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Comparison between genetic and physical maps in *Zea mays* L. of molecular markers linked to resistance against *Diatraea* spp.

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Abstract In the pachytene stage, chromosomes are maximally extended and can easily be distinguished. Therefore, by applying fluorescence in situ hybridization (FISH) to pachytene chromosomes, it is possible to generate a high-resolution physical map of chromosome 9 in maize. Molecular markers (*umc105a* on the short arm of chromosome 9, *csu145a* on the long arm) were used that flank quantitative trait loci (QTL) for sugarcane borer (SCB) and southwestern corn borer (SWCB) resistance. As reference markers, a centromere-specific probe (CentC) and a knob-specific probe (pZm4-21) were utilized. Two fluorescent dyes with four probes were used to physically position these markers. Signals of repetitive DNA sequences in cosmid probes were suppressed by chromosome in situ suppression (CISS) hybridization. FISH signals were strong and reproducible for all probes. We measured the distances in micrometers for four subchromosomal regions and estimated the corresponding number of base pairs. The physical locations of the markers were compared on mitotic metaphase and pachytene chromosomes to the genetic map of chromosome 9. Genetic analysis positioned the two markers for SCB resistance in a central interval representing approximately 33.7% of the genetic length. However, the physical distance between these probes was determined to encompass about 70% of the physical length of chromosome 9. The two markers were located at distal positions on opposite arms of chromosome 9. Physical maps provide valuable information for gene isolation and understanding recombination.

Keywords *Zea mays* · Physical and genetic maps · Chromosome in situ suppression hybridization · Southwestern corn borer · Sugarcane borer

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Introduction

In tropical and subtropical areas of maize (*Zea mays* L.) cultivation, the southwestern corn borer (SWCB) (*Diatraea grandiosella* Dyar) and sugarcane borer (SCB) (*D. saccharalis* Fabricius) are destructive pests. These insects are responsible for annual losses of crops up to 600 million $US in Brazil and Mexico alone (CIMMYT 1988). Feasible measures against these pests have been achieved by breeding for resistances, and quantitative trait loci (QTL) for resistance to SCB (Bohn et al. 1996) and SWCB (Khairallah et al. 1998) have been mapped using molecular markers.

Genetic maps, constructed on the basis of recombination frequencies, are important tools in plant genetics; for example, the map of maize (Davis et al. 1999). However, discrepancies have been demonstrated between genetic and physical maps, with variations between chromosomal regions (e.g., in barley; Künzel et al. 2000). Therefore, plant geneticists, instead of studying a genomic region by depending only on its genetic map, would find it highly valuable to be able to correlate to a physical map for mapping and isolating genes.

The physical mapping of markers or genes can be accomplished with various means – using terminal deficiencies (Lin et al. 1997), translocation lines (Künzel et al. 2000), pulsed field gel electrophoresis (Bonnema et al. 1997), BAC or YAC contiguous DNA sequences (Kurata et al. 1997), genomic introgression (Humphreys et al. 1998) and fluorescence in situ hybridization (FISH) (Jiang and Gill 1994). The latter is being increasingly applied to physical mapping.

Being a powerful tool, a physical map may be employed for gene isolation; for example, by using microdissection and microcloning (Stein et al. 1998). Genome structure and function may be elucidated when comparing the genetically mapped positions of genes with their physical locations. Furthermore, physical distances between genes on chromosomes, in particular in meiosis at the pachytene stage, could shed light on the mechanism of chromosome recombination and rearrangement in higher plants.

For constructing high-resolution physical maps using FISH, large chromosomes are needed. This can be achieved by using species with large mitotic metaphase chromosomes, like barley (Lapitan et al. 1997). However, many other plant species have relatively small metaphase chromosomes (e.g., rice; Jiang et al. 1995) or very small ones (e.g., *Arabidopsis*; Murata and Motoyoshi 1995). As an alternative, chromosomes from the pachytene stage of meiosis can be used. Pachytene chromosomes, unlike those from mitotic metaphase, are less condensed and show a higher resolution with FISH (de Jong et al. 1999). In general, plant chromosomes isolated at the pachytene stage from pollen mother cells are 7- to 40-fold longer than those from the mitotic metaphase stage. The maize genome at the pachytene stage has, on average, a compactness of 5 Mb/μm (see methods), which is about one-tenth as compact as a chromosome at mitotic metaphase (de Jong et al. 1999). Pachytene chromosomes are more accessible to probes due to their decondensed chromatin (Peterson et al. 1999) and, in addition, for diagnostic purposes, they possess unique cytological landmarks – chromomeres, heterochromatic blocks, and knobs (Chen et al. 1998). Moreover, four copies of the DNA sequence of interest are present in pachytene chromosomes in a homozygous genotype as opposed to the two copies present in mitotic metaphase chromosomes.

