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PLANT GENETICS

A Comparative Cytogenetic Study of the Tetraploid Oat Species with the A and C Genomes: *Avena insularis*, *A. magna*, and *A. murphyi*

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Abstract—C-banding of chromosomes and in situ hybridization with the probes pTa71 and pTa794 were used for a comparative cytogenetic study of the three tetraploid oat species with the A and C genomes: *Avena insularis, A. magna,* and *A. murphyi.* These species were similar in the structure and C-banding patterns of several chromosomes as well as in the location of the loci 5S rRNA genes and major NOR sites; however, they differed in the number and localization of minor 45S rDNA loci as well as in the morphology and distribution of heterochromatin in some chromosomes. According to the data obtained, *A. insularis* is closer to *A. magna*, whereas *A. murphyi* is somewhat separated from these two species. Presumably, all the three studied species originated from the same tetraploid ancestor, and their divergence is connected with various species-specific chromosome rearrangements. The evolution of *A. murphyi* is likely to have occurred independently of the other two species.

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INTRODUCTION

The tetraploid species of the genus *Avena* are classified into four groups according to their genomic composition. The first group is formed by the species containing the A and B genomes: *A. barbata* Pott., *A. vaviloviana* (Malz.) Mordv., and *A. abyssinica* Hochst. [1]. They are closely related genetically, as indicated by fertility of their interspecies hybrids [2].

The second group contains the single perennial autopolyploid species *A. macrostachya* Balan. [3]. Analysis of the karyotype structure has suggested that this species has genomic formula AA [2]; however, the level of homology of its chromosomes with the diploid species *A. damascena* Rajh. et Baum (Ad), *A. prostrata* Ladiz. (Ap), *A. atlantica* Baum (As), and *A. canariensis* Baum (Ac), according to meiotic analysis, is very low [4, 5]. As comparative studies of the internal transcribed sequences ITS1 and ITS2 of ribosomal RNA genes and 5.8S sequences of nuclear rRNA indicated closer relationship of the genome of *A. macrostachya* with the group of C genomes, this species was ascribed genomic formula CmCm [6].

The third group contains one endemic Moroccan species *A. agadiriana* Baum et Fedak, whose systematic position is not yet finally determined [7]. Analysis of the meiotic pairing of chromosomes in hybrids with other *Avena* species [7] as well as the study of its karyotype by C-banding [8] suggests a closer relation of *A. agadiriana* to representatives of the first group, although their genomes are structurally differentiated as a result of chromosome rearrangements.

The fourth group contains the species *A. magna* Murphyi et Terr. (synonym, *A. maroccana* Gdgr.), *A. murphyi* Ladiz. [9–12], and the recently discovered tetraploid species *A. insularis* Ladiz. [13]. The species of this and other groups either do not intercross or give completely or partially sterile offspring, indicating a distant relationship between their genomes [2]. These data support the results of karyotype studies by differential staining.

Analysis of heterochromatin (HC) distribution in the chromosomes of tetraploid oat species has demonstrated that the euchromatin regions in the species with the A and B genomes are uniformly stained, with small heterochromatin blocks predominantly localized to centromeric and telomeric regions. Characteristic of the species with the A and C genomes are two types of staining: seven chromosome pairs display a weaker staining of the euchromatin regions with small heterochromatin blocks localized telomerically and pericentrically, whereas the remaining chromosomes look more condensed and dark, resembling the staining pattern of the diploid species with the C genomes [14, 15].

The meiotic chromosome pairing of the pentaploid hybrids *A. magna* × *A. sativa* suggested that *A. magna* could have been involved in formation of hexaploid oat species [16]. The significance of the species from this group in the evolution of polyploid oats is confirmed by

Table 1. The studied oat accessions and their origin

Species	VIR catalog no.	Species	Site of collection	
			latitude	longitude
A. insularis	k-2067	Italy, Sicily, Gela	37°10′ N	14°20′ E
A. insularis	k-2102*	Italy, Sicily, Gela	37°10′ N	14°20′ E
A. murphyi	k-1986	Spain, Zahara de los Atunes	36°17′ N	5°86′ W
A. murphyi	k-2088*	Spain, Gaucin	36°53′ N	5°15′ W
A. murphyi	k-2101	Spain, Tarifa	36°01′ N	5°50′ W
A. magna	k-144	Morocco, Quazzane	34°58′ N	5°23′ W
A. magna	k-161	Morocco, Khemisset	33°70′ N	6°31′ W
A. magna	k-1786	Morocco, Maazia	35°20′ N	5°53′ W
A. magna	k-1787*	Morocco, Maazia	35°20′ N	5°53′ W
A. magna	k-1852	Morocco, Ar Rommani	33°53′ N	6°48′ W
A. magna	k-1863	Morocco, Tiflet	33°72′ N	6°30′ W
A. magna	k-1896	Morocco, Tetouan	34°81′ N	6°13′ W
A. magna	k-2100	Morocco, Maazia	35°20′ N	5°53′ W

* The samples used for in situ hybridization.

isozyme analysis [17], study of satellite DNA [18], and meiotic analysis of the hybrids of other species from this group with *A. sativa* L. [19–21]. For example, up to 23.2 chiasms are formed in meiosis of the hybrids *A. insularis* × *A. sativa*, which exceeds essentially the number of chiasms in the meiosis of cultivated oat with *A. magna* (21.0) or *A. murphyi* (16.8) [13]. These data suggest a closer relationship of *A. insularis* with the cultivated species *A. sativa* than with *A. magna* or *A. murphyi.*

Analysis of the meiotic chromosome pairing in the interspecies hybrids between *A. murphyi, A. magna*, and *A. insularis* has demonstrated that these species had originated from the same tetraploid ancestor and then diverged from one another as a result of several large chromosome rearrangements and other changes in their chromosomes that decreased their homology level [14, 22, 23]. Genomic in situ hybridization also confirms the role of chromosome rearrangements in the evolution of *A. murphyi* and *A. magna* [24, 25]. Although the diploid oats that were involved in formation of the species with the A and C genomes are not identified yet, the most probable ancestors that donated their genomes are *A. canariensis*, carrying the Ac genome, and *A. ventricosa* Balan., donor of the C genome [26, 27]. However, it cannot be excluded that these tetraploids originated independently of one another from the same or different diploid ancestors.

In this work, we used differential C-banding to perform a comparative cytogenetic analysis of several accessions of three tetraploid oat species with the A and C genomes to specify their phylogenetic relations and assess the intraspecies polymorphism. In addition, the genomes of *A. magna, A. insularis*, and *A. murphyi* were characterized by in situ hybridization with the probes of 45S and 5S rRNA genes.

MATERIALS AND METHODS

Two accessions of *Avena insularis*, three accessions of *A. murphyi,* and eight accessions of *A. magna* of various origins, obtained from the collection of the Vavilov All-Russia Institute of Plant Industry, were examined (Table 1).

DNA probes. The clone pTa794 is a *Bam*HI fragment of wheat 5S rDNA 410 bp in size, cloned in the plasmid pBR322 [28]. The clone pTa71 is a *Eco*RI fragment of 18S–26S rDNA with a length of 9 kbp isolated from wheat and subcloned in the plasmid pUC19 [29]. The DNA probes were labeled with biotin or digoxygenin by nick translation according to manufacturer's instructions (Roche, Germany). In situ hybridization was conducted according to the protocol described earlier [30] with minor modifications.

