Behavior of Parental Genomes in the Hybrid Hordeum vulgare × H. bulbosum

K. Ananthawat-Jönsson, T. Schwarzacher, and J. S. Heslop-Harrison

In situ hybridization of labeled total genomic DNA with unlabeled blocking DNA enabled the parental origin of all chromosomes to be established in root tips of the mature sexual hybrid plant Hordeum vulgare × H. bulbosum. The parental genomes tended to remain spatially separated throughout the cell cycle, with the chromosomes of H. vulgare origin lying in a more central domain than those of H. bulbosum origin. During anaphase and telophase, chromosomes of H. bulbosum origin tended to lag. Although the chromatin usually separated, they did not have the V shape characteristic of anaphase chromatids. Aneuploid nuclei, missing H. bulbosum origin chromosomes, arose when the lagging chromatids were not incorporated into the daughter nuclei, although most cells remained diploid. Some interphase cells contained micronuclei, all of which were of H. bulbosum origin. Information about chromosome disposition and movement is important to enable the understanding of chromosome stability.

Interspecific hybrids between cereals and their wild relatives are important because they enable the transfer of genes from one species into another, and hence broaden the genetic base of cereal crops (e.g., Blan- co et al. 1986; Lapitan et al. 1986). In barley breeding, the cross Hordeum vulgare (cultivated barley) × H. bulbosum (a wild species) is used widely to produce haploid barley, because the H. bulbosum genome is eliminated in the first divisions of the zygote when some genotypes are used; the chromosome number in the haploid plant can then be doubled to produce a true-breeding variety in a single step (Kasha and Kao 1970). In crosses between genotypes such as those studied here, the H. bulbosum genome is usually retained, and the hybrid provides a good model for studying genome interactions and chromosome behavior.

The spatial positioning of chromosomes in mitotic metaphases has been studied in both spread preparations and reconstructions of hybrid plants (see Heslop-Harrison and Bennett 1990; Linde-Laursen and Jensen 1991). Metaphase studies per se are important because aberrations leading to aneuploidy occur during division, and both the three-dimensional position and identity of all chromosomes can be established in reconstructions (Schwarzacher et al. 1992). The study of chromosome disposition at interphase is required because chromosomes are active in gene expression and DNA replication (Jackson 1991) at this stage of the cell cycle, and thus any influence of position on chromosome activity would be important then.

In our present work, we aimed to understand aspects of the behavior of the two paternal genomes in the hybrid H. vulgare × H. bulbosum. First, we improved in situ hybridization methods to enable the identification of the parental origin of chromosomes in the hybrid between the two closely related species in the same taxonomic section of the genus (von Bothmer et al. 1991); second, we used the method to demonstrate where the chromosomes lay in the nuclei of the hybrid throughout the cell cycle; and third, we studied the physical behavior of the chromosomes, including their loss from the main nucleus during division.

Materials and Methods

We studied (1) H. vulgare L. cv. Tuleen 346 (barley; 2n = 2x = 14), (2) H. bulbosum L. clone L6 (2n = 2x = 14), and (3) the sexual F1 hybrid (2n = 2x = 14, code number C244 80/15) between the two species (kindly given by Dr. R. A. Finch). Ramets of the hybrid plant used here were maintained in growth cabinets and glasshouses for about 10 years before fixation. We transferred plants from soil to hydroponic culture for 3 to 4 days and used new root tips for experiments. Fresh leaves were collected from glasshouse-grown plants for DNA extraction.

For in situ hybridization, we pretreated root tips in ice water for 24 h, fixed them in ethanol: acetic acid (3:1), partially digested them with 2% cellulase (Calbiochem) and 20% pectinase (liquid from Aspergillus niger, Sigma) for 60–75 min at 37°C, and squashed them in 45% acetic acid onto glass slides, as described by Schwarzacher et al. (1989). We refixed the preparations on slides with 3:1 fixative for 10 min and dehydrated them through an ethanol series before treating slides with RNase (100 µg/ml) at 37°C for 1 h, washing in 2× SSC (0.3 M sodium chloride and 0.03 M sodium citrate), and dehydrating through an ethanol series again.