The construction of physical maps using pachytene chromosomes will deliver directly visible physical evidence of the order and physical position on a chromosome of molecular markers or genes of interest. Repetitive sequences have been localized physically using FISH on pachytene chromosomes of tomato (Zhong et al. 1996), maize (Chen et al. 1998), rye (Albini and Schwarzacher 1992), *Brassica* (Armstrong et al. 1998), and *Arabidopsis* (Fransz et al. 2000). However, only a few single-copy sequences of important markers or genes have been localized on pachytene chromosomes of higher plants (Shen et al. 1987; Peterson et al. 1999; Fransz et al. 2000; Song et al. 2000). FISH has recently been employed to visualize single-copy DNA sequences on maize pachytene chromosomes (Sadder et al. 2000).

Here we present the physical locations of single-copy molecular markers (*umc105a* and *csu145a*) flanking QTL for SCB and SWCB resistance on maize chromosome 9 by applying in situ hybridization to mitotic metaphase as well as to pachytene chromosomes. The locations of these markers were related to the position of the centromere, which enabled a comparison of their positions on the genetic map versus their physical locations.

Materials and methods

Plant material and chromosome preparation

The maize inbred line KYS, obtained from Dr. D.F. Weber (Illinois State University, Normal, Ill., USA), was used for chromosome spreads. For mitotic metaphase chromosomes, fixed root tips were enzymatically macerated (Pan et al. 1993) and used for chromosome preparation (Jewell and Islam-Faridi 1994). Pachytene chromosomes were obtained from plants grown in the greenhouse for 8 weeks (summer) or 10 weeks (winter). Immature tassels were fixed in ethanol:glacial acetic acid (3:1) and stored at –20 °C (Dempsey 1994). Pachytene chromosomes were spread on microscopic slides according to Zhong et al. (1996), and the slides were pretreated with RNase A (DNase free) (Serva, Germany) and pepsin (Sigma, USA) (Zhong et al. l996). The chromosomes were then fixed on slides, denatured, and dehydrated (Stein et al. 1998).

Preparation and labeling of molecular probes

Individual cosmid clones (approx. 38 kb) (Llaca and Messing 1998) homologous to restriction fragment length polymorphism (RFLP) markers (*umc105a* and *csu145a*) were used for chromosome in situ suppression (CISS) hybridization. As reference markers, the maize centromere-specific probe, CentC (Ananiev et al. 1998), and the knob-specific probe, pZm4-21 (Dennis and Peacock 1984), were used.

Plasmid DNA was isolated with a kit (Maxiprep, Qiagen, Germany). Cosmids (*umc105a* and *csu145a*) were labeled using biotin-14-dATP (Life Technologies, Germany), and reference probes (CentC and pZm4-21) were labeled with digoxigenin-11-dUTP (Roche, Germany) by nick translation (Life Technologies).

In situ hybridization and probe detection

Maize genomic DNA was extracted from ground lyophilized leaves according to Hoisington et al. (1994). A modified protocol was used to prepare competitor DNA (C_o *t*-DNA; C_o t = mol/l × T_s; C_o : initial DNA concentration (mol/l); T_s : time in seconds) from maize to suppress hybridization signals caused by repetitive DNA sequences (Zwick et al. 1997). Instead of C_o *t*-1, C_o *t*-100 was isolated and used for CISS hybridization. According to Hake and Walbot (1980), this fraction contained high- and mid-repetitive DNA sequences. Digestion of single-stranded DNA with S1-nuclease was extended to 30 min.