Karyotypes were analyzed using conventional C-banding [31]. As the homology between individual oat chromosomes is unknown, the chromosomes of each of the species in question were classified according to the cytological nomenclature. It is known that the chromosomes of the A and C genomes differ in the content and distribution of heterochromatin and are easily differentiable according to these characteristics. Therefore, we first separated the chromosomes of the studied species into the A and C genomes and then ranged the chromosomes according to their lengths within each genome.

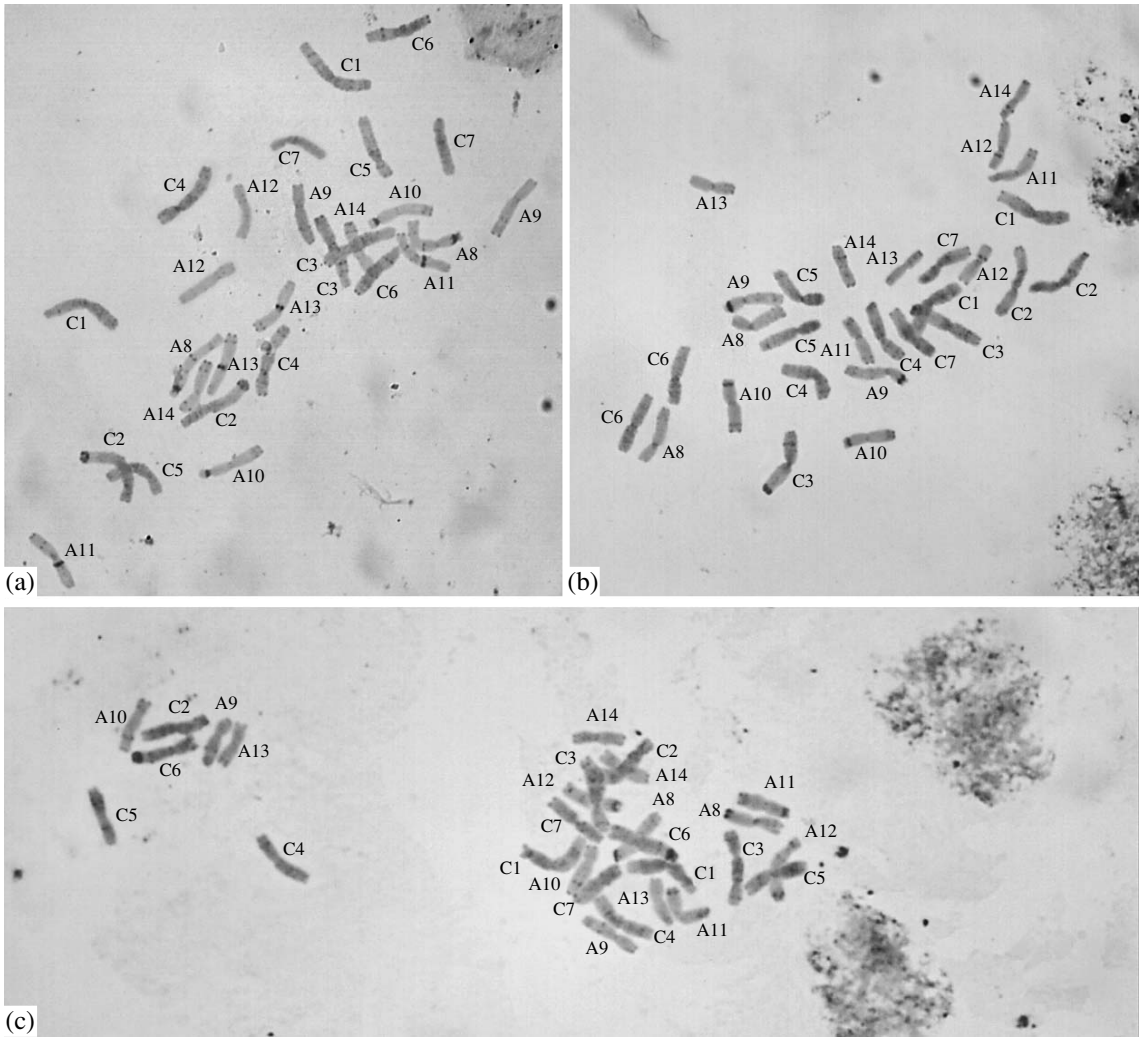

Fig. 1. Differentially stained metaphase plates of oat species: (a) *A. magna* (k-1787), (b) *A. murphyi* (k-2088), and (c) *A. insularis* (k-2102).

RESULTS

Cytogenetic examination confirmed that all the studied accessions of *A. murphyi, A. magna*, and *A. insularis* are tetraploids $2n = 4x = 28$ with the genomic formula AC. The chromosomes of the A genome display a low content of heterochromatin, represented by small but distinct C bands localized to the interstitial chromosome regions. The chromosomes of the C genome are as a rule larger than the chromosomes of the A genome. Their main part is represented by the so-called "diffuse" heterochromatin, which has a darker color compared with euchromatin regions and fuzzy boundaries. More brightly stained and distinct heterochromatin blocks are observable on its background (Fig. 1).

Avena insularis Ladiz.

The stems are ascending; leaves are vertical and hairless; internodes and nodes are naked. The ligula is extended; the panicle is short, with small number of spikes, unilateral, drooping, and loose. The spikelets are large and develop three or more florets. Glumes are long, almost equal, with nine–ten veins. The flower glumes are of medium size and pronouncedly pubescent. The lemma has two awl-shaped teeth at its top. The awn is attached below one-third from the top of the lemma. Only one lowest floret in the spikelet has articulation and readily falls when mature. The callus is elliptic [32].

A. insularis was first discovered in Sicily in 1996. Four populations were found between the cities of Gela and Butera in the south part of the island, where they grew in undisturbed cenoses on the hills at an altitude of 50–150 m above the sea level on alluvial clay soils with sand–clay and conglomerate–stony subsoil. Later the populations of this species were found in Tunis (Temime and Bargon) (Fig. 2).

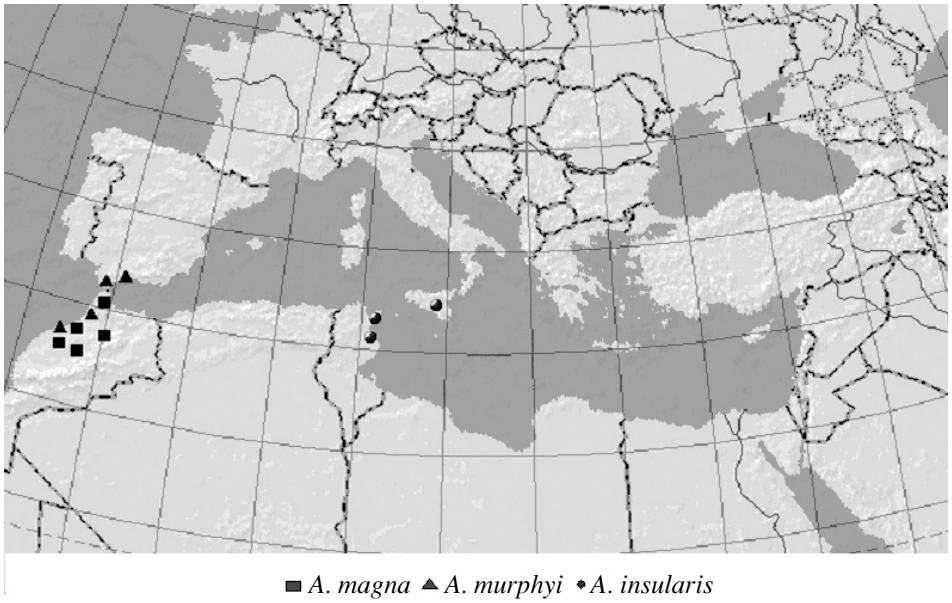

Fig. 2. Habitats of the wild tetraploid species *A. magna, A. murphyi*, and *A. insularis.*

