Theor Appl Genet (1996) 93: 123–135

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Cytological and molecular characterization of oat × maize partial hybrids

Received: 5 March 1996 / Accepted: 8 March 1996

Abstract In cereals, interspecific and intergeneric hybridizations (wide crosses) which yield karyotypically stable hybrid plants have been used as starting points to widen the genetic base of a crop and to construct stocks for genetic analysis. Also, uniparental genome elimination in karyotypically unstable hybrids has been utilized for cereal haploid production. We have crossed hexaploid oat (2n=6x=42, *Avena sativa* L.) and maize (2n=2x=20, *Zea mays* L.) and recovered 90 progenies through embryo rescue. Fifty-two plants (58%) produced from oat×maize hybridization were oat haploids (2n=3x=21) following maize chromosome elimination. Twenty-eight plants (31%) were found to be stable partial hybrids with 1–4 maize chromosomes in addition to a haploid set of 21 oat chromosomes (2n=21+1 to 2n=21+4). Ten of the ninety plants produced were found to be apparent chromosomal chimeras, where some tissues in a given plant contained maize chromosomes while other tissues did not, or else different tissues contained a different number of maize chromosomes. DNA restriction fragment length polymorphisms (RFLPs) were used to identify the maize chromosome(s) present in the various oat-maize progenies. Maize chromosomes 2, 3, 4, 5, 6, 7, 8, and 9 were detected in partial hybrids and chromosomal chimeras. Maize chromosomes 1 and 10 were not detected in the plants analyzed to-date. Furthermore, partial self-fertility, which is common in oat haploids, was also observed in some oat-maize hybrids. Upon selfing, partial hybrids with one or two maize chromosomes showed nearly complete transmission of the maize chromosome to give self-fertile maize-chromosome-addition oat plants. Fertile lines were recovered that contained an added maize chromosome or chromosome pair representing six of the ten maize chromosomes. Four independently derived disomic maize chromosome addition lines contained chromosome 4, one line carried chromosome 7, two lines had chromosome 9, one had chromosome 2, and one had chromosome 3. One maize chromosome-8 monosomic addition line was also identified. We also identified a double disomic addition line containing both maize chromosomes 4 and 7. This constitutes the first report of the production of karyotypically stable partial hybrids involving highly unrelated species from two subfamilies of the Gramineae (Pooideae – oat, and Panicoideae – maize) and the subsequent recovery of fertile oat-maize chromosome addition lines. These represent novel material for gene/marker mapping, maize chromosome manipulation, the study of maize gene expression in oat, and the transfer of maize DNA, genes, or active transposons to oat.

Key words *Avena sativa* · *Zea mays* · Wide hybridization · Genomic in situ hybridization · Cytogenetics

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Communicated by F. Salamini

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Introduction

Interspecific and intergeneric hybridization between crop plants and between crops and their wild relatives has been termed "wide crosses". In cereals, where wide crosses have been particularly useful, karyotypically stable crosses which produce hybrid plants have been used as starting points to widen the genetic base of a crop by the introduction of germplasm from related wild or cultivated species

(Fedak 1985; Mujeeb-Kazi and Kimber 1985; Knott 1987) or to construct stocks for genetic analysis (Islam et al. 1981; Driscoll 1983). Also, uniparental genome elimination in karyotypically unstable hybrids has been utilized for cereal haploid production (Kasha and Kao 1970; Barclay 1975; Fedak 1977).

Recently, sexual hybridization between distantly related species of two subfamilies (Pooideae and Panicoideae) of the Gramineae (Poaceae) family (Clayton and Renvoize 1986) has attracted research interest for potential gene transfer between such divergent gene pools and as an alternate means for the production of haploid plants (for review see Laurie et al. 1990). A decade ago, reports that crosses involving hexaploid wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) resulted in the production of globular embryos suggested that fertilization in these remarkably wide crosses had occurred (Zenkteler and Nitzsche 1984). In 1986, cytological preparations of zygotes at metaphase showing the expected combination of 21 wheat chromosomes and ten maize chromosomes provided convincing evidence in wheat×maize crosses that hybrid zygotes resulted from fertilization (Laurie and Bennett 1986). In later studies, successful fertilization in crosses involving hexaploid wheat with sorghum (*Sorghum bicolor* L. Moench) and pearl millet (*Pennisetum glaucum* R. Br.) were also documented cytologically (Laurie and Bennett 1988a, b; Laurie 1989). Fertilization in crosses involving barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), tetraploid wheat (*Triticum turgidum* L.), and some wild wheat relatives (*Triticum* and *Aegilops* spp.) with maize also have been reported (Laurie and Bennett 1988a; Laurie et al. 1990; O'Donoughue and Bennett 1988, 1994a).

In crosses involving Pooideae and Panicoideae, species of the Pooideae were used as female parents while species of the Panicoideae were the male parents. During embryo development, the chromosomes of the Panicoideae were rapidly eliminated, while chromosomes of the Pooideae species were retained. Regeneration of plants from embryos produced by these and other distant hybridizations has allowed the production of haploid cereal plants. Cross combinations that have resulted in the production of cereal haploids include: *T. aestivum*×maize (Suenaga and Nakajima 1989; Inagaki and Tahir 1990; Riera-Lizarazu and Mujeeb-Kazi 1990; Laurie and Reymondie 1991; Comeau et al. 1992; Kisana et al. 1993), *T. turgidum*×maize (Riera-Lizarazu et al. 1992; Amrani et al. 1993; O'Donoughue and Bennett 1994b), wheat×pearl millet (Ahmad and Comeau 1990; Inagaki and Bohorova 1995), wheat×*Zea mays* ssp. *mexicana* (Ushiyama et al. 1991), wheat×sorghum (Comeau et al. 1992; Ohkawa et al. 1992; Inagaki and Mujeeb-Kazi 1995), wheat×*Tripsacum dactyloides* L. (Riera-Lizarazu and Mujeeb-Kazi 1993), and barley×maize (Chen et al. 1991; Furusho et al. 1991; Inagaki et al. 1991).

The production of haploids from different Triticeae species and a near total lack of oat (*Avena sativa* L.) haploid plant recovery from anther culture (Rines 1983) motivated researchers in our laboratory to try oat×maize crosses as an alternative means to produce oat haploids. In 1990, Rines and Dahleen reported the production of oat haploid plants from oat×maize crosses. Oat haploid embryo formation in oat×maize hybridization most likely involves a process of maize chromosome elimination similar to that described for wheat haploid embryo formation in wheat×maize crosses. This conclusion stemmed from the observation of chromosome laggards and micronuclei during mitosis in 48-h post-pollination endosperm (Rines and Dahleen 1990). The production of oat haploids by oat×maize crosses has also been achieved by other researchers (Machan et al. 1995; Matzk 1996). Oat haploids have also been produced using oat×pearl millet crosses (Matzk 1996; H. Rines, O. Riera-Lizarazu, and S. Maquieira, unpublished results).

Embryo rescue is required to recover haploid oat plants from oat×maize crosses because there is little or no accompanying endosperm development, just as with wheat haploid plant recovery from wheat×maize crosses (Suenaga and Nakajima 1989). Post-pollination treatments with 2,4-D or other growth regulators does not seem to be as critical with oat as with wheat (Rines and Dahleen 1990; Rines et al. 1996). An attractive feature of maize-mediated oat haploid production that is analogous to wheat haploid production is the apparent lack of genotypic restrictions on either the pollen donor or the recipient maternal plant (Rines and Dahleen 1990; Rines et al. 1996). Oat haploid plants produced from oat×maize crosses do differ, however, from wheat haploids in that oat haploids are partially self-fertile. A process of meiotic restitution that produces viable gametes results in seed set in up to 40% of primary and secondary florets of main tillers in these haploids (Davis 1992). Furthermore, among plants grown from seed produced by the self-fertilization of oat haploids, 20–30% are aneuploid with monosomic and double monosomic plants being quite common (Rines and Dahleen 1990; Davis 1992).

