Funct Integr Genomics (2002) 2:60–69
DOI 10.1007/s10142-002-0052-8

ORIGINAL PAPER

R.G. Kynast · R.J. Okagaki · H.W. Rines
R.L. Phillips

# Maize individualized chromosome and derived radiation hybrid lines and their use in functional genomics

Received: 7 January 2002 / Accepted: 12 February 2002 / Published online: 9 April 2002
© Springer-Verlag 2002

**Abstract** The duplicated and rearranged nature of plant genomes frequently complicates identification, chromosomal assignment and eventual manipulation of DNA segments. Separating an individual chromosome from its native complement by adding it to an alien genetic background together with the generation of radiation hybrids from such an addition line can enable or simplify structural and functional analyses of complex duplicated genomes. We have established fertile disomic addition lines for each of the individual maize chromosomes, except chromosome 10, with oat as the host species; DNA is available for chromosome 10 in a haploid oat background. We report on instability and transmission in disomic additions of maize chromosomes 1, 5, and 8; the chromosome 2, 3, 4, 6, 7, and 9 additions appear stable. The photoperiodic response of the two recovered maize chromosome 1 addition lines contrasts to the long-day flowering response of the oat parents and the other addition lines. Only when grown under short days did maize chromosome 1 addition lines set seed, and only one line transmitted the maize chromosome 1 to offspring. Low resolution radiation hybrid maps are presented for maize chromosomes 2 and 9 to illustrate the use of radiation hybrids for rapid physical mapping of large numbers of DNA sequences, such as ESTs. The potential of addition and radiation hybrid lines for mapping duplicated sequences or gene families to chromosome segments is presented and also the use of the lines to test interactions between genes located on different maize chromosomes as observed for ectopic expression of cell fate alterations.

**Keywords** *Avena sativa* L. · Oat-maize chromosome addition line · Oat-maize radiation hybrid line · *Zea mays* L.

Genomic DNA and seed in limited amounts of oat-maize addition lines are currently available to the scientific community and DNA of radiation hybrid lines will become available; for information see http://www.agro.agri.umn.edu/rp/genome/

R.G. Kynast · R.J. Okagaki · R.L. Phillips (✉)
Plant Molecular Genetics Institute,
Department of Agronomy and Plant Genetics,
University of Minnesota, 411 Borlaug Hall, 1991 Buford Circle,
St. Paul, MN 55108, USA
e-mail: phill005@umn.edu
Fax: +1-612-6251268

H.W. Rines
Plant Science Research Unit, USDA-ARS,
Department of Agronomy and Plant Genetics,
University of Minnesota, 411 Borlaug Hall, 1991 Buford Circle,
St. Paul, MN 55108, USA

## Introduction

The very large size of the genomes of many higher plants combined with the highly duplicative nature of the sequences comprising their genomes have made the identification and assignment of function to the genes of these species a formidable task. In maize, the genome size is 1 C ≅2,671 million base pairs (Bennett and Leitch 2001). These sequences are distributed among the ten chromosomes. As in other higher organisms there is evidence for duplication of many genes in maize, and the genome also is highly duplicated on a macro scale being essentially an ancient tetraploid (Ahn and Tanksley 1993). These duplicated forms may provide duplicative function, may have nonfunctional products (pseudogenes) or be silenced, or may have been modified either to new catalytic activity or developmental expression in timing, level, or tissue specificity. However, the basic sequence can be highly conserved; thus, there exists the challenges of assigning duplicated sequences to map location and of identifying the specific sequence expressed when analyzing randomly isolated DNAs, such as ESTs. The sequence multiplicity becomes even more abundant and confusing when one considers the high degree of sequence duplication in the heterochromatic regions distributed across the genome including retrotransposons, telomeric sequences and others that may be present in hundreds or even multi-thousands of copies of identical or near identical sequences (Bennetzen et al. 1994;

SanMiguel et al. 1996). This size and duplicity of the maize genome makes efforts to sequence and analyze expression on a whole-genome level a major challenge.

To simplify structural and functional genome analysis, we have described in this paper an update on a system of maize chromosome addition and radiation hybrids in an oat background. Adding individual maize chromosomes to oat allows, in principle, the cloning of whole maize chromosomes. Subsequent fragmentation of these maize chromosomes using radiation allows isolation of a maize chromosome segment in an oat background to simplify the physical mapping of specific maize sequences. Expression analyses and phenotypic effects of such isolated segments in a living alien system may provide clues regarding specific functions of genes located on these segments. Also, the system of physical mapping provides the added feature that monomorphic sequences can be associated to genome position without the requirement for polymorphisms and development of a segregating population.

The initial step in generation of the individualized maize chromosome lines is crossing cultivated oat (*Avena sativa* L., 2n = 6x = 42, AACCDD) with maize (*Zea mays* L., 2n = 2x = 20) to generate interspecies embryos that undergo uniparental chromosome elimination during early phases of embryogenesis (Rines and Dahleen 1990; Machan et al. 1995; Rines et al. 1996, 1997). Because maize genome loss is often incomplete, embryo development can lead to haploid oat plants with 21 oat chromosomes and 1 or more maize chromosome(s) in the complement (Rines et al. 1995; Riera-Lizarazu et al. 1996). To date, all 10 maize chromosomes are represented as monosomic additions to haploid oat plants (Kynast et al. 2001a, b). Such haploid oat-maize chromosome addition plants (n = 3x +1 = 22) produce unreduced gametes at a frequency which is similar to that observed in haploid oat plants (n = 3x = 21) (Kynast et al. 2000). Depending on the oat genotype, the frequency of unreduced gametes can produce as much as 40% seed set after self-pollination (Davis 1992). If the added maize chromosome is transmitted, the haploid plant can produce doubled haploid (diploid) $F_2$ oat with disomic (totally homozygous) maize chromosome additions (2n = 6x +2 = 44). By propagating the generated oat-maize addition line, identical copies of maize chromosomes are reproduced in vivo and multiplied throughout the offspring. Hence, whole maize chromosomes become cloned in oat. Kynast et al. (2001b) reported fertile disomic oat-maize chromosome addition lines for the maize chromosomes 1, 2, 3, 4, 6, 7, and 9 and a fertile monosomic addition line for maize chromosome 8. Herein we report the recovery of disomic additions for maize chromosomes 5 and 8; hence, only the monosomic maize chromosome 10 addition to haploid oat remains to be converted into a disomic addition.

The maize chromosome addition lines in oat have been shown to be an effective tool for assigning markers to a specific maize chromosome or chromosomes (Okagaki et al. 2001). For more precise localization of markers, we are developing an approach based on radiation hybrid mapping. Radiation hybrid mapping was first used in the 1970s (Goss and Harris 1975), and has developed into a powerful mapping tool (reviewed in Walter and Goodfellow 1993). In radiation hybrid mapping, chromosome breaks induced by radiation exposure substitute for genetic crossovers. The frequency of chromosome breakage between two markers is related to physical distance. Thus, radiation hybrid maps are physical maps of the genome. The number of breaks induced, which is determined by the level of radiation exposure, largely determines the resolution of the map. Radiation hybrid maps have been published for human (Hudson et al. 1995; Stewart et al. 1997; Olivier et al. 2001), mouse (McCarthy et al. 1997; Van Etten et al. 1999), canine (Priat et al. 1998), rat (Watanabe et al. 1999), zebrafish (Geisler et al. 1999), pig (Hawken et al. 1999), and horse (Kiguwa et al. 2000). Radiation hybrid lines for these projects were produced by fusing irradiated donor cell lines with a rodent host cell line. Use of oat-maize chromosome addition lines offers an alternative approach to cell fusion for making radiation hybrid maps (Riera-Lizarazu et al. 2000), with several added features including that they are chromosome-specific. This chromosome specificity provides physical separation of many of the duplicated sequences. In addition, these radiation hybrids may serve as a source for isolating and cloning individual members of duplicated sets or small families of genes. Together, these oat-maize addition lines and radiation hybrids provide not only powerful physical mapping tools but also unique genetic materials to observe the function of genes in a background that integrates genetic information of two disparate species.