CISS hybridization and probe detection were performed as described by Lichter et al. (1988) and Sadder et a1. (2000). For suppression, a 12- to 36-fold excess of C_o *t*-l00 DNA was used. Cosmid probes (*umc105a* and *csu145a)* were detected by avidin-FITC (Vector Laboratories, Calif.), and reference probes (CentC and pZm4-21) by anti-digoxigenin-rhodamine (Roche). Signal enhancement was facilitated by biotinylated anti-avidin (Vector Laboratories) followed by avidin-FITC. Chromosomes were counterstained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI). Slides were mounted in Vectashield (Vector Laboratories).

Southern blot verification of probe identity

For quality control and to rule out any contamination, hybridization was performed with the genomic cosmid probes used for CISS hybridization. Both RFLP markers (*umc105a* and *csu145a*) were labeled with digoxigenin-11-dUTP using nick translation (Life Technologies). Southern hybridization of blots (Southern 1975) containing DNA of the corresponding cosmid and RFLP in-

Table 1 Size comparison of the genetic map of chromosome 9 to the physical map of mitotic and pachytene chromosomes of four subchromosomal regions in maize

Maps of chromosome 9 (mean ± SD)	*n*	Subchromosomal region				Total	Arm ratio[a]
		9S telomere-*umc105a*	*umc105a*-centromere	centromere-*csu145a*	*csu145a*-9L telomere		
Genetic map: absolute size (cM)[b]	–	54.7	13[d]	37.7[d]	45	150.4	1.2
Genetic map: relative size (%)[b]	–	36.4	8.6[d]	25[d]	30	100	1.2
Mitotic physical map: relative size (%)	25	17.3 ± 2.3	24.5 ± 3.2	45.5 ± 4.0	12.7 ± 3.1	100	1.4 ± 0.16
Pachytene physical map: relative size (%)	38	16.2 ± 1.3	20.1 ± 1.4	49.8 ± 2.4	13.9 ± 1.6	100	1.76 ± 0.16
Pachytene physical map: absolute size (μm)	38	7.8 ± 1.5	9.7 ± 1.6	24.1 ± 4.8	6.7 ± 1.3	48.3 ± 8.5	1.76 ± 0.16
Molecular size (Mb)[c]	–	35.04	43.69	107.72	29.85	216.3	–
Mb/cM ratio	–	0.64	3.36	2.86	0.66	–	–

[a] The ratio of the long arm to the short arm
[b] Genetic map of chromosome 9 in maize (Davis et al. 1999)
[c] Molecular sizes of the subchromosomal regions in megabases were calculated on the basis of their relative sizes on pachytene chromosomes (see methods for more details)
[d] Centromere position estimated (Davis et al. 1999)

sert (positive control), as well as of a non-homologous cosmid (negative control), was applied according to Hoisington et al. (1994).

Photography, image processing, and calculations

Images were recorded on films (Kodak EliteChrom ASA-400) using a Zeiss Axiovert 135 epi-fluorescence microscope equipped with a FITC-rhodamine-DAPI three-way filter set (Zeiss, Germany). Slides were digitized (CanoScan 2700F, Canon), and images were processed digitally. Chromosome measurements were carried out using Optimas 6.0 (Optimas, USA). Relative measurements, means, and standard deviations (SD) were performed using MS-Excel (Microsoft, USA). Relative sizes were related to the length of the whole chromosome, giving relative values between 0 and 100%. Based on a DNA content of 2.865 pg per haploid maize genome of the KYS line (Rayburn et al. 1989) and assuming 1 pg of DNA to be equivalent to 965 Mb (Bennett and Leitch 1995), the number of megabases per haploid maize genome was estimated to be 2,764.731 Mb. At the pachytene stage, the combined length of the ten chromosomes of maize equals 552.66 μm, and chromosome 9 spans 43.24 μm (Rhoades 1950). Accordingly, the relative length of chromosome 9 is 7.82%, which is equal to approximately 216.3 Mb. The compactness is on average 5 Mb/μm for pachytene chromosomes. The entire genome of maize has a genetic length of 1,727.4 cM (Davis et al. 1999) and the genetic length of chromosome 9 equals 150.4 cM. The ratio of megabase pairs to centiMorgans (Mb/cM) was calculated assuming a constant DNA density along chromosomes. Average values of 1.44 Mb/cM and 1.6 Mb/cM may be estimated for chromosome 9 or the entire genome of maize, respectively. For individual subchromosomal regions of chromosome 9, the Mb/cM estimates were calculated on the basis of their relative sizes (Table 1).

