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Recent progress in chromosome painting of *Arabidopsis* and related species

Martin A. Lysak, Ales Pecinka & Ingo Schubert*
Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany; Tel: (+49) 39482 5239; Fax: (+49) 39482 5137; E-mail: schubert@ipk-gatersleben.de
**Correspondence*

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Abstract

This paper reports on state-of-the-art achievements of chromosome painting in *Arabidopsis thaliana* ($2n = 10$). *Arabidopsis* chromosomes 1, 2 and 4 were painted using chromosome-specific BAC contigs. We consider technical aspects of the painting approach and document major applications, such as the tracing of *Arabidopsis* chromosomes as interphase chromosome territories and during mitotic and meiotic cell cycles as well as comparative chromosome painting in related species. This is the first report of successful interspecific chromosome painting in plants. The evolutionary history of chromosomes homeologous to *Arabidopsis* chromosome 4 was reconstructed by hybridization of chromosome-4-specific painting probes to karyotypes of *Brassicaceae* species with $x = 8$ chromosomes. Future perspectives of chromosome painting in *A. thaliana* and its wild relatives are outlined.

Introduction

The term chromosome painting (CP) was coined by Pinkel *et al.* (1988) to describe *in-situ* visualization of specific chromosomes or chromosome segments within chromosome complements by fluorescence *in-situ* hybridization (FISH). Although CP has brought forth dramatic progress in animal and human cytogenetics (for review see Ferguson-Smith 1997, Chowdhary & Raudsepp 2001), attempts to establish CP in euploid plants have failed. This is probably due to the large amounts of complex dispersed repeats that are homogeneously distributed over all chromosomes (Fuchs *et al.* 1996, Schwarzacher *et al.* 1997, Schubert *et al.* 2001).

The situation changed when *Arabidopsis thaliana* became a suitable subject for CP due to its small genome (~125 Mb/1C), its small amount of dispersed repetitive sequences (The Arabidopsis Genome Initiative 2000), and the public availability of BAC contigs covering nearly the entire chromosome complement (Scholl *et al.* 2000). The breakthrough was accomplished by taking advantage of high-resolution FISH on pachytene chromosomes (Fransz *et al.* 1998, 2000) and the application of BAC contig pools as probes. *Arabidopsis* chromosome 4 became the first entirely painted chromosome of a euploid plant karyotype (Lysak *et al.* 2001).

Here we present the recent progress of CP in *A. thaliana* and several related species. We show

CP of *Arabidopsis* chromosomes 1 (29.1 Mb), 2 (19.6 Mb) and 4 (17.5 Mb) and demonstrate major technical aspects and potential applications. Then, we focus on the first successful comparative CP in plants to search for chromosome homeology and to elucidate karyotype evolution within the *Brassicaceae* family.

Materials and methods

A. thaliana accessions Columbia (Col), C24 and Wassilewskija (WS) were used. The other seven analyzed species and their origin are listed in Table 1. Herbarium voucher specimens of these species are deposited at the IPK, Gatersleben.

Flower buds for chromosome spreads were harvested from plants in the field or from plants grown from collected seeds. Chromosome spreading technique, DNA isolation and labeling, FISH protocol and image processing were as described previously (Lysak *et al.* 2001) with the following modifications. For interspecific CP, the hybridization times have been prolonged up to 65 h and lower stringency conditions were applied for post-hybridization washing (20% formamide in $2 \times$ SSC). The BAC clones were provided by the Arabidopsis Biological Resource Center (ABRC, Columbus, OH). BACs included in the contigs for chromosome 4 were the same as listed in Lysak *et al.* (2001). The list of individual BACs of the contigs for chromosomes 1 and 2 may be requested from the authors.

The terminology for *Arabidopsis* chromosome arms follows that of The Arabidopsis Genome Initiative (2000). In the case of chromosomes 2 and 4, 'top arm' and 'bottom arm' are synonymous with short and long arm, respectively.

Results and discussion

Selection of BAC clones suitable for CP

Although the *Arabidopsis* genome consists of only ~10% repetitive DNA arrays (Leutwiler *et al.* 1984), the presence of dispersed DNA repeats within BAC clones has to be considered carefully when painting probes are arranged since such sequences could interfere with painting of individual chromosomes by cross-hybridization. In *Arabidopsis*, repeats are particularly abundant within the (peri)centromeric heterochromatin (chromocenters) and the nucleolus organizing regions (NORs). Therefore, BACs containing (peri-)centromeric or 45S rDNA sequences were omitted from painting probes. Although chromosome arms of most *Arabidopsis* accessions lack

Table 1. Taxa included in interspecific CP experiments.