The karyotype of *A. insularis* comprises 14 pairs of chromosomes distinctly differentiable in the morphology and C-banding patterns (Fig. 1c), which allowed for their precise identification. According to the karyotype, chromosomes 1–7 were ascribed to the C genome and designated, correspondingly, as C1–C7; chromosomes 8–14 were ascribed to the A genome (A8–A14; Fig. 3). Two chromosome pairs from A genome, A11 and A12, carry satellites at their short arms. Below are the descriptions of all the individual chromosomes of this species (Table 2).

C1 is a large metacentric chromosome. The main part of the short arm and approximately two-thirds of the long arm are occupied by diffuse HC, which is more intensely stained in the proximal part of the chromosome. Two marker brightly stained bands are distinctly seen on this background in the short arm and divide this arm approximately into three equal parts. A set of two to three small bands is evident in the pericentric part of the long arm. In addition, another distinct marker band is seen it its distal third. Small telomeric bands can be seen in both arms.

C2 is a metacentric chromosome only slightly shorter than chromosome C1. The diffuse heterochromatin occupies the entire short arm and the proximal half of the long arm. The large intensely stained bands, characteristic of this chromosome, are located in the telomere of short arm and pericentrically, in the middle part, and in the distal region of the long arm approximately equidistant from one another. In addition, several smaller intercalary bands can be observed in the short arm of this chromosome.

C3 is a large subacrocentric chromosome. The diffuse HC occupies its entire length. The characteristic features of chromosome C3 are a bright subtelomeric band in the short arm and a set of intensely stained C bands in the proximal part of the long arm, near a bright marker C band. The distal part of the long arm contains an intercalary C segment, varying considerably in its size. Smaller polymorphic HC blocks may be observed in both arms.

C4 is a submetacentric chromosome of a medium size. The diffuse HC is located over its entire length. One large intercalary block is localized to the distal third of the short arm; the marker bands in the middle of the long arm are double. A distinct C band is adjacent to the centromere. The short arm sometimes carries small telomeric blocks.

C5 is a submetacentric chromosome. The diffuse HC is located over the entire length of the chromosome; however, the staining intensity is higher in the proximal part of the short arm. Bright marker C bands are localized to the middle of the long arm. A number of large HC blocks are observable in the middle of the short arm approximately at the boundary of diffuse HC.

C6 is a submetacentric chromosome of a medium size. Characteristic of it is a large intensely stained double marker block in the telomeric region of the long arm. The diffuse HC occupies the entire short arm and a larger part of the long arm. Distinct bright blocks located in the subtelomeric region of the short arm and distal third of the long arm are at the boundaries of diffuse HC. A number of interstitial blocks differing insignificantly from diffuse HC in the staining intensity may be present in the proximal part of this chromosome.

C7 is a submetacentric chromosome. The diffuse HC is located along the entire length of the short arm and the proximal third of the long arm. The telomere in

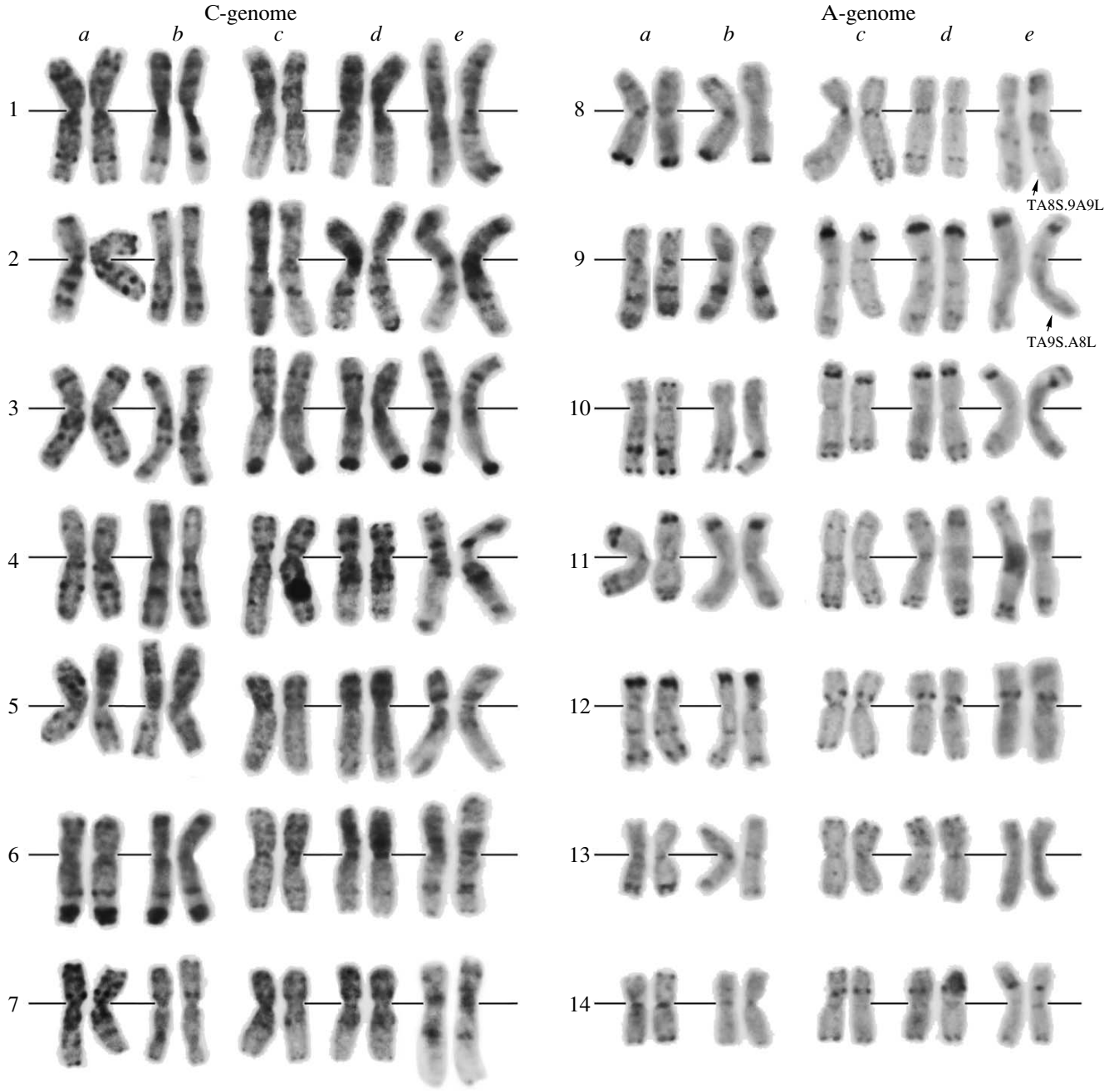

Fig. 3. C-banding patterns of chromosomes in karyotypes of various accessions of *A. insularis*—(*a*) k-2102 and (*b*) k-2067 and *A. murphyi*—(*c*) k-2088, (*d*) k-2101, and (*e*) k-1986.

the short arm contains a bright HC segment, and two large C bands divide the short arm into three approximately equal parts. The proximal part of the long arm contains a set of two-to-three HC blocks of a medium size. Small marker C bands are located in the middle of the long arm and its telomeric region.