Results

Identification of chromosome 9

The main objective of this study was to determine the physical locations of single-copy RFLP markers of maize related to resistance against SCB. Cosmid clones with homologous sequences to RFLP markers (*umc105a* and *csu145a)* were applied to chromosomes by CISS hybridization. A prerequisite for the physical localization of these markers was the identification of chromosome 9. In maize, this can be accomplished by determining the chromosome length, ratio of relative arm length, presence of specific amounts of centric heterochromatin, and characteristic patterns of enlarged chromomeres or knobs (Dempsey 1994). In this study, maize inbred line KYS was used for its superior capability of spreading and the quality of its pachytene preparations. Chromosome 9 was identified by its relative length and arm ratio (1.8). Furthermore, using FISH, we were able to detect its large easily identifiable centromere (probe CentC) in mitotic metaphase (Fig. 1a, b) and in pachytene chromosomes (Fig. 1c, d). Moreover, the prominent telomeric knob on the short arm of chromosome 9 was evident. This knob was visible as a bright fluorescent region on pachytene chromosomes 9 after counterstaining with DAPI (Fig. 1c). On mitotic metaphase chromosome 9, this knob may be easily recognized by the knob-specific probe pZm4-21 (Fig. la, b), which is also able to identify the knob of chromosome 9 at the pachytene stage (Fig. 1c). Hybridization of the pZm4-21 probe to the unique telomeric knob confirmed the identity of chromosome 9 (Fig. 1d).

In situ hybridization

Southern blot hybridization verified that each cosmid used contained its respective RFLP marker (data not shown). Direct hybridization of fluorescently labeled cosmids resulted in completely labeled chromosomes (data not shown). Only when CISS hybridization was used were specific signals obtained. On mitotic metaphase as well as on pachytene chromosomes, cosmids *umc105a* and *csu145a* produced in situ hybridization signals near telomeres on chromosome arms 9S or 9L, respectively (Fig. 1c, d).

When mitotic metaphase chromosomes were used, cosmid *umc105a*, cosmid *csu145a*, probe CentC, and probe pZm4-21 produced specific signals when they were hybridized simultaneously (Fig. 1a, b). Likewise on pachytene chromosomes, cosmid *umc105a*, cosmid