Another dramatic way in which progenies from oat×maize hybridization differ from those of wheat×maize hybridization was our discovery that oat×maize crosses not only result in the production of oat haploids but also result in the production of partial hybrids with 21 oat chromosomes plus 1–4 maize chromosomes. The present paper details the cytological and molecular characterization of these oat×maize progenies.

Materials and methods

Oat×maize crosses

A combination of the oat lines Starter-1, SunII-1, Kanota-1, and GAF/Park-1 (re-selected lines of the oat cultivars Starter, SunII, Kanota, and GAF/Park, respectively) were hand-emasculated and pollinated with freshly shed pollen of the inbred maize line A188 or the F_1 hybrid Seneca 60. The crossing, embryo rescue, and plantlet regeneration manipulations were performed as described by Rines and Dahleen (1990). When recovered plants were 6–10 cm in height, they were transferred into a 2:1 mixture of soil and potting mix in plastic pots and placed in a growth chamber with a 12-h photoperiod and day/night temperatures of 20/15°C. After 6–8 weeks the photoperiod was increased to 14 h to promote reproductive development.

Chromosome counting

Chromosome counts were performed using a modified procedure from that described by Mujeeb-Kazi and Miranda (1985). Root tips were collected from pots in the morning (10:00 am) and placed on filter papers saturated with a pre-treatment solution of 0.5 g l^{-1} colchicine, 25 g l^{-1} 8-hydroxyquinoline, and 1.5% (v/v) dimethyl sulfoxide (DMSO) for 3.5 h at room temperature in the dark. Root tips were transferred to a solution of 2% (w/v) acetic orcein solution and stored in the refrigerator for at least a week. Root tips were squashed in 45% acetic acid and chromosomes counted.

Slide preparation for in situ hybridization

Root tips were collected and pre-treated as described by Mujeeb-Kazi and Miranda (1985). Root tips were then fixed in 3:1 (absolute ethanol: glacial acetic acid) and stored in a –20°C freezer until they were used. Root tips were placed in 45% acetic acid 5 min before squashing in 45% acetic acid. After squashing, slides were placed on dry ice, coverslips removed, and transfered to absolute ethanol in the freezer (–20°C) for 24 h. Slides were then moved to a dessicator at room temperature where they remained for at least 24 h.

Isolation of genomic DNA

Ground samples of 200–400 mg of freeze-dried tissue or 2–5 g of fresh tissue from the oat cultivars Starter-1, SunII-1 and GAF/Park-1, the maize lines Seneca 60, A188, Tx303 and CO159, and oat-maize derivatives were used to isolate total genomic DNA following the methods described by Hu and Quiros (1991).

Genomic in situ hybridization

Genomic DNA preparations of the oat and maize lines were digested to completion with the restriction endonuclease *Eco*RI. The *Eco*RI-digested maize genomic DNA was labeled by random primed incorporation of digoxigenin-labeled deoxyuridine-triphosphate (Dig-dUTP) using a commercial kit (Genius, Nonradioactive DNA Labeling and Detection Kit, Boehringer Mannheim, Indianapolis, Ind.).

In situ hybridization, with labeled genomic DNA from one of the species used as the probe and unlabeled genomic DNA of the other as the block or competitor, was used to differentiate oat from maize chromosomes (Le et al. 1989; Anamthawat-Jonsson et al. 1990; Mukai and Gill 1991). The unlabeled DNA was added at a higher concentration (10:1).

Desiccated slides were treated with 0.2 ml of a 0.1 mg ml^{-1} RNase A solution in 2×SSC (0.3 M NaCl, 0.03 M sodium acetate) at 37°C for 1 h. Following RNase A treatment, slides were washed twice in 2×SSC for 10 min each at room temperature (rt). For denaturation of chromosomal DNA, slides were placed in a solution of 70% (v/v) formamide in 2×SSC at 70°C for 2 min. Slides were then washed twice in 2×SSC for 5 min each time at rt. Slides were serially dehydrated in 70% (v/v), 95% (v/v), and absolute ethanol for 5 min each and air dried. Following dehydration, a 35-µl solution of digoxigenin-labeled genomic maize DNA (3 µg ml^{-1}), unlabeled blocking genomic oat DNA (30 µg ml^{-1}), sonicated salmon sperm DNA (0.6 mg ml^{-1}), sodium dodecyl sulfate [SDS, 0.1% (w/v)], dextran sulfate [10% (w/v)] and deionized formamide [50% (v/v)] in 2×SSC was applied to each slide and covered with a coverslip. Slides were incubated in a humidity chamber at 37°C overnight. After hybridization, slides were washed in 2×SSC at rt for 5 min, 2×SSC at 37°C for 10 min, 50% formamide in 2×SSC at 37°C for 5 min, 2×SSC at 37°C for 5 min, twice in 0.5×SSC at 37°C for 5 min each, 0.5×SSC at rt for 5 min, 0.1% Triton X-100 (t-octylphenoxypolyethoxyethanol) in PBS (10×PBS=1.3 M NaCl, 0.07 M Na_2HPO_4, and 0.03 M NaH_2PO_4) at rt for 5 min, and PBS at rt for 5 min.

Probe detection using a fluorescein-conjugated anti-digoxigenin antibody

Procedures for in situ hybridization and probe detection using a fluorescein-conjugated anti-digoxigenin antibody were similar to those described by Schwarzacher et al. (1992). A fluoresceinated anti-digoxigenin antibody from sheep (Boehringer Mannheim, Indianapolis, Ind.) was used to detect probe hybridization. The antibody was diluted to 20 µg ml^{-1} in PBS with 2% bovine serum albumen (BSA). The antibody solution (0.1 ml) was placed on each slide and a coverslip added. Slides were placed in a humidity chamber at 37°C for 30–60 min. Unbound antibody conjugate was removed by washing slides twice in 0.1% Triton X-100 in PBS at rt for 5 min each, and PBS at rt for 5 min. Signal amplification was obtained by applying 0.1 ml of fluoresceinated rabbit anti-sheep immunoglobulin (Boehringer Mannheim, Indianapolis, Ind.) diluted (1:100) in PBS with 5% rabbit serum to each slide and then incubated in a humidity chamber at 37°C for 30 to 60 min. Slides were washed twice in 0.1% Triton X-100 in PBS at rt for 5 min each, and PBS at rt for 5 min. Chromosomes were counterstained with propidium iodide (5 µg ml^{-1}). Slides were mounted in an antifade solution (10 mg ml^{-1} p-phenylenediamine, 90% glycerol in PBS) and analyzed on a Zeiss Axioscop microscope equipped with an epifluorescence attachment (Zeiss filter sets 02, 09, and 15). Photographs were taken with Fujicolor Super G 400 and Kodak Gold 400 color print film.

Probe detection using a peroxidase-conjugated anti-digoxigenin antibody

Probe detection using a peroxidase-conjugated anti-digoxigenin antibody was adapted from published procedures (Mukai and Gill 1991; Schwarzacher et al. 1992). Anti-digoxigenin antibodies from sheep conjugated with horse radish peroxidase (POD) (Boehringer Mannheim, Indianapolis, Ind.) were diluted to 7.5 U/ml in tris buffer (100 mM tris-HCl, and 150 mM NaCl; pH 7.5) and 100 µl was applied to each slide. Slides were then placed in a humidity chamber at 37°C for 30 min. Unbound antibody conjugate was removed by washing in tris buffer twice for 15 min. A solution of 4.67 mM DAB [diaminobenzidine (3-,4-,3-,4-tetraaminobiphenyl)] and 0.01% (v/v) H_2O_2 in PBS was applied to each slide (500 µl). Slides were incubated in the dark at room temperature for 5 min. The reaction was stopped by washing slides in PBS for 5 min. Chromosomes were counterstained in 2% (v/v) Giemsa for 1 min. Slides were mounted in Permount (Fisher Scientific, New Jersey). Photographs were taken with Kodak technical Pan 2415 black and white print film.