Taxon	Ploidy level and chromosome number	Origin/donor
Arabidopsis lyrata (L.) O'Kane & Al-Shehbaz **subsp. *lyrata*** [=*Arabis lyrata* L., *Cardaminopsis lyrata* (L.) Hiitonen]	$2n = 2x = 16$	Bash-Bish, MA, USA; T. Mitchell-Olds
Arabidopsis halleri (L.) O'Kane & Al-Shehbaz [=*Cardaminopsis halleri* (L.) Hayek]	$2n = 2x = 16$	Západné Tatry Mts., Zuberec, Slovakia; leg. M. Lysak & A. Pecinka
Cardaminopsis carpatica Měsíček nom. prov.	$2n = 2x = 16$	Bešeňová, Slovakia; leg. M. Lysak & A. Pecinka
Crucihimalaya wallichii (Hook. f. & Thoms.) Al-Shehbaz, O'Kane & Price [=*Arabidopsis wallichii* (Hook. f. & Thoms.) Busch.]	$2n = 2x = 16$	Shurob, Uzbekistan; leg. M. Hoffmann
Arabis alpina L.	$2n = 2x = 16$	Belanské Tatry Mts., Tatranská Javorina, Slovakia; leg. M. Lysak & A. Pecinka
Capsella rubella Reuter	$2n = 2x = 16$	H. Hurka, Osnabrück, Germany
Capsella bursa-pastoris (L.) Med.	$2n = 4x = 32$	Gatersleben, Germany; leg. M. Lysak

larger blocks of repetitive DNA, visible microscopically as interstitial heterochromatin, mobile elements and their derivatives are scattered along euchromatic regions. Initial painting experiments with chromosome 4 have shown that the use of the complete set of all BACs from the tiling path results in cross-hybridization signals on other chromosomes in addition to painting of chromosome 4. We therefore analyzed each BAC clone for the presence of repetitive DNA sequences in the TIGR database (The Institute for Genomic Research, Rockville, MD; http://www.tigr.org/). BACs containing >5% mobile elements within annotated sequences were considered to be unsuitable for CP. Nevertheless, the presence of repeats in a BAC sequence does not necessarily hamper the specificity of CP and, conversely, some apparently suitable BAC clones may yield additional FISH signals at other regions. Thus, annotation analysis does not unambiguously indicate the suitability of BAC clones for CP. For further testing of the suitability of individual BACs for CP, these were spotted on filters and hybridized with radioactively labeled genomic DNA (Figure 1). BACs that revealed strong hybridization signals were excluded from painting probes.

Misaligned BAC clones as revealed by CP

Painting experiments with BAC clones which should belong to the top arm of chromosome 2 yielded signals at other positions than those expected. We have found, on the basis of FISH signals present elsewhere in the complement, that at least 14 BACs anchored on the map of the top arm of chromosome 2 were misaligned. These BAC clones were differently labeled and hybridized in pairs to pachytene chromosomes for determination of their correct location. Two major groups of misaligned BAC clones from the top arm of chromosome 2 were identified: (1) BACs giving a signal on the bottom arm of chromosome 2, and (2) BACs localized on another chromosome than

