Dissecting the maize genome by using chromosome addition and radiation hybrid lines


*Department of Agronomy and Plant Genetics and Center for Microbial and Plant Genomics, University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108; and **Plant Science Research Unit, Department of Agriculture–Agricultural Research Service, St. Paul, MN 55108

Contributed by Ronald L. Phillips, May 13, 2004

We have developed from crosses of oat (Avena sativa L.) and maize (Zea mays L.) 50 fertile lines that are disomic additions of individual maize chromosomes 1–9 and chromosome 10 as a short-arm telosome. The whole chromosome 10 addition is available only in haploid oat background. Most of the maize chromosome disomic addition lines have regular transmission; however, chromosome 5 showed diminished paternal transmission, and chromosome 10 is transmitted to offspring only as a short-arm telosome. To further dissect the maize genome, we irradiated monosomic additions with γ rays and recovered radiation hybrid (RH) lines providing low- to medium-resolution mapping for most of the maize chromosomes. For maize chromosome 1, mapping 45 simple-sequence repeat markers delineated 10 groups of RH plants reflecting different chromosome breaks. The present chromosome 1 RH panel dissects this chromosome into eight physical segments defined by the 10 groups of RH lines. Genomic in situ hybridization revealed the physical size of a distal region, which is represented by six of the eight physical segments, as being ~20% of the length of the short arm, representing ~one-third of the genetic chromosome 1 map. The distal ~20% of the physical length of the long arm of maize chromosome 1 is represented by a single group of RH lines that spans >23% of the total genetic map. These oat–maize RH lines provide valuable tools for physical mapping of the complex highly duplicated maize genome and for unique studies of interspecific gene interactions.

Plants with one chromosome (monosomic) or one pair of homologous chromosomes (disomic) of an alien donor species added to the entire recipient species chromosome complement serve to dissect the donor genome into individual chromosome entities and separate them from their own genome remnant. The transfer liberates the added chromosome (pair) from the interactive gene expression network of the donor genome and puts the chromosome’s genes into the environment of the host genome. This new structural and functional situation can create novel orthologous and nonhomologous gene-to-gene interactions and, hence, helps to answer fundamental questions about gene expression control, inheritance, and syntenic correspondence among different plant species, especially those with large genomes, including maize, with a 1C content of ~2.7 billion base pairs [Plant DNA C-Values Database (Release 2.0, January 2003), M. D. Bennett and I. J. Leitch, http://rbgkew.org.uk/eval/homepage.html] and a subgenomic structure reflecting ancient tetraploidy (1).

By crossing maize to oat, (oat × maize)F1 proembryos were generated, of which ~5–10% could be rescued in vitro. Molecular and cytological analyses showed retention of one or more maize chromosomes in addition to the haploid oat genome in 34% of the F1 plants (2–7). Because haploid oat frequently develops unreduced gametes (8), subsequent self-fertilization of (oat × maize)F1 plants with one maize chromosome added to the haploid oat genome (n = 2x + 1 = 22) can produce F2 offspring with one homologous maize chromosome pair added to the doubled haploid (hexaploid) oat genome (2n = 6x + 2 = 44) among other euploid and aneuploid types (9).

A complete series of oat–maize chromosome addition lines (10) has enabled markers and genes to be physically allocated to maize chromosomes without a need for detectable polymorphisms. These unique plant materials confirmed interchromosomal duplicate loci on a large scale, one of the obstacles to whole-genome sequencing. Numerous locus duplications complicate the reassembly of a set of shotgun DNA sequences. Oat–maize chromosome addition lines, however, physically separate these interchromosomal maize orthologs and paralogs from each other and make them accessible to mapping, sequencing, and cloning, even in cases where the duplicated loci of interest carry genes with monomorphic allelic sequences.

A second obstacle that impedes sequencing of the complete maize genome by today’s technology is the repetitive nature of ~85% of the maize DNA. Thus, sequencing strategies are being tested that accomplish the targeted sequencing of less repetitive gene-rich regions (11, 12). These strategies must involve technologies that are capable of arranging those gene islands along the chromosomes and bridging long gaps between contigs. Generating random breaks in the maize chromosome in an identified monosomic oat–maize chromosome addition line and maintaining diminutive maize chromosomes or pieces translocated into oat can provide DNA panels of radiation hybrid (RH) lines, which allow for a presence vs. absence test of markers without the need for polymorphisms (13). With sufficient resolution that is determined by the number and distribution of breaks along the maize chromosomes, RH lines can contribute to placing contigs in the correct order. A panel of RH lines for maize chromosome 9 has demonstrated the efficient mapping of molecular markers (14).