Genomic DNA restriction digestion, agarose electrophoresis and Southern transfer

Genomic DNA of oat (15–20 µg), maize (5–8 µg), and oat-maize derivatives (15–20 µg) was digested in a total volume of 40 µl per sample with 2 units of restriction enzyme [*Dra*I or *Hin*dIII for RFLP assays and *Hae*III for 185-bp (maize knob-specific sequence) probings] per µg of DNA at 37°C overnight. After DNA digestion, 4 µl of 10× gel loading buffer [0.25% bromophenol blue and 25% Ficoll (type 400)] were added to each DNA sample prior to electrophoresis in agarose TBE (0.09 M tris-borate, 0.02 M EDTA, pH 8.0) gels (0.8% gels for RFLP assays and 1–2% gels for 185-bp probings). Gels were soaked in a DNA-nicking solution (0.25 N HCl) for 10 min, a DNA denaturation solution (0.5 N NaOH and 1.5 M NaCl) for 30 min, and a neutralization solution [1 M tris (pH 8.0) and 1.5 M NaCl] for 30 min. DNA was then blotted onto membranes (Hybond-N, Amersham International, UK or Immobilon-N, Millipore Corp., Belsford, Mass.) following the manufacturers' recommendations. Membranes were baked at 80°C for 3 h.

Probe sources

For maize chromosome identification, umc (University of Missouri-Columbia) probes (Coe et al. 1990; Gardiner et al. 1993) were kind-

ly provided by Dr. E. H. Coe (USDA-ARS and University of Missouri, Columbia, Mo.) and bnl (Brookhaven National Laboratory) probes (Burr et al. 1988) by Dr. B. Burr (Brookhaven National Laboratory, Upton, N.Y.). pZmr-1 containing a maize 17S/5.8S/26S rDNA 9.1-kb repeat (McMullen et al. 1986) was used as a maize chromosome-6 marker (*NOR*). For maize chromosome detection, the 185-bp maize knob-specific sequence (Peacock et al. 1981; Dennis and Peacock 1984) in pZM4-14 (a 185-bp *Hae*III fragment cloned into the *Hin*dIII site of pBR322) was kindly provided by Dr. W. J. Peacock (CSIRO, Canberra, Australia).

Plasmid DNA isolation, DNA probe radiolabeling, and hybridization

Plasmids were isolated from 10-ml cultures using a method similar to that described by Birnboim (1983). Inserts were obtained by digesting each plasmid with the appropriate enzyme (*Pst*I for umc and bnl probes; *Hin*dIII for the 185-bp probe; and *Eco*RI, *Bam*HI, or both, for the maize 17S/5.8S/26S rDNA probe), electrophoresing in TAE (40 mM tris, 5 mM sodium acetate, and 0.5 mM EDTA, pH 8.0) low-melting-point (LMP) agarose gels, and excising the desired DNA fragment. Insert DNA (50–100 ng) in LMP was labeled using the random primer oligolabeling method (Feinberg and Vogelstein 1984) described by Sambrook et al. (1989). The radiolabeled probe was denatured in a heating block (100°C) for 6 min and chilled on ice before use.

Membranes were pre-hybridized overnight at 65°C in a buffer consisting of 5×SSPE (20×SSPE=3.0 M NaCl, 0.2 M NaH_2PO_4, and 0.02 M EDTA), 5× Denhardt's solution [50× Denhardt's=1% Ficoll (type 400), 1% polyvinylpyrrolidone, 1% bovine serum albumin (fraction V)], 0.5% SDS, 5% dextran sulfate, and 0.2 mg ml^{-1} of denatured sonicated salmon sperm DNA. After pre-hybridization, denatured radiolabeled probe was added and hybridized overnight at 65°C. Membranes were washed in a 0.1% SDS solution in 2×SSC at rt and 65°C for 30 min each and in a solution of 0.1% SDS in 0.1×SSC for 30 min each. After washing, membranes were exposed to X-ray film (RX Fuji Medical X-ray Film, Fuji Photo Film Co., Japan) with the aid of intensifying screens.

Results and discussion

From a series of seven crossing experiments involving cultivated hexaploid oat and maize, we recovered a total of 90 plants. Their production involved the emasculation and maize pollination of about 15 000 oat florets and the excision and plating of over 1000 embryos, with between 0.2 to 1.0% of florets pollinated resulting in a plant. Chromosomal analysis of root-tip cells revealed that plants with 21 chromosomes (oat haploid chromosome number) and plants with 21 plus 1–4 extra smaller chromosomes were produced in each set of crosses. Of the 90 plants produced, 60 (67%) were found to contain 21 chromosomes (Fig. 1C) and the remaining 30 (33%) were found to contain from 22 to 25 chromosomes (Table 1). The smaller size of the extra chromosomes (Fig. 1D, E, and F) suggested that they were of maize origin. Differences in chromosome number and genome size between oat (2n=6x=42; about 26 pg DNA/2C) (Fig. 1A) and maize (2n=2x=20; about 5 pg DNA/2C) (Fig. 1B) (Bennett and Smith 1976; Laurie and Bennett 1985) predicted that oat chromosomes, on the average, should be about twice the size of a maize chromosome.

To show that the extra chromosomes in our oat×maize derived plants were of maize origin and not simply fragmented oat chromosomes, a series of chromosome in situ hybridization experiments using total genomic DNA as a probe (genomic in situ hybridization or GISH) were conducted on root-tip cells of progeny plants with 22–25 chromosomes. The use of genomic DNA as a hybridization probe to identify chromosomes in interspecific hybrids has been widely utilized in wheat wide-cross derivatives (Le

Table 1 Chromosome constitution of plants recovered from oat×maize crosses based on root-tip chromosome preparations

Crossing set	Number of plants analyzed	Distribution of plants according to root-tip chromosome content (2n)					Percentage of plants with maize chromatin
		21	21+1	21+2	21+3	21+4	
1	16	13[a]	1		2		25%
2	10	8[b]		1		1	60%
3	4	3		1			25%
4	13	6	6	1			54%
5	11	5[c]	2[e]	2		2	72%
6	11	8[d]	2	1			36%
7	25	17	5[f]	2		1	32%
Total	90	60	16	8	2	4	

[a] One of these individuals (SN10-1) was found to have an oat-maize chromosome translocation (Fig. 2D)
[b] Four of these individuals had 21 chromosomes in root-tip analyses but Southern analyses with the maize chromosome knob-specific 185-bp repeat probe of leaf tissue DNA revealed the presence of maize DNA
[c] Two of these individuals had 21 chromosomes in root-tip analyses but Southern analyses with the maize chromosome knob-specific 185-bp repeat probe of leaf tissue DNA revealed the presence of maize DNA
[d] One of these individuals had 21 chromosomes in root-tip analyses but Southern analyses with the maize chromosome knob-specific 185-bp repeat probe of leaf tissue DNA revealed the presence of maize DNA
[e] One of these individuals had 22 chromosomes in root-tip analyses but Southern analyses with maize RFLP probes of leaf tissue DNA revealed the presence of maize chromosomes 4 and 8
[f] One of these individuals had 22 chromosomes in root-tip analyses but Southern analyses with maize RFLP probes of leaf tissue DNA revealed the presence of maize chromosomes 7 and 8

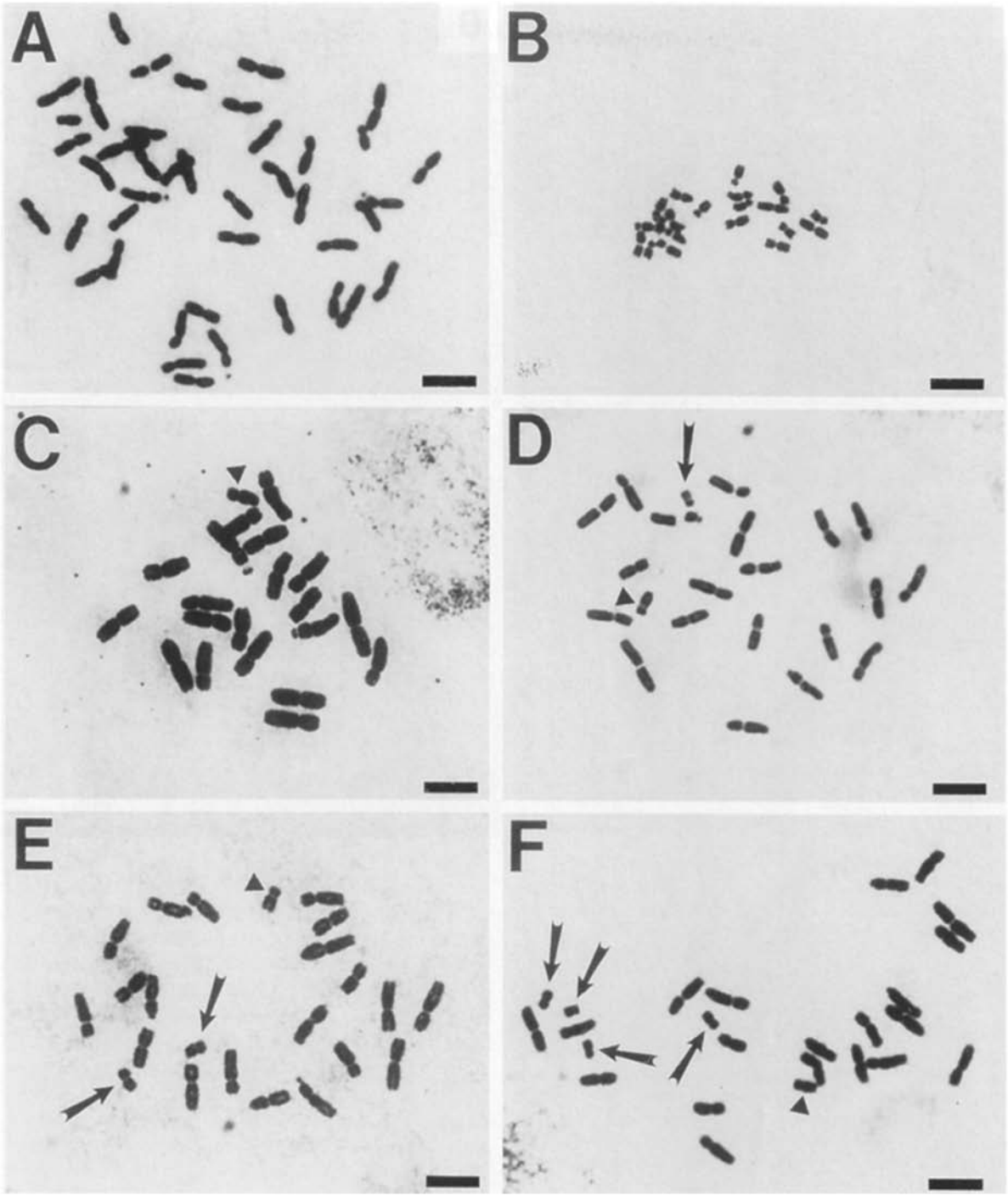

Fig. 1A–F Aceto orcein-stained root-tip chromosome preparations of oat, maize, and four oat-maize derivatives. Metaphase spreads of a 42-chromosome oat (**A**), a 20-chromosome maize (**B**), and oat-maize derivatives with 21 (**C**), 22 (**D**), 23 (**E**), and 25 chromosomes (**F**). *Triangles* mark the smallest chromosome of oat and *arrows* mark maize chromosomes. Bars: 6 μm

et al. 1989; Schwarzacher et al. 1989; Mukai and Gill 1991; Schwarzacher et al. 1992). In our studies, a mixture of labeled genomic DNA from maize and an excess (10×) of unlabeled genomic DNA of oat (the block or hybridization competitor) was denatured and hybridized to denatured DNA of mitotic chromosome spreads of plants with 22–25 chromosomes. Hybridization of labeled DNA to the smaller chromosomes resulted in uniformly labeled chromosomes while no labeling of the larger oat chromosomes was observed (Fig. 2A and B). When genomic DNA of oat was labeled and mixed with an excess of unlabeled genomic DNA of maize, the opposite labeling pattern was observed; i.e., hybridization of labeled DNA was observed in the 21 larger oat chromosomes with no labeling of the smaller extra chromosomes (data not shown). These ex-