A8 is a small metacentric chromosome with a characteristic large telomeric block in the long arm and a weak interstitial polymorphic band in the proximal third of the short arm. A small distinct band is located in the pericentric region of the short arm.

A9 is a submetacentric chromosome with a weak telomeric band in the short arm and near the centromere. A bright interstitial block with a medium size is located in the middle of the long arm, and the distal quarter of it contains diffuse HC.

A10 is an acrocentric chromosome with distinct telomeric blocks in both arms, a large marker block in the distal third of the long arm, and a rather weak C band in its middle part. The proximal third of the short arm contains a small marker HC block. Weak polymor-

Table 2. Morphometric parameters of the chromosomes of oat tetraploid species with the AC genome (chromosome classification by Jellen et al. [23] is used for comparison)

Chromosome	*A. insularis*			*A. murphyi*			*A. magna*		
	relative length, %	arm ratio	classification according to [23]	relative length, %	arm ratio	classification according to [23]	relative length, %	arm ratio	classification according to [23]
C1	9.63	1.08	M1	9.79	1.05	1	9.43	1.04	1
C2	8.55	1.08	M2	9.48	1.18	3	8.21	1.37	3
C3	8.31	1.66	SM3	8.70	1.12	2	8.04	1.19	2
C4	7.76	1.38	M3	7.53	1.81	5	7.86	1.86	4
C5	7.38	1.38	SM2	7.45	1.97	4	7.62	1.80	6
C6	7.28	1.72	SM1	6.99	1.36	6	7.04	0.99	5
C7	7.11	1.45	SM4	6.86	1.60	7	6.97	1.86	7
A8	7.05	1.18	M4	6.91	1.91	8	7.12	1.66	8
A9	7.04	1.02	SM2	6.90	1.88	9	6.81	1.57	10
A10	6.82	2.26	ST1	6.53	1.21	12	6.64	1.24	12
A11	6.50	1.07	SAT2	6.21	1.21	11	6.43	1.07	9
A12	6.06	2.08	SAT1	6.14	1.27	14	6.39	2.17	14
A13	5.54	1.16	SM6	5.57	1.18	13	6.19	1.17	11
A14	4.96	1.24	M5	4.94	1.19	10	5.25	1.24	13

phic interstitial bands are located pericentrically in the long arm.

A11 is a metacentric satellite chromosome with a characteristic large perinucleolar HC block. The long arm carries a telomeric C band of medium size and a slightly larger subtelomeric block.

A12 is submetacentric chromosome with a medium-sized satellite in the short arm. The region of a secondary constriction contains a large C block virtually similar in its size to the satellite. Distinct C blocks are located in the middle of the long arm and in its subtelomeric regions.

A13 is a small metacentric chromosome virtually free of heterochromatin: weak bands are seen only in the regions of both telomeres and the subtelomeric region of the long arm,

A14 is a small submetacentric chromosome. The telomeres and centromere carry hardly visible polymorphic blocks.

Double fluorescent in situ hybridization (FISH) with the probes pTa71 and pTa794 detected eight signals of pTa794 probes on the chromosomes of *A. insularis.* Two chromosomes contained two bright signals each and two other chromosome pairs, one signal each at a considerably weaker site (Fig. 4a). The hybridization with the probe pTa71 gave ten signals; four of them were very large and six were point (Fig. 4b). Thus, the genome of *A. insularis* contains four loci of 5S rDNA as well as two major and three minor loci of 45S rDNA. One of the chromosomes that carry the major NOR (45S rDNA2) also contains two large loci of 5S rRNA genes in the short arm (5S rDNA1) and in the proximal part of the long arm (5S rDNA2, Fig. 1a), whereas 5S rDNA loci were undetectable in the other nucleolus-forming chromosome.

One more chromosome in the genome of *A. insularis* carried simultaneously the minor loci of 5S and 45S rRNA genes. They were located approximately in the middle of the long arm of a submetacentric chromosome, and the 5S (5S rDNA4) site was located distally of the minor 45S rDNA (45S rDNA3) locus. The fourth 5S rDNA locus and two minor 45S rDNA loci were distributed between different chromosomes: 5S rDNA3 was detected in the proximal quarter of a large metacentric chromosome; 45S rDNA4, approximately in the middle of the arm of a small metacentric chromosome; and 45S rDNA5, in the proximal region of an acrocentric chromosome.

Avena murphyi Ladiz.

The stems are angular, later straightening; leaves are horizontal, slightly scabrous, pubescent or nude; leaf sheathes are smooth. The ligula is shortened or extended. The panicle is spreading, with small number of spikes, with loosen and wide floral spikelets. Spikelets are large. Glumes are almost equal with eight veins and wide, equal, mainly nude floral glumes. The awe attaches to the upper quarter part from the top of the lemma. The lemma has two teeth on its top. Only one

