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E. N. Jellen · B. S. Gill

C-banding variation in the Moroccan oat species *Avena agadiriana* (2n = 4x = 28)

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Abstract The C-banding technique was used to describe the chromosomes of a relatively recently-discovered Moroccan oat species, *Avena agadiriana* (2n = 4x = 28). A substantial amount of polymorphism for arm ratios and C-banding patterns was observed among five accessions of this species. However a common set of ten putatively homologous chromosomes was identifiable among the five accessions. The chromosomes of *A. agadiriana* do not closely match those of any of the previously described diploid or tetraploid oat species in terms of their arm ratios and C-banding patterns. However, their overall C-banded appearance generally resembles the A/B/D groups of chromosomes of *Avena* species, rather than the more hetrochromatic C genomes. Implications of these findings in terms of chromosome evolution in the genus *Avena* are discussed.

Key words *Avena agadiriana* · Oat · C-banding · *Avena hispanica*

Introduction

Two oat species from Coastal Morocco, *Avena atlantica* Baum et Fedak (Baum and Fedak 1985a) and *A. agadiriana* Baum et Fedak (Baum and Fedak 1985b), were first identified in 1985. The former species, a diploid (2n = 2x = 14), was determined to have chromosomes of the As genome based on karyotypic similarities and regular bivalent pairing with the As-genome taxa *A. strigosa* Schreb., *A. wiestii* Steud. and *A. hirtula* Lag. (Leggett 1987). The fact that researchers were able to construct an RFLP-based linkage map having seven linkage groups using segregating progeny from an *A. atlantica* × *A. hirtula* cross further confirmed the relatedness of these two taxa (O'Donoughue et al. 1992).

For *A. agadiriana*, the holotype for accession CAV 6743 described by Baum and Fedak (1985b) was unique among tetraploid *Avena* species in that it consisted of two satellited, two median, seven submedian, and three subterminal pairs of chromosomes. However, Leggett (1988) observed three pairs of satellited chromosomes in this and three out of four other *A. agadiriana* accessions he examined. In addition, he reported occasional meiotic pairing abnormalities such as univalents and, more frequently, rod bivalents in most of the *A. agadiriana* accessions he studied. This aberrant pairing was hypothesized to be due to the influence of genes affecting synapsis. With most of his inter-accession, intraspecific *A. agadiriana* hybrids, Leggett reported increased frequencies of univalents, rod bivalents, and multivalents up to pentavalents in one cross combination indicative of chromosomal rearrangement.

Leggett (1988) also examined interspecific hybrids with *A. agadiriana* in an attempt to identify relationships between the genomes of this and other *Avena* species. His examination of pairing in triploid hybrids between *A. agadiriana* and A-genome diploid *Avena* species revealed the formation of 2–4 rod bivalents along with 3–7 univalents and complex multivalents, indicative of residual homology with extensive chromosomal rearrangement. Nevertheless, *A. agadiriana* was observed by Leggett to have some increased pairing affinity with *A. barbata* Pott. ex Link. (AABB), a species presumed to have arisen via A-genome autoploidy (Ladizinsky 1973), including a mean 5–6 bivalents (mostly rods) and 2–3 trivalents per PMC. However, an average of more than 19 univalents per PMC was reported by Leggett in interspecific hybrids with *A. maroccana* (AACC) and less than 12% of chromosomes were paired in hybrids with *A. sativa* L. (AACCDD). These results indicated that *A.*

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E. N. Jellen (✉) · B. S. Gill
Department of Plant Pathology, Wheat Genetics Resource Center, Kansas State University, Manhattan, KS 66506, USA

agadiriana was not a likely candidate as an ancestral species of cultivated oat (Leggett et al. 1992).

The objectives of the present study were to (1) describe the C-banding patterns of chromosomes of *A. agadiriana*; and (2) examine the amount of variation for C-bands among five accessions of this species.

Materials and methods

Seed for *A. agadiriana* (2n = 4x = 28) accessions CAV 6729, CAV 6730, CAV 6743, CAV 6757 and CAV 6758, as well as CAV 6633 (*A. hispanica* A_sA_s), were obtained from Guy Baillargeon, Plant Gene Resources, Ottawa, Canada. The *A. agadiriana* accessions were originally collected at locations spanning 280 kilometers near the Moroccan coast from El-Jadida in the north to approximately 60 kilometers south of Essaouira (Baum and Fedak 1985b).

Techniques for chromosome squash preparations and C-banding are detailed elsewhere (Jellen et al. 1993); for this study Giemsa stain (Fisher, liquid) was used. Karyotypic measurements were based on analyses of chromosomes in three cells per line. Chromosomal preparations were photographed using a Zeiss photomicroscope.