## Materials and methods

### Plants

Single plants of spring oat cultivars GAF-Park, Kanota, Preakness, Starter, Stout, Sun II and the experimental $F_1$ (MN97201–1 × MN841801–1) hybrid and the maize lines Seneca 60, *bz1-mum9*, A188 and the $F_1$ (A188 × W64A) hybrid were grown and crossed as described in detail by Kynast et al. (2001b). In brief, oat plants were grown for 7 weeks under a short-day regime and shifted to a long-day regime for shooting and flowering. Maize was germinated and grown for the first 4 weeks under a long-day regime and then shifted to a short-day regime. To analyze photoperiodic responses, tiller-clones of the haploid oat-maize chromosome 1 additions (OMAm1.1 and OMAm1.7) were cultivated in growth chambers set for long-day (16 h light, 8 h dark) and for short-day (11 h light, 13 h dark) regimes, respectively.

### Cytology

Root tips (1.5–2 cm) of oat, maize, and oat-maize chromosome addition lines were pretreated and fixed as described by Kynast et al. (2001b). For chromosome counting, root tips were rinsed in distilled water at room temperature for 20 min, hydrolyzed in 1 N HCl at 60°C for 12 min and stained in modified Schiff reagent (SS32–500, Fisher Scientific, Fair Lawn, N.J.) at room temperature for 20 min (Feulgen and Rössenbeck 1924). The meristem cells were squashed in 2% (w/v) aceto-orcein (84–1453, Carolina

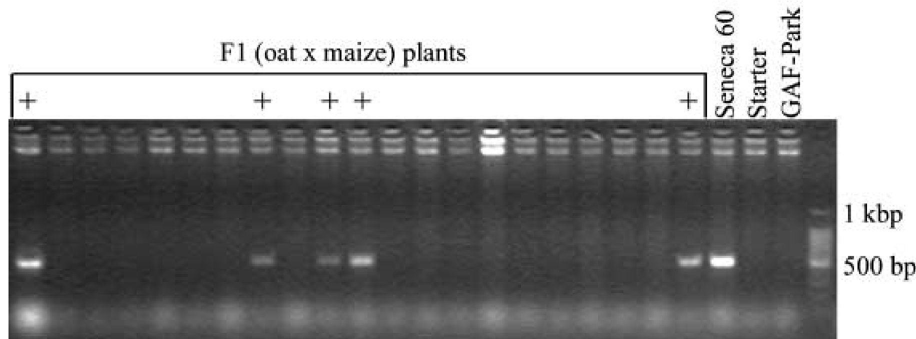


**Fig. 1** PCR of $F_1$ (oat × maize) plant DNA using maize-specific Grande 1-LTR primers. A 500-bp long PCR product indicates the presence of maize chromosome(s) in the corresponding $F_1$ plant (labeled +). The positive control (maize Seneca 60 DNA) shows a band. Negative controls (oat Starter and GAF-Park DNAs) show no bands. Electrophoresis was in 1.5% agarose

Biological Supply, Burlington, N.C.). Chromosomes were counted from four to six metaphase cells per slide.

For genomic in situ hybridization (GISH), root tips were washed in distilled water at 4°C overnight to entirely remove fixative from tissues. Root tips were incubated in 10 mM citrate buffer, pH 4.5, at room temperature for 20 min and in maceration solution [225 mU/ml cellulase (21947, Calbiochem, La Jolla, Calif.), 75 mU/ml pectinase "Macerase" (441201, Calbiochem, La Jolla, Calif.) in 10 mM citrate buffer, pH 4.5] at 37°C for 120 min. Digestion was stopped by equilibrating root tips in 45% (v/v) acetic acid at room temperature for 3–5 min. A small number of meristem cells from each root tip were gently stroked out and squashed onto a glass slide in a drop of 45% (v/v) acetic acid. Further steps of RNase treatment, postfixation, and hybridization were as described by Pickering et al. (1997) except that total genomic DNA of maize cv. Seneca 60 was labeled with ChromaTide Oregon Green 488 (C-7630, Molecular Probes, Eugene, Ore.) and probed onto slides without using an unlabeled competitor DNA. The hybridization and washes were at 80% and 85% stringency, respectively. Chromosomes were counterstained with propidium iodide. Signals were visualized using an Axioskop microscope equipped for epifluorescence (Carl Zeiss, Oberkochen). Images were captured with a Magnafire camera (Optronics, Goleta, Calif.) and processed with PhotoShop 5.5 software (Adobe Systems, San Jose, Calif.).

### DNA extraction and PCR

Seedlings were screened for the presence of maize sequences using maize-specific Grande 1 or CentA primers (Ananiev et al. 1998). DNA micro-extraction and PCR were accomplished by using REDExtract-N-Amp Plant PCR Kit (XNAR, Sigma, St. Louis, Mo.). Either 0.6-cm disks of leaf tissue were incubated in 100 µl extraction solution, or 0.3-cm disks in 50 µl, at 95°C for exactly 10 min. After cooling to room temperature an equal volume of dilution buffer was added. The mix was stored together with the leaf disk at 4°C until further use. The PCR mix (20 µl) was made of 10 µl 2×PCR ready-mix plus 6 µl of 1 µM (2×500 nM) F/R primer-mix plus 4 µl leaf disk extract. The PCR program was set according to the manufacturer's recommendation.

The identity of individual maize addition chromosomes was determined by PCR analysis using previously described SSR primers (Kynast et al. 2001b). Primers were selected from the MaizeDB (http://www.agron.missouri.edu/) and tested as described by Kynast et al. (2001b).

To perform a large number of PCR reactions on plants, DNA was isolated using either the DNeasy Plant Mini Kit (69106, Qiagen, Valencia, Calif.) or a CTAB procedure (Saghai-Maroof et al. 1984). PCR primer sequences are available upon request. Primers were designed using the program Primer-3 and the conditions described previously, as were PCR conditions (Okagaki et al. 2001).

### Production of radiation hybrid lines

Monosomic oat-maize chromosome additions are produced by backcrossing disomic oat-maize chromosome addition lines to their parental oat lines. To produce radiation hybrid lines, the $BC_1$ seed are irradiated with 30–40 krad γ-rays (gamma-rays) to induce breaks in the added maize chromosome but without seriously damaging the vitality of the embryos ($M_0$ generation). The subsequent cultivation of the $M_0$ plants acts as a filter against severely deleterious chromosome aberrations. Vigorous plants were self-pollinated. Radiation hybrid lines possessing different fragments of a maize chromosome including intergenomic translocations and modified maize chromosomes with terminal and interstitial deletions were recovered in the $M_1$ ($BC_1F_2$) offspring at a frequency of about 5% (Riera-Lizarazu et al. 2000).

## Results

### $F_1$ (oat × maize) hybrids and disomic oat-maize additions

To date, a total of 400 $F_1$ plants recovered from over 60,000 oat × maize pollinated florets have been tested for presence versus absence of maize chromatin in their genomes. Earlier data (Kynast et al. 2001a, b) and recent results concordantly show that about 35% (139/400) of the tested $F_1$ plants possessed one or more maize chromosome(s) in their complements as indicated by a PCR product generated using maize-specific primers for Grande 1-LTR (Fig. 1). Grande 1 is a highly repetitive LTR-type retrotransposon, which is dispersed on each of the ten maize chromosomes (Monfort et al. 1995; SanMiguel et al. 1996). The specific maize chromosomes present in each of the 139 maize chromatin-positive $F_1$ plantlets were identified by maize chromosome-specific SSR-markers (Fig. 2) and visualized by GISH. Though all ten maize chromosomes could be retained with different frequencies in $F_1$ plants as single additions and in combination with other maize chromosomes, previous (Riera-Lizarazu et al. 1996; Kynast et al. 2001a, b) and recent data together show no obvious preferential combinations of specific maize chromosomes in multiple additions. In general, the fewer maize chromosomes retained, the more vigorous the $F_1$ plants. Partial hybrids with only one maize chromosome often set seed following self-pollination. Plants with two different added maize chromosomes also occasionally set seed. Of the 139 maize chromatin-positive $F_1$ plantlets recovered, 39 plants transmitted maize chromosomes to $F_2$ offspring (Table 1). Individualized maize chromosomes could be recovered as disomic additions to the hexaploid oat (2n =

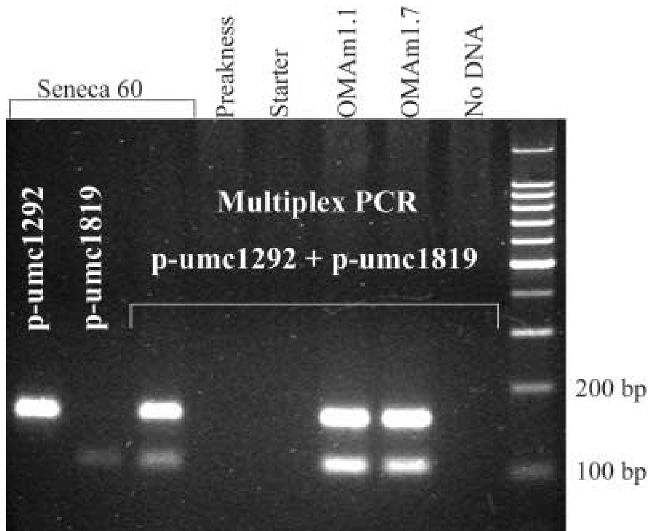


**Fig. 2** Maize chromosome 1 identification using chromosome-specific SSR markers. SSR-marker *p-umc1292* is located on the short arm of maize chromosome 1 (bin 1.00). SSR-marker *p-umc1819* is located on the long arm of maize chromosome 1 (bin 1.12). Electrophoresis in 3.75% agarose enables the PCR products to be clearly separated from each other after being generated by multiplex PCR (*third lane*). Both markers are absent from both oat cultivars Preakness and Starter (*lanes 4 and 5*). The plant $F_1$–9808–2 retained a chromosome 1 of Seneca 60 in haploid Preakness (*OMAm1.1*). The plant $F_1$–0143–1 retained a chromosome 1 of Seneca 60 in haploid Starter (*OMAm1.7*)

6x +2 = 44) among offspring of 36 of these $F_1$ plants. One further $F_1$ plant produced an $F_2$ offspring with a monosomic substitution of an oat chromosome by a maize chromosome (2n = 6x –1+1 = 42). Screening of the $F_3$ offspring of this $F_2$ plant showed that besides monosomic (2n = 6x –1 = 41) and euploid (2n = 6x = 42) oat plants, a monosomic maize chromosome 8 addition (2n = 6x +1 = 43) occurred. Neither a disomic substitution nor a disomic addition were generated from this monosomic substitution plant. Screening of the $F_4$ offspring from the monosomic 8 addition revealed about 10% transmission of the added maize chromosome, as expected for monosomic plant chromosomes in an alien genetic background. No disomic addition was detected in this line, which probably indicates a lack of paternal transmission. In two of the other $F_1$ addition plants, $F_2$ offspring showed the transmission of two maize chromosomes by producing double disomic oat-maize chromosome addition plants (2n = 6x +2+2 = 46). These included combinations of the maize chromosomes 1 plus 9 and 4 plus 6, respectively (Kynast et al. 2001b). Two more fertile $F_1$ plants with maize chromosome combinations, one with 4 plus 7 and one with 6 plus 8, produced $F_2$ offspring but did not transmit their maize chromosomes.

Each of the maize chromosomes, except chromosome 10, have been recovered as fertile disomic oat-maize chromosome additions. The phenotypes of most of the lines have been described in detail elsewhere (Kynast et al. 2001b). Two disomic additions of maize chromosome 5 in different oat backgrounds (Table 1) have most recently been recovered and added to the set. These represent the first recovery of transmitted maize chromosome 5. Although chromosome 5 has been the maize chromosome most frequently retained among all 139 $F_1$ plants, somatic sectoring activity and possibly low chromosome 5 tolerance at generative stages have caused a very low transmission frequency for this addition chromosome as indicated by cytological analyses of $F_2$ and $F_3$ offspring populations. The $F_2$ plants with disomic addition of maize chromosome 5 are characterized by stalk branching, as it was also observed in their corresponding parental $F_1$ plants. These $F_2$ plants show reduced tillering intensity when compared to their corresponding $F_1$ parents. A newly recovered disomic addition of maize chromosome 6, this one in a new oat background (Table 1), shows the phenotypic character of disease lesion mimic like the two chromosome 6 additions recovered earlier in a Starter oat background, except with less severity on leaf sheaths. New disomic addition lines in different oat backgrounds were also recovered for maize chromosomes 2 and 9 (Table 1). Both lines revealed the respective typical phenotypes, bluish leaf color and erratic premature senescence syndrome, observed by Kynast et al. (2001b) for oat-maize addition 2 and 9 lines in the Starter oat background.

**Table 1** Fertile oat-maize chromosome addition lines

| Added maize chromosome | Donor maize line | Oat background | Addition type | Number of lines |
|---|---|---|---|---|
| 1 | Seneca 60 | Starter | Disomic | 1 |
| 2 | Seneca 60 | Starter | Disomic | 7 |
| 2 | Seneca 60 | Sun II | Disomic | 1 |
| 3 | Seneca 60 | Sun II | Disomic | 1 |
| 3 | Seneca 60 | Preakness | Disomic | 1 |
| 4 | Seneca 60 | Starter | Disomic | 5 |
| 4 | A188 | Starter | Disomic | 2 |
| 5 | Seneca 60 | Starter | Disomic | 1 |
| 5 | Seneca 60 | $F_1$ hybrid | Disomic | 1 |
| 6 | Seneca 60 | Starter | Disomic | 2 |
| 6 | Seneca 60 | $F_1$ hybrid | Disomic | 1 |
| 7 | Seneca 60 | GAF-Park | Disomic | 1 |
| 7 | Seneca 60 | Starter | Disomic | 3 |
| 8 | Seneca 60 | GAF-Park | Monosomic | 1 |
| 8 | *bz1-mum9* | Starter | Disomic | 1 |
| 9 | Seneca 60 | Starter | Disomic | 7 |
| 9 | Seneca 60 | GAF-Park | Disomic | 1 |
| 1+9 | Seneca 60 | Starter | Double disomic | 1 |
| 4+6 | Seneca 60 | Starter | Double disomic | 1 |

## Individualized chromosomes – irregularities in transmission

Disomic oat-maize chromosome addition lines are the foundation for the development of chromosome-specific panels of radiation hybrid lines. By backcrossing these disomic lines to their corresponding oat parent, seed are produced with monosomic additions of a defined maize

**Table 2** Photoperiodic response of haploid oat-maize chromosome 1 additions

| OMAxy.z | $F_1$ plants | | Photoperiod treatment | Number of $F_2$ offspring | |
|---|---|---|---|---|---|
| | Tiller-clones | Panicles | | Total | Maize-positive |
| OMAm1.1 | $F_1$–9808–2-A | 35 | Long-day | 0 | 0 |
| OMAm1.1 | $F_1$–9808–2-B | >100 | Short-day | 0 | 0 |
| OMAm1.1 | $F_1$–9808–2-C | 20 | Short-day | 49 | 0 |
| OMAm1.7 | $F_1$–0143–1-A | 35 | Long-day | 0 | 0 |
| OMAm1.7 | $F_1$–0143–1-B | >100 | Short-day | 0 | 0 |
| OMAm1.7 | $F_1$–0143–1-C | 30 | Short-day | 400 | 7[a] |

[a] Three maize-positive seeds were formed in one panicle ($F_1$–0143–1-C/d), four maize-positive seeds were formed in another panicle ($F_1$–0143–1-C/u)

chromosome. A high level of transmission and somatic stability of the added maize chromosome in the oat background are useful prerequisites for generating the monosomic additions and subsequent radiation hybrids. The incorporation in progeny of radiation breakage segments from a maize monosomic addition, rather than from a disomic addition, simplifies the possible cytogenetic outcome and enables detection and marker characterization of the segments.

Maize chromosomes 2, 3, 4, 6 and 9 in disomic condition show somatic stability and nearly 100% transmission in an oat background from the $F_2$ through subsequent generations. Maize chromosome 7 had shown transmission problems. Kynast et al. (2001b) reported 16% monosomic and 12% no addition offspring from a disomic chromosome 7 addition $F_2$ plant. We observed a loss of the maize chromosome in more than 75% of screened $F_3$ offspring of another disomic chromosome 7 addition $F_2$ plant. However, by propagating selected plants with highest frequencies of disomic addition offspring in $F_4$ and $F_5$ genotypes, we have "stabilized" the transmission of this maize chromosome and produced almost 100% disomic offspring in the $F_6$ generation.

Maize chromosomes 1, 5 and 8 were the most recently recovered disomic additions to oat and have in common a certain level of somatic instability. Maize chromosome 1 additions in $F_1$ plants of two different oat cultivars (Preakness and Starter) were identified. The $F_1$ plants ($F_1$–9808–2 and $F_1$–0143–1) tillered readily when grown under short-day conditions and could be vegetatively propagated (tiller-cloned) by breaking the plants apart at their crowns. A lack of seed set was initially encountered when tiller-clones of the two $F_1$ plants were shifted to a long-day regime (Table 2) in order to initiate shooting and flowering. Thus, we tested tiller-clones of both $F_1$ plants continuously grown in a short-day regime. Shooting and heading of all tillers were delayed by about 4 weeks. Although both genotypes produced more than 100 panicles with no seed, some tillers did set seed. From the $F_1$–9808–2 genotype we recovered 49 $F_2$ seeds in 20 panicles, but none of the offspring inherited the maize chromosome 1. The $F_1$–0143–1 genotype produced 400 $F_2$ seeds in 30 panicles. Three maize-positive seeds were formed in one panicle ($F_1$–0143–1-C/d) out of a total of 3 seeds. Furthermore, 4 maize-positive seeds were formed in another panicle ($F_1$–0143–1-C/u) out of a total of 95 seeds (Table 2). All of the maize-positive $F_2$ plants were disomic additions of maize chromosome 1,

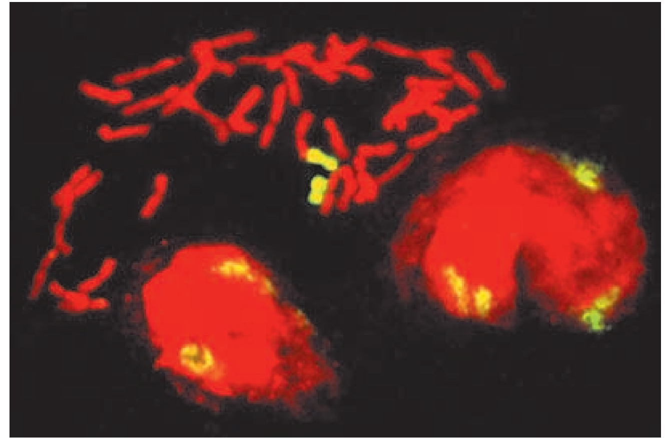

**Fig. 3** Genomic in situ hybridization (GISH) of OMAd1.7 root meristem cells. In situ hybridization of ChromaTide Oregon Green 488-labeled genomic DNA of maize cv. Seneca 60 to root tip cells of an $F_2$ offspring plant from $F_1$–0143–1 visualizes the maize chromosome pair by *bright green-yellow* colorization at metaphase and as *two stripes and spots* in interphase nuclei. Oat chromosomes are counterstained with propidium iodide (*red* colorization)

indicating both maternal and paternal transmission (Fig. 3). The maize chromosome 1 showed a transmission of 1.75% (7/400) in the Starter background. Analyses of $F_3$ offspring showed that a disomic maize chromosome 1 addition plant can produce 100% disomic offspring in one panicle, mixed offspring of disomic and nullisomic additions in another panicle, and 100% nullisomic addition in yet another panicle, all from the same plant. Similar segregating chromosome constitutions of $F_4$ offspring from among different panicles of the same disomic $F_3$ addition plant indicate instability as a result of somatic sectoring activity. The majority of $F_4$ offspring, however, show regular transmission of maize chromosome 1 in the Starter background provided that the disomic addition plants are cultivated under a short-day regime.