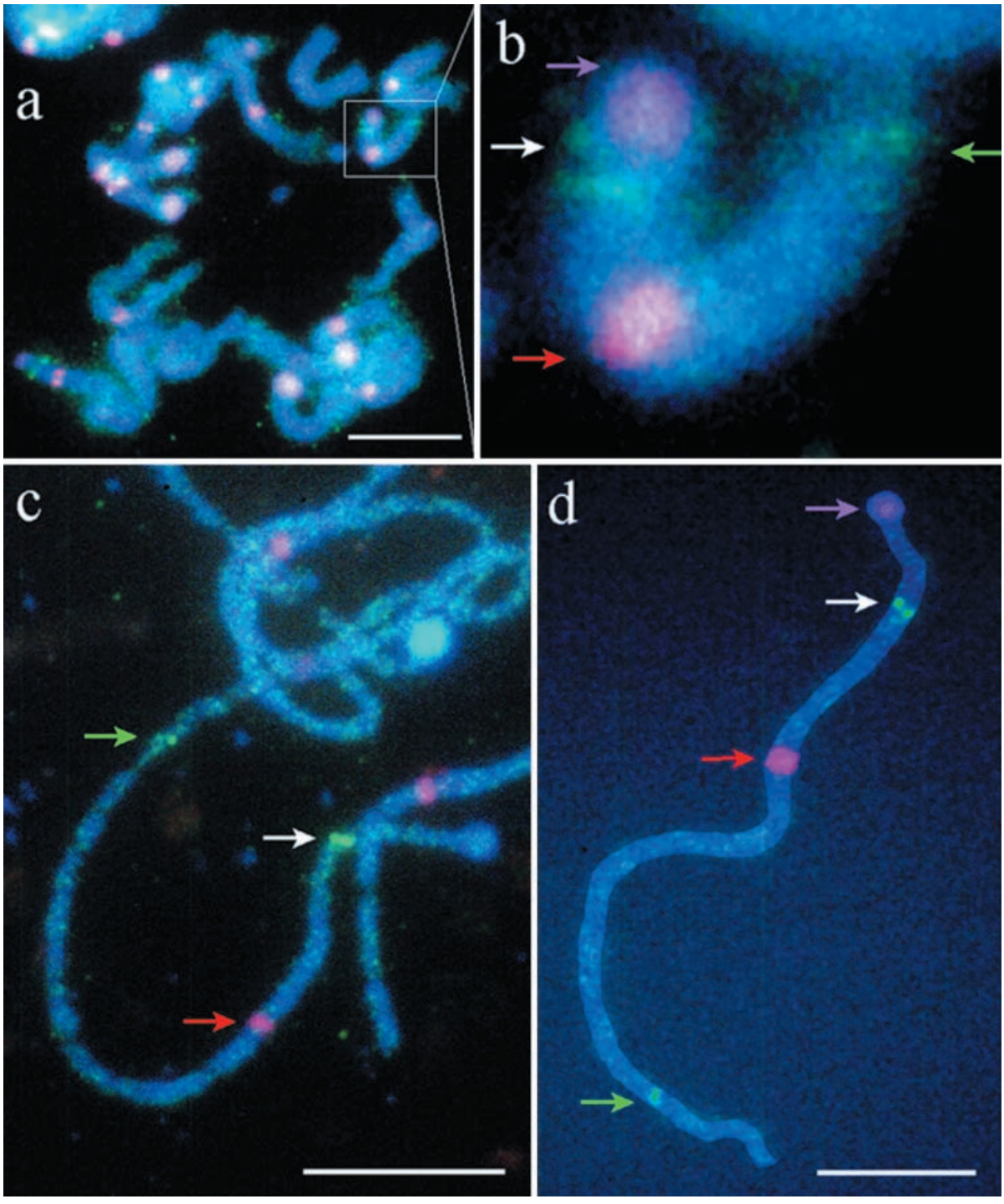

Fig. 1a–d Multicolor in situ suppression hybridization on maize chromosomes. Chromosomes were counterstained with DAPI. In all cases scale *bar* = 10 μm. **a,b** Hybridization of *umc105a* cosmid (*white arrow*), *csu145a* cosmid (*green arrow*), CentC (*red arrow*), and pZm4-21 (*purple arrow*) to mitotic metaphase chromosomes. **c** Hybridization of *umc105a* cosmid (*white arrow*), *csu145a* cosmid (*green arrow*), and CentC (*red arrow*) to pachytene chromosomes. **d** Hybridization of *umc105a* cosmid (*white arrow*), *csu145a* cosmid (*green arrow*), CentC (*red arrow*), and pZm4-21 (*purple arrow*) to pachytene chromosomes

csu145a, and CentC were hybridized successfully by themselves (Fig. 1c) or additionally with pZm4-21 (Fig. 1d). The probe CentC generated clear signals on centromeres of most chromosomes (Fig. la, b). A prominent fluorescence of knobs of both mitotic metaphase and pachytene chromosomes was clearly visible on chromosomes 5, 6, 7, and 9 after counterstaining with DAPI (data not shown). These observations were consistent with the expected knob positions of the KYS karyotype (Dempsey 1994).

Table 2 Comparison of distances of the genetic map of chromosome 9 to the physical map of mitotic and pachytene chromosomes of the chromosomal markers (*umc105a*, centromere, *csu145a*, and 9L telomere) to the 9S telomere

Maps of chromosome 9 (mean ± SD)	*n*	Chromosome marker					Arm ratio[a]
		9S telomere	*umc105a*	Centromere	*csu145a*	9L telomere	
Genetic map: absolute distance (cM)[b]	–	0	54.7	67.4[c]	105.4	150.4	1.2
Genetic map: relative distance (%)[b]	–	0	36.4	45[c]	70	100	1.2
Mitotic physical map: relative distance (%)	25	0	17.3 ± 2.3	41.8 ± 2.7	87.3 ± 3.1	100	1.4 ± 0.16
Pachytene physical map: relative distance (%)	38	0	16.2 ± 1.3	36.3 ± 2.2	86.1 ± 1.6	100	1.76 ± 0.16
Pachytene physical map: absolute distance (μm)	38	0	7.8 ± 1.5	17.53	41.6 ± 7.5	48.3 ± 8.5	1.76 ± 0.16
Molecular distance (Mb)	–	0	35.04	78.73	186.45	216.3	–