We used genomic in situ hybridization (Le and Armstrong 1991; Schwarzacher et
al. 1989), with blocking DNA (Anamthawat-Jönsson et al. 1990) and digoxigenin probe labeling and detection (Leitch et al. 1991). For use as a probe, we sheared total genomic DNA from *H. bulbosum* to give 3- to 10-kb fragments, and labeled them with digoxigenin-11-dUTP (Boehringer) using a standard nick-translation protocol. Blocking DNA was autoclaved genomic DNA from *H. vulgare* (15 psi, 5 min, to give DNA (fragments between 200 and 500 bp long). Our probe hybridization mixture (50 μl per slide) included 200- to 300-ng labeled probe, 20-40 times that amount of blocking DNA, 60% formamide, 2× SSC, 10% dextran sulfate, and 0.2% SDS (sodium dodecyl sulfate). We denatured the hybridization mixture at 70°C for 10 min, placed it on the slide with a plastic cover slip over it, and denatured the mixture and preparation in a humid chamber at 90°C for 10 min. After hybridization overnight at 37°C, we washed the slides in 50% formamide in 2× SSC at 40°C for 10 min and two or three times more in 2× SSC. These conditions allowed sequences with more than 80% homology to form stable hybrids. We detected sites of probe hybridization by incubating slides in sheep anti-digoxigenin conjugated to fluorescein (FITC, Boehringer, 20 μg ml⁻¹; 37°C, 1 h), washed in 4× SSC with 0.2% Tween 20, and amplified signal in rabbit anti-sheep secondary antibody conjugated to FITC (Dakopatts, 10 μg ml⁻¹). We countersigned DNA with propidium iodide (5 μg ml⁻¹) and examined the preparations by epifluorescence microscopy. Photographs were taken on Fujicolor 400 print film.

**Results**

Root tip chromosome spreads from the hybrid *H. vulgare* × *H. bulbosum* following in situ hybridization of labeled genomic DNA from *H. bulbosum* are shown in Figure 1. A hybridization signal of similar strength was detected as yellow-green fluorescence on all 14 chromosomes when no blocking DNA was used (Figure 1A). Some chromosomes showed unlabeled bands that were stained only with the orange-fluorescing propidium iodide counterstain. The addition of unlabeled blocking DNA from *H. vulgare* revealed seven chromosomes that showed a strong yellow-green in situ hybridization signal and seven with the orange counterstain predominant (Figure 1B,C). The chromosomes that originated from the *H. vulgare* cv. Tuleen 346 parent have morphologies unlike those of *H. bulbosum* (see Schwarzacher et al. 1992). Orange chromosomes that showed little labeling with *H. bulbosum* genomic DNA had the *H. vulgare* morphology, whereas chromosomes that were labeled strongly had morphologies that corresponded to chromosomes originating from the *H. bulbosum* genome.

In most metaphases, the two parental genomes were not intermixed but occupied spatially separated domains. For example, in Figure 1B, the genomes lie next to each other (side-by-side), while in Figure 1C the labeled chromosomes from *H. bulbosum* lie around the seven *H. vulgare* origin chromosomes. At other stages of the cell cycle (Figure 1D-H), genomic probing with blocking enabled labeled, *H. bulbosum* origin, and unlabeled, *H. vulgare* origin, chromatin or chromosomes to be distinguished clearly by yellow-green or orange fluorescence, respectively. At anaphase, the labeled *H. bulbosum*-origin chromosomes separated into chromatids, but many chromatids did not have the V shape characteristic of anaphase (Figure 1E). In a late anaphase (Figure 1F), the *H. vulgare*-origin chromosomes were at the spindle poles, while many *H. bulbosum*-origin chromosomes were lagging. At interphase, domains of labeled and unlabeled chromatin could be distinguished (Figure 1F). At prophase (Figure 1G,H), chromosomes were recognizable, with the yellow-green chromosomes of *H. bulbosum* origin tending to be spatially separated from the orange chromosomes of *H. vulgare* origin.