Maize chromosome 5 has been added to two different oats [Starter and the experimental $F_1$ (MN97201–1 × MN841801–1) hybrid], and the respective $F_1$ plants ($F_1$–0102–1 and $F_1$–0003–1) tiller-cloned. Both of the haploid oat-maize chromosome 5 addition plants had a high tillering capacity and produced more than 500 $F_2$ seeds. The $F_1$–0102–1 genotype had a total of six maize-positive $F_2$ offspring tracing to 3 panicles, but only one addition offspring was disomic. The other $F_2$ plants were

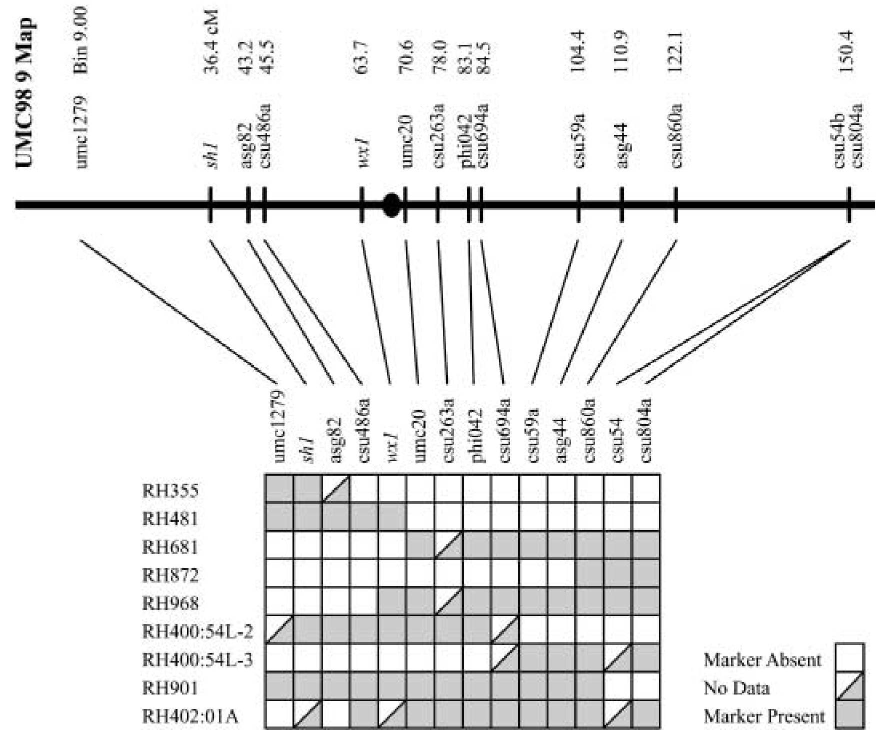


**Fig. 4** Chromosome 9 low-resolution map. Low resolution maps are intended to facilitate the rapid placement of markers to a chromosome region. Nine lines that could be used to place markers on chromosome 9 are illustrated together with some of the markers used to characterize the lines. The *circle* on the genetic map gives the approximate position of the centromere. *Lines RH400:54L-2 and RH400:54L-3* represent the two halves of a reciprocal translocation

either monosomic for the maize chromosome 5 or chimerical among root meristem cells and between root and shoot for the same plant. The $F_1$–0003–1 genotype had 11 maize-positive $F_2$ offspring tracing to 3 panicles; 4 of these offspring were disomic additions. The other $F_2$ plants were either monosomic or chimerical for the maize chromosome 5 as described for the $F_1$–0102–1 genotype. The transmission of maize chromosome 5 was 3.4% (17/500) in both oat backgrounds, but included chimeras. Consecutive offspring generations of both chromosome 5 addition genotypes showed segregation patterns similar to those observed in the $F_2$ generation for oat-maize chromosome 1 additions. However, the appearance of monosomic additions for maize chromosome 5 indicated that besides somatic sectoring there was also instability at the generative stage with possibly differential transmission in pollen versus ovules. In contrast to the photoperiodic response observed with the maize chromosome 1 addition, the chromosome 5 addition lines – like the other maize chromosome addition lines – showed no alteration in the long-day flowering response of the oat host.

## Radiation hybrid materials from oat-maize addition lines

Radiation treatment of oat-maize monosomic addition seed induces breakage and loss of maize sequences in progenitor cells of monosomic addition plants and can produce mosaic plants. Selfing $M_0$ plants allows segregation and provides genetically uniform material. As individual panicles may arise from separate progenitor cells, screening individual panicles permits recovery of multiple radiation hybrid lines from a single plant. Four or five seedlings per panicle were tested for the presence of maize sequences, because transmission of maize chromosomes was at a low frequency as expected. Maize-specific primers for Grande 1, a repetitive sequence widely dispersed in the genome (SanMiguel et al. 1996), and CentA, a repetitive sequence concentrated in centromeric regions (Ananiev et al. 1998), were used to detect maize chromosomes or chromosome segments. The probe Grande 1 assesses the presence versus absence of maize chromosome segments but cannot provide an estimate of the amount of maize chromosome that is left after irradiation. Between 5% and 10% of the progeny retained maize sequences. Seedlings testing positive for one or both of Grande 1 and CentA sequences were saved for further analysis. These lines are referred to as "candidate radiation hybrid lines", because further analysis is required to determine their constitution and suitability for radiation hybrid mapping. A total of 239 chromosome 2 plants, 202 chromosome 9 plants, 101 chromosome 6 plants and 78 chromosome 4 plants have been identified as candidate radiation hybrid lines. Fifty-five of the chromosome 9 lines have been previously described (Riera-Lizarazu et al. 2000).

Over 50 markers have been tested against a subset of the original 55 chromosome 9 candidate radiation hybrid lines (Riera-Lizarazu et al. 2000; Okagaki et al., unpublished data). Four classes of candidate lines may be identified. Class 1 lines retained all, or almost all, of the tested markers. These lines appear to have an intact maize chromosome, a maize chromosome with small deletions or a reciprocal translocation of the maize with an oat chromosome. Class 2 lines retained maize centromeric

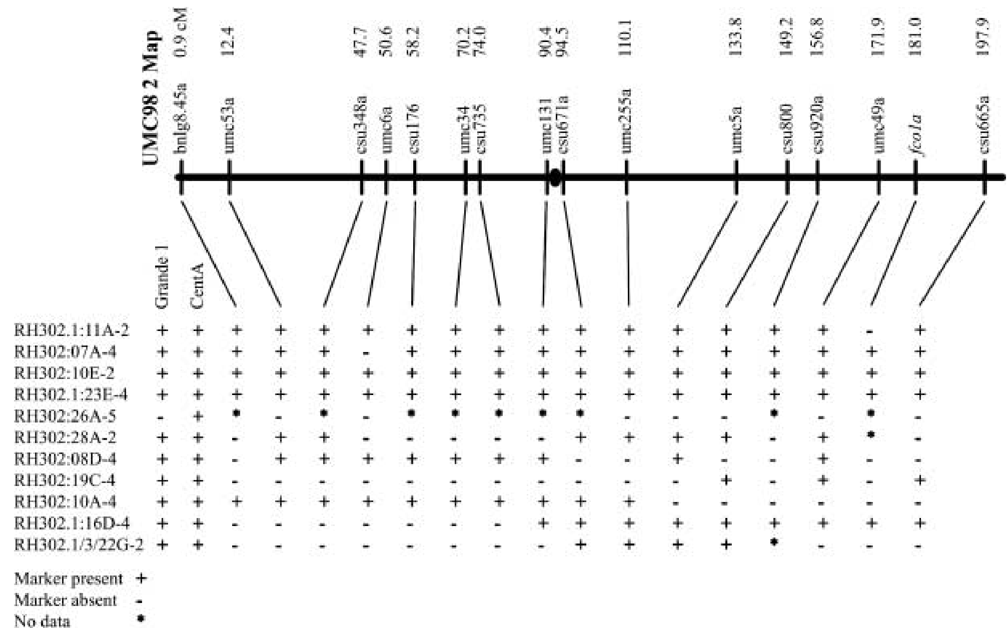


**Fig. 5** Chromosome 2 candidate radiation hybrid lines. Eleven representative candidate radiation hybrid lines are presented to illustrate the range of chromosome rearrangements recovered. Lines *RH302.1:11A-2*, *RH302:07A-4*, *RH302:10E-2*, and *RH302.1:23E-4* have either small interstitial deletions or an intact maize chromosome. Line *RH302:26A-5* has maize centromere sequences, as detected by the CentA probe, but other sequences were not present. Lines *RH302:28A-2*, *RH302:08D-4*, and *RH302:19C-4* have complex rearrangements, and lines *RH302:10A-4*, *RH302.1:16D-4*, and *RH302.1/3/22G-2* have simple rearrangements. These last six lines would be suitable for radiation hybrid mapping

sequences, as determined by the presence of CentA sequences, but have lost most, or all, of the other tested markers. Class 3 lines have complex rearrangements of the maize chromosome, such as multiple deletions. Class 4 lines have a simple rearrangement such as a terminal deletion or a translocation consistent with a single break in the maize chromosome.