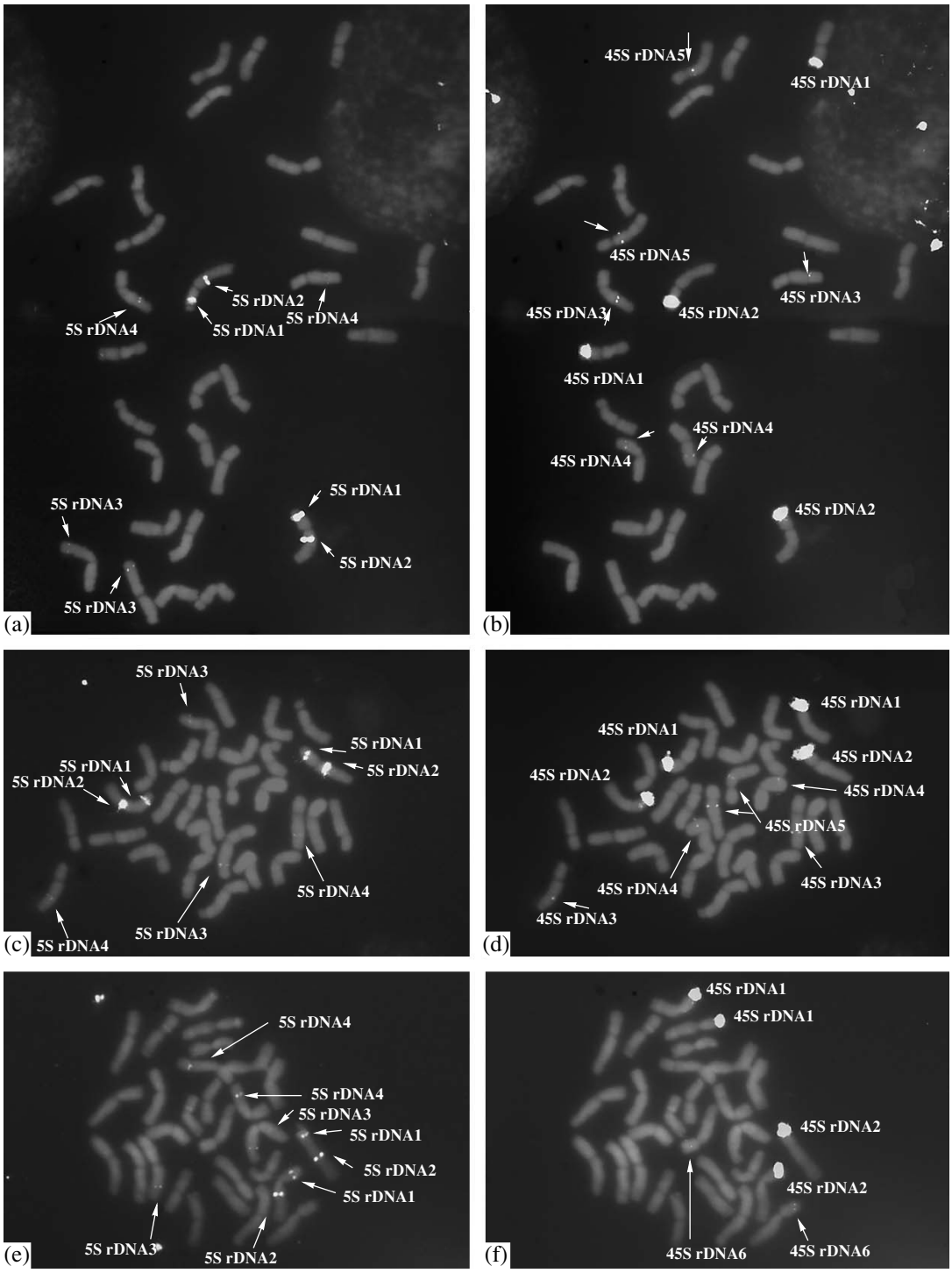

Fig. 4. In situ hybridization of the probes (b, d, and f) pTa71 and (a, c, and e) pTa794 to the chromosomes of (a, b) *A. insularis* k-2102, (c, d) *A. magna* k-1787, (e, f) and *A. murphyi* k-2088. The loci of rRNA genes are indicated by arrows and designated 5S rDNA1–5S rDNA4 and 45S rDNA1–45S rDNA6.

lowest floret in the spikelet has articulation and readily falls when mature. The callus is round [32].

A. murphyi originates from a local area in the south of Spain (between Tarifa and Vejer de la Frontera), where it grows in a typically Mediterranean climate on rich alluvial soils in undamaged associations with the hexaploid oat species *A. sterilis*, which is morphologically similar. In addition to sampling of this species in the southwestern Spain (coastal zone), where a number of *A. murphyi* specimens were found, not numerous forms of this species were discovered in the northern part of Morocco near Tangier (coast; Fig. 2). The

Moroccan population of *A. murphyi* inhabits rich alluvial soils with intensive agricultural activities, which may result in eradication of this species or its displacement by a more aggressive member of this community, the hexaploid *A. sterilis*, which intensely weeds the cultivated crops. Recently, only several *A. murphyi* plants were found in southern Spain in the Cadiz province, confirming the assumption about a drastic decrease in the population of this species or its partial disappearance from the territory of Spain (Fig. 2).

The karyotype of *A. murphyi* comprises 14 chromosome pairs distinct in their morphology (Table 2) and C-banding patterns (Fig. 1b). The presence of diffuse HC is a characteristic feature of the C genome (pairs C1–C7), whereas the chromosomes of the A genome (A8–A14) lack it (Fig. 3c–3e).

C1 is a large metacentric chromosome. The diffuse heterochromatin is located along the entire length of this chromosome except for the distal quarter of the long arm; however, it is stained more intensely in the proximal part of the chromosome. The short arm contains a large marker block with indistinct boundaries at a distance of one-third from the telomere. A set of three rather small blocks is located in the middle of the long arm on the background of a small diffuse HC region. The telomeres of both arms carry weak blocks.

C2 is a large metacentric chromosome. The diffuse heterochromatin is localized to the short arm and a larger part of the long arm except for its distal third. Bright marker C bands in the pericentric region and approximately in the middle of the long arm are seen on its background. A relatively small C block is evident between the central block and the telomere, which divides this region into two approximately equal parts. The distal quarter of the short arm contains a characteristic HC block with indistinct boundaries.

C3 is a metacentric chromosome with a characteristic distinct and very large telomeric block in the long arm, resembling the marker block of *A. insularis* chromosome C6. The region of the diffuse HC occupies the proximal region of the long arm and the entire short arm. The short arm contains a set of two–three marker C blocks, located approximately equidistantly from one another, and a small telomeric band. A relatively weakly stained HC region with fuzzy boundaries is observed in the middle of the long arm.

C4 is a pronouncedly submetacentric chromosome. Diffuse HC occupies the entire short arm and a proximal half of the long arm. Characteristic of the short arm are bright large blocks in the telomere, subtelomeric region, and near the centromere. The proximal third of the long arm contains a set of C bands polymorphic in their size, and a very small block limiting diffuse HC is seen in its middle. A small polymorphic C block is located subterminally in the long arm.

C5 is a submetacentric chromosome with a characteristic gradual decrease in the intensity of diffuse HC staining from the telomere of the short arm to the telomere of the long arm. The short arm contains two indistinct characteristic blocks (located near the centromere and subterminally) differing insignificantly from diffuse HC in the staining intensity. The distal third and telomeric region of the long arm contain two weakly stained small blocks.

C6 is a small presumably metacentric chromosome. The darkly stained diffuse HC is observed in the short arm except for subtelomeric region and in the proximal quarter of the long arm. A distinct marker block is located in the distal third of the long arm, and two-to-three small bands are located pericentrically and in the telomere of the same arm. The short arm contains several large indistinctly outlined blocks, which differ insignificantly from diffuse HC in the staining intensity.

C7 is a relatively small submetacentric chromosome. The diffuse HC is localized to the proximal third of the long arm and to the short arm. The marker blocks are located near the centromere and subtelomerically in the short arm. A set of blocks varying in their size is observed in the proximal third of the long arm, and its telomere carries a distinct C segment.

A8 is a submetacentric chromosome with distinctly stained centromeric HC and a bright C band in the distal third of the long arm. The block in its proximal third is polymorphic and pronounced only in one sample of the three studied. Both arms carry small telomeric blocks.

A9 is a submetacentric chromosome with a satellite. A large brightly stained region of perinucleolar HC is localized to the region of secondary constriction. Small yet distinct blocks are observed approximately in the middle of the long arm and in its subtelomeric and telomeric regions. The centromere is distinctly strained.

A10 is a metacentric chromosome with a satellite. A large marker block is present in the region of secondary constriction. The subtelomeric and telomeric regions of the long arm carry small distinctly stained HC segments.

A11 is a medium-sized metacentric chromosome similar to chromosome A10 in the distribution of HC blocks but lacking satellite. The subterminal and telomeric regions of its long arm also contain bright HC blocks, and a small subtelomeric C block is observed in the short arm at a distance slightly larger than that of the block in the long arm.