Results

The C-banded karyotype of the *A. agadiriana* holotype CAV 6473 is presented in Fig. 1, with accompanying arm ratios in Table 1. The chromosomes have been numbered 1 to 14, from longest and shortest. Chromosomes 3 (arm ratio 1.1), 9 (1.1), 10 (1.1), and 11 (1.2) were identified as metacentric. Chromosomes 1 (1.4), 4 (1.4), 7 (1.4), 8 (1.6), 12 (1.5), and 14 (1.5) were identified as submetacentric. Subtelocentric chromosomes were 2 (2.0) and 5 (2.1). Satellites, as determined under phase contrast prior to C-banding, were observed on chromosomes 6 (1.6) and 13 (1.8). Prominent heterochromatic C-bands were subsequently observed at the nucleous organizer regions (NORs). Leggett (1988) had reported observing three pairs of satellited chromosomes in this accession, in contrast to Baum and Fedak (1985b) who had only reported two pairs of satellited chromosomes in the CVR 6743 holotype. Chromosomes 7 and 12 also possessed prominent C-bands near the tip of the short arm, though no secondary constrictions were observed. All of the chromosomes possessed at least one telomeric C-band and multiple interstitial C-bands. The most prominent interstitial C-bands were observed on chromosome arms 1L, 3S, 5L, 7S, 8L, 9L, 12S, and 14L. Diffuse bands of hetrochromatin were located near the termini of chromosome arms 10L, 13L, and 14L.

The C-banded karyotype of *A. agadiriana* CAV 6729 is presented in Fig. 2. In this figure the chromosomes are again numbered from longest to shortest. Chromosomes 2 and 5 were metacentric, with respective long:short arm ratios of 1.1 and 1.2 (Table 1). Chromosomes 3 (1.4) and 9 (2.1) were satellited, having prominent bands of NOR heterochromatin in their short arms. Chromosome 7 was subtelocentric, with an arm ratio of 2.1, while chromosomes 4 and 6 were near-subtelocentrics with arm ratios of 1.9. The remaining eight chromosome pairs were found to be submetacentric. Chromosomes 4 and 6 had similar banding patterns but were

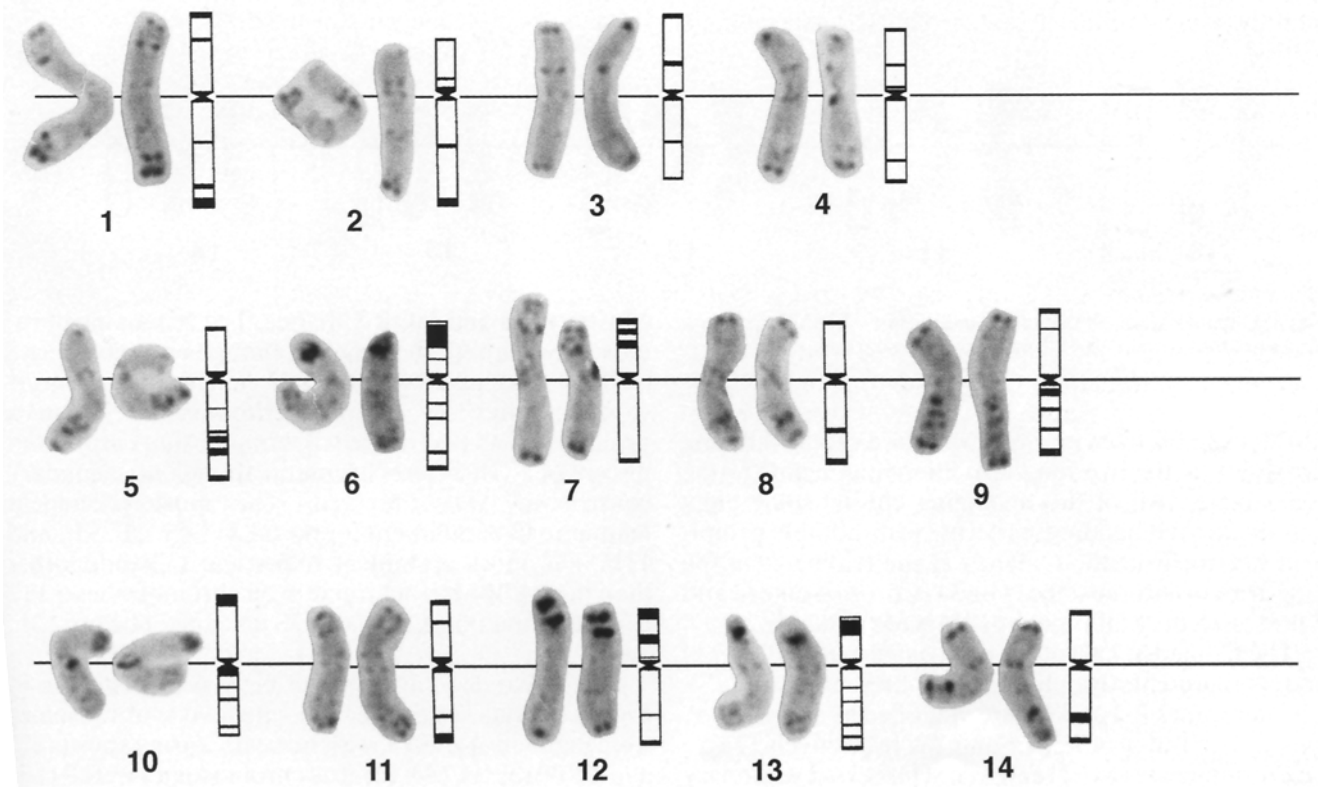

Fig. 1 C-banded karyotype and idiogram of *A. agadiriana* CAV 6743. Chromosomes are numbered 1 to 14, from longest to shortest

Table 1 Arm-ratios of chromosomes of *A. agadiriana* accessions CAV 6729, CAV 6730, CAV 6743, CAV 6757 and CAV 6758

Chromosome	CAV 6729	CAV 6730	CAV 6743	CAV 6757	CAV 6758
1	1.5	1.2	1.4	1.6	1.5
2	1.1	1.1	2.0	1.1	1.0
3	1.4	1.8	1.1	1.8	1.2
4	1.9	1.3	1.4	1.3	1.4
5	1.2	1.9	2.1	1.3	1.4
6	1.9	1.7	1.6	1.4	1.9
7	2.1	1.6	1.4	2.2	1.2
8	1.4	1.8	1.6	1.8	1.2
9	2.1	1.2	1.1	1.6	1.7
10	1.7	1.9	1.1	1.1	1.3
11	1.5	1.3	1.2	1.6	1.4
12	1.8	1.3	1.5	1.6	1.7
13	1.7	1.4	1.8	1.8	2.0
14	1.6	1.9	1.5	1.7	1.5

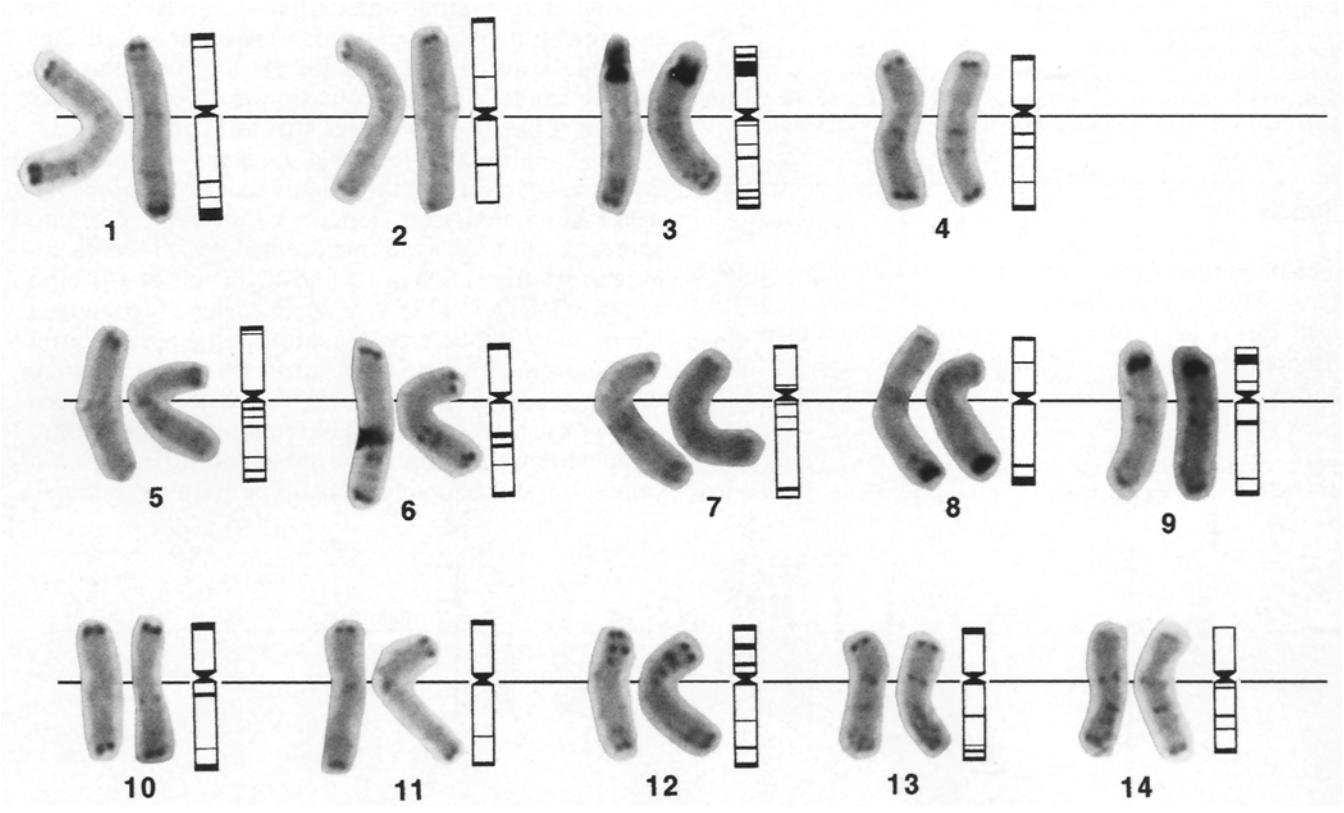

Fig. 2 C-banded root-tip cell of *A. agadiriana* CAV 6729. Chromosomes are numbered 1 to 14, from longest to shortest