Radiation hybrid lines with a terminal deletion or translocation are the ideal material for low resolution deletion maps. Using a panel of eight lines, a low resolution map for chromosome 9 has been developed (Fig. 4). This map divides chromosome 9 into seven regions, and markers may be rapidly located to a chromosome region. More precise localization of markers may be attained by incorporating additional lines into this panel. The eight lines comprising the low resolution chromosome 9 panel showed no detectable phenotypic variation. The erratic premature senescence (EPS) syndrome characterized in the disomic chromosome 9 additions requires the growing of many plants of each genotype to score the trait because of the erratic occurrence (Kynast et al. 2001b). Apart from the EPS syndrome, chromosome 9 addition plants develop normal phenotypes with high similarity to their oat parents.

Sixteen markers were used to screen 239 chromosome 2 candidate radiation hybrid lines (Fig. 5). These lines derived from material irradiated with 30 krads rather than the 40 krads used for chromosome 9. This lower radiation dose was expected to yield fewer breaks per line which would increase the proportion of lines suitable for deletion mapping – those with a single break. Class 1 lines RH302.1:11A-2 and RH302:07A-4 had a single missing marker. Line RH302.1:11A-2 was missing marker *fco1a*, but adjacent markers were present (Fig. 5). The size of the interstitial deletion cannot be determined from the data; it may be relatively small. Chromosome breaks were not detected in lines RH302:10E-2 and RH302.1:23E-4, although small deletions would be difficult to detect. The class 2 line RH302:26A-5 only retained the centromeric CentA sequences suggesting that most of both chromosome arms were lost. Lines RH302:28A-2, RH302:08D-4, and RH302:19C-4 had multiple chromosome breaks; these lines resembled chromosome 9 class 3 lines. Such lines would be ideal for developing standard radiation hybrid maps. Class 4 lines RH302:10A-4, RH302.1:16D-4 and RH302.1/3/22G-2 retained large contiguous stretches of chromosome 2. These lines have either terminal deletions or translocations with the maize chromosome and could be used for deletion mapping. The types of chromosome rearrangements found among chromosome 2 candidate radiation hybrid lines were similar to those found with chromosome 9.

## Discussion

### Chromosome addition lines

Genomes of most species are complex. Even the model plant *Arabidopsis thaliana* is extremely complex with much of its genome present as duplicated or higher order segments (Vision et al. 2000). In addition to the expected complexity of haploid or diploid genomes, maize has a subgenome structure (Ahn and Tanksley 1993). Major genetic regions in maize are syntenic among different chromosomes owing to the presence of two subgenomes. The importance of potential interactions among these subgenomes is not clear at the present time. The addition of individual maize chromosomes to oat enables the relatively large maize genome to be separated into ten defined subunits. Reducing the amount of total genomic maize DNA to approximately one-tenth offers advanta-

**Table 3** Common phenotypes of oat-maize additions

| Maize Addition | Characters |
|---|---|
| 1 | Erect leaf blade, photoperiod neutral response, sectoring among shoots |
| 2 | Bluish leaf, waxy stem |
| 3 | Liguleless, crooked panicle, growth of aerial axillary buds |
| 4 | Lighter green leaf, small seed, earlier maturing |
| 5 | Branched stem, sectoring among shoots, generative instability |
| 6 | Disease lesion mimic, NOR amphiplasty |
| 7 | Small stature, instability in some offspring |
| 8 | Small stature, sectoring among roots, irregular transmission |
| 9 | Erratic premature senescence |
| 10 | Grassy type, ovule and pollen sterility |

ges to many applications, especially large scale mapping and functional genomics. The disomic oat-maize chromosome addition lines have been generated in a manner that ensures a doubled haploid character to the offspring, i.e. from a functional point of view all disomic addition plants are totally homozygous and may possess the different maize chromosomes in a common genetic background. The benefit of homozygous plants – especially a homozygous background of alien addition lines – is that responses to different test conditions can be directly measured and compared among different addition lines without the interference of a segregating background. In particular, the expression pattern of alleles can be correlated to phenotypes much as in (near) isogenic lines. For instance, morphological examination of the disomic oat-maize chromosome 3 addition line reveals several morphological changes including crooked panicles, abnormal blade-to-sheath transformation and outgrowth of aerial axillary buds. By genetic analyses of $BC_1F_2$ offspring segregating for presence versus absence of maize chromosome 3, Muehlbauer et al. (2000) showed that the expression of these cell fate abnormalities in oat is correlated with the presence of maize chromosome 3. Transcription analyses of leaf sheath tissues from the offspring clearly showed that maize *liguleless3* (*lg3*) homeobox gene expression is associated with the particular phenotypes (Muehlbauer et al. 2000). *Lg3* is known for its ectopic expression of cell fate alterations and was physically mapped to maize chromosome 3 by the use of oat-maize chromosome addition lines. These results illustrate that the maize chromosome addition lines are useful genetic stocks for studying expression of maize genes in an oat background.

We have generated disomic oat-maize chromosome additions in more than one genetic oat background for most of the maize chromosomes (Table 1) allowing analysis of maize gene function in different host genomes. The addition of maize chromosome 1 to two different oat cultivars, Preakness and Starter, has shown an apparent background impact. Maize chromosome 1 was transmitted in the Starter-derived line and was not transmitted in the Preakness-derived line. Future cytogenetic analyses of those hybrids may provide the genetic basis of this distinct behavior of the maize chromosome 1 in different oat backgrounds. Different expressions of the same maize chromosome in different backgrounds may enable the cloning of key genes with regulatory significance.