[a] The ratio of the long arm to the short arm
[b] Genetic map of chromosome 9 in maize (Davis et al. 1999)
[c] Centromere position estimated (Davis et al. 1999

Measurements

For determining the physical positions of the molecular markers, we had to measure the distances on chromosomes between the signals generated by FISH. Therefore, hybridization signals for all markers were reproduced in five independent experiments, each comprising two to four slides with chromosome spreads. On each slide, 50–70 spreads of pachytene or 30–50 spreads of mitotic metaphase chromosomes were analyzed, respectively; signals were observed in 80–90% of them. Only properly spread chromosomes with visible signals of the probes *umc105a*, *csu145a*, and CentC were considered further. Measurements were taken from individuals of chromosome 9 (25 samples of mitotic metaphase and 38 samples of pachytene chromosomes).

For further analysis, chromosome 9 was subdivided into four topographical regions, each being defined by its respective flanking chromosomal markers: (1) 9S telomere to *umc105a*, (2) *umc105a* to centromere, (3) centromere to *csu145a*, and (4) *csu145a* to 9L telomere (Tables 1 and 2). The sizes of these subchromosomal regions were arranged according to centiMorgans based on the genetic map (Davis et al. 1999). A rough estimate for the centromere position was also provided, although it could not be mapped genetically. Size measurements of the subchromosomal regions facilitated the construction of physical maps in two ways. One approach was based on the ratio of the size of each measured region relative to the total length of the chromosome. Physical maps for mitotic metaphase or pachytene chromosomes were calculated on the basis of these relative size measurements (Table 1). Relative size data obtained for mitotic metaphase chromosomes compared well with those of pachytene chromosomes. Alternatively, absolute size measurements (μm) were conducted for pachytene chromosome 9 only (Table 1). Due to varying degree of condensation of mitotic metaphase chromosomes only relative size calculations were used (Table 1).

Discussion

In situ hybridization

At the pachytene stage in maize, pollen mother cells have ten bivalent chromosome complements. Meiotic chromosomes of maize have been well studied and show distinct cytological characteristics, thereby permitting easy identification (McClintock 1929; Rhoades 1950; Dempsey 1994). Therefore, chromosome 9 at the pachytene stage in maize genotype KYS was identified on the basis of some unique features. The chromosome has an arm ratio of 1.8 (long:short) (Dempsey 1994). This was easily recognized by applying multicolor FISH using a centromere-specific probe (CentC) (Fig. 1c, d). Furthermore, chromosome 9 displayed a prominent knob at the telomere of 9S (Fig. 1c, d). It was interesting to note that the intensity of the CentC signal was not equal for all chromosomes (data not shown).

The detection limit of FISH on chromosomes is variable. Several factors, including chromosome material and the FISH procedure applied, are highly influential. For human chromosomes, DNA probes as small as 0.5 kb have been successfully used in FISH (Jhanwar et al. 1983). In plants, probes as short as 4 kb could be used for FISH in maize (Jiang et al. 1996) and petunia (Ten Hoopen et al. 1996), while in rice (Ohmido et al. 1998), probes as small as 1.29 kb have been reported. Nevertheless, a typical molecular marker is too short (<1 kb) to generate a detectable signal by FISH. Therefore, homologous cosmid clones (approx. 35 kb) with RFLP markers *umc105a* (54.7 cM) and *csu145a* (105.4 cM) (Davis et al. 1999) were used for FISH on maize chromosome 9.