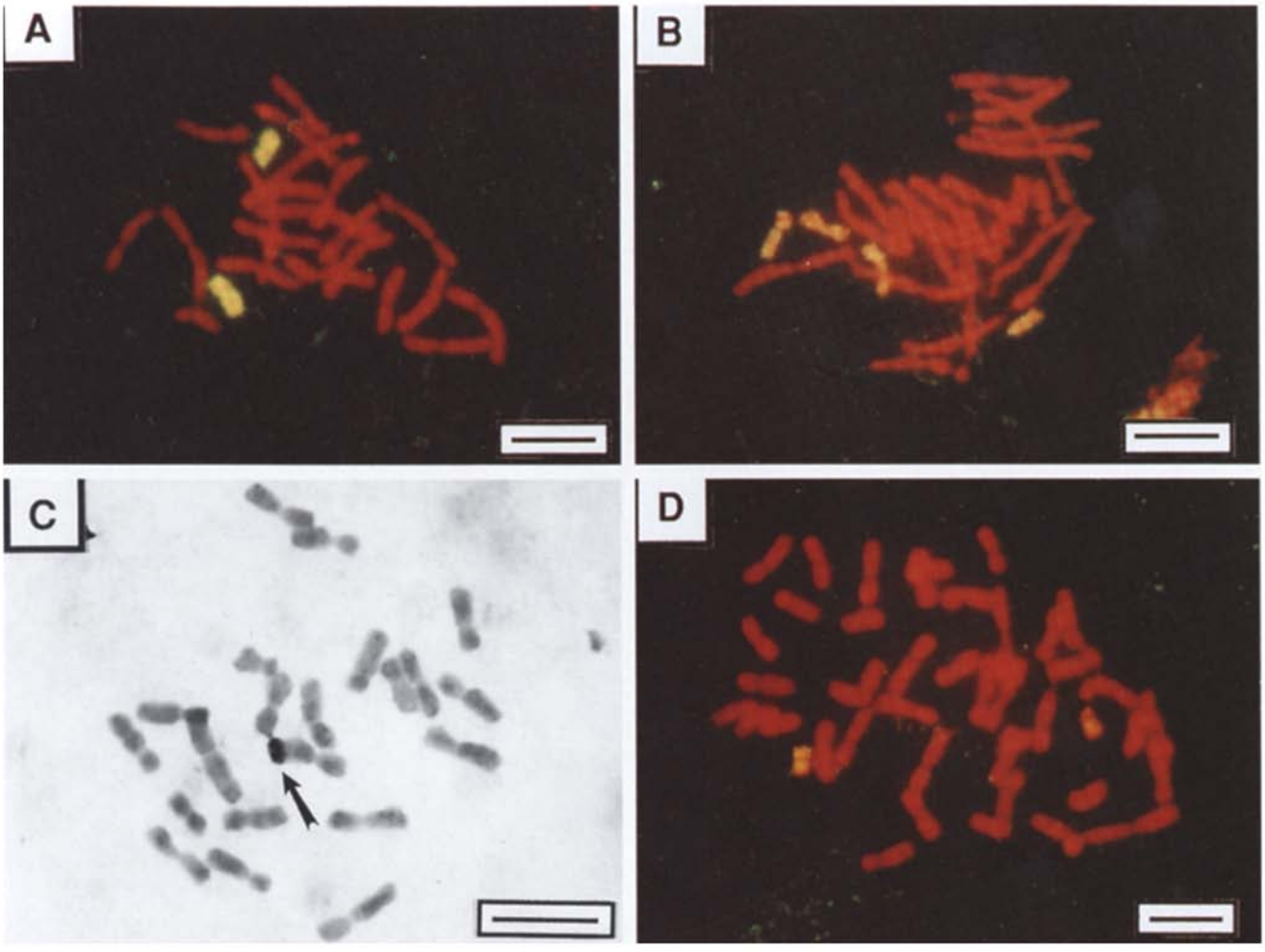

Fig. 2A–D Genomic in situ hybridization (GISH) of oat×maize progenies, an oat-maize derivative with an oat/maize chromosome translocation, and a disomic maize chromosome addition line. **A** Fluorescence in situ hybridization (FISH) of a chromosome spread of a 23-chromosome individual with 21 oat chromosomes (*red-orange*) and two maize chromosomes (*yellow*) using digoxigenin-labeled maize genomic DNA as a probe and fluorescence detection with fluorescein-conjugated anti-digoxigenin antibodies under blue light. Fluorescein-labeled chromosomes fluoresce yellow-green under blue light. Unlabeled chromosomes were counterstained with propidium iodide which fluoresces red-orange under blue light. **B** FISH of a chromosome spread of a 25-chromosome individual as in **A** with 21 oat chromosomes (*red-orange*) and four maize chromosomes (*yellow*). **C** GISH of a 21-chromosome individual with an oat/maize translocation chromosome using digoxigenin-labeled maize genomic DNA as a probe and peroxidase-conjugated anti-digoxigenin antibodies and diaminobenzidine tetrachloride (DAB) detection under normal light. Labeled maize DNA is detected by the formation of an insoluble precipitate (*black*). Unlabeled oat chromosomes were counterstained with Giemsa (*gray*). An *arrow* points to the translocation site. **D** FISH of a chromosome spread of a 44-chromosome individual as in (**A**) with 42 oat chromosomes (*red-orange*) and a pair of maize chromosomes (*yellow*). Bars: 10 μm

periments provided evidence that maize chromosomes were present in plants with 22–25 chromosomes.

In addition to genomic in situ hybridization experiments, the 185-bp maize chromosome knob-specific repetitive sequence (Peacock et al. 1981) was used in Southern analysis to show that plants with more than 21 chromosomes contained maize-specific DNA. The 185-bp highly repeated DNA sequence is a major constituent of chromosome knob heterochromatin, which can occur at 23 locations in maize chromosomes (Peacock et al. 1981). Southern hybridization studies demonstrated that this sequence is found in species related to maize, such as *Zea diploperennis* and *Tripsacum dactyloides,* but is absent in *Coix* and *Sorghum* (Dennis and Peacock 1984). Our work has shown that this 185-bp sequence is not detected in wheat, rye, and oat under regular hybridization stringencies (O. Riera-Lizarazu, personal observation). The 185-bp knob-specific repeats were detected in all 23 individuals analyzed that had chromosome numbers ranging from 22 to 25. Differences in the amount of hybridization of the 185-bp probe were observed between individuals (Fig. 3). These differences most likely reflect differences in the copy number of the 185-bp sequence in different maize chromosomes in these individuals. Maize knob-specific sequences were not detected in 43 of 51 individuals studied that had only 21 chromosomes. Unexpectedly, this maize-specific sequence was detected in eight individuals that cytologically appeared to contain only 21 oat chromosomes. GISH analyses showed that one of these individuals (SN10-1) had an oat-maize chromosome translocation (Fig. 2C). This plant senesced

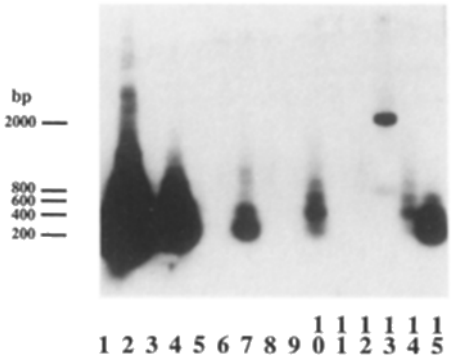

Fig. 3 Southern-blot hybridization of oat-maize derivatives with the 185-bp knob-specific sequence as the probe. DNA samples were digested with *Hae*III. Starter-1 oat (*lane 1*); A188 maize (*lane 2*); 21-chromosome plants (*lanes 3, 5, 6, 8, 9, 11, 12, and 15*); 22-chromosome plants (*lanes 4 and 10*); 23-chromosome individuals (*lanes 7 and 14*); 2-kb DNA marker (*lane 13*). 185-bp sequences were detected in maize (*lane 2*), plants with 22 and 23 chromosomes (*lanes 4, 7, 10 and 14*), and one plant with 21 chromosomes (*lane 15*).

Table 2 Fertility and flowering of oat haploids with and without retained maize chromosomes

Chromosome content[a]	Number of plants analyzed	Sterile plants	Senesced before flowering	Partially fertile plants
2n=21	60[b]	10	3[d]	47
2n=21+1	16[c]	3	3	10
2n=21+2	8	2	3	3
2n=21+3	2	0	2	0
2n=21+4	4	0	4[e]	0
Total	90	15	15	60

[a] Chromosome content was based on root-tip chromosome preparations. Plants with 21 oat chromosomes are 2n=21. Plants with 21 chromosomes plus one, two, three, and four maize chromosomes are 2n=21+1, 21+2, 21+3, and 21+4, respectively

[b] Eight of these individuals had 21 chromosomes in root-tip analyses but Southern analyses with the maize chromosome knob-specific 185-bp repeat probe of leaf tissue DNA revealed the presence of maize DNA

[c] Two of these individuals had 22 chromosomes in root-tip analyses but Southern analyses with maize RFLP probes of leaf tissue DNA revealed the presence of two different maize chromosomes in each individual

[d] One of these plants (SN10-1) was found to have an oat-maize chromosome translocation

[e] Two of these individuals flowered precociously and produced a single small panicle

before flowering. Progenies from the remaining 21-chromosome plants in which maize DNA was detected with the 185-bp probe were grown and examined by GISH and Southern analyses. No maize DNA was detected in these progenies by either method. We believe that the 21-chromosome individuals in which maize knob-specific sequences were detected in DNA extracts of sampled leaf tissues were probably chromosomally chimeric plants with some tissues containing maize chromosomes and other tissues that gave rise to flowers and gametes containing only oat chromosomes.

The presence of maize chromosomes had deleterious effects on the growth and vigor of some plants. Plants with three or four maize chromosomes were visibly less vigorous and less viable than haploid plants with no maize chromosomes (Fig. 4A). Of four plants with four maize chromosomes, two senesced as seedlings and two flowered precociously but were extremely stunted and sterile. All plants with three maize chromosomes senesced as seedlings. In general, plants with one or two maize chromosomes appeared more normal and similar to 21-chromosome individuals (Fig. 4B). The reduced viability of plants from oat×maize crosses was not strictly associated with the presence of maize chromosomes, but the presence of maize chromosomes was associated with an increase in the proportion of plants that senesced as seedlings (Table 2). A larger proportion of plants with one or two maize chromosomes were sterile (21 and 25%, respectively) or senesced as seedlings (21 and 37%, respectively) when compared to oat haploids with no maize chromosomes (19% were sterile and 4% senesced as seedlings).

Genomic sequences of maize that had been previously mapped to specific maize chromosomes (Burr et al. 1988; Coe et al. 1990; Gardiner et al. 1993) were used in Southern analyses to identify the maize chromosomes present in plants with 21, 22, 23 and 25 chromosomes (Table 3). Maize RFLP probes were pre-screened on hybridization blots against DNA extracted from oat and maize lines used in the crosses to select probes that either only detected sequences in maize (maize-specific; Fig. 5) or detected sequences of different molecular size in maize compared to oat. Also, a conscious effort was made to select probes for maize sequences distributed over the maize genome in order to include at least two markers from each chromosome with both chromosomal arms usually represented (Fig. 6).