A12 is a submetacentric chromosome with a bright relatively large band in the proximal region of the short arm. Both telomeres carry small HC blocks. In addition, a very weak point HC block is localized to the middle of the long arm.

A13 is a small metacentric chromosome with characteristic C bands in the proximal and distal regions and telomere of the short arm. A very weak band is observable in the subtelomeric region of the long arm.

A14 is a small metacentric chromosome with a bright block approximately in the middle of the long

arm and distinct telomeric blocks. A very small weakly stained C band is seen between the marker proximal and telomeric bands in the short arm.

In situ hybridization on the chromosomes of *A. murphyi* detected four loci of 5S rRNA genes (per haploid genome). Two of them are major and are localized on one satellite chromosome near the secondary constriction in the short arm (5S rDNA1) and in the proximal quarter of the long arm (5S rDNA2). The two other loci are minor and are located in different chromosomes: the distal third of one of the arms of a large metacentric chromosome (5S rDNA3) and the distal region of the long arm of an acrocentric chromosome (5S rDNA4). The loci of 45S rRNA genes (probe pTa71) were detected in three chromosome pairs. The major signals are located in the NOR region of two pairs of satellite chromosomes. One of them (45S rDNA2) carries also the major sites of 5S rDNA in the short and long arms. A minor locus of 45S rDNA displaying a very low intensity is recorded in the distal third of the short arm of an acrocentric chromosome. As this locus is unique for *A. murphyi*, it was designated 45S rDNA6 (Fig. 4f).

Avena magna Murphy et Terr.

The stems are ascending; leaves are arranged differently, pubescent or hairless; internodes and nodes are naked or pubescent. The ligula is extended; the panicle is with small number of spikes, unilateral, drooping, and loose. The spikelets are large, with three-to-four florets. Glumes are wide, long, membranous, slightly unequal, with eight-to-ten veins. The awn is attached in the first half from the top of the lemma. The flower glumes are long and pronouncedly pubescent with colorless or dark colored hairs. The lemma has two teeth at its top. Only one lowest floret in the spikelet has articulation and readily falls when mature. The callus is pronouncedly pubescent and round [32].

The first samples of the endemic species *A. magna* (synonym, *A. maroccana* Gdgr.) were collected in 1964 on the Moroccan coast; later more numerous populations were found to the south of Rabat at an altitude of 1000–1300 m above the sea level, to the southeast of Casablanca at an altitude of 500 m above the sea level, and to the northwest of Fes on the slopes of Atlas mountains at an altitude to 600 m above the sea level (Fig. 2). In all its habitats, this species grows on fertile loose reddish-brown alluvial loam soils, frequently in ditches together with *A. sterilis*; however, unlike this species *A. magna* less frequently weeds cultivated crops. However, a large population of *A. magna* was described, where the plants reached 2 m in height and successfully weeded cereal cultures, forming a crown layer over them. As the population of *A. magna* was found in the region of rich alluvial soils intensely used in farming, this may lead to eradication or displacement of this oat species by a more aggressive member of this community, *A. sterilis.* The communications about discovery of tetraploid samples similar to *A. magna* in Sardinia were not confirmed.

Similar to the two species described above, *Avena magna* is a tetraploid ($2n = 4x = 28$) with the A and C genomes. The chromosomes of the A and C genomes were classified according to the presence of diffuse HC (Fig. 1a) and ranged within each genome in the order of descending length (Table 2). The individual chromosomes are described below (Fig. 5).

C1 is the largest metacentric chromosome. The diffuse HC is located along virtually entire chromosome length; however, the distal thirds of both arms are stained weaker. Bright marker blocks are located near the centromere in the long arm and in its distal third, limiting the region of dark diffuse HC. A set of smaller interstitial bands are localized between these distinct blocks. The short arm contains one marker block approximately in its middle and a double block in the subtelomeric region. In addition, smaller interstitial blocks are also observable in the short arm.

C2 is a submetacentric chromosome with a very large bright marker block in the telomere of the long arm. The diffuse HC is more intensely stained in the short arm and the proximal region of the long arm. Two marker blocks are localized to approximately middle and distal third of the short arm. Small polymorphic HC blocks are observed in the middle of the long arm and its pericentric region.

C3 is an approximately metacentric chromosome. The diffuse HC is stained evenly along the entire chromosome length. Characteristic large C bands are located pericentrically and in the middle of the long arm; a constant block in the telomeric region is considerably smaller than these bands. The majority of accessions also contain a rather large C band located in the distal third of the long arm. Small blocks are detectable approximately in the middle of the short arm and near its telomere. The accession k-1863 displayed a pericentric inversion in chromosome C3.

C4 is a submetacentric chromosome. The diffuse HC in the proximal region of the chromosome and the distal third of the long arm is stained more intensely. A distinct marker block is located approximately in the middle of the short arm and near the centromere. The long arm carries a large HC block in the subtelomeric region and a smaller C band in its middle. In addition, the long arm carries a set of weak interstitial bands.

C5 is a metacentric chromosome of a medium size. The diffuse HC in the pericentric region of the short arm and proximal third of the long arm is stained darker than in the distal regions. Bright marker C bands are located approximately in the middle of the short arm and at its telomere. A characteristic large block is localized to the distal third of the long arm; a set of small polymorphic C blocks are observable in its proximal half on the background of diffuse HC.

C6 is a small metacentric chromosome. The diffuse HC is localized predominantly to its short arm. Sets of