distinguishable from one another based on the differing proximity of the two long-arm interstitial bands to the centromeres. All of the remaining chromosome pairs had distinctive banding patterns, with notable prominent heterochromatic C-bands at the telomeres of the long arms of chromosomes 1 and 8. Chromosomes 2 and 7 possessed only faint heterochromatic C-bands.

The C-banded karyotype of *A. agadiriana* CAV 6730 and its representative idiogram are presented in Fig. 3. Chromosomes 1, 2 and 9 were identified as metacentric, having arm ratios of 1.2, 1.1 and 1.2, respectively (Table 1). Chromosomes 6 (1.7) and 8 (1.8) possessed secondary constrictions and NOR C-bands. The remaining chromosomes were submetacentric, though chromosomes 5 (1.9), 10 (1.9), and 14 (1.9) could be classified as near-subtelocentrics. In addition, chromosome 7 had a prominent C-based in the subterminal short arm reminiscent of NOR heterochromatin, though no secondary constriction was observed. The most prominent telomeric C-bands were found on 1L, 4S, 5L, 9L, and 11L. The most prominent interstitial C-bands, other than the NOR heterochromatin on chromosomes 6 and 8, were found on 3L, 5L, 6L, 7S (as noted above), 12L, and 14L.

The C-banded karyotype of CAV 6757 is shown in Fig. 4. In this accession, the satellited chromosomes were numbers 4 and 13, with respective arm ratios of 1.3 and 1.8 (Table 1). Metacentric chromosomes were 2 (1.1)