Cosmid *umc105a* showed consistently more intense signals and a larger labeled area than cosmid *csu145a* in both mitotic metaphase (Fig. la, b) and pachytene chromosomes (Fig. 1c, d). These observation could be explained by assuming that cosmid *umc105a* contained a larger proportion of single-copy sequences than cosmid *csu145a.*

The maize genome contains about 78% repetitive sequences (Flavell et al. 1974). To physically localize the cosmids, we had to suppress hybridization caused by re-

petitive sequences in these probes. Therefore, aliquots of labeled cosmid probes were re-annealed with an excess amount of C_o *t*-100 DNA before being hybridized to chromosomes. An 18–22-fold excess of C_o *t*-l00 DNA was necessary to reduce the unspecific fluorescent background sufficiently to obtain signals for the molecular markers. Different proportions of C_o *t*-1 DNA have been used for suppressing unspecific signals by CISS hybridization for different plant genomic clones; e.g., 20-fold for barley (Lapitan et al. 1997) or 100-fold for rice (Jiang et al. 1995). The amount of C_o *t*-1 DNA needed for CISS hybridization was found to be directly proportional to the amount of repetitive DNA sequences present in the probes (Zwick et al. 1997).

Comparison between physical and genetic maps

Based on the measurements of the lengths of the arms of pachytene chromosome 9, we calculated, a ratio of 1.76. This value is in close agreement with data obtained by others: 1.8 (Rhoades 1950), 1.89 (Gillies 1973), and 2.09 (Gillies 1981). For mitotic metaphase chromosome 9, we obtained a value of 1.4 (Table 1); Bennett and Laurie (1995) previously obtained a ratio of 1.49. The total length of pachytene chromosome 9 was measured to be 48.3 ± 8.5 μm. Variation in the absolute length of pachytene chromosome 9 is likely due to different degrees of condensation. In fact, highly diverging measurements have been reported with respect to the length of pachytene chromosome 9–43 μm (Rhoades 1950), 28.1 μm (Gillies 1973), and 32.1 μm (Gillies 1981). These variations depend on a number of parameters, i.e. genotype, pachytene chromosome substage, method of chromosome preparation, and microscopy (Gillies 1981). However, the close agreement of the arm ratio revealed that the absolute values did not influence the arm ratios and that whatever causes these variations acts uniformly within bivalents. The molecular size of pachytene chromosome 9 was estimated to be 216.3 Mb (see Methods). However, for mitotic metaphase chromosome 9 in cv. Seneca 60 of maize, the size was estimated to be 191 Mb (Bennett and Laurie 1995).

The physical map of chromosome 9 was compared to its genetic map based on relative sizes (Table 1) and relative distances (Table 2). Relative to the genetic map, molecular markers *umc105a* and *csu145a* were physically located to more distal positions of the short or long arms, respectively. Because these markers are not the most distal loci on the genetic map, it is likely that other genes or markers will be clustered within the most distal subtelomeric regions of this chromosome. This suggests that the recombination frequencies in distal subchromosomal regions might be higher, explaining the decreased Mb/cM ratios of these regions (Table 1). The estimated Mb/cM ratios were 0.64 or 0.66, respectively, for the distal subchromosomal regions 9S telomere to *umc105a* and *csu145a* to 9L telomere. In contrast, the ratios for the proximal inner subchromosomal regions *umc105a* to centromere and centromere to *csu145a* were 3.36 or 2.86, respectively.

The two RFLP markers *umc105a* and *csu145a* are linked to QTL for resistance against SCB (Bohn et al. 1996) and SWCB (Khairallah et al. 1998), respectively. The two markers flank the QTL and were mapped genetically to central positions (Tables 1 and 2). However, they were physically localized to distal positions (Fig. 1d; Tables 1 and 2). The genetic distance between these markers on chromosome 9 is 50.7 cM (Davis et al. 1999) and comprises 33.7% of the length of its genetic map. Surprisingly, the measured physical distance between these probes was larger, spanning about 70% of the physical map (Table 1). Using radioactive in situ hybridization, Shen et al. (1987) reported data showing a close correlation between physical position and the genetic map for the *Waxy* locus of chromosome 9 in maize. It seems likely that in maize there are also regions of high recombination that are randomly distributed along chromosomes. Recently, these were reported for barley with a recombination frequency of lower than 1.0 Mb/cM (Künzel et al. 2000). Furthermore, our data revealing high recombination frequencies in the distal subchromosomal regions (between 0.64 and 0.66 Mb/cM; Table 1) were comparable to those for the telomeric region for chromosome 5S in barley (1 Mb/cM), where the average value for the entire genome was 4 Mb/cM (Pedersen and Linde-Laursen 1995). In contrast, the proximal subchromosoml regions of maize revealed lower recombination frequencies with ratios of 3.36 and 2.86 Mb/cM (Table 1). In barley, subchromosomal regions with suppressed recombination have been recorded with ratio of greater than 4.4 Mb/cM (Künzel et al. 2000). It is interesting to note that a significant fluctuation in recombination frequencies may occur even within short stretches of DNA. Within a genetic interval of 0.09 cM, corresponding to about 140 kb in maize, recombination frequencies were found to vary by a factor of about seven. This suggests that genes might be located in regions where higher recombination frequencies were measured (Civardi et al. 1994).