This report summarizes the status for the oat–maize chromosome addition line production and characteriation, including the irregular transmission behavior of maize chromosomes 5 and 10 in oat, the last two maize chromosomes recovered as fertile oat–maize addition lines. We also illustrate the development of oat–maize RH lines from addition plants for maize chromosome 1. We show the use of these RH lines for physical mapping and relating genetic map distances to physical chromosome segment sizes.

Materials and Methods

Plant Material. Plants of oat (Avena sativa L.) cultivars GAF-Park, Kanota, Preakness, Starter, Stout, Sun II, and the MN-hybrid (MN97201-1 × MN841801-1) were grown and crossed by maize

This report was presented at the International Congress “In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution,” held May 21–31, 2003, at the University of Bologna, Bologna, Italy. The scientific organizers were Roberto Tuberosa, University of Bologna, Bologna, Italy; Ronald L. Phillips, University of Minnesota, St. Paul, MN; and Mike Gale, John Innes Center, Norwich, United Kingdom. The Congress web site (www.doublehelix.too.it) reports the list of sponsors and the abstracts.

Abbreviations: BC, backcross; GISH, genomic in situ hybridization; RH, radiation hybrid; SSR marker, simple-sequence repeat marker.

To whom correspondence should be addressed. E-mail: phil005@umn.edu.

© 2004 by The National Academy of Sciences of the USA

www.pnas.org/cgi/doi/10.1073/pnas.0403421101

PNAS | June 29, 2004 | vol. 101 | no. 26 | 9921–9926
formamide in 1.5
Posthybridization stringency washes were carried out in 40%
Hybridization was carried out in 40% formamide in 1.5
probed on slides without using unlabeled competitor DNA.
Further steps of RNase treatment, postfixation, and
some addition plants back to their corresponding parental oat
various tissues, and maize chromosome transmission to F2
Hybridization were as described earlier in ref. 21 except that total
meristem cells were
chromosome addition and RH lines were pretreated, fixed, and
BC1F2 offspring with transmitted maize chromosome deficien-
classes or oat–maize chromosome translocations (RH plants) were
PCRs were accomplished by the use of the REDExtract-N-Amp Plant PCR kit (Sigma). For larger numbers
acetyltrimethylammonium bromide procedure (18). For labeling
of PCRs (75 or
Genomic DNA Extraction. For a limited number of PCRs (75 or
dispersed retrotransposon Grande 1 (16) and for the highly
maize chromosome translocations in F2 plants were
maize chromosome elimination (GISH), and chromosome
counting for presence, stability, and transmission of maize
hybridization were recovered and
analyzed for vigor, seed set, maize chromosome retention in
various tissues, and maize chromosome transmission to F2
offspring (5, 10). F2 plants were tested by PCR-based markers,
test crosses, genomic in situ hybridization (GISH), and chromosome
counting for presence, and maize chromosome retention to

(P. mexicana L.) lines Seneca 60, A188, A619, B73, Mo17, the
(A188 × W64A)F1 hybrid, and a line carrying the allele b21-
mum9. More than 200 putative F1-hybrid plants indicating successful (oat × maize) hybridization were recovered and
analyzed for vigor, seed set, maize chromosome retention in
various tissues, and maize chromosome transmission to F2
offspring (5, 10). F2 plants were tested by PCR-based markers,
test crosses, genomic in situ hybridization (GISH), and chromosome
counting for presence, stability, and transmission of maize
hybridization were as described earlier in ref. 21 except that total
meristem cells were
chromosome addition and RH lines were pretreated, fixed, and

Cytology. Root tips (1.5–2 cm) of oat, maize, and oat–maize
chromosome addition and RH lines were pretreated, fixed, and
stored as described in ref. 10. Root tips for chromosome counting
were prepared as described in ref. 20. Meristem cells were
squashed in 2% wt/vol Aceto-Orcein (Carolina Biological Sup-
ply). Root tips for GISH were prepared as described in ref. 10.
Many steps of RNase treatment, postfixation, and in situ
hybridization were as described earlier in ref. 21 except that total
genomic maize DNA was labeled by the use of the ULYSIS
Alexa Fluor 488 nucleic acid labeling kit (Molecular Probes)
and probed on slides without using unlabeled competitor DNA.
Hybridization was carried out in 40% formamide in 1.5× SSC
(225 mM NaCl/22.5 mM trisodium citrate, pH 7.0) at 37°C.
Posthybridization stringency washes were carried out in 40%
formamide in 1.5× SSC at 42°C. Chromosomes were counter-
stained with propidium iodide. Signals were visualized and
captured by using an Axioskop microscope equipped for epi-
fluorescence (Zeiss) and a Magnafire charge-coupled device
camera (Optronics International, Chelmsford, MA).