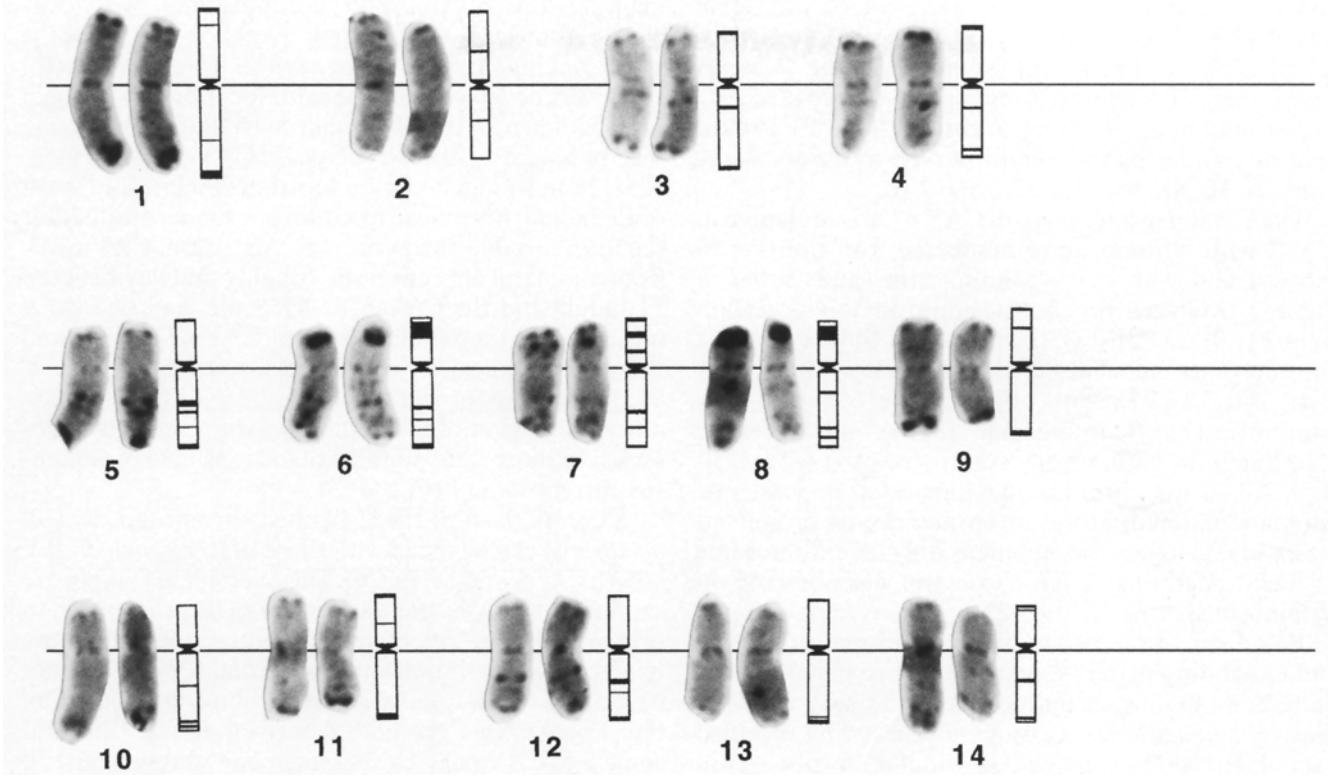

Fig. 3 C-banded karyotype and idiogram of *A. agadiriana* CAV 6730. Chromosomes are numbered 1 to 14, from longest to shortest

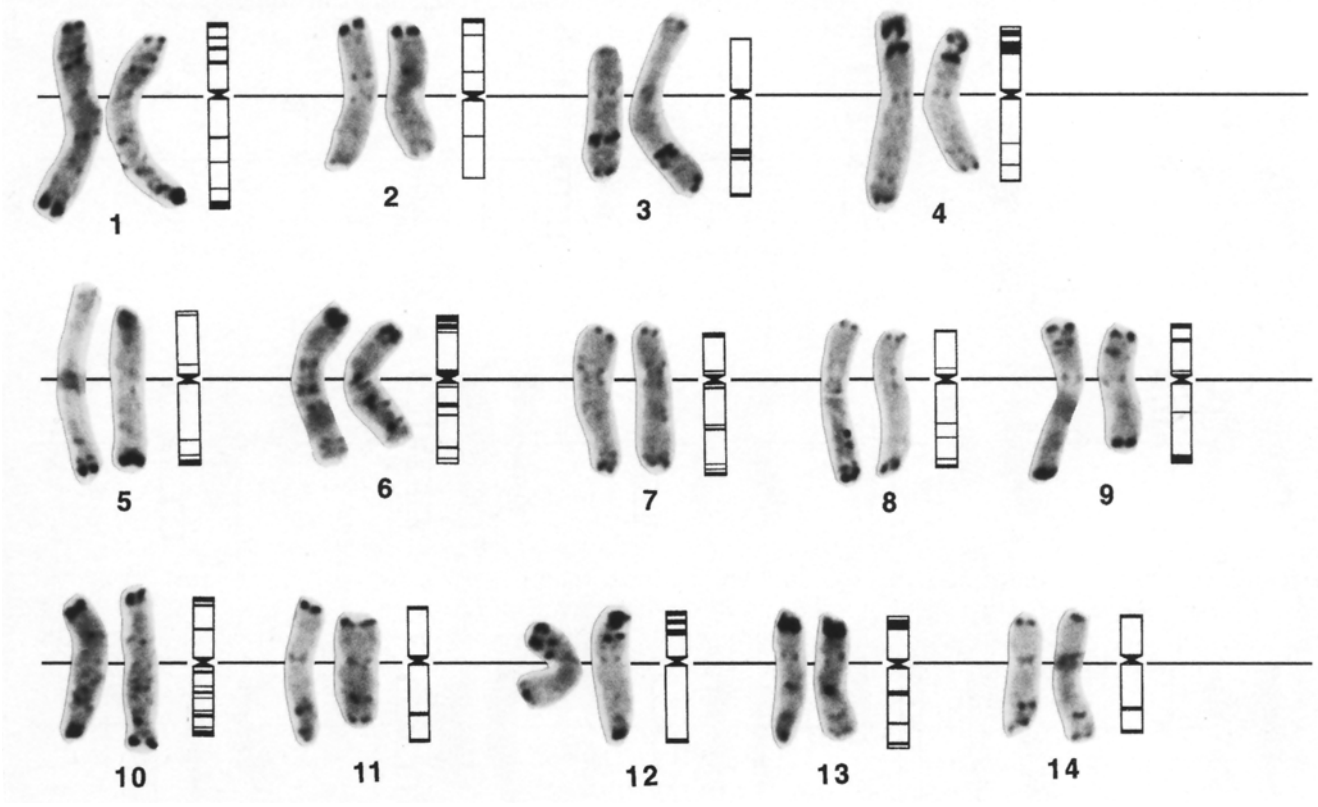

Fig. 4 C-banded root-tip cell of *A. agadiriana* CAV 6757. Chromosomes are numbered 1 to 14, from longest to shortest

and 10 (1.1) and the only subtelocentric chromosome was 7 (2.2). The remaining nine chromosomes were submetacentric. Prominent heterochromatic C-bands were found at the telomeric regions of 1L, 2S, 5L, 6S, 9L, and around both telomeres of chromosome 10. Prominent interstitial bands were observed in chromosome arms 1S, 3L, 6L, 9S, 11L, 12S, and 13L.