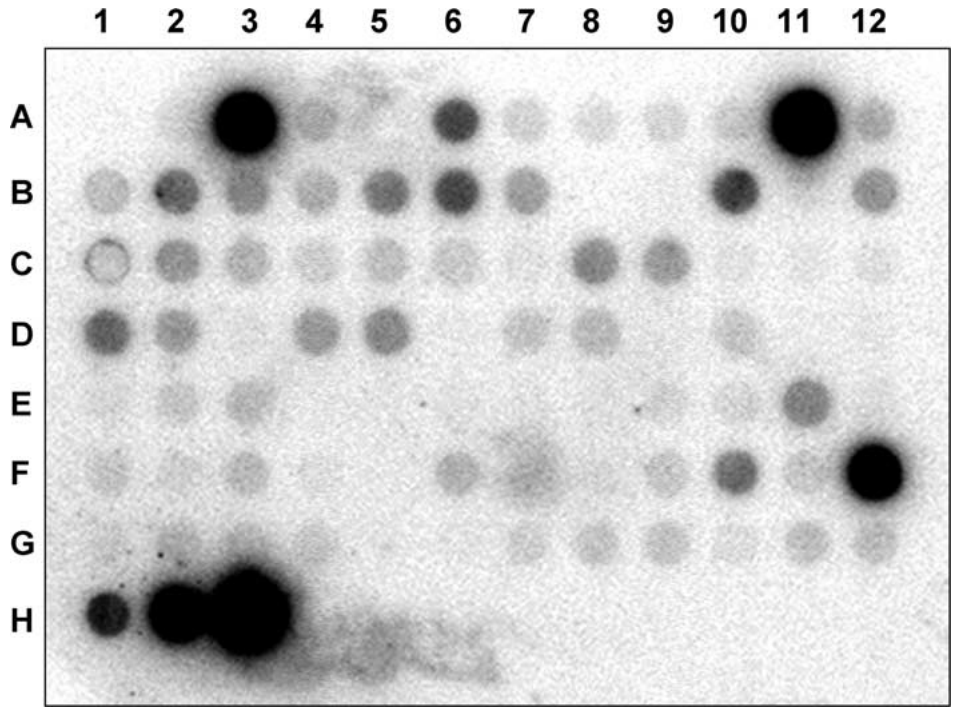
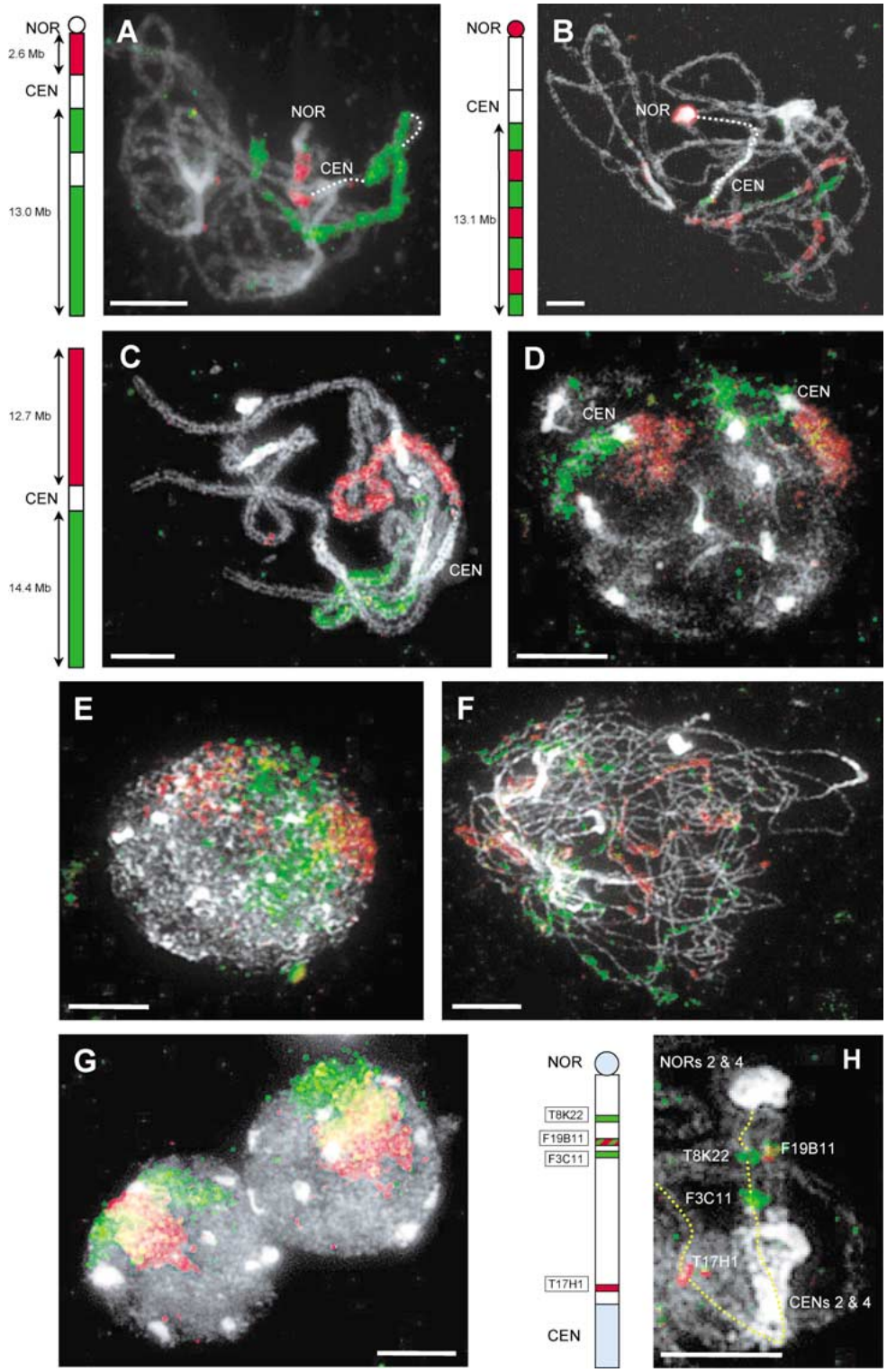