Results and Discussion
Maize Chromosome Elimination in (Oat × Maize)F1 Hybrids. In oat ×
maize crosses, maize chromosomes are occasionally retained (2).
This situation is distinct in that the maize genome is completely
eliminated in hybridizations between maize and wheat or barley.
There is only one report of a maize chromosome being retained
in wheat; however, the maize chromosome was not transmitted
to offspring (23). The timing of elimination differs as well. The
maize chromosome elimination process in oat sometimes ex-
tends over longer periods of time compared with that in F1
hybrids generated from wheat × maize and barley × maize
crosses (24). In 70% of (wheat × maize)F1 embryos, one or more
maize chromosomes were eliminated at the first mitosis. By the
eight-cell stage, the embryos had lost all maize chromosomes
(24). Maize chromosome elimination from (oat × maize)F1
embryos starts at an early stage in embryogenesis as well (4).
However, as an example of the extended time of maize chro-
mosome elimination from oat, in the (oat × maize)F1 plant
F1-5133-1 with maize chromosomes 4, 7, and 10 all detected at
a young plant stage, a consecutive elimination of individual
maize chromosomes was detected by the PCR analysis of
genomic DNA extracted from tissues of flag leaves of different
tillers from the same plant shortly after meiosis. The first tiller
retained only maize chromosome 4, thus eliminated chromo-
somes 7 and 10. The second tiller eliminated chromosome 10,
thus retained chromosomes 4 and 7. The third and fourth tillers
eliminated chromosome 7, thus retained chromosome 4 and
chromosome 10 as a short-arm telosome (Fig. 1). We observed
further instances where maize chromosomes were lost in later
growth stages, particularly from plantlets with two or more
originally retained maize chromosomes in their complements
(results not shown).

(Oat × Maize)F1 Hybrids and F2 Offspring in Different Genetic Back-
grounds. Our initial work was conducted with the maize chro-
mosome donor Seneca 60 and the oat recipient Starter. In the
last 2 years we have tested several other combinations of maize
and oat lines. A total of 201 (oat × maize)F1 plants have been
generated from various maize and oat backgrounds, of which 68
F1 hybrids retained one or more maize chromosome(s) in their
complements. All 10 maize chromosomes could be recovered,
with each occurring at different frequencies as single additions
and in combination with other maize chromosomes. No obvious
preferential combination for two or more specific maize chro-
mosomes was detected in multiple additions in haploid oats.
The frequency of recovery of a particular maize chromosome, the

![Fig. 1.](image-url)
fertility of that plant, and the stability of a maize chromosome appeared to be primarily dependent on the particular maize chromosome interacting with the oat background. Furthermore, the ability to recover a particular maize chromosome in F$_1$ hybrids was not correlated with the ability to produce a fertile addition line for that chromosome. For instance, 20 F$_1$ hybrids with maize chromosome 5 were produced, making the chromosome 5 the most frequently recovered chromosome, either as a single chromosome addition or in combination with other maize chromosomes; chromosome 2 addition plants were the next most frequent with 15 F$_1$ plants produced. Yet, only one of the chromosome 5 plants was fertile and transmitted the chromosome 5 to offspring resulting in the fertile disomic addition OMAd5.59 in Sun II oat background. This finding can be contrasted with chromosome 4, which has been recovered as an addition line nine times, with three of these addition plants being fertile and transmitting the chromosome 4 to offspring. With respect to transmission of maize chromosomes, fertile disomic addition lines fall into different categories. Addition lines carrying chromosomes 2, 3, 4, 6, and 9 exhibited little or no problems with transmission (nearly 100% maternal and paternal transmission rate). Lines carrying chromosomes 1 and 7 initially had poor transmission of the maize chromosome, but after several generations they show good transmission of the maize chromosome (>80% transmission rate), possibly due to selective breeding for stable diploid offspring. Chromosomes 1, 5, and 8 additions have fertility problems even after several generations of selection, and chromosome 10 additions have transmitted only short-arm derivatives to offspring. Eleven disomic additions for different maize chromosomes, including 2, 4, 5, 6, 7, 8, and 9 in different oat backgrounds, have been added to the previously reported set (15), making a total of 50 fertile addition lines (Table 1).

### Table 1. Fertile oat–maize chromosome addition lines

<table>
<thead>
<tr>
<th>Added maize chromosome</th>
<th>Oat host</th>
<th>Maize donor</th>
<th>Addition</th>
<th>No. of lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Starter</td>
<td>bz1-mum9</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Sun II</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Sun II</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Preakness</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Starter</td>
<td>A188</td>
<td>Disomic</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Starter</td>
<td>B73</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>MN-hybrid</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Sun II</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>MN-hybrid</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Sun II</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>GAF-Park</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Starter</td>
<td>bz1-mum9</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>GAF-Park</td>
<td>Seneca 60</td>
<td>Monosomic</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>GAF-Park</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Sun II</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>10S</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Disomic</td>
<td>1</td>
</tr>
<tr>
<td>10S</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Double</td>
<td>1</td>
</tr>
<tr>
<td>10S</td>
<td>Starter</td>
<td>Seneca 60</td>
<td>Double</td>
<td>1</td>
</tr>
</tbody>
</table>

This table lists all available addition lines and is an update of an earlier list that involved fewer lines (15).

### Table 2. Seed set from fertile oat–maize chromosome 10S additions

<table>
<thead>
<tr>
<th>F$_1$-plant panicle</th>
<th>Total</th>
<th>Tested</th>
<th>Maize-positive</th>
<th>Disomic</th>
<th>Monosomic</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_1$-0289-1/a</td>
<td>51</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>F$_1$-0289-1/b</td>
<td>59</td>
<td>1</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>F$_1$-0289-1/c</td>
<td>48</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F$_1$-0289-1/d</td>
<td>31</td>
<td>0</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>F$_1$-5133-1/a</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>F$_1$-5133-1/b</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F$_1$-5133-1/c</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>F$_1$-5133-1/d</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1*</td>
<td>1*</td>
</tr>
</tbody>
</table>

*Monosomic for maize chromosome 4 and monosomic for maize chromosome 10S derivative.

1Disomic for maize chromosome 4.

Irregular Maize Chromosome 5 Transmission in Oat. Disomic maize chromosome 5 additions recovered earlier in two different oat backgrounds (OMA5d.09 in Starter and OMA5d.17 in MN-hybrid) show significantly diminished paternal transmission, whereas maternal transmission of the maize chromosome 5 is only moderately reduced. Crossing a OMA5d.09 plant as male back to Starter produced four monosomic maize additions among 58 BC$_1$ offspring, giving a paternal transmission rate of 6.9% (4 of 58). In 30 F$_4$ offspring of a disomic addition from the line OMA5d.09, only 2 plants were disomic additions, which corresponds to a minimal paternal transmission of 6.7% (2 of 30). Twenty-one plants were monosomic additions, which indicates a probable maternal transmission of 76.7% (23 of 30), although one or two of the monosomic additions could be from paternal transmission. In a further experiment analyzing 25 F$_4$ offspring of a disomic addition from the line OMA5d.17, only two plants were disomic additions, indicating a paternal transmission of 8% (2 of 25). Seventeen plants were monosomic additions, which indicates a probable maternal transmission of 76% (19 of 25). Taking data for both oat backgrounds together, the irregular low paternal transmission of the maize chromosome 5 (6.7–8%) clearly accounts for the low frequency of disomic addition offspring from disomic chromosome 5 addition plants.

Maize Chromosome 10 Transmission. Chromosome 10 is the smallest chromosome, with 190 Mb in the Seneca 60 complement. Yet, it was the most frequently eliminated chromosome in (oat × maize)F$_1$ hybrids, indicating a low tolerance for its presence in oat (4, 10). The first recovered monosomic addition of chromosome 10 occurred in a haploid of GAF-Park oat background (10). Since that time, the plant has been vegetatively propagated by tiller cloning under short-day conditions. Periodically, tiller clones have been moved to a long-day regime to induce flowering for self-pollination or backcrossing with GAF-Park pollen. After screening thousands of spikelets over a period of ≈3 years, we recently recovered one seed. This one seed, however, did not possess maize chromatin; thus, a maize chromosome addition offspring has not been produced by this plant.

In this new series of oat × maize crosses, we recovered 11 plantlets that retained maize chromosome 10 as single or multiple chromosome additions, 9 plantlets in Starter oat background, and 2 plantlets in Sun II background. One chromosome 10-positive F$_1$ plant (F$_1$-0289-1, Seneca 60 × Sun II) set 189 F$_2$ seeds in its first four panicles (Table 2). The PCR assay with Grande 1 showed that 28 F$_2$ plants originating from three panicles were maize-positive, and 2 F$_2$ plants were maize-negative. GISH analyses revealed 16 disomic and 12 monosomic addition plants. All 10 F$_2$ plants originating from the fourth panicle were maize-negative. In all 28 maize-positive plants, only
chromosome 10 short-arm-specific SSR markers (p-phi041, p-phi117, and p-umc1293) were present; none of the long-arm-specific markers (p-umc1249, p-umc1196, p-umc1176, and p-umc1084) tested was detected (22). We, therefore, assume that the chromosome transmitted to offspring in every case is a short-arm telocentric derivative of chromosome 10 (22). The derived line disomic for the chromosome 10 short-arm telocentric was labeled OMAdt10S.20 and seeds (F3 offspring) have already been distributed (Table 1).

The F1 hybrid F1-5133-1 originated from crosses of Starter oat with maize B73. This hybrid possessed the three maize chromosomes 4, 7, and 10 in addition to the haploid oat complement at a young growth stage. In DNA samples from both the third and fourth tillers (F1-5133-1/c and F1-5133-1/d), SSR markers were present for chromosome 4 and chromosome 10. Although all three short-arm-specific markers for chromosome 10 were present in the two DNA samples, neither sample showed evidence for long-arm-specific markers. Therefore, we assume that chromosome 10, which was accompanied by maize chromosome 4, also was a telocentric short-arm derivative of chromosome 10.

PCR analysis of the three F2 offspring from the panicles of the third and fourth tiller of plant F1-5133-1 (Table 2) showed that only two F2 plants had the short-arm-specific SSR markers for chromosome 10, and none had any of the long-arm-specific SSR markers for chromosome 10. All three F2 plants had the chromosome 4-specific SSR markers. All those F2 offspring from the F1-5133-1/a panicle were positive for B73 chromosome 4, three as monosomic and three as disomic additions. The tiller F1-5133-1/b did not set seed.

The generation of a fertile disomic telocentric addition for chromosome 10 (OMAdt10S.20) is a major breakthrough in our efforts to develop a complete series of fertile oat–maize addition lines (22). However, this observation raises the question of why does only the short arm of an added maize chromosome 10 transmit in oat. Does the long arm possess a gene that prevents transmission in this alien background? High sterility occurs in the highly stable whole chromosome 10 addition in GAF-Park oat and the two independent events of short-arm derivatives of chromosome 10 in Sun II and Starter oat, where the long-arm telocentrics could not be established. The situation appears similar to the difficulties of generating a disomic euplasmic addition line for Betzes barley chromosome 1H and for its long-arm telosome 1HL in Chinese Spring wheat (25). The difficulties in wheat (26–28) appear to be caused by the interaction of the gene Shw (sterility in hybrid with wheat) with the wheat background causing sterility. However, the sterility was alleviated by the simultaneous addition of monosomic or disomic chromosome 6H to the 1H addition (29). Perhaps we could select (oat × maize)F1 hybrids for the simultaneous additions of other chromosomes with chromosome 10 to possibly allow fertility and transmission of the whole chromosome 10. In addition, it may be feasible to use additional maize genotypes that possess a different allel of the presumed gene on chromosome arm 10L responsible for the sterility. In the corresponding wheat–barley addition situation, chromosome 1H of the closely related wild barley (Hordeum vulgare L. subsp. spontaneum) was added to wheat without causing a severe effect of sterility (30).