The C-banded karyotype of CAV 6758 is presented in Fig. 5 with chromosomes numbered from longest to shortest and with corresponding arm ratios listed in Table 1. Metacentric chromosomes in this accession were 2 (1.0), 3 (1.2), 7 (1.2), and 8 (1.2). Submetacentric chromosomes included 1 (1.5), 5 (1.4), 9 (1.7), 10 (1.3), 11 (1.4), and 12 (1.7), with chromosome 6 a near-subtelocentric (1.9). Chromosome 13 was subtelocentric (2.0). Satellited chromosomes consisted of 4 (1.4) and 14 (1.5). All of the chromosomes possessed at least one telomatic C-band; however, an absence of prominent interstitial C-bands was apparent only on chromosome 11. Diffuse terminal heterochromatin was observed on chromosome arms 3L and 13L.

Based on comparisons of chromosome arm ratios and C-banding patterns, we were able to match potentially homologous chromosomes in the karyotypes of the five *A. agadiriana* accessions (Table 2). This included a set of ten chromosomes having similar morphology in all five accessions. The karyotypes of CAV 6729 and CAV 6730 closely resembled one another for the remaining four polymorphic chromosomes, with only chromosomes 13 of the former accession and 11 of the latter lacking close correspondence. These two accessions were collected from the same location 10 km north of El-Jadida near the Moroccan coast (Baum and Fedak 1985b). Similarly, the karyotypes of CAV 6757 and CAV 6758, both from a common location 280 km southwest of El-Jadida, bore close resemblance to one another for the four variable chromosomes. Accession, CAV 6743, from an inland collection site roughly midway between El-Jadida and the CAV 6757/6758 site, had two chromosomes that resembled those of CAV 6729/6730 and two others that closely resembled chromosomes of CAV 6757/6758. Among the set of recognizable homologues, a notable region of C-banding polymorphism was observed in the distal one-third of the long arm of consensus chromosome 1 (Figs. 1–5).

A comparison of the C-banded chromosomes of *A. agadiriana* can be made with those of *A. hispanica* CAV 6633 (A_sA_s genomes, Fig. 6). The overall karyotypes are similar in terms of the location of heterochromatin in primarily discrete telomeric, interstitial, and pericentric bands. However, the chromosomes of CAV 6633 cannot be matched with a set of seven chromosomes of any of the *A. agadiriana* genomes described above. Chromosome 5 of CAV 6633 closely resembles chromosome 10 of CAV 6729 and chromosome 3 of CAV 6730. In addition, consensus satellited chromosome 12 bears similarity to satellited chromosome 2 of CAV 6633. However, it is obvious that a considerable degree of chromosomal rearrangement differentiates the As-