Eight of the ten maize chromosomes were represented among the addition chromosomes in the 27 oat×maize derivative plants which were analyzed (Table 3). Maize chromosomes 4, 5, 7, and 8 were detected in five plants which had only 21 chromosomes based on root-tip cytological analyses but contained the 185-bp maize-specific DNA repeat in Southern hybridization analysis of leaf-tissue DNA (Table 3). As discussed earlier, these individuals were believed to be chimeric. Maize chromosomes 2, 3, 4, 5, 6, 7, 8, and 9 were identified among 14 plants cytologically found to contain 22 (21 oat+1 maize) chromosomes (Table 3). Three other individuals that cytologically were found to contain 22 (21 oat+1 maize) chromosomes appeared to each contain two different maize chromosomes based on RFLP analyses of leaf DNA. One of these individuals (GP1229-1) possessed maize chromosomes 7 and 8, another (GP340B2) had chromosomes 5 and 8, while the third plant (ST619-1) had maize chromosomes 4 and 8. These individuals, like the 21-chromosome plants that contained 185-bp sequences, were believed to be chromosomal chimeras. Among four individuals with 23 (21 oat+2 maize) chromosomes, one had maize chromosomes 4 and

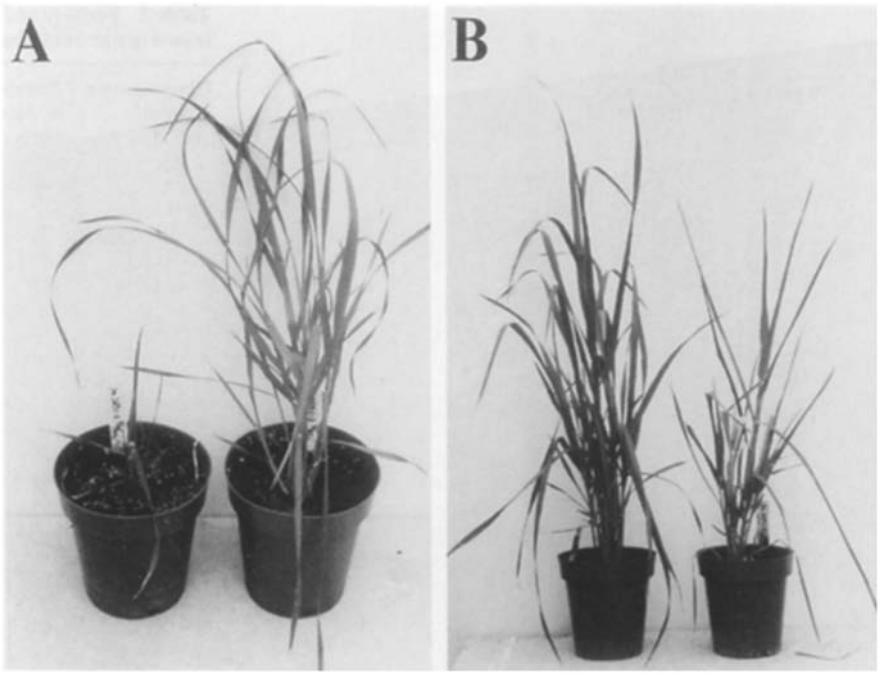

Fig. 4A, B Plant morphology of some oat×maize progenies. **A** Left: plant with 25 (21 oat+4 maize) chromosomes. Right: plant with 21 chromosomes. **B** Left: plant with 21 chromosomes. Right: plant with 23 (21 oat+2 maize) chromosomes

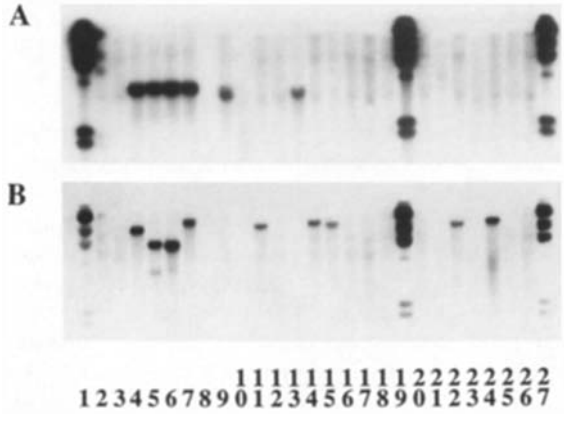

Fig. 5A, B Maize chromosome identification by Southern hybridization of gel blots with maize RFLP markers. *Lanes 1, 19, and 27* contained Lambda phage DNA digested with *Hin*dIII. *Lanes 2 and 3* contained DNA from GAF/Park-1 and Starter-1 oat, respectively. *Lanes 4, 5, 6 and 7* contained DNA from CO159, Tx303, A188, and Seneca 60 maize, respectively. *Lane 8* contained no DNA. *Lanes 9 to 18* and *20 to 26* contain DNA from 17 oat-maize partial hybrids. In **A**, *Hin*dIII-digested genomic DNA was probed with the maize chromosome-9 marker umc109. Two partial hybrids were found to contain the maize chromosome-9 marker (*lanes 9 and 13*). In **B**, *Hin*dIII-digested genomic DNA was probed with the maize chromosome-7 marker umc110. Five individuals were found to contain the maize chromosome-7 marker (*lanes 11, 14, 15, 22 and 24*)

7, one had chromosomes 2 and 7, one had chromosomes 5 and 9, and one had two copies of chromosome 4. Maize chromosomes 4, 5, 6, and 9 were identified in one individual with 25 (21 oat+4 maize) chromosomes (Table 3). Maize chromosomes 1 and 10 were not found among the plants analyzed. There did not appear to be strong preferential retention of any given maize chromosome found in oat. However, it is not clear if the failure to recover plants with maize chromosomes 1 and 10 is due simply to chance, or if the experimental scheme we have used to-date tends to select against them.

Partial self-fertility in oat haploids has been attributed to a meiotic restitution process (Davis 1992). Similarly, partial self-fertility resulted in seed set in some oat×maize partial hybrids with maize chromosomes 2, 3, 4, 7, 8, or 9. Seed set was also observed in a plant with both maize chromosomes 4 and 7, and in one plant with two representatives of chromosome 4. Three individuals with a combination of maize chromosomes 2 and 7, 5 and 9, and 5 and 8, respectively, were sterile (Table 3).

Selfing of 22-chromosome plants resulted in the production of progenies with 42, 43, and 44 chromosomes. All progenies, except one from GP1029-5, had a pair of maize chromosomes, suggesting that the maize chromosomes had been transferred through both male and female gametes (Table 3; Fig. 2D). Plants with 42 and 43 chromosomes were deficient for two and one oat chromosome, respectively. Selfing of ST1053-1, a 23-chromosome plant, resulted in progenies with 45 and 46 chromosomes. All plants had two maize chromosome pairs suggesting that two different maize chromosomes (4 and 7) had been transmitted through both male and female gametes (Table 3). Selfing of ST888 (with two copies of maize chromosome 4) resulted in progenies with 42, 43, and 45 chromosomes. These progenies contained either two or three maize chromosomes. Multivalent pairing and abnormal distribution of this chromosome into gametes during meiosis, or else reduced viability of some gametes with two doses of maize chromosome 4, could account for the failure to produce progenies with four maize chromosomes.

Two plants believed to have been chromosomally chimeric (ST619-1 and GP1229-1) were also partially self-

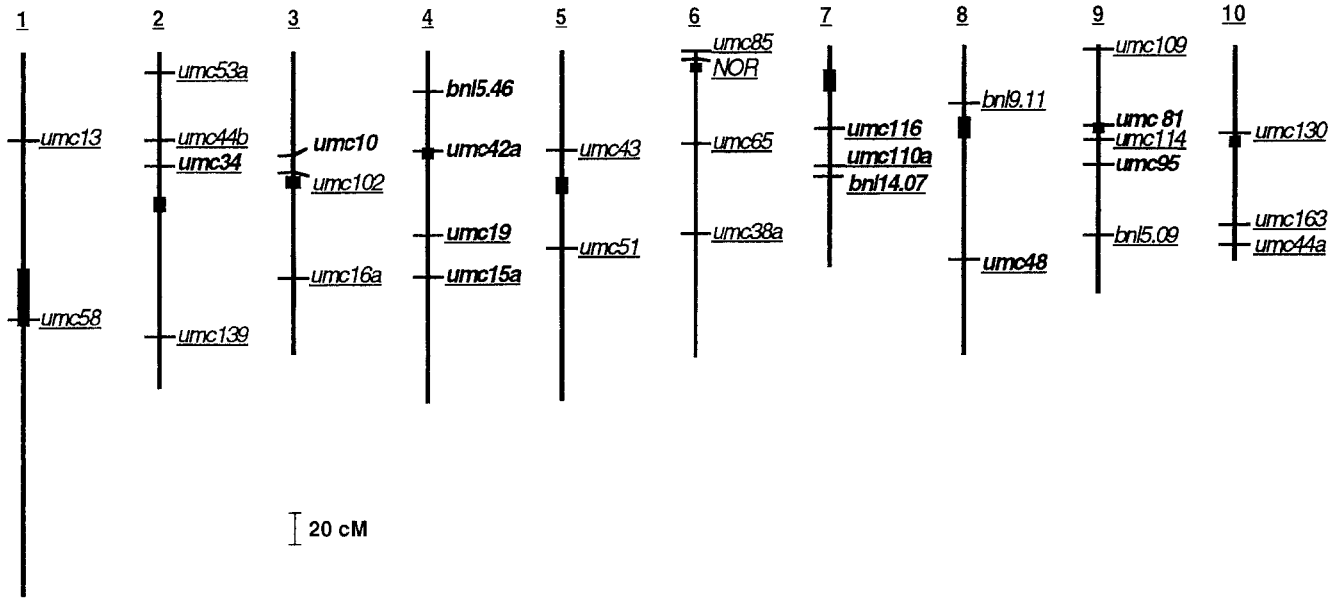

Fig. 6 RFLP linkage map of maize showing the distribution and approximate location of the markers used in maize chromosome identification. Markers which detected loci that are *underlined* were used in maize chromosome identification of oat×maize derivatives. Markers which detected loci that are in *bold* were used to confirm chromosome identification of self-fertile maize addition oat lines. The approximate position of centromeres are indicated by *heavy bars*. The map was drawn according to Gardiner et al. (1993) and the approximate locations of the centromeres are based on Burr et al. (1988)

fertile and produced seed. RFLP analysis of leaf DNA of ST619-1 indicated that maize chromosomes 4 and 8 were present in this individual. Cytologically, ST619-1 had maize chromosome 4 in the root-tip tissues analyzed. Self-fertilization of ST619-1 resulted in the production of 32 seeds. Eight seeds were planted; seven plants were obtained with 44 (42 oat+2 maize) chromosomes and one plant with 43 (41 oat+2 maize) chromosomes (Table 3). RFLP analysis of two 44-chromosome individuals showed that only maize chromosome 4 had been inherited. Since maize chromosomes 4 and 8 are morphologically distinct, cytological analyses suggest that all eight progenies grown from ST619-1 inherited a pair of maize chromosome 4. We believe that ST619-1 was a chimeric individual where some tillers (or tissues) contained either maize chromosome 4 or 8, or both, but only tillers with maize chromosome 4 were partially self-fertile. RFLP analysis of leaf DNA of GP1229-1 indicated that maize chromosomes 7 and 8 were present in this individual. Cytologically, GP1229-1 was found to contain one maize chromosome in the root-tip tissues that were analyzed. Since maize chromosomes 7 and 8 are indistinguishable in root-tip chromosome spreads, either of these chromosomes could have been present in the root-tip tissues that were analyzed. Self-fertilization of GP1229-1 resulted in the production of two seeds. The two resulting plants both possessed 44 (42 oat+2 maize) chromosomes (Table 3). One of these plants senesced as a seedling but the other grew, flowered, and produced seed upon selfing. RFLP analysis showed that this plant had inherited only maize chromosome 7. Like ST619-1, GP1229-1 apparently was a chimeric individual where some tillers (or tissues) contained either maize chromosome 7 or 8, or both, but only tillers with maize chromosome 7 were partially self-fertile.

Self-fertility in some partial hybrids resulted in the production of fertile maize-chromosome-addition oat lines containing six of the ten maize chromosomes. Four independently derived disomic maize-chromosome-addition lines contained chromosome 4, one line carried chromosome 7, two lines had chromosome 9, one had chromosome 2, and one had chromosome 3. One maize chromosome-8 monosomic addition line was also identified. We identified a double disomic addition line containing both maize chromosomes 4 and 7. Phenotypic effects in these plants range from slight variations of normal oat growth rate and morphology in the maize chromosome-2, -4, -8, and -9 addition lines to much reduced growth in the maize chromosome-7 addition, and abnormal stem and panicle morphology in the maize chromosome-3 addition line. Fertility in general, however, remains high with full transmission of the added maize chromosome pair to the next generation (data not shown).

The rate and mechanism of maize chromosome elimination has not been described in oat×maize crosses; however, the presence of maize chromosomes in oat×maize derivatives indicates that maize chromosome elimination in oat×maize crosses is not always complete. Also, the identification of chimeras may indicate that maize chromosome elimination is more gradual in comparison to wheat×maize crosses. Variation in karyotypic stability has been observed in other wide hybrids. In wheat×maize crosses, elimination of one or more maize chromosomes occurred at the first division of about 70% of hybrid embryos and all maize chromosomes appeared to be lost by the time the embryo had eight cells (Laurie and Bennett 1989). This rapid loss of maize chromosomes was attributed to the failure of maize chromosome centromeres to

Table 3 Maize chromosome constitution of oat×maize partial hybrids and their progenies

Plant ID	Chromosome number[a]	Maize RFLP loci detected[b]	Maize chromosome(s)[b]	Number of progeny with various chromosome numbers[af]					
				41	42	43	44	45	46
ST703[d]	21	*umc15a, umc19*	4						
ST582-1[d]	21	*umc43, umc51*	5	2	2				
ST712-1[cd]	21	*umc43, umc51*	5						
ST758-2[d]	21	*umc116, bnl14.07*	7	1	2				
GP340C3	21	*umc48, bnl9.11*	8						
ST1090-2	22	*umc53a, umc44b, umc34, umc139*	2		1		1		
GP324B2[c]	22	*umc53a, umc44b, umc34, umc139*	2						
ST535-1[c]	22	*umc53a, umc44b, umc34, umc139*	2						
SN3	22	*umc102, umc16a*	3		2	3	3		
ST506-1	22	*umc19, umc15a*	4			4	12		
ST786	22	*umc19, umc15a*	4			2	6		
ST619-1[d]	22	*umc19, umc15a, bnl9.11, umc48*	4, 8			1	7		
GP1029-3[c]	22	*umc43, umc51*	5						
GP1223-3[c]	22	*umc43, umc51*	5						
GP340B2[cd]	22	*umc43, umc51, bnl9.11, umc48*	5, 8						
ST727[c]	22	*NOR, umc85, umc65, umc38a*	6						
GP328B3[c]	22	*umc116, umc110a, bnl14.07*	7						
ST524-2[c]	22	*umc116, bnl14.07*	7						
GP1229-1[d]	22	*umc110a, bnl14.07, bnl9.11, umc48*	7, 8				2		
GP1029-5	22	*bnl9.11, umc48*	8		1				
ST633	22	*umc109, umc114, bnl5.09*	9			3	9		
ST505-5	22	*umc109, umc114*	9			2	3		
ST888[e]	23	*umc19, umc15a*	4		2		10	2	
ST1053-1	23	*umc19, umc15a, umc110a, bnl14.07*	4, 7					5	8
ST619-2[c]	23	*umc53a, umc44b, umc34, umc110a*	2, 7						
SN269C1[c]	23	*umc43, umc51, umc109, bnl5.09*	5, 9						
ST1034-1[c]	25	*umc15a, umc43, umc51, umc85, umc65, umc109, umc114*	4, 5, 6, 9						