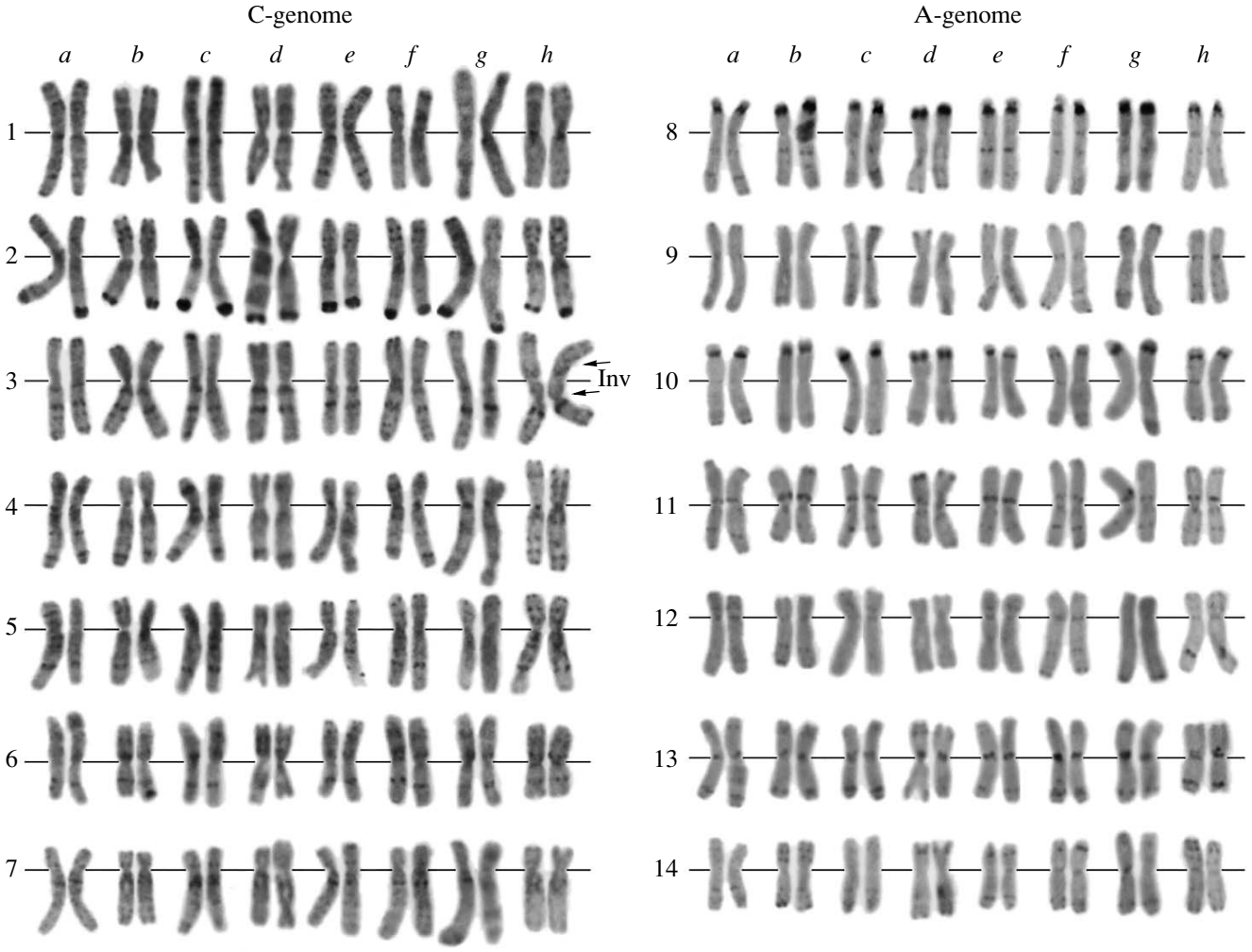

Fig. 5. C-banding patterns of the karyotypes of various *A. magna* accessions: (a) k-2100, (b) k-1852, (c) k-1786, (d) k-1896, (e) k-1787, (f) k-144, (g) k-161, and (h) k-1863.

several large distinct C bands are located in the pericentric region and in the middle of the short arm. A bright marker block is observed approximately in the middle of the long arm.

C7 is a submetacentric chromosome. The most evident region of diffuse HC is in the proximal region of the long arm. A characteristic distinct block is located in the middle of the short arm; the long arm carries several marker blocks: one near the centromere and weaker bands in its middle and subtelomeric region. The long arm also contains several polymorphic interstitial blocks.

A8 is a submetacentric chromosome with a satellite. A very large marker block is located near the secondary constriction. Another small polymorphic block is adjacent to it in the short arm. Two distinct marker blocks are localized in the proximal and distal thirds of the long arm.

A9 is a submetacentric chromosome. The centromere and telomeres of both arms contain small but distinct C bands. The long arm carries two small HC blocks in its distal third.

A10 is an approximately metacentric chromosome carrying the satellite with a large block of perinucleolar HC. The telomere and subterminal region of the long arm contain small C bands.

A11 is a metacentric chromosome with a characteristic large brightly stained C band adjacent to pericentric HC in the short arm and marker blocks located in the distal third of the long arm. A small but distinct C band is also observed in the pericentric region of the long arm. Both telomeres carry bright HC blocks.

A12 is an acrocentric chromosome. An indistinct marker HC segment is observable approximately in the middle of the short arm. The distal third of the long arm contains a characteristic bright C band. The telomeres of both arms carry small HC blocks. The long arm also displays polymorphic blocks in its middle and proximal regions.

A13 is a metacentric chromosome with characteristic distinct large blocks located subtelomerically and near the centromere in the short arm. The subtelomeric blocks in the long arm are usually stained brighter than in the short arm.

A14 is an approximately metacentric chromosome carrying a set of one–two small polymorphic blocks in the short arm and one–two small interstitial bands in the distal half of the long arm. The telomere of the long arm sometimes contains small HC segments.

The labeling pattern of *A. magna* chromosomes obtained with the probes pTa71 and pTa794 by double FISH was analogous to the pattern displayed by another species of this group, *A. insularis* (Figs. 4a, 4b), and somewhat differed from *A. murphyi.* In particular, *A. magna* contains four 5S rDNA loci. Of them, two larger loci were localized to the same chromosome in the satellite distally of the main NOR and in the proximal region of the long arm. Smaller signals of pTa794 probe were detected in two different chromosome pairs. Note that 5S rDNA4 locus in the long arm of a submetacentric chromosome was linked to the minor locus of 45S rDNA (45S rDNA3), as was observed in *A. insularis.* One major and two minor 45S rDNA loci were detected in three different chromosome pairs, and the relative locations of hybridization signals were similar to that of *A. insularis* (Fig. 4).

DISCUSSION

Cytogenetic analysis of three tetraploid oat species—*Avena insularis, A. magna*, and *A. murphyi*—by C-banding and in situ hybridization with the probes pTa794 (5S rDNA) and pTA71 (45S rDNA) has demonstrated their similarity in the structure of karyotypes, patterns of chromosome differential staining, and, partially, in the location of the loci of rRNA genes. These facts suggest a close evolutionary relation of the genomes of the studied species.

Specific features of their morphology and patterns of HC distribution in chromosomes allowed us to identify all individual chromosomes of *A. insularis, A. magna*, and *A. murphyi*. The banding patterns of the accessions studied on the whole complied with the patterns published earlier [14, 23, 25, 33], despite that we studied new material and used another variant of C-staining. Compatibility of the used methods allowed us to compare not only the studied accessions with one another, but also the data obtained with the results of other authors. This is especially important as the number of published papers in this field is rather limited.

Interestingly, the level of HC polymorphism of the chromosomes of *A. insularis, A. magna*, and *A. murphyi* appeared rather low. An insignificant variation in C-banding patterns of geographically distant *A. insularis* populations was reported earlier [33]. Presumably, the studied oat species also display a low frequency of chromosome aberrations. For example, only two chromosome rearrangements were detected in the studied samples. The reciprocal translocation between chromosomes A8 and A9 was detected in the karyotype of *A. murphyi* line k-1986, and a pericentric inversion of chromosome C3, which considerably changed its morphology (Fig. 5h), was identified in *A. magna* accession k-1863. Another pericentric inversion was identified in the Tunisian sample of *A. insularis* based on the analysis of meiotic conjugation in hybrids between Tunisian and Sicilian populations [33]. Unlike the inversion that we described, the rearrangement found in *A. insularis* did not change the morphology and C-banding of chromosomes, which allowed the authors to assume that the breakpoints during this inversion were located at the same distance from the centromere [33].