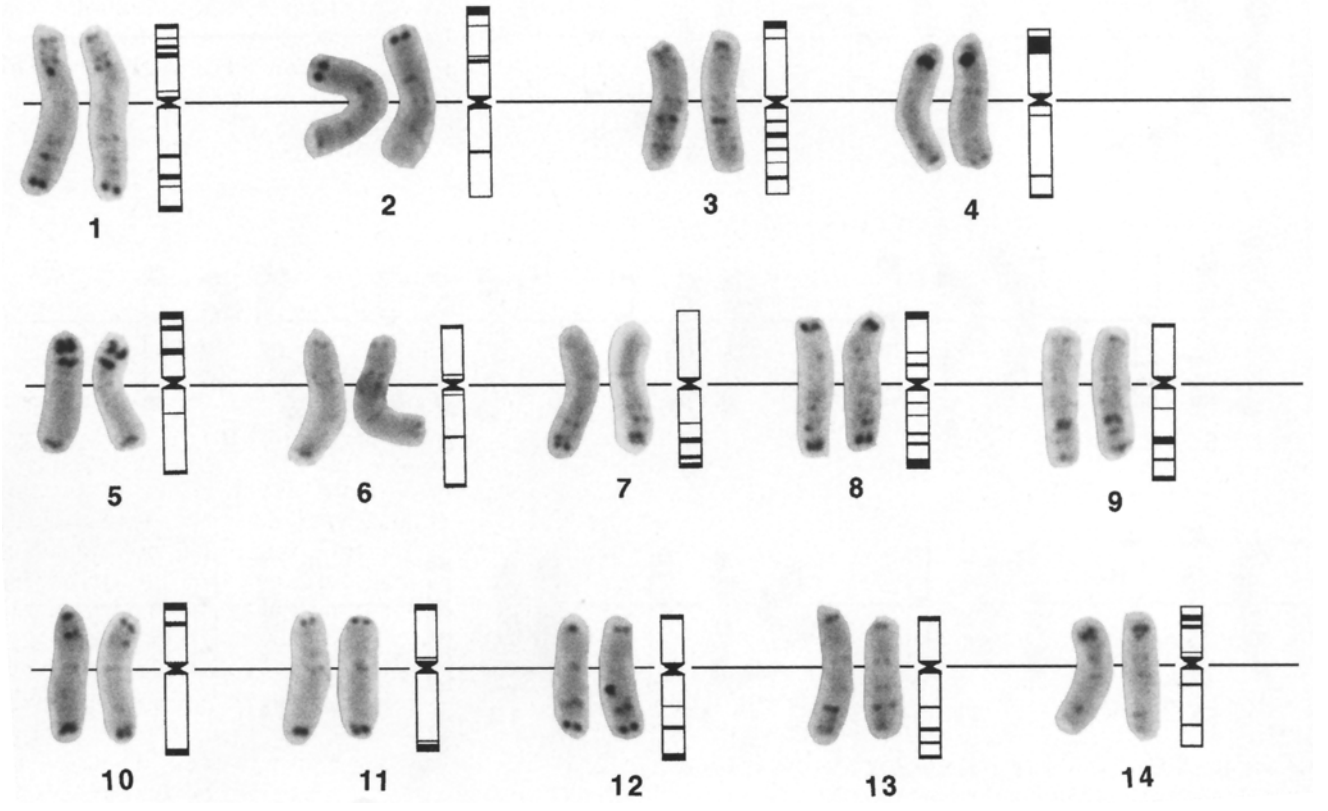

Fig. 5 C-banded karyotype and idiogram of *A. agadiriana* CAV 6758. Chromosomes are numbered 1 to 14, from longest to shortest

Table 2 Putative homologous chromosomes of *A. agadiriana* accessions CAV 6729, CAV 6730, CAV 6743, CAV 6757 and CAV 6758 based on C-banding and arm-ratio similarities

CAV 6729	CAV 6730	CAV 6743	CAV 6757	CAV 6758	Consensus #	Centromere position
1	1	1	1	1	1	Submetacentric
2	2	3	2	2	2	Metacentric
5	4	10	6	3	3	Metacentric
6	5	5	3	9	4	Subtelocentric
3	6	6	4	4	5	Satellited
7	10	2	7	6	6	Subtelocentric
12	7	7	12	5	7	Submetacentric
8	9	4	5	7	8	Metacentric
9	8	13	13	14	12	Satellited
4	12	–	–	–	–	Submetacentric
10	3	–	–	–	–	Submetacentric
11	13	–	8	11	11	Submetacentric
13	–	14	11	12	13	Submetacentric
14	14	8	14	13	14	Submetacentric
–	11	11	–	–	–	Metacentric
–	–	9	10	8	9	Metacentric
–	–	12	9	10	10	Submetacentric

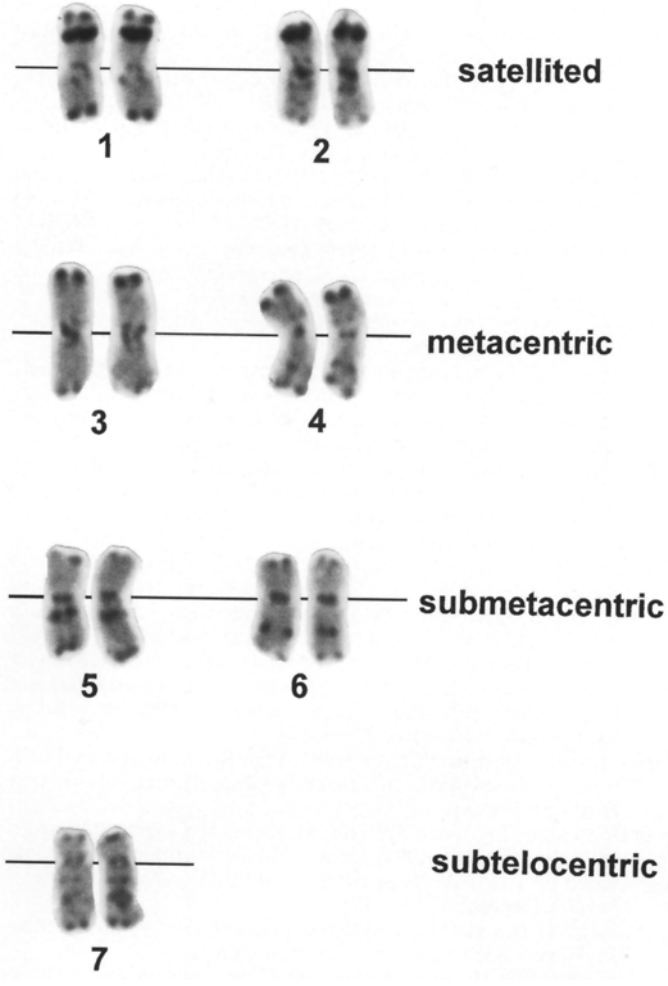

Fig. 6 C-banded cell of *A. hispanica* CAV 6633 (A_sA_s genomes)

genome diploid species and the two constituent genomes of *A. agadiriana*, as was previously demonstrated in interspecific hybrid pairing studies (Leggett 1988).