Fertile disomic oat-maize addition lines open the opportunity of combining two selected maize chromosomes in the same oat background to test for specific maize gene interactions. The possibility to stably combine maize chromosomes in oat already has been demonstrated by the recovery of two double disomic addition lines (Table 1). Specific chromosome combinations allow the reconstitution of interaction of genes from different maize chromosomes. These interactions are separated from those of the rest of the maize genome and can be compared to single chromosome addition lines. A model for this type of work is the maize *lg3* gene located on chromosome 3. In a dominant mutant (*Lg3*) of maize the *Lg3* allele has been associated with ectopic expression of liguleless leaves and a crooked tassel (Fowler and Freeling 1996; Muehlbauer et al. 1999, 2000). Among the specific phenotypic characters of oat-maize chromosome 3 additions (Table 3) are liguleless leaves and crooked panicles. One possible explanation for ectopic *Lg3*-type expression of the *lg3* gene in the oat-maize chromosome 3 addition line is a lack of regulation by a gene located on one of the absent maize chromosomes. In a maize plant the maize *lg3* gene is normally under the control of the *rough sheath2* (*rs2*) gene located on maize chromosome 1. In maize the *rs2* gene suppresses ectopic expression of the *lg3* allele, but does not suppress ectopic expression of the *Lg3* allele. The maize chromosome 3 addition of oat lacks maize chromosome 1 (the location of *rs2*) and hence the regulatory action of *rs2*. If oat orthologues of *rs2* do not exist, or exist but can not regulate the maize *lg3* gene in the maize chromosome 3 addition line, then ectopic *Lg3*-type expression of the maize *lg3* gene may occur. Combining the maize chromosome 1 and maize chromosome 3 by crossing between the respective disomic addition lines for these two chromosomes would allow evaluation of the *rs2*-*lg3* interaction separate from the remaining chromosomes of the maize genome. Extending the crossing program of the chromosome 3 addition line to further maize chromosome addition lines may contribute to finding paralogous or other loci on other maize chromosomes with regulatory action on *lg3*. Isolating individual maize chromosomes from the remainder of their genome and systemat-

ically producing new combinations with other individual maize chromosomes in oat can provide a valuable source for finding regulatory genes by observation of expression or suppression of phenotypes. With a systematic involvement of radiation hybrid lines (translocations and deletions) a dissection of relevant chromosome segments becomes feasible and new suppressors found may open alternative ways of engineering chromosomes that control undesirable phenotypes.

The introgression of alien chromosomes or chromosome segments provides plant material that allows investigation of the extent to which alien genetic material can add new characters to the recipient genotype. The oat-maize addition plants generally grow well but show characters that make them distinct from their oat parent. However, there are environmental interactions, erratic appearances and occasional inconsistencies in the phenotypes depending on the oat genomic background and the maize chromosome donor. Apart from the variability among the additions there are also maize chromosome-specific characters with remarkable stability through consecutive generations and consistency across oat genomic backgrounds (Table 3).

Another question is: can alien chromosomes release the suppression of normally nonexpressed intrinsic characters in the host? Such questions can be raised with respect to disease resistance. Breeding strategies need to be created that distinguish between adding resistance to a susceptible plant and removing susceptibility from a susceptible plant. Phenotype and expression analysis of oat-maize additions may help find answers. For instance, oat crown rust caused by *Puccinia coronata* f. sp. *avenae* and oat stem rust caused by *P. graminis* f. sp. *avenae* are major oat diseases (Ohm and Shaner 1992). These rust species tend to be highly species-specific. Maize is not susceptible to oat rust and oat is not susceptible to corn rust (caused by *P. sorghi*). Of interest would be rust resistance genes present in maize that are introduced into oat as part of a maize chromosome segment. The potential gain of host resistance or, alternatively, susceptibility to oat or maize diseases by an oat plant due to the addition of maize genes would allow further insights into host-pathogen interaction phenomena and related genes and mechanisms. One advantage to this system is that once a trait such as resistance is identified in an oat-maize addition line, radiation hybrid lines derived from that oat-maize addition line might be used to map the gene in maize, and thus provide a step toward its identification or cloning.

The generation of radiation hybrids from oat-maize chromosome addition lines provides the ability to dissect duplicated chromosome regions into different strains containing only one of the paralogous segments even from the same chromosome. Mapping a family of related sequences to chromosome location is difficult by standard methods. The use of addition and radiation hybrid lines allows the mapping of related sequences to chromosome and chromosome segment. The cloning of specific members of a gene family also will be made easier with these materials. Syntenic regions and evolutionary relationships in maize should be more clearly defined as such mapping efforts proceed.

Traits expressed in addition lines may be mapped using radiation hybrid lines (Rasko et al. 2000). Most chromosome 6 addition lines express a disease lesion mimic phenotype (Kynast et al. 2001b). Unpublished preliminary data we have recently generated involve results of 20 radiation hybrid lines that have been derived from chromosome 6 additions and scored for the lesion trait and for nine chromosome 6 markers. Based on which radiation hybrids exhibit the trait, data indicate that the lesion phenotype is associated with the proximal half of maize chromosome 6L. *Les13*, a dominant lesion mimic mutation, has been mapped to this region; further work will be required to determine if *Les13* is responsible for the lesion trait in chromosome 6 addition lines.

The disease lesion mimic example, like the *Lg3* gene expression discussed earlier, illustrates the exciting value of the individualized maize chromosome addition lines and derived radiation hybrids to study maize gene expression and interactions in an alien background. The addition and radiation hybrid lines provide powerful tools for physical mapping of the thousands of maize genes now being isolated and sequenced. A knowledge of the physical map location of these genes will provide valuable help in sorting, assignment of function, and delineating evolutionary relationships among the numerous duplicated DNA sequences of maize.

**Acknowledgements** We thank C.D. Russell, A.O. Stec and H.A. Zaia for excellent technical assistance. For discussion of the manuscript and valuable suggestions, we thank Prof. Rudi Appels, WA Agriculture/PMB-CRC, Munoch University, Perth, Western Australia. This material is based upon work supported by the National Science Foundation under Grant No. 9872650.

## References

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ananiev EV, Phillips RL, Rines HW (1998) Complex structure of knob DNA on maize chromosome 9: retrotransposon invasion into heterochromatin. Genetics 149:2025–2037

Bennett MD, Leitch IJ (2001) Plant DNA C-values database (release 1.0, September 2001). http://www.rbgkew.org.uk/cval/homepage.html

Bennetzen JL, Schrick K, Springer P, Brown WE, SanMiguel P (1994) Active maize genes are unmodified and flanked by diverse classes of modified, highly repetitive DNA. Genome 37:565–576

Davis DW (1992) Characterization of oat haploids and their progeny. MSc thesis, University of Minnesota, St. Paul, Minn.

Feulgen R, Rössenbeck M (1924) Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende selektive Färbung von Zellkernen in mikroskopischen Präparaten. Z Physiol Chem 135:203–248

Fowler J, Freeling M (1996) Genetic characterization of the dominant *Liguleless* mutations in maize. Dev Genet 18:198–222

Geisler R, Rauch GJ, Baier H, van Bebber F, Brobeta L, Dekens MP, Finger K, Fricke C, Gates MA, Geiger H, Geiger-Rudolph S, Gilmour D, Glaser S, Gnugge L, Habeck H, Hingst K, Holley S, Keenan J, Kirn A, Knaut H, Lashkari D, Maderspacher F, Martyn U, Neuhauss S, Haffter P, et al (1999) A radiation hybrid map of the zebra fish genome. Nat Genet 23:86–89

Goss SJ, Harris H (1975) New method for mapping genes in human chromosomes. Nature 255:680–684

Hawken RJ, Murtaugh J, Flickinger GH, Yerle M, Robic A, Milan D, Gellin J, Beattie CW, Schook LB, Alexander LJ (1999) A first-generation porcine whole-genome radiation hybrid map. Mamm Genome 10:824–830

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, et al (1995) An STS-based map of the human genome. Science 270:1945–1954

Kiguwa SL, Hextall P, Smith AL, Critcher R, Swinburne J, Millon L, Binns MM, Goodfellow PN, McCarthy LC, Farr CJ, Oakenfull EA (2000) A horse whole-genome-radiation hybrid panel: chromosome 1 and 10 preliminary maps. Mamm Genome 11:803–805