Suppressed recombination frequencies in proximal chromosomal regions as opposed to more recombination events in distal regions have also been reported in several mapping projects. Markers and genes have been mapped physically at more distal locations on chromosomes as compared to their location on the genetic map for barley (Pedersen and Linde-Laursen 1995), tomato (Peterson et al. 1999; Zhong et al. 1999), maize (Jiang et al. 1996), and wheat (Kota et al. 1993; Hohmann et al. 1994). In barley, heterogeneous distribution of recombination rates along individual chromosomes has been recorded. Recombination was restricted to few small regions, with most of the regions with high recombination frequencies being located at more distal positions (Künzel et al. 2000). Likewise, areas with a high density of genes have been reported for wheat, representing 86% of group 1 markers and covering but 10% of the chromosome (Gill et al. 1996). Using FISH in petunia the central region of chromosome II showed at least a tenfold

suppression in recombination frequency (Ten Hoopen et al. 1996). Song and Gustafson (1995) showed that two rice RFLP markers covering a short genetic distance were physically distant or vice versa.

A change in the linear order of some markers has also been reported; for example, when using terminal deficiencies in mapping RFLP markers physically in maize (Lin et al. l997) and in applying FISH to spreads of tomato synaptonemal complex (Peterson et al. 1999). This inconsistency of the position of loci may be due to variations between studied genotypes and those used for construction of the genetic maps (Peterson et al. 1999). Differences between genetic and physical maps may well be due to errors in calculating genetic distances because genetic maps have been derived mainly from crosses involving distantly-related parents (Song and Gustafson 1995).

The disparity between the recombination frequencies observed along the length of chromosomes could be related to varying distributions of heterochromatin blocks along chromosomes (Gustafson and Dillé 1992). Heterochromatin has been shown to be widely distributed in the genome (Hennig 1999). In maize, heterochromatin contributes up to 25.2% of the total chromatin (Bashir et al. 1995). Furthermore, it was implicated in representing chromatin areas of inactive genes (Hennig 1999) as well as in having very few recombination nodules (Zhong et al. 1999). In these regions, housekeeping genes may be located with their spatial arrangement being pertinent for the fitness of the organism. Therefore, they might have evolved and concentrated into regions not commonly involved in recombination (Gustafson and Dillé 1992). Alternatively, recombination may take place evenly along the chromosome. Due to selection pressure, gametes with recombinant centromeric regions would not survive, as has been shown in barley (Künzel et al. 2001).

With respect to the isolation of genes, the feasibility of chromosome walking will be highly affected by the Mb/cM ratio (Civardi et al. 1994) and the prevalence of repetitive DNA sequences (Putterill et al. 1993). Therefore, a detailed knowledge of the physical localization of DNA sequences will be crucial. Likewise, they provide important hints concerning physical position of molecular markers linked to desired QTL, enabling their isolation (Alpert and Tanksley 1996). The data presented here located single-copy DNA sequences (*umc105a*, *csu145a*) physically, which were used as molecular markers to identify QTL conveying resistance against SCB (Bohn et al. 1996) and SWCB (Khairallah et al. 1998). The interval between these markers encompassed 70% of the physical length of chromosome 9. The physical locations of further DNA sequences have to be identified before attempting to isolate these QTL, for instance, by microdissection of chromosomes (Stein et al. 1998). Therefore, developing additional markers which are more closely linked to the resistance is of significant importance in cloning these genes. Comparisons between the genetic map and integrative physical map could elucidate mechanisms, frequency, and the distribution of recombination and rearrangement in defined regions along chromosomes.

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