MEIOTIC CHROMOSOME PAIRING IN TWO INTERSPECIFIC HYBRIDS AND A CRITICISM OF THE EVOLUTIONARY RELATIONSHIP OF DIPLOID AVENA

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In recent years the authors have obtained many interspecific hybrids of *Avena* during a study of a mechanism of cross-incompatibility, especially due to endospermic abortion of seeds. Some of them were cytologically studied (Yabuno and Nishiyama (1975)). This paper deals with meiotic chromosome behavior of two diploid hybrids, *A. pilosa* crossed with *A. strigosa* and *A. ventricosa*. The former is a new hybrid which is obtained only when *A. pilosa* is used as a male parent. The cytological investigation has revealed the poor relationship between *A. strigosa* and *A. pilosa* as presumed by Thomas and Rajhathy (1967). Based on the available cytogenetic information the phylogenetic relationships among diploid species of *Avena* are discussed.

MATERIALS AND METHODS

Two diploid hybrids, *Avena strigosa* × *A. pilosa* and *A. pilosa* × *A. ventricosa*, have been cytologically studied. The former was successful when *A. strigosa* was crossed with *A. pilosa* pollen although crossed seeds poorly developed. The reciprocal hybridization gave non-viable empty and shrivelled seeds at maturity. Reciprocal crosses were successfully made between *A. pilosa* and *A. ventricosa*. Seed germination was tried in petri dishes under temperature of $20^{\circ} \pm 0.5^{\circ}\text{C}$, and potted plants were mostly grown in the green house. In the meiotic observation of PMC's material was fixed in Carnoy's solution (6:3:1), stored in 70% alcohol and stained in aceto-carmine. Slide preparation was made by the squash method.

RESULTS

Morphological observation: Morphological characteristics of the hybrid *A. strigosa* × *A. pilosa* and its parents are briefly summarized in Table 1. It is generally said that most of *A. pilosa* characteristics (weed type) are dominant over the cultivated type of *A. strigosa* but a few intermediate. Pubescence of leaf blades and sheaths was ob-

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Table 1. Morphology of *A. strigosa*, *A. pilosa* and their hybrid

Characteristics	<i>A. strigosa</i>	<i>A. pilosa</i>	Hybrid
Juvenile growth habit	Erect	Prostrate	Semi-prostrate
Hairs on leaf blades			
Upper surface	Glabrous	Glabrous	Glabrous
Under surface	Glabrous	Slightly hairy	Slightly hairy
Leaf margins	Glabrous	Ciliate	Ciliate
Hairs on sheaths	Glabrous	Slightly hairy	Slightly hairy
Time of anthesis	About 7:00 p.m.	About 4:30 p.m.	About 5:30 p.m.
Spikelet type	Cultivated type	Semi-wild type	Semi-wild type
Hairs on lemma			
Upper part	Glabrous	Short, fine hairs	Short, fine hairs
Hairs of scar and rachilla	Glabrous	Densely hairy	Densely hairy
Hairs of awn (twisted part)	Glabrous	Short, fine hairs	Short, fine hairs
Ratio of empty glume length	1 : 1.19	1 : 1.90	1 : 1.36

served in a young stage of the hybrid, but might be easily removed in mid to adult stages.

Time of daily anthesis of *A. strigosa* and *A. pilosa* was observed to be around 7:00 p.m. and 4:30 p.m., respectively, under regular weather condition (Nishiyama 1970). The F₁ hybrid showed intermediate, about 5:30 p.m., being rather close to an early flowering parent. *A. strigosa* indicates nonshattering of spikelets and florets at maturity. *A. pilosa* is of a typical semi-wild-type separation, that is, the spikelet easily separates from its pedicel by abscission leaving a long narrow basal scar but the florets remain attached together. The semi-wild type is completely dominant in the hybrid and the basal scar is long rounded to elliptic cavity, being similar to that of *A. barbata*. First and second empty glumes of *A. strigosa* are approximately equal in length, 1:1.19, while *A. pilosa* indicates unequal length, 1:1.90. The F₁ hybrid expressed an intermediate ratio 1:1.36, or rather close to the equal glume type.

A. ventricosa is morphologically similar to *A. pilosa*, except a few characteristics. In their hybrid the equal glume type, 1:1.24, of *A. ventricosa* was almost completely dominant. *A. ventricosa* is especially characterized by a very elongated grain-base with very sharp, pointed narrow scar. The basal scar of the F₁ hybrid was almost similar to that of *A. pilosa*.

