is not characterized by genetic differences sufficient to segregate the hexaploids from their putative AACC tetraploid progenitors.

It is noteworthy that in both dendrograms (Fig. 1) the AACC tetraploids and AACCDD hexaploids are clustered to the A genome diploids rather than to the C genome diploids. Although both the A and C genomes are present, it seems that the C genome of the hexaploids and AACC tetraploids has undergone more changes during the formation of the tetraploid than has the A genome. Song et al. (1988; 1995) studied the natural and synthetic allopolyploids in *Brassica* and observed that the higher the degree of divergence between the parental diploid genomes, the greater the subsequent genomic change in the resulting polyploid. They also provided evidence that the nuclear genome with a maternal origin undergoes fewer changes than the nuclear genome with a paternal origin. Although similar studies have not been conducted in *Avena*, this may as well be the case in *Avena* species, since a number of studies have provided evidence, either direct or indirect, to support the hypothesis that an A genome diploid was the female parent of the AACC tetraploids (Nishiyama and Yabuno 1975; Leggett 1984b; Nishiyama 1984; Murai and Tsunewaki 1987; Rines et al. 1988).

According to estimated crossabilities among A genome diploids and AC genome tetraploids using the polar nuclei activation (PNA) hypothesis (Katsiotis et al. 1995), *A. longiglumis* crosses readily with AACC tetraploids when the former is used as the pollinator. Such an AAC-$CA_lA_l$ hexaploid hybrid would have type I chloroplast DNA (contributed by the AACC tetraploid), while the $A_l$ genome chromosomes would undergo substantial changes (due to their paternal origin) that would differentiate them from the original $A_l$ karyotype. This modified $A_l$ genome could be the D genome of the hexaploids. This is an appealing theory on the evolution of the hexaploid oats that needs a more thorough investigation since very little is known on the origin of the D genome of the hexaploids.

In conclusion, the present study extends our knowledge of the phylogenetic relationships within the genus *Avena* and the relationships among *Avena* genomes. *Avena matritensis* and *A. lusitanica* are designated as $A_s$ genome species, and the possible role of the latter in the evolution of the AABB tetraploids is discussed. The data re-emphasizes the major genomic divergence between the A and C genomes and their potential change during the formation of the tetraploids and the hexaploids. Further investigation is required to clarify the link of the recently discovered tetraploid *A. insularis* and the perennial autotetraploid *A. macrostachya* to the evolution of genus *Avena*.

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