We observed the *A. agadiriana* accessions to possess relatively minor phenotypic variation when grown in the greenhouse. CAV 6758 tended to be shorter and more highly tillered than the other four accessions. The primary difference among accessions was for hull color, with CAV 6729 being light golden yellow with medium lemma tips, CAV 6730 medium brown with dark lemma tips, CAV 6743 light brown with black lemma tips, CAV 6757 dark reddish brown with medium lemma tips, and CAV 6758 medium brownish red with light lemma tips. Karyopses of CAV 6757 and CAV 6758 were smaller and less than the other accessions, though noticeably more plump than seed of diploid taxa of the section Tenuicarpa which we grew in the same greenhouse. This latter group included accessions of *A. lusitanica*, *A. hirtula*, *A. wiestii*, and *A. atlantica* (Baum 1977). Seed for all *A. agadiriana* accessions was dispersed via shattering at the base of the spikelet.

Discussion

Although all 14 chromosome pairs of *A. agadiriana* can be identified using C-banding the chromosomes cannot be definitively assigned to its two component genomes and the origin of this species remains undefined. It is obvious that the chromosomes of the five accessions described in this study are continuing to evolve via rearrangement, as a set of only ten out of the possible 14 chromosomes were identifiable as being in common among the five karyotypes described above. Intraspecific polymorphism for C-bands has been observed previously in polyploid species of the genus *Avena* (Jellen et al. 1993), and genomic differentiation via chromosomal rearrangement has been well-documented for this genus (Rajhathy and Thomas 1974). These intraspecific karyotypic differences are also in harmony with the findings of Leggett (1988), wherein certain hybrid cross combinations of *A. agadiriana* parents displayed increased frequencies of univalents. This finding was at-

tributed to an operating desynaptic mechanism. In addition, Leggett observed multivalents up to pentavalents in these crosses, indicative of translocations, with concomitant decreases in bivalents per pollen mother cell (PMC). Univelent misdivision has been implicated as a possible mechanism in the generation of centric interchanges in the Triticeae tribe (Sears 1952; Lukaszewski and Gustafson 1983).

We were also interested to note that Leggett (1988) invoked the pivotal genome hypothesis of Zohary and Feldman (1962) to explain pairing similarities in pentaploid hybrids derived from a set of differentiated *A. agadiriana* parents that had been crossed with hexaploid *A. sativa*. If a subset of the ten less-differentiated chromosomes we have identified in these five *A. agadiriana* accessions constitute a "pivotal" genome that has remained more or less intact through evolutionary time, then these data would tend to support the pivotal/differential genome evolutionary hypothesis. Unfortunately, attempts to assign chromosomes to the euchromatic genomes of *Avena* using molecular techniques such as genomic in situ hybridization (GISH) have been unsuccessful in polyploids where these genomes are found together in the same nucleus. This is apparently due to a lack of repetitive sequence dissimilarity at the DNA level (Chen and Armstrong 1994; Jellen et al. 1994; Leggett et al. 1994). Nevertheless, in our view the structural dissimilarities among chromosomes of the two genomes of *A. agadiriana* warrants their designation as separate genomes. Such designations would follow the precedent for *Avena* established by Ladizinsky (1969) in setting apart the structurally-differentiated B genome from its progenitor, the A genome, by assigning it a unique genome designation (Rajhathy and Thomas 1974).

In terms of a general distribution of euchromatic and heterochromatic bands, the chromosomes of *A. agadiriana* are more like the A/B/D genome group than the highly heterochromatic C genomes. There is no evidence from C-banding patterns that *A. agadiriana* evolved via autopolyploidy. Although there had been some evidence from hybrid chromosome pairing studies that this species might be related to *A. barbata* (Leggett 1988), C-banding results show the chromosomes of *A. agadiriana* to be distinct from those the AsAs diploids (Fig. 6; Fominaya et al. 1988a) as well as *A. barbata* (Fominaya et al. 1988b and our unpublished observations). It is interesting to note that the geographically widespread and phenotypically diverse taxa of the *A. barbata* biological species group have retained a considerable degree of chromosomal homology, as evidenced by hybrid pairing studies (for a review see Rajhathy and Thomas 1974). In contrast, *A. agadiriana* accessions found within a relatively small area of western Morocco have undergone an appreciable amount of chromosomal divergence with relatively minor variation for plant morphological traits (Baum and Fedak 1985b; Leggett 1988). At any rate, it is clear that the extensive amount of chromosomal structure divergence and low overall pairing affinity with chromosomes of *A. sativa* will greatly complicate the utilization of this species as a genetic resource for improving cultivated oats.

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