Figure 1. DNA–DNA dot-blot hybridization of *Arabidopsis* (Col) genomic DNA to 84 BAC clones aligned from A1 to G12 according to their physical position on *A. thaliana* chomosome 1 (A1 to B4: BAC T22A15 to T28N5, representing the top arm; B5 to G12: BAC F25O15 to T7N22 representing the bottom arm); H1 to H3: *Arabidopsis* (Col) genomic DNA (10, 100, 1000 ng); H11,12: water controls. Clones showing signal intensity ⩾C2 were omitted from the painting probe because their signal indicated the presence of repeated sequences.


NOR
2.6 Mb
CEN
13.0 Mb
A
NOR
CEN
NOR
CEN
13.1 Mb
B
NOR
CEN
12.7 Mb
CEN
14.4 Mb
C
CEN
D
CEN
CEN
E
F
G
NOR
T8K22
F19B11
F3C11
T17H1
CEN
NORs 2 & 4
H
T8K22
F19B11
F3C11
T17H1
CENs 2 & 4

chromosome 2 (Figure 2H). Thus, CP provides a tool to confirm identity and/or chromosomal location of individual BAC clones (see also Schubert *et al.* 2001). It might even be more effective and less time-consuming to verify the map position of a given BAC by CP on pachytene chromosomes than by PCR-based approaches.

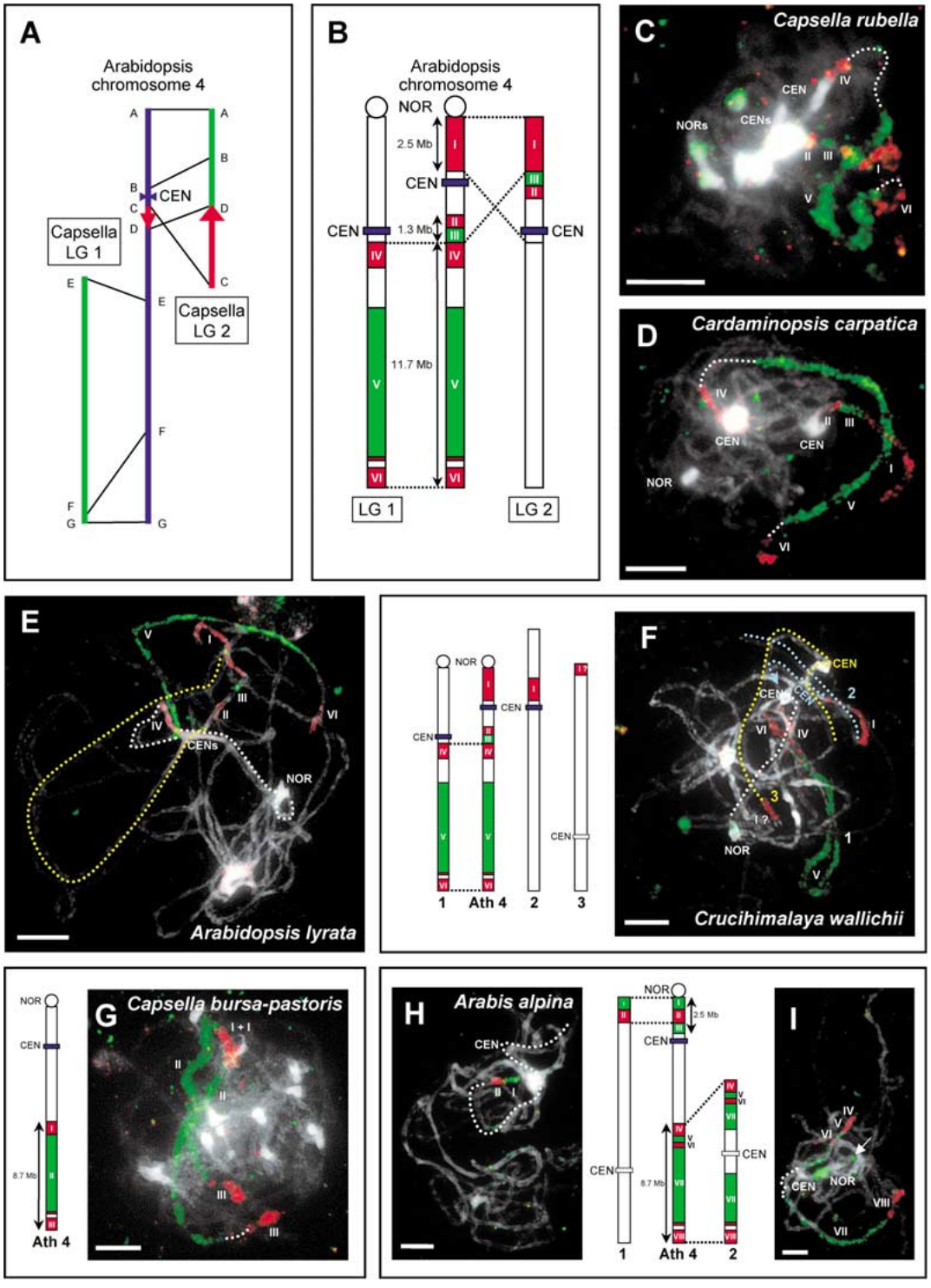
Tracing chromosome morphology and dynamics during mitotic and meiotic cell cycles