Oat–Maize Chromosome 1 RHs. Monosomic oat–maize chromosome 1 addition seeds, the foundation for the development of oat–maize chromosome 1 RH lines, were treated with γ rays at two levels. These levels were 180 BC1 seeds treated with 40 krad and 120 BC1 seeds treated with 35 krad. A total of 46 maize-positive plants, as indicated by the presence of the markers Grande 1 and/or CentA, were recovered from the 40-krad treatments. The 35-krad treatment generated 54 maize-positive plants. These 100 BC1 plants were allowed to self-pollinate. Of these, 91 panicles produced 340 BC1:F2 offspring that tested negative and 171 BC1:F2 offspring that tested positive for maize chromatin in their genomes, indicating successful transmission of maize segments. It is notable that after the γ radiation treatment of 300 monosomic addition seeds, only 100 plants retained their maize chromosomes or a diminutive maize chromosome derivative. This finding indicates that a majority of the breaks generated maize fragments that were eliminated from somatic tissues. Earlier results showed a certain level of somatic instability for whole chromosome 1 addition plants resulting in chromosome loss (7, 10).

A set of 45 SSR markers distributed along maize chromosome 1 was used to determine by a presence vs. absence test for each marker approximate points of maize chromosome breakage in the 171 BC1:F2 plants. All 45 SSR markers were present in 98 BC1:F2 plants, which represent 50 families. These plants were considered as possessing either a whole maize chromosome without a break or a reciprocal oat–maize translocation. These plants will be self-pollinated, and offspring of those with reciprocal translocations will be selected for the segregating translocated chromosomes. Nine BC1:F2 plants, forming five families, showed complex rearrangements, including interstitial deletions and multiple translocations with oat. Forty-four BC1:F2 plants constituted 21 families, each representing one likely independent (chromosome rearrangement) event (Table 3). These 44 RH plants were placed into 10 panel groups, with plants within a group resulting from similar maize chromosome breaks based on marker analysis (Table 3 and Fig. 2). Fig. 2 illustrates the definition of eight segments by seven breaks in selected RH lines for maize chromosome 1 representing the 10 groups (Table 3). Plants with only one break in their maize chromosome, and thus possessing only one deficiency or one oat–maize translocation, are shown in the first panel (Fig. 2). The markers shown in the left column are the first and last marker present or absent and frame the breakpoints. The points define six segments on the short arm (p-umc1354 to p-umc168), one large segment spanning the centromere region (p-umc1626 to p-umc1604), and one additional segment on the long arm (p-bnlq1720 to p-umc2244).

The apparently single breakpoint in the long arm of chromo-
The physical sizes of the segments differ remarkably as shown by GISH experiments (Fig. 3). In the line 1.07.3-001.3-03 (a sibling from group 7), the maize chromosome shows a primary constriction that defines the deficient short arm to ~80% of its regular WT metaphase length. This result would mean that the missing element (20%) represents a genetic size of at least 445 map units separated into 6 distinct segments by 8 of 10 groups. On the other hand, GISH of line 1.07.2-007.3-04 (a sibling from group 9) shows the distal maize chromosome fragment translocated to an oat chromosome. The fragment length corresponds to ~20% of the long-arm WT length in metaphase and visualizes segment 8. Even considering that the definition of the single segment by two markers varies over a considerable genetic distance, the segment 7 spans approximately the proximal 80% of the short and the proximal 80% of the long arm of the genetic map of maize chromosome 1. The line 1.07.1-020.3-01 (sibling of group 3) shows by GISH analysis a fragment of ~15% of the WT short-arm length translocated to an oat chromosome. This fragment visualizes the length of the segments 1 and 2 together marked by SSRs p-umc1397 and p-umc1479.

Summary

The current set of disomic oat–maize addition lines involves all maize chromosomes in different oat backgrounds with the exception of chromosome 10. The maize chromosome 10 addition progeny has only the short arm; a fertile disomic telocentric addition line is available. The whole chromosome 10 added to haploid GAF-Park oat does allow the availability of DNA. Although not fertile, and therefore not capable of producing disomic addition offspring, we continue to maintain the original plant vegetatively by tiller cloning under short-day growing conditions. The leaves show remarkable somatic stability for the added maize chromosome over a period of >3 years. The plant serves as a source for chromosome 10 genomic DNA and RNA.

The complete series of DNAs made from each maize chromosome addition has been used as a powerful tool to allocate genes and markers to chromosome. Ananiev et al. (31) used the oat–maize chromosome 9 addition line as the DNA source to construct a chromosome-specific cosmid library allowing the isolation of maize-specific repetitive DNA families. The low level of cross-hybridization under standard conditions between oat and maize genomic DNA makes it possible to screen libraries for maize species-specific sequences (31).