Both of the two interspecific hybrids were highly sterile, only three seeds being found in *A. strigosa* × *A. pilosa* under free pollination. Of them one germinated but died in a seedling stage.

Chromosome observation: Very poor pairing of chromosomes was observed at metaphase I of PMC's in *A. strigosa* × *A. pilosa*. A total of 256 PMC's indicates 0-4 bivalents, with a mean number of 0.75 bivalents per cell (Table 2). All bivalents were of rod shape. No complex association of chromosomes was observed.

The F₁ hybrid *A. pilosa* × *A. ventricosa* is characterized by irregular pairing of chromosomes at metaphase I. Although seven bivalents were found in an extreme

Table 2. Chromosome pairing of a hybrid,
A. strigosa $\times$ *A. pilosa*

Chromosome configuration	Frequency
$4_{II} + 6_I$	3
$3_{II} + 8_I$	11
$2_{II} + 10_I$	35
$1_{II} + 12_I$	78
14_I	129
Total	256
Mean 0.75_{II} per cell	

case, chromosome configurations usually consisted of some univalents, bivalents and complexes of 3-5 chromosomes. $1_{III} + 4_{II} + 3_I$ or $1_{III} + 3_{II} + 5_I$ were most frequently observed (Table 3). Most of bivalents were of rod shape, ring shaped pairs being only 0-2 per cell. When associations of 3, 4, and 5 chromosomes are treated as 1, 2 and 2 bivalents, respectively, the revised number of bivalents ranges 3-7, with a mean

Table 3. Chromosome pairing of a hybrid,
A. pilosa $\times$ *A. ventricosa*

Chromosome configuration	Frequency
7_{II}	1
$1_{IV} + 4_{II} + 2_I$	2
$1_{III} + 5_{II} + 1_I$	4
$6_{II} + 2_I$	8
$1_V + 1_{IV} + 1_{II} + 3_I$	1
$1_{IV} + 1_{III} + 2_{II} + 3_I$	1
$1_{IV} + 3_{II} + 4_I$	1
$3_{III} + 2_{II} + 1_I$	1
$2_{III} + 3_{II} + 2_I$	7
$1_{III} + 4_{II} + 3_I$	11
$5_{II} + 4_I$	6
$1_{IV} + 1_{III} + 1_{II} + 5_I$	1
$1_{IV} + 2_{III} + 4_I$	1
$3_{III} + 1_{II} + 3_I$	3
$2_{III} + 2_{II} + 4_I$	7
$1_{III} + 3_{II} + 5_I$	11
$4_{II} + 6_I$	2
$2_{III} + 1_{II} + 6_I$	1
$1_{III} + 2_{II} + 7_I$	2
$3_{II} + 8_I$	1
Total	72
Mean 4.78_{II} per cell	

number of 4.78 bivalents per cell.

DISCUSSION

The hybrid *A. strigosa* $\times$ *A. pilosa* indicated 0-4 bivalents with a mean number of 0.75 bivalents at metaphase I. It accords an estimated number of bivalents, 0.89, between *A. hirtula* and *A. pilosa* from the data found in the hybrid between two amphiploids, *A. pilosa-sativa* and *A. hirtula-sativa* (Thomas and Rajhathy 1967). Complex configurations consisting of uni-, bi-, tri- and quadrivalents were usually found in *A. pilosa* $\times$ *A. ventricosa*. The revised mean number of bivalents amounts to 4.78 per cell. This result is in fairly well agreement with extensive observations of Rajhathy and Thomas (1967), showing 4.35 to 5.04 bivalents per cell.

Based on the raw data of chromosome configurations in Tables 2 and 3 genome relationships between the three diploid species are tentatively illustrated in Fig. 1. It is presumed that *A. strigosa* has a standard genome consisting of 7 chromosomes numbered on both ends. Four *A. pilosa* chromosomes are partially homologous to those of *A. strigosa*, but the remainder has differentiated to *A. pilosa* proper.

Fig. 1. Diagrams showing structural divergences between three genomes, As, Cp and Cv. As (tentative standard type): *A. strigosa*, Cp: *A. pilosa*, Cv: *A. ventricosa*.