In *Arabidopsis*, CP allows us to trace mitotic and meiotic chromosomes as well as interphase chromosome territories (CTs) as previously exemplified for chromosome 4 (Figure 2A; Lysak *et al.* 2001). Here we extend these data by differential painting of chromosomes 1 and 2 (Figure 2B–G). Identification of individual chromosomes during meiosis had been difficult for a long time. CP now makes it possible to trace distinct chromosomes and/or specific chromosome regions throughout meiotic divisions. In pollen mother cells, chromosomes can be visualized as CTs (Figure 2E). Particularly, the course of homologous chromosome pairing can be established from early leptotene, when homologs start presynaptic alignment, to pachytene, when homologs are fully paired. Due to differential painting of chromosome regions, it is now possible to find out at which positions chromosomes initiate and complete pairing. The highest resolution of CP is achieved at leptotene when chromosomes form thin and extended threads. However, due to frequent tangles, unambiguous tracing of leptotene chromosomes is rather difficult (Figure 2F). Fully paired pachytene chromosomes, although clearly shorter, are therefore easier to analyze as shown for chromosomes 1, 2, and 4 (Figure 2A–C). Chromosomes can be traced also at later stages of meiosis, in spite of the decreasing possibility to assign hybridization signals to distinct BAC pools due to increasing chromatin condensation of meiotic and mitotic chromosomes (Figure 2D).

In interphase nuclei, individual chromosomes are discernible as CTs occupying discrete nuclear domains as known from mammalian chromosome painting studies (Cremer & Cremer 2001). FISH with differentially labeled single BACs or BAC pools revealed chromatin loops in *Arabidopsis* interphase nuclei (Fransz *et al.* 2002). Even within painted CTs, differently labeled BACs can be recognized (Lysak *et al.* 2001); however, contrary to the situation in pachytene nuclei, the sequential order of individual BACs or BAC pools cannot be assessed. Figure 2G shows *Arabidopsis* nuclei with the territories occupied by the top (red) and the bottom (green) arms of chromosome 1.

Because it is now feasible to explore in detail the spatial arrangement of CTs of individual chromosomes by CP in *Arabidopsis* nuclei, we can address the question of somatic pairing of homologs in nuclei of different tissues and developmental stages of this species. The association patterns of homologous CTs will be compared with those obtained from random-based computer simulations on the one hand and with the association frequency of FISH signals for individual BAC sequences from various positions along the chromosomes on the other. These will be the first data for a euploid plant that allow a comparison with corresponding data reported for vertebrates (Cremer *et al.* 2001, Habermann *et al.* 2001), insects (Hiraoka *et al.* 1993) and for alien

Figure 2. Chromosome painting of *A. thaliana* chromosomes 1, 2 and 4. (**A**) CP of chromosome 4 using a BAC contig spanning 2.6 Mb of the top arm (red) and two contigs (green) covering most of the bottom arm (13.0 Mb). (**B**) CP of the bottom arm of chromosome 2 with 7 differentially labeled pools of 145 BAC clones spanning 13.1 Mb. The distal NOR on the top arm is labeled by 45S rDNA (red). (**C–G**) CP of chromosome 1 with 183 chromosome-specific BAC clones. BACs from the top arm (12.7 Mb) were labeled by biotin-dUTP (red) and BACs from the bottom arm (14.4 Mb) by digoxigenin-dUTP (green). (**C**) pachytene, (**D**) mitotic prophase, (**E**) nucleus of a pollen mother cell, (**F**) leptotene, (**G**) interphase nuclei showing association of territories of both arms (right) or only of the top arms of chromosome 1 homologs (left). (**H**) *In-situ* localization of two misaligned BAC clones from the top arm of chromosome 2 on pachytene chromosomes. The scheme shows the expected positions deduced from the physical map of *Arabidopsis* chromosome 2 of four tested BAC clones (left); FISH localization of differentially labeled BACs (right). The correctly aligned BACs T8K22 and F3C11 (both in green) appear on the top arm of chromosome 2, the misaligned BACs F19B11 (green/red) and T17H1 (red) hybridize on the top arm of chromosome 4 (between the NOR and the heterochromatic knob which is visible proximally to the chromocenter) and the bottom arm of chromosome 2, respectively. Bars = 5 μm.


A
Arabidopsis chromosome 4
CEN
Capsella LG 1
Capsella LG 2
B
Arabidopsis chromosome 4
NOR
2.5 Mb
CEN
1.3 Mb
11.7 Mb
LG 1
LG 2
C
Capsella rubella
NORs
CENs
D
Cardaminopsis carpatica
E
Arabidopsis lyrata
CENs
NOR
F
Crucihimalaya wallichii
Ath 4
G
Capsella bursa-pastoris
8.7 Mb
H
Arabis alpina
I

chromosomes in plants painted by GISH (e.g. Schwarzacher *et al.* 1992, Aragón-Alcaide *et al.* 1997). The comparison with data obtained for *Drosophila* will be of special interest since this insect model species has roughly the same genome size as *Arabidopsis* and a significant frequency of somatic pairing of homologs has been described for that species (Hiraoka *et al.* 1993, Csink & Henikoff 1998).

Furthermore, CP on pachytene and possibly also on interphase nuclei will make it possible to detect spontaneous and experimentally induced chromosome rearrangements, such as translocations, inversions, duplications and deletions when suitable painting probes are applied.

Comparative CP in Brassicaceae

The availability of BAC contigs covering the *Arabidopsis* chromosomes offers a unique possibility for cross-mapping of individual BACs to pachytene chromosomes of other *Brassicaceae* by FISH (Jackson *et al.* 2000, Ziolkowski & Sadowski 2002) and also for revealing homeologous chromosomes or chromosome regions within closely related species by interspecific CP.

In spite of considerable morphological differences, *Capsella rubella* ($2n = 2x = 16$) was found to be closely related to *A. thaliana* by comparing rDNA sequences (Koch *et al.* 1999) and by genetic mapping of markers from *A. thaliana* to *C. rubella* (Acarkan *et al.* 2000, Schmidt *et al.* 2001). For instance, markers of *Arabidopsis* chromosome 4 display collinearity with two linkage groups in *C. rubella*. The larger part of the bottom arm belongs to one linkage group (LG 1), the top arm and a proximal part of the bottom arm (in inverted orientation) to another one (LG 2).

In order to identify the chromosomes corresponding to these linkage groups in *C. rubella*, we have designed a set of differentially labeled BAC contigs from *Arabidopsis* chromosome 4 (Figure 3B) according to the collinearity breaks within the genetic map of *C. rubella* (Acarkan *et al.* 2000, R. Schmidt personal communication). These BAC contigs were hybridized together to pachytene chromosomes of *C. rubella*, *Arabidopsis halleri*, *A. lyrata*, *Cardaminopsis carpatica* and *Crucihimalaya wallichii* (all $2n = 2x = 16$). The hybridization patterns obtained were similar for the analyzed species and in close accordance with the genetic map of *C. rubella*. Large parts of one bivalent were found to be labeled by probes for LG 1 and the short arm of another homeolog by the probes for LG 2 (Figure 3C–E). The continuous BAC contig identified on the first homeolog corresponds to 11.7 Mb of the bottom arm of *Arabidopsis* chromosome 4. The second homeolog was labeled by the contig corresponding to 3.8 Mb of chromosome 4 (2.5 Mb of the top arm, 1.3 Mb of the inverted region from the bottom arm).