[a] Chromosome numbers were based on analysis of root-tip chromosome preparations
[b] Maize chromosome identity in oat×maize partial hybrids was established by positive Southern-blot hybridization for all RFLP probes tested (2–4) specific for a given maize chromosome and absence of signal for probes specific for each of the other maize chromosomes
[c] These plants were sterile
[d] These were chromosomally chimeric individuals (see text). Progenies from ST703 were not studied. Progenies from ST582-1 and ST758-2 did not contain maize chromosomes. Progenies from ST619-1 contained only maize chromosome 4. Progenies from GP1229-1 contained only maize chromosome 7
[e] This individual had two copies of maize chromosome 4
[f] All progenies from ST1090-2, SN3, ST506-1, ST786, ST619-1, GP1229-1, ST633, and ST505-5 had a pair of a specific maize chromosome plus 40, 41, or 42 oat chromosomes. All progenies from ST1053-1 had two pairs of maize chromosomes plus 41 or 42 oat chromosomes. The progeny from GP1029-5 had 41 oat chromosomes plus a single maize chromosome

attach to spindle microtubules in wheat, resulting in the inability of maize chromosomes to migrate during the embryonic cell divisions. In contrast, maize chromosomes had well-defined centromeres in barley×maize hybrid zygotes and some maize chromosomes were retained for a minimum of four cell cycles (Laurie and Bennett 1988a). There has also been a report of the retention of what appeared to be a maize chromosome in wheat (Comeau et al. 1992). Comeau et al. (1992) described a small chromosome present in root tips of a wheat×maize plant but not found in the colchicine-doubled progeny. In another report, Ahmad and Comeau (1990) found that a small chromosome observed in root tips of a wheat×pearl millet derivative was present in only 2 of 55 microsporocytes but was not detected in any gamete. However, in neither case were the extra chromosomes definitively identified as being of pollen-donor origin. Still, maize chromosome retention in wheat and barley appears to be uncommon. About 500 progenies from hexaploid wheat×maize crosses that were cytologically studied by four different research groups were found not to contain maize chromosomes (Suenaga and Nakajima 1989; Inagaki and Tahir 1990; Comeau et al. 1992; Riera-Lizarazu et al. 1992). Similarly, about 150 plants from durum wheat×maize crosses that were cytologically studied by two research groups showed that no maize chromosomes were present in any individual (Riera-Lizarazu et al. 1992; O'Donoughue and Bennett 1994b). Ninety-five progenies from barley×maize crosses produced by three different groups were also shown not to contain maize chromosomes (Chen et al. 1991; Furusho et al. 1991; Inagaki et al. 1991).

If karyotypic stability is directly related to the affinity of maize centromeres for binding foreign spindle microtubules, then maize chromosome retention in oat haploids would suggest that maize chromosome centromeres have greater affinity for oat spindle microtubules than for those of wheat and barley. On the other hand, maize chromosome retention may be related to the expression of factors nec-

essary for the proper function of a given maize chromosome in a foreign genetic background. Thus, differences in maize chromosome retention in oat×maize versus wheat×maize crosses may reflect differences in the expression of maize genes necessary for proper chromosome movement during mitosis. It will be interesting to test whether maize chromosome stability in oat is related to the similarity between maize and oat centromere structure, maize gene expression in oat, or a combination of both. These genetics materials may help clarify the processes involved in chromosome elimination or retention in wide hybrids.

The ability to produce plants from remarkably wide crosses opens the possibility of gene transfer between these highly divergent gene pools. The observation that maize chromosomes were not so rapidly eliminated in barley was explored by Chen et al. (1991) in an attempt to transfer maize DNA to barley. In their study, various two-rowed barley cultivars were crossed to maize stocks carrying active transposable elements (*Mu* and *Ac*). Maize chromosome instability resulted in the recovery of barley haploids with no maize DNA introgressions. Faced with karyotypic instability, researchers have suggested that maize DNA transfers might be induced by exposing zygotes and two-celled embryos to ionizing radiation to induce intergenomic translocations before maize chromosome elimination. Another approach employed in attempts to transfer genes from maize to Triticeae species involves protoplast fusion. Wang et al. (1993) reported the production of somatic hybrid cell lines between maize and a perennial hybrid of *T. durum*×*Elitrigia intermedium* (Host) Nevski. This development, although promising, was faced with limitations such as failure to regenerate plants from these protoplast hybrids and the elimination of maize chromosomes (Wang et al. 1993). Our discovery that maize chromosomes are retained in oat is in itself the first example of DNA transfers (whole chromosomes) from sexual crosses between such highly divergent gene pools.

Karyotypically stable oat-maize derivatives, including self-fertile maize-chromosome-addition oat plants, represent unique material which could be a source for gene transfer from maize to oat. These addition lines, which are grown isolated from our standard genetic and breeding materials to prevent uncharacterized transfers, are currently being evaluated for disease response reactions as well as changes in morphology and growth parameters. Also, efforts are underway where maize lines carrying *Mu* transposons are being crossed to oat in an attempt to transfer maize transposons. Once an interesting trait or gene in a maize chromosome has been identified in these materials, their transfer via chromosome engineering may offer a desirable alternative to plant transformation.

The availability of these unique oat lines with added maize chromosomes creates novel opportunities to exploit them in gene mapping and cloning. For example, any previously unmapped sequence from maize could presumably be located to its chromosome by simply hybridizing the sequence to a blot made with DNA from a series of chromosome addition lines if that sequence is either maize specific or shows a banding pattern distinguishable from oat. Also, stocks in the same oat background containing the same maize chromosome but polymorphic for various markers could be used to generate mapping populations for a specific maize chromosome. In addition, DNA libraries for single maize chromosomes might be produced by physically separating maize from oat chromosomes by size using flow cytometric chromosome sorting (Arumuganathan et al. 1991), chromosome microdissection (Kao and Yu 1991), or genomic subtraction techniques (Rosenberg et al. 1994).

We used a combination of mitotic chromosome, GISH, and Southern analyses with the 185-bp and RFLP probes to show that oat×maize hybridization results in the production of oat-maize partial hybrids containing one or various combinations of eight of the ten maize chromosomes. These analyses also revealed the production of chromosomally chimeric first-generation plants where some tissues apparently contained maize chromosomes while others did not, and some cases where different tissues contained different maize chromosomes. Interestingly, an individual with a spontaneous oat-maize chromosome translocation was also observed. Self-fertility in some oat-maize partial hybrids resulted in the production of fertile maize-chromosome-addition oat lines containing six of the ten maize chromosomes. These maize-chromosome-addition oat plants represent unique material for maize gene/marker mapping, maize chromosome manipulation, maize gene expression studies in oat, and the transfer of DNA, genes, or transposons to oat. Undoubtedly, these plants will be of use in a wide array of other studies as we investigate further the expression, transmission, and interaction of these novel genomic combinations.

Acknowledgements We thank Drs. E. H. Coe, B. Burr, I. Rubenstein, and W. J. Peacock for providing the probes used in this study. We also thank Dr. S. Kianian and Dr. B.-C. Wu for their helpful suggestions on the molecular analyses and D. Davis, R. Halstead, S. Livingston, and L. Gulbranson for their technical support. Funding from the Quaker Oats Company and the Midwest Plant Biotechnology Consortium (USDA Prime/Purdue Univ. sub# 593-0120-13) is gratefully acknowledged.

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