Three chromosome interchanges may be enough to explain meiotic pairing in *A. pilosa* $\times$ *A. ventricosa*. Rare occurrence of 1-2 ring shaped bivalents is suggestive that their partners also are partially nonhomologous, probably in interstitial segments as illustrated in two pairs from the left in Fig. 1. In short, on the basis of meiotic chromosome behavior of the hybrids it is confirmed that *A. pilosa* is obviously closer to *A. ventricosa* than *A. strigosa*. Of course, *A. ventricosa* also appears to relate poorly to *A. strigosa*.

Since establishment of the genus *Avena* by Tournefort in 1700 there have been several opinions on the origin and classification of the species (see Coffman 1961). On the morphological grounds Malzew (1930) reported comprehensive investigation on the phylogenetic relationship of the section *Euavena*. In a recent decade the genus including newly discovered species was extensively studied by many authors from stand points of karyotype, genome affinity, morphology, cross-compatibility, and geographical distribution. Reflecting all information now available Rajhathy and Thomas (1974) reexamined the phylogenetic situation of *Avena* and revised Malzew's evolutionary

sequence, especially in three problems as follows:

(1) All of seven diploid biological species were classified into two groups with AA and CC genomes. The latter has *A. pilosa* (Cp Cp genomes) and *A. ventricosa* (Cv Cv), and the former includes the remainder, *A. strigosa* (As As), *A. longiglumis* (Al Al), *A. damascena* (Ad Ad), *A. prostrata* (Ap Ap) and *A. canariensis* (Ac Ac).

(2) *A. barbata* (AABB), including *A. vaviloviana* and *A. abyssinica*, is a biological species but not a tetraploid donor to the hexaploid species.

(3) *A. murphyi* has AACC genomes and is a tetraploid ancestor of hexaploids (AACDD).

At present there are still several opinions on the relationship of diploid oats, especially in the AA genome group. Based on evidence from cytogenetics and morphology Holden (1966) suggested that *A. strigosa* did not arise from *A. longiglumis* and that both were probably diverging lines from a common ancestor. Ladizinsky (1971, 1973) who discovered a new diploid *A. prostrata* mentioned that the new species could be regarded a more ancient type from which evolution proceeded in two different pathways, (1) *A. longiglumis* and (2) *A. strigosa* and *A. damascena*. From stand points of karyotype evolution and structural divergence of chromosomes Rajhathy and his co-workers finally concluded that the $Ap \rightarrow Al \rightarrow Ad \rightarrow Ac \rightarrow As$ sequence is most probable (Rajhathy and Morrison 1959, 1960, Rajhathy and Baum 1972, Rajhathy and Thomas 1974).

The ploidy status of *Avena* should be discussed on the grounds of three basic evolutionary mechanisms, i.e. (1) structural divergence of chromosomes, (2) formation of ploidy, and (3) genetic differentiation with no or a few minor structural changes of chromosomes which appear to promote speciation of *Avena*. Either of these or strictly speaking a combination of them might actually result in the differentiation of species.

The present paper, however, deals with a further criticism of a phylogenetic development of diploid species. Diploid *Avena* appears to be classified into three groups having unique genetic systems under which speciation took place. Five well known diploids, *A. hirtula*, *A. wiestii*, *A. strigosa*, *A. brevis*, and *A. nudibrevis*, can be grouped into the same category. They show a wide differentiation of plant morphology from wild to naked type, and all of them have the same genomes AsAs, characterized by almost no structural difference of chromosomes. They are completely interfertile. Then we trend to a speculative assumption that the karyotype of this group is unchangeable and remains at the primitive type or is close to it. It is probably most striking to find a similar successful evolution of cultivated barley.

On the basis of cytological evidence presented by several authors it appears that genomes of the four species, *A. longiglumis*, *A. prostrata*, *A. damascena* and *A. canariensis*, still retain high pairing affinity to that of *A. strigosa*, although tolerably diverged in chromosome structure. For instance, $A. prostrata \times A. strigosa$ indicated $3_{III} + 1_V$ most frequently (Ladizinsky 1973). On the other hand, some of them were hardly crossed with AsAs species and highly sterile when hybridized. Thus their genomes are distinctly distinguished from the A (or As) genome, and should be deservedly designated as $^T A$. Interspecific hybrids within $^T A^T A$ genome group were sterile and indicated some structural divergences between parent genomes. Then *A.*