In addition to diploid taxa, two tetraploid species were analyzed applying the same probe. Pachytene nuclei of *Arabidopsis arenosa* ($2n = 4x = 32$) revealed the same labeling pattern on two quadrivalents (see Comai *et al.*, this issue), indicating an autopolyploid origin of this species. Within the tetraploid species *Capsella bursa-pastoris* ($2n = 4x = 32$), the long arms of two

Figure 3. Interspecific painting of *A. thaliana* chromosome 4-specific probes to chromosome complements of related *Brassicaceae* species. (**A**) Scheme of *Arabidopsis* chromosome 4 and its correspondence to linkage groups LG 1 and LG 2 of *Capsella rubella*. (A–G) Positions of selected RFLP markers and EST sequences used for genetic mapping in *Capsella*. The inversion between markers C and D on the bottom arm of *Arabidopsis* chromosome 4 is shown by a red arrow. Modified after Schmidt *et al.* 2001. (**B**) Scheme of labeled BAC contigs of *Arabidopsis* chromosome 4 and of two homeologous chromosomes found in (**C**) *C. rubella*, (**D**) *Cardaminopsis carpatica* and (**E**) *Arabidopsis lyrata*. Red and green regions (I–VI) represent differentially labeled BAC contigs. (**F**) Pachytene of *Crucihimalaya wallichii* showing labeled homeologous chromosomes 1–3 after applying the same probe as shown in (**B**). The labeled part of homeolog 1 corresponds to LG 1 of *Capsella* (contigs IV–VI), homeolog 2 bears a large part of the top-arm contig I and homeolog 3 is labeled at terminal position due to a translocation involving the distal part of contig I. Contigs II and III could not be identified. (**G**) Pachytene nucleus of the tetraploid *Capsella bursa-pastoris* showing two bivalents labeled by bottom-arm contigs I–III. (**H** and **I**) Pachytene nuclei of *Arabis alpina* labeled by BAC contigs I and II from the top arm (**H**) and IV–VIII from the bottom arm (**I**) of *Arabidopsis* chromosome 4, respectively. A minor green signal (arrow) was observed additionally to the labeled bivalent in (**I**). The contig III was not detectable in *Arabis*. The unintentional labeling of NORs in (**C**), (**F**) and (**I**) (green) is likely due to sequences with similarity to intergenic spacers of rDNA within BACs from the corresponding species (Schubert *et al.* 2001). For correspondence and composition of the BAC contigs (roman numbers) see Table 2. Bars = 5 μm.

Table 2. BAC clones of *Arabidopsis* chromosome 4 delimiting the contigs used for comparative CP (see Figure 3). All BAC contigs comprise BAC clones listed in Lysak *et al.* 2001. Contigs V and VI in Figure 3B–F correspond to contigs I–III in Figure 3G and IV–VIII in Figure 3H, I; contig I in Figure 3B–F corresponds to contigs I–III in Figure 3H, I.

Contig	Border BAC clones	BAC accession numbers (EMBL)
C. rubella, C. carpatica, A. lyrata, C. wallichii (Figure 3B–F)		
I	F6N15–T1J1	AF069299–AF128393
II	T32A17–T25P22	AL161813–AL161831
III	F17A8–T22B4	AL049482–AL049876
IV	F25E4–F18A5	AL050399–AL035528
V	T6K21–F4B14	AL021889–AL031986
VI	T19K4–T5J17	AL022373–AL035708
***Capsella bursa-pastoris* (Figure 3G)**		
I	T6K21–T16H5	AL021889–AL024486
II	F18F4–F4B14	AL021637–AL031986
III	T19K4–T5J17	AL022373–AL035708
***Arabis alpina* (Figure 3H, I)**		
I	F6N15–T7B11	AF069299–AC007138
II	T10M13–T7M24	AF001308–AF077408
III	T25H8–T1J1	AF128394–AF128393
IV	T6K21–T16H5	AL021889–AL024486
V	F18F4–T13K14	AL021637–AL080282
VI	F7J7–T8O5	AL021960–AL021890
VII	F1N20–F4B14	AL022140–AL031986
VIII	T19K4–T5J17	AL022373–AL035708

bivalents were labeled with the BAC contig comprising a large part (8.7 Mb) of the bottom arm of *Arabidopsis* chromosome 4 and corresponding to LG 1 of *C. rubella* (Figure 3 G). The question of whether *C. bursa-pastoris* is an allotetraploid of *C. rubella* and *C. grandiflora* as postulated (Mummenhoff & Hurka 1990) or an autotetraploid of *C. rubella* could be answered only if the *Arabidopsis* chromosome-4 homeolog(s) potentially contributed by *C. grandiflora* yielded a painting pattern deviating from that of *C. rubella*.