Oat–maize addition lines are ideal for mapping gene families and markers that have more than one copy on different chromosomes likely because of the duplicative nature of maize. For example, Okagaki et al. (32) mapped 350 ESTs and sequence tagged sites to chromosomes by a presence vs. absence test and

### Table: Relationship of maize chromosome 9 RHs to marker positions

<table>
<thead>
<tr>
<th>SSR Marker</th>
<th>Map Coordinates According to Homoeologous Pair Map</th>
<th>Genetic Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>punc1354</td>
<td>0.60 1.00</td>
<td>1</td>
</tr>
<tr>
<td>punc1071</td>
<td>0.60 1.01</td>
<td>2</td>
</tr>
<tr>
<td>punc1727</td>
<td>0.97 1.00</td>
<td>3</td>
</tr>
<tr>
<td>punc1997</td>
<td>0.40 2.00</td>
<td>4</td>
</tr>
<tr>
<td>punc1479</td>
<td>0.40 2.01</td>
<td>5</td>
</tr>
<tr>
<td>punc1914</td>
<td>0.30 3.01</td>
<td>6</td>
</tr>
<tr>
<td>p-bnlg2328</td>
<td>1.04 4.00</td>
<td>7</td>
</tr>
<tr>
<td>p-bnlg2171</td>
<td>0.97 5.01</td>
<td>8</td>
</tr>
<tr>
<td>p-bnlg1611</td>
<td>0.90 6.01</td>
<td>9</td>
</tr>
<tr>
<td>p-bnlg2025</td>
<td>0.20 7.00</td>
<td>10</td>
</tr>
<tr>
<td>p-bnlg1669</td>
<td>0.25 8.01</td>
<td>11</td>
</tr>
<tr>
<td>p-bnlg1626</td>
<td>0.15 9.01</td>
<td>12</td>
</tr>
</tbody>
</table>

Fig. 2. Panel of the first RH lines for maize chromosome 1. Shown are the 15 SSR markers that frame the seven breakpoints, hence define the RH segments between p-umc1354 (most distal on the short arm) and p-umc2244 (most distal on the long arm) markers representing a genetic distance of more than 1,120 map units according to the IBM2 map.

Fig. 3. GISH of metaphase chromosomes from root tips of three RH plants of the maize chromosome 1 panel. (A) Plant BC$_{F_2}$ 1.07.3-001.3-3 (sibling of group 7), arrow points to the deficient short arm of maize chromosome 1; the chromosome lost ~20% of its short arm. p-umc1626 is the most distal present marker tested (see also Fig. 2). The yellow-painted chromosome visualizes the segments 7 and 8 representing the genetic distance of 656–675 map units. (B) Plant BC$_{F_2}$ 1.07.2-007.3-4 (sibling of group 9), arrow points to the translocation fragment visualizing the RH segment 8. The translocation fragment accounts for ~20% of the long-arm length representing the genetic distance of 261–332 map units. (C) Plant BC$_{F_2}$ 1.07.1-020.3-1 (sibling of group 3), arrow points to the translocation fragment visualizing the RH segments 1 and 2 accounting for ~15% of the short-arm length representing the distance of 226–257 map units.
demonstrated the usefulness of the complete addition line set. However, the true power of the addition lines as a tool for maize genomics and genetics may be that no marker polymorphism is required for large-scale mapping. The value of the plant material for gene expression studies has already been shown in an analysis of interchromosomal interaction with respect to expression of the maize gene liguleless 3 on chromosome 3 (33) or the reduced susceptibility against the fungal pathogen *Puccinia coronata* f. sp. *avenae* in the oat–maize chromosome 5 addition lines (unpublished data).

With the development of RH lines from the oat–maize additions, markers can be placed to chromosome regions. Visualization by GISH of the rearranged maize chromosome fragments together with marker data helps to relate physical sizes to genetic distances along the chromosome arms. Besides the use of addition and radiation hybrid lines for mapping purposes, the extensive dissection of the maize genome provides powerful material for the targeted cloning of chromosome-specific DNA and to study chromosome-specific structures and their behavior in an alien background.

This is a joint contribution of the Minnesota Agricultural Experiment Station and the Department of Agriculture–Agricultural Research Service. This material is based upon work supported by the National Science Foundation Grant 011134.