longiglumis, *A. prostrata*, *A. damascena* and *A. canariensis* should be designated as modified symbols such as TAl , TAp , TAd , and TAc , respectively. Accordingly further details of relative genomic homology may be demonstrated with the number of bivalents, except associations of three or more chromosomes, in their hybrids. The following scheme shows mean frequencies of bivalents per cell in interspecific hybrids which were cited from Table 3 in an article of Rajhathy and Thomas (1974). It is clear that *As* relates poorly to TAp and TAl , both of which have the closest relationship, and TAp resembles TAc and TAd closely. According to Ladizinsky (1971, 1973), Rajhathy and Baum (1972), Rajhathy and Thomas (1974) and Craig, Murray and Rajhathy (1974) the morphology of *A. prostrata*, *A. damascena* and *A. canariensis* are very similar to that of *A. wiestii* or *A. hirtula* which are of the most primitive type in the *AsAs* group. These results tend to support a notion that the genetic system of all species in the TATA group, except *A. longiglumis*, showed a frequent occurrence of chromosome structural changes but a few or no morphological divergence. In other words it means that the TATA group, especially *A. prostrata* maintains the primitive type in most of plant characteristics but hardly of the karyotype.

Comparative demonstration of karyotypes in *Avena* was briefly summarized by Rajhathy and Thomas (1974). Based on the data the karyotype similarity in the *AsAs* and TATA groups can be roughly shown as follows when *As* is tentatively a standard type.

The karyotype sequence accords closely with the chromosome affinity relationship mentioned above. As suggested by previous authors, however, a general concept of karyotype evolution appears to be hardly applied to these karyotypes.

A. longiglumis might evolve from *A. prostrata* and progress toward a remarkable specialization with some unique characteristics, e.g. daily anthesis about 4:00 a.m., formation of giant anthers, strong activity for initiating mitosis of fertilized polar nuclei, growth habit, especially almost full-elongation of the peduncle before beginning of anthesis in the spike (Yabuno and Nishiyama 1975).

The karyotype and genome of *A. pilosa* including *A. clauda*, and *A. ventricosa* were designated as Cp and Cv , respectively (Rajhathy 1966, Rajhathy and Dyck 1963, Thomas and Rajhathy 1967, Rajhathy and Thomas 1974). Difficulty of interspecific

crossing or a few meiotic pairs in F_1 hybrids suggest a very poor relationship between the CC group and $TAT A$ as well as AsAs groups (Rajhathy and Dyck 1963, Rajhathy and Thomas 1967, 1974, present study). The establishment of the C genome might be due to accumulation of rather small segmental rearrangements of chromosomes, probably associated with differentiation of some morphological and physiological characters. Spikelets or florets of the CC group, especially of *A. ventricosa*, indicate very elongated sharp, pointed base with narrow small scars. The base type is more effective for penetration of grains into ground. It is interesting to note that *A. pilosa* crossed with *A. strigosa* showed elongated elliptical scars, similar to those of *A.*

Fig. 2. A tentative genealogical tree of diploid *Avena*. Thick line indicates the same genome constitution.

barbata. It seems that the CC group has made progressive speciation toward the superwild type of base. The same may be true in *A. longiglumis*, showing the same base type as *A. pilosa*.

In short, based on our understanding cytogenetic data Fig. 2 illustrates a tentative evolutionary sequence of diploid species in *Avena*. It appears to be supported by evidence from plant morphology, cross-compatibility, geographical and ecological distribution (Ladizinsky 1971, 1973, Ladizinsky and Zohary 1971, Rajhathy and Thomas 1974).

Rajhathy and Thomas (1974) have preliminarily given an interesting summary of the ploidy evolution of tetraploid and hexaploid species. As cytogenetic evidence for this concept is still insufficient it is difficult to make a further discussion on this problem.

SUMMARY

A new cross, *A. strigosa* $\times$ *A. pilosa*, was successfully made when *A. pilosa* was a pollen parent. The hybrid indicated 0-4 bivalents with an average 0.75 bivalents per cell.

In *A. pilosa* $\times$ *A. ventricosa* chromosome configurations of $1_{III} + 4_{II} + 3_I$ and $1_{III} + 3_{II} + 5_I$ were most frequently observed although they usually varied widely.

Based on the available cytogenetic evidence the authors arranged all diploid oats in three groups with AsAs (or AA), TAT_A and CC genomes. They might have a common ancestor and make progress in different trends of their unique specialization.

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