Comparison of differentially stained karyotypes of *A. insularis, A. magna,* and *A. murphyi* demonstrates considerable similarity of certain chromosomes (Fig. 6). First and foremost, these are the satellite chromosomes (A12 of *A. insularis*–A9 of *A. murphyi*–A8 of *A. magna* and A11 of *A. insularis*–A10 of *A. murphyi*–A9 of *A. magna*) ascribed to the A genome according to the mapping using the repetitive sequence pAm1, specific of C genome, and genes of rDNA on the chromosomes of *A. murphyi* and *A. magna* [15]. An essential similarity between the morphology and C-banding patterns is also evident for several chromosomes of the C genome. For example, the morphological characteristics (Table 2) and C-banding patterns of the largest chromosomes of these three species, C1, are very similar. Close patterns of the HC distribution were also detected for the large approximately metacentric chromosomes C2 of *A. insularis*–C2 of *A. murphyi*–C3 of *A. magna*, a small metacentric C5 of *A. insularis*–C6 of *A. murphyi*–C6 of *A. magna*, and a small submetacentric chromosome C7 of *A. insularis*–C7 of *A. murphyi*–C5 of *A. magna.* The chromosome C6 of *A. insularis* contains a very bright large telomeric HC block in its long arm. Such marker blocks are also characteristic of *A. magna* C2 and *A. murphyi* C3 chromosomes; moreover, they display similar patterns of HC distribution in the long arm and, to a smaller degree, in the short arm. Despite that the relative lengths and arm ratios of the cytogenetically similar chromosomes of these species differ, we can assume that each of the groups mentioned above originated from one chromosome of the common ancestor and that their divergence is connected with accumulation of repetitive sequences and/or species-specific chromosome rearrangements.

Close morphological parameters and patterns of HC distribution were recorded also for several other chromosomes; however, the similarity between them was less pronounced than for the chromosomes mentioned above. In several cases, similar chromosomes were found only in two of the three species studied, and many chromosomes lacked the similarity, i.e., they are unique for particular species.

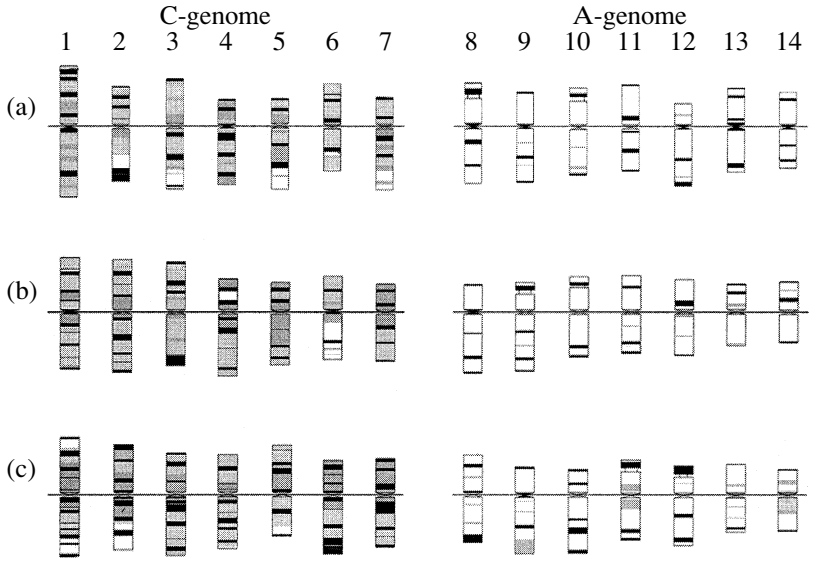

Fig. 6. Karyotypes of the differentially stained chromosomes of (a) *A. magna*, (b) *A. murphyi,* and (c) *A. insularis.*

Pronounced differences between the chromosomes of related species are likely to result from chromosome aberrations that occurred during their divergence. The presence of intergenome translocations in *A. maroccana* (=*A. magna*) and *A. murphyi* was demonstrated by genomic in situ hybridization (GISH) [24, 25]. These papers report at least four translocations of C genome chromosomes to A genome chromosomes in *A. maroccana* (two of them, satellite chromosomes) and one translocation of genetic material from the A genome to chromosome C7. In several cases, intergenome translocations are also observable in C-stained chromosomes. In particular, the distal regions of the long arm of *A. insularis* chromosome A9 and of the chromosomes A11 of *A. insularis*–A10 of *A. murphyi*–A9 of *A. magna* were stained darker compared with the chromosome body, i.e., contained diffuse HC, a trait characteristic of the C genome. Quite probably, the species-specific rearrangements may also involve the chromosomes belonging to the same genome (A–A or C–C chromosome translocations).

Comparison of these three species according to their morphology and C-banding patterns fails to give an unambiguous answer whether they originated from one common ancestor due to chromosome rearrangements or were formed by crossing of various diploid ancestors. To specify the phylogenetic relations of these species, we compared the relative location of the clusters of ribosomal genes. It appeared that the distribution of 5S and 45S rDNA loci on the chromosomes of *A. insularis* and *A. magna* is completely identical, and the similarity in the location of 18S–26S rDNA (45S rDNA) on the chromosomes of *A. magna* and *A. murphyi* was reported earlier [15].

However, according to our data, the similarity between these species is not absolute. Despite that the localization of 5S rDNA loci and major NOR sites completely coincided for these three species, the number and positions of minor 45S rRNA loci were different. In particular, we detected only one very weak site for pTA71 in the short arm of an acrocentric chromosome in the genome of *A. murphyi*, whereas the two rest species contained three minor NOR each and their location differed from that of *A. murphyi.*

The chromosomes carrying major loci of 45S rRNA genes were ascribed to the A genome based on the simultaneous FISH mapping of a satellite DNA sequence specific of the C genome (pAm1) and rDNA on *A. magna* chromosomes [15]. Comparison of our results with the data on localization of 45S and 5S rDNA probes in the chromosomes of *A. strigosa* Schreb. [34] confirms the data of Fominaya et al. [15]. The similarity between the HC distributions on the satellite chromosomes of all the three studied tetraploid species and the diploid ancestors of the A genome [35], detected by C banding, also favors this assumption.

The similarity in distribution of 45S and 5S rRNA genes on the satellite chromosomes of *A. insularis, A. magna*, and *A. murphyi* (Fig. 4) with that in *A. strigosa* [33] implies that the two large 5S rDNA loci mapped on a nucleolus-forming chromosome originate from the A genome. The remaining two loci are located on chromosomes of the C genome. The first chromosome, which contains weak signals in the distal quarter (5S rDNA3), corresponds presumably to C1, as it is a very large metacentric. The second, a small submetacentric chromosome, containing the 5S rDNA locus linked to minor NOR in *A. insularis* and *A. magna*, was ascribed to the C genome according to the results of the simultaneous mapping of pAm1 sequence and 45S rDNA probe on *A. magna* chromosomes [15]. The morphological parameters suggest that this is chromosome C7. As only two

major 45S rDNA loci were detected in *A. strigosa* [34], we can assume that one or even two chromosome pairs of *A. insularis* and *A. magna* that carry minor NOR belong to the C genome.

Possibly, the only minor NOR detected in *A. murphyi* genome, 45S rDNA6, is also located on a chromosome of the C genome. Presumably, it is homologous to the 45S rDNA5 locus of *A. insularis* and *A. magna*, which was transferred into the chromosome short arm due to the pericentric inversion. The absence of two minor 45S rDNA sites (45S rDNA3 and 45S rDNA4) in *A. murphyi* may result from a decrease in the copy number of rRNA genes in the corresponding loci to the level below the FISH threshold.

The similarity in distribution of the loci for rRNA genes on *A. insularis, A. magna,* and *A. murphyi* chromosomes suggests that these species diverged from one common tetraploid ancestor; however, the differences in the number of minor 45S rDNA loci demonstrate that the evolution of *A. murphyi* proceeded somewhat separately from the other species.

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