In six tested species (*C. rubella*, *A. arenosa*, *A. halleri*, *A. lyrata*, *C. carpatica* and *C. wallichii*), the homeolog comprising the larger part of the bottom arm of *Arabidopsis* chromosome 4 is submetacentric with the long arm entirely labeled by the corresponding set of BACs. The NOR-bearing short arm of these chromosomes was free of signals. The second homeolog is most probably also submetacentric. Its short arm, not bearing a NOR, appeared to be entirely labeled by the probes covering the top arm (except the 45S rDNA) and the inverted proximal region of the bottom arm of *Arabidopsis* chromosome 4. The centromere positions on both painted homeologs are in favor of a pericentric inversion involving the proximal region of the bottom arm of chromosome 4 (Figure 3B). Only *C. wallichii* revealed an unlabeled terminal segment on the second homeolog and instead a signal at the terminal position on another bivalent (Figure 3 F), apparently due to a translocation involving this part of LG 2. Further experiments are needed to address the location of the inverted region in this species.

Phylogenetically, *Arabis alpina* ($2n = 2x = 16$) is more distantly related to *A. thaliana* than the other tested species (Koch *et al.* 1999, 2001). Since preliminary experiments applying the same BAC pools to *A. alpina* chromosomes as those used for the other diploid species resulted in a deviating hybridization pattern, we tested the identity of particular BAC pools by hybridizing two probes comprising a large part of the bottom arm (8.7 Mb) and the entire top arm (2.5 Mb) of *Arabidopsis* chromosome 4 separately to pachytene nuclei of *A. alpina*. The bottom-arm contig labeled in the preserved order of BAC sequences an entire apparently metacentric bivalent. The

central part of this chromosome showed an unlabeled DAPI-positive region, possibly representing the centromere, and a few minor gaps without a signal. Additionally, a small region of another chromosome became labeled by BACs of the middle part of the contig (Figure 3I, arrow). The probe corresponding to the top arm of chromosome 4 yielded a signal at the terminal position of a chromosome arm not bearing a NOR. However, a signal corresponding to the proximal part of this contig could not yet be observed (Figure 3H).

Taken together, three patterns of chromosome homeology to *Arabidopsis* chromosome 4 have been observed which are in agreement with the phylogenetic trees based on comparative analyses of rDNA and other gene sequences (Koch *et al.* 1999, 2001). The pattern obtained for *Capsella* (Figure 3B) was also found for the closest relatives of *Arabidopsis thaliana* with $x = 8$ such as *A. halleri*, *A. lyrata*, *A. arenosa* and *Cardaminopsis carpatica*. Therefore, the two identified homeologs are most likely identical to the precursors of chromosome 4 of *A. thaliana*. The additional translocation involving linkage group 2 within *C. wallichii* (Figure 3F) might reflect the separate lineage of *C. wallichii* within *Arabidopsis sensu lato* and its close relationship to the North American $x = 7$ *Arabis* and *Halimolobus* (Price *et al.* 1994, Koch *et al.* 1999). The pattern observed for *A. alpina* (Figure 3H, I) is more different with respect to the chromosome representing linkage group 1. This reflects the most distant phylogenetic position of *Arabis alpina* to *A. thaliana* among the analyzed taxa. Comparing *Arabis alpina* and *Arabidopsis thaliana* karyotypes, the presence of a centromere splitting LG 1 and the apparent absence of the proximal parts of LG 2 suggest additional structural rearrangements within the complement of *A. alpina*. Further testing of more species will decide whether the *A. alpina* pattern preceded that of *Capsella* or whether it represents an independent lineage of karyotype evolution.

Nevertheless, the presented data suggest that the combination of large parts of two ancestral chromosomes in *Arabidopsis* chromosome 4 (accompanied by a pericentric inversion) might have contributed to the reduction of the chromosome number from $x = 8$ to $x = 5$ during the evolution of *A. thaliana*. Further investigations including painting probes for the other *Arabidopsis* chromosomes, arranged according to the collinearity breaks within the *C. rubella* genome, should elucidate the fate of the remaining parts of the two ancestral linkage groups not present within chromosome 4 of *A. thaliana*. Interspecific CP using chromosome-specific BAC contigs of all *Arabidopsis* chromosomes to pachytene nuclei of species with 6, 7 or 8 chromosome pairs should allow detection of homeologs of *A. thaliana* chromosomes within these chromosome complements and to elucidate the sequence of events which have led to the evolutionary reduction of chromosome number eventually resulting in the karyotype of *A. thaliana*.

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