Kynast RG, Okagaki RJ, Odland WE, Russell CD, Livingston SM, Rines HW, Phillips RL (2000) Towards a radiation hybrid map for maize chromosomes. Maize Genet Coop Newslett 74:60–61

Kynast RG, Okagaki RJ, Odland WE, Stec A, Russell CD, Zaia H, Livingston SM, Rines HW, Phillips RL (2001a) Oat-maize chromosome manipulation for the physical mapping of maize sequences. Maize Genet Coop Newslett 75:54–55

Kynast RG, Riera-Lizarazu O, Vales MI, Okagaki RJ, Maquieira SB, Chen G, Ananiev EV, Odland WE, Russell CD, Stec AO, Livingston SM, Zaia HA, Rines HW, Phillips RL (2001b) A complete set of maize individual chromosome additions to the oat genome. Plant Physiol 125:1216–1227

Machan F, Nesvadba Z, Ohnoutkova L (1995) Production of haploid plants of new wheat and oat donors through wheat × maize and oat × maize crosses. Genet Slecht 31:1–10

McCarthy LC, Terrett J, Davis ME, Knights CJ, Smith AL, Critcher R, Schmitt K, Hudson J, Spurr NK, Goodfellow PN (1997) A first-generation whole genome-radiation hybrid map spanning the mouse genome. Genome Res 7:1153–1161

Monfort A, Vicient CM, Raz R, Puigdomenech P, Martinez-Izquierdo JA (1995) Molecular analysis of a putative transposable retroelement from the *Zea* genus with internal clusters of tandem repeats. J DNA Res 2:255–261

Muehlbauer GJ, Fowler JE, Girard L, Tyers R, Harper L, Freeling M (1999) Ectopic expression of the maize homeobox gene *Liguleless3* alters cell fate in the leaf. Plant Physiol 119:651–662

Muehlbauer GJ, Riera-Lizarazu O, Kynast RG, Martin D, Phillips RL, Rines HW (2000) A maize-chromosome 3 addition line of oat exhibits expression of the maize homeobox gene *liguleless3* and alterations of cell fate. Genome 43:1055–1064

Ohm HW, Shaner GS (1992) Breeding oat for resistance to diseases. In: Marshall HG, Sorrells ME (eds) Oat science and technology. American Society of Agronomy, Madison, Wis. pp 657–698

Okagaki RJ, Kynast RG, Livingston SM, Russell CD, Rines HW, Phillips RL (2001) Mapping maize sequences to chromosome using oat-maize chromosome addition materials. Plant Physiol 125:1228–1235

Olivier M, Aggarwal A, Allen J, Almendras A, Bajorek ES, Beasley EM, Brady SD, Bushard JM, Bustos VI, Chu A, Chung TR, De Witte A, Denys ME, Dominguez R, Fang NY, Foster BD, Freudenberg RW, Hadley D, Hamilton LR, Jeffrey TJ, Kelly L, Lazzeroni L, Levy MR, Lewis SC, Liu X, Lopez FJ, Louie B, Marquis JP, Martinez RA, Matsuura MK, Misherghi NS, Norton JA, Olshen S, Perkins SM, Perou AJ, Piercy C, Piercy M, Qin F, Reif T, Sheppard K, Shokoohi V, Smick GA, Sun WL, Stewart EA, Tejeda JF, Tran NM, Trejo T, Vo NT, Yan SCM, Zierten DL, Zhao S, Sachidanandam R, Trask BJ, Myers RM, Cox DR (2001) A high-resolution radiation hybrid map of the human genome draft sequence. Science 291:1298–1302

Pickering RA, Hill AM, Kynast RG (1997) Characterization by RFLP analysis and genomic in situ hybridization of a recombinant and a monosomic substitution plant derived from *Hordeum vulgare* L. × *H. bulbosum* L. crosses. Genome 40:195–200

Priat C, Hitte C, Vignaux F, Renier C, Jiang Z, Jouquand S, Cheron A, Andre C, Galibert F (1998) A whole-genome radiation hybrid map of the dog genome. Genomics 54:361–378

Rasko JEJ, Battini J-L, Kruglyak L, Cox DR, Miller AD (2000) Precise gene localization by phenotypic assay of radiation hybrid cells. Proc Natl Acad Sci USA 97:7388–7392

Riera-Lizarazu O, Rines HW, Phillips RL (1996) Cytological and molecular characterization of oat × maize partial hybrids. Theor Appl Genet 93:123–135

Riera-Lizarazu O, Vales MI, Ananiev EV, Rines HW, Phillips RL (2000) Production and characterization of maize-chromosome 9 radiation hybrids derived from an oat-maize addition line. Genetics 156:327–339

Rines HW, Dahleen LS (1990) Haploid oat plants produced by application of maize pollen to emasculated oat florets. Crop Sci 30:1073–1078

Rines HW, Riera-Lizarazu O, Phillips RL (1995) Disomic maize chromosome-addition oat plants derived from oat × maize crosses. In: Oono K, Takaiwa F (eds) Modification of gene expression and non-Mendelian inheritance. NIAR, Tsukuba, pp 235–251

Rines HW, Riera-Lizarazu O, Maquieira SB, Phillips RL (1996) Wide crosses for haploids. In: Scoles G, Rossnagel B (eds) Proc 5th International Oat Conference and 8th International Barley Genetics Symposium, Saskatoon, Saskatchewan, Canada, pp 207–212

Rines HW, Riera-Lizarazu O, Nunez VM, Davis DM, Phillips RL (1997) Oat haploids from anther culture and from wide hybridizations. In: Jain SM, Sopory SK, Veilleux RE (eds) In vitro production of haploids in higher plants, 4. Kluwer, Dordrecht, pp 205–221

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL (1996) Nested retrotransposons in the intergenic regions of the maize genome. Science 274:765–768

Stewart EA, McKusick KB, Aggarwal A, Bajorek E, Brady S, Chu A, Fang N, Hadley D, Harris M, Hussain S, Lee R, Maratukulam A, O'Connor K, Perkins S, Piercy M, Qin F, Reif T, Sanders C, She X, Sun WL, Tabar P, Voyticky S, Cowles S, Fan JB, Cox DR, et al (1997) An STS-based radiation hybrid map of the human genome. Genome Res 7:422–433

Van Etten WJ, Steen RG, Nguyen H, Castle AB, Slonim DK, Ge B, Nusbaum C, Schuler GD, Lander ES, Hudson TJ (1999) Radiation hybrid map of the mouse genome. Nat Genet:384–387

Vision TJ, Brown DG, Tanksley SD (2000) The origins of genomic duplications in *Arabidopsis*. Science 290:2114–2117

Walter MA, Goodfellow PN (1993) Radiation hybrids: irradiation and fusion gene transfer. Trends Genet 9:352–356

Watanabe TK, Bihoreau MT, McCarthy LC, Kiguwa SL, Hishigaki H, Tsuji A, Browne J, Yamasaki Y, Mizoguchi-Miyakita A, Oga K, Ono T, Okuno S, Kanemoto N, Takahashi E, Tomita K, Hayashi H, Adachi M, Webber C, Davis M, Kiel S, Knights C, Smith A, Critcher R, Miller J, James MR, et al (1999) A radiation hybrid map of the rat genome containing 5,255 markers. Nat Genet 22:27–36