Some chromosome preparations had fewer than 14 chromosomes. Of these, 30 metaphases and prophase from five slides had a mean of 5.5 chromosomes of *H. bulbosum* origin and 6.7 chromosomes of *H. vulgare* origin. At prophase, where the nuclear envelope was still largely intact, chromosomes are unlikely to be lost during spreading; hence the presence of fewer than 14 chromosomes indicated that the nucleus was aneuploid as it entered division. In Figure 1G, one *H. bulbosum*-origin chromosome has been lost. In some metaphases, Figure 1C, a micronucleus of *H. bulbosum* origin was present. Thus cells that were aneuploid or contained micronuclei could enter division.

**Discussion**

**Genomic Probing**

In situ hybridization of labeled total genomic DNA from *H. bulbosum* without addition of competitive blocking DNA to metaphases of the hybrid *H. bulbosum* × *H. vulgare*, labeled all chromosomes (Figure 1A). Most chromosomes of *H. vulgare* origin (identified by their morphology) showed unlabeled bands, which presumably consisted of tandemly repeated sequences that were not represented in the labeled DNA probe from *H. bulbosum*. Paracentromeric and some intercalary C bands in *H. vulgare* cv. Tuleen 346 (Finch and Bennett 1982) correspond to the unlabeled bands. Many species have tandem repeats at the centromere, and these repeats tend to be divergent in sequence between closely related species (e.g., Maluszynska and Heslop-Harrison 1991) because of their rapid evolution. In *Hordeum*, too, the centromeric and noncentromeric tandem repeats have apparently evolved and diverged between the two related species, giving the unlabeled bands, whereas interspersed sequences show high levels of homology between the genomes.

At stages of the cell cycle other than metaphase, the parental origin of the chromatin could not be identified without addition of blocking DNA in chromosome spreads. When blocking DNA from *H. vulgare* was added to the hybridization mix, the parental origin of all chromosomes in the hybrid *H. vulgare* × *H. bulbosum* could be easily distinguished throughout the cell cycle (Figure 1B-H). The DNA sequences that are common to both species, and hence are blocked, presumably consist largely of interspersed and tandemly repeated sequences. About 5% of the *Hordeum* genome consists of one interspersed sequence family, BIS 1, which is dispersed uniformly along most of the chromosomes, excluding the paracentromeric, nucleolar organizing, and telomeric regions (Moore et al. 1991). Members of the BIS 1 sequence family presumably are represented by the hybridization signal detected in the unblocked chromosomes (Figure 1A), but are probably blocked in the other spreads (Figure 1B-H). The *H. bulbosum* genome must include a substantial proportion of essentially species-specific sequences since the chromosomes are uniformly labeled from telomere to telomere in the blocked spreads except for a slightly less labeled region at the centromere. The regions with strong cross-hybridization in Figure 1A are likely to include groups of genes that are conserved, but the ability of the blocking DNA to allow discrimination of the two species shows that the conserved sequences are interspersed with sequences essentially specific to one genome.
Nuclear Architecture
The micrographs of chromosomes following in situ hybridization show that the nucleus is not randomly organized, but that complete parental sets of chromosomes, detected by presence or absence of an in situ hybridization signal, occupy spatially separate domains not only at metaphase (Figure 1B,C) but throughout the cell cycle (Figure 1D–H). We had expected the result at metaphase based on knowledge from reconstructions (Schwarzacher et al. 1992): the metaphase in Figure 1B shows side-by-side parental genome separation, while Figure 1C shows the chromosomes of H. bulbosum origin surrounding the chromosomes of H. vulgare origin. Analysis of the intergeneric hybrid H. vulgare × Secale africanum has established that the impression of interphase genome separation (Figure 1) is not an artifact but is present in reconstructions of serially sectioned nuclei (Leitch AR et al. 1991). Therefore we can conclude that, in the intrageneric hybrid H. vulgare × H. bulbosum, parental genome separation is present at all stages of the cell cycle, and the results presented here are likely to represent the in vivo situation.

To examine nuclear architecture, knowledge of both the identity and position of all chromosomes is required. In hybrids between species that are closely related, in situ hybridization is the method of choice to identify each chromosome, and it is likely that multiple labeling systems (Leitch IJ et al. 1991) will be developed to allow identification of genomes in hybrids as well as of individual chromosomes. This will enable detailed studies of the higher order organization of individual chromosomes within the nucleus (Heslop-Harrison 1991).

Stability of the Hybrid and Chromosome Elimination
In many hybrids between cereals, including Hordeum species, a complete parental genome may be eliminated during early embryo development, so that embryo culture regularly recovers plants with a complete haploid genome from one parent and no chromosomes from the other parent (Kasha and Kao 1970). The degree of such instability varies within a given pair of species and depends on the genotypes involved (Simpson et al. 1980). For example, crosses between diploid H. vulgare and diploid H. bulbosum clone L1 gave almost 100% haploid and no diploid progeny, but H. vulgare × H. bulbosum clone L6 (used in our study) gave about 70% diploid hybrids in its progeny.

Chromosome counts in divisions indicated that many cells had remained diploid, with seven chromosomes from each parent (Figure 1A–C), over the 10 years since the hybrid was produced. In divisions, lagging chromosomes were often observed (Figure 1E). Such chromosomes might not be incorporated into daughter nuclei at telophase, and thus they give rise to micronuclei. The micronuclei may eventually degrade, but in some cases they remain until the next metaphase (Figure 1C); however, most aneuploid cells may not divide or cycle more slowly than euploid ones. Despite the numbers of aberrant nuclei, it is unlikely that the plant is becoming increasingly aneuploid since it has not become haploid over 10 years.

The H. bulbosum chromosomes may be unstable because they fail to initiate congression at metaphase or to migrate to the poles at anaphase. In mammalian cells, a similar differential behavior of chromosomes has been reported. In hamster-human cell fusion hybrids, Zelesco and Marshall Graves (1988) found that the human chromosomes were preferentially lost during division (segregant) and tended to be central within a ring of hamster chromosomes. The authors suggested that the human centromeres attached aberrantly, or simply less efficiently, to the spindle in hybrid cells. If so, then differential centromere function alone would be unlikely to cause the parental genome separation seen in both the plant and mammalian hybrids, since the less stable chromosome set is peripheral in one case and central in the other. In the human disease Roberts syndrome, Labs et al. (1991) reported that aneuploidy and micronuclei (in 5–11% of cells) arise as a direct result of lagging chromosomes, although all chromosomes congress onto the plate. Thus congression and anaphase movement seem to be independent events, although defects in microtubule attachment might be expected to affect both processes.

Maintenance of Genome Separation and Centromere Activity
Wide hybrids enable analysis of genome interactions and the genetical control of chromosome behavior. In the hybrid H. vulgare × H. bulbosum, the differences between the two genomes may enable the maintenance of genome separation throughout the cell cycle, along with differential expression and functioning of the two sets of centromeres. The activity of the centromeric structures, and the rate or timing of their becoming active, must be under genetic control since the two parental genomes show disparate behavior in the hybrid. However, the lagging of one parental set of chromosomes is not the only mechanism for maintaining genome separation, since the hybrid between H. vulgare and S. africanum shows genome separation but the chromosomes segregate together (Leitch AR et al. 1991). Comparative, timed studies of chromosome behavior in different hybrids and quantitative measures of centromere activity will advance understanding of the control of chromosome elimination and may lead to more general conclusions about centromere activity and chromosome movement.

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References
Genetics of Skin Color, Flowering Group, and Anise Scent in Avocado

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The genetic information available on subtropical fruit trees in general, and on avocado in particular, is quite limited. The genetics of skin color, flowering group, and anise scent in avocado (Persea americana Mill) have been studied. Progeny distribution of seedlings originating from crosses between all possible phenotypes in the above-mentioned three traits have been presented. The results rule out a model of one or two loci for any of these traits. It is quite probable that these traits are coded by several loci having several alleles. 

The parent and progeny plots were located at the Akko Experiment Station in Israel. We collected seed from crosses and selfings by caging trees under a net, using bees as the pollen vector (Lavi et al. 1991). The harvested seed was sown in a nursery, and one year later we transplanted the seedlings into breeding plots. The progeny of each cage were planted randomly in one block. Progenies of different cages were randomized in the orchard. The juvenile period was shortened by the use of autumn girdling (Lahav et al. 1986).

We recorded fruit skin color, flowering group, and leaf anise scent traits over a 2-year period. Skin color was classified as green or purple, flowering group as A or B, and leaf anise scent as present or absent. The cultivars used were Anaheim, Fuerte, Irving, Nabal, Regina, Rincon, Wurtz (Rounds 1950), Ettinger (Storey and Bergh 1963), Hass (Griswold 1945), Pinkerton, Reed (Platt 1976), Rosh-Hanikra II (Lavi et al. 1991), Horshim, and Tova (Slor and Spodheim 1971–1972).

To distinguish between hybrids and self-pollinated seedlings, we characterized the progeny by isozyme analyses of leaf tissue (Degani and Gazit 1984). However we cannot rule out the possibility that a few individuals were wrongly classified.

The number of observations in the various crosses varied from four to 387, for a total of 1,688 seedlings. Significance was determined by the chi-square tests.

### Results

#### Fruit Skin Color

There were nine selfing crosses of the green x green type. The ratio of green/purple among the progeny varied from 3-20 to one, (3-20:1) and on the average was 8.6. There were also 19 crosses between various cultivars with green skin color. The ratio of green/purple among these progeny varied from 3-20 to one, (3-20:1) and on the average was 13.6. No significant differences were found between the two ratios (8.6 and 13.6) (Table 1).

There was only one cross of green x purple, resulting in 10 green and four purple progeny. There were three crosses of the purple x green type, resulting in a progeny distribution ranging from 1.1 to 1.2 (Table 1).

#### Materials and Methods

The parent and progeny plots were located at the Akko Experiment Station in Israel. We collected seed from crosses and selfings by caging trees under a net, using bees as the pollen vector (Lavi et al. 1991). The harvested seed was sown in a nursery, and one year later we transplanted the seedlings into breeding plots. The progeny of each cage were planted randomly in one block. Progenies of different cages were randomized in the orchard. The juvenile period was shortened by the use of autumn girdling (Lahav et al. 1986).

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#### Table 1. Progeny distribution in the fruit skin color trait

<table>
<thead>
<tr>
<th>Parent phenotype</th>
<th>Green</th>
<th>Purple</th>
<th>No. of families</th>
<th>Green/purple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green x green (selfings)</td>
<td>121</td>
<td>14</td>
<td>9</td>
<td>8.6</td>
</tr>
<tr>
<td>Green x green (crosses between cultivars)</td>
<td>273</td>
<td>36</td>
<td>19</td>
<td>13.6</td>
</tr>
<tr>
<td>Green x green (total)</td>
<td>394</td>
<td>46</td>
<td>28</td>
<td>11.6</td>
</tr>
<tr>
<td>Green x purple</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Purple x green</td>
<td>71</td>
<td>48</td>
<td>13</td>
<td>1.5</td>
</tr>
<tr>
<td>Purple x purple (selfings)</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Total</td>
<td>480</td>
<td>90</td>
<td>33</td>
<td>5.3</td>
</tr>
<tr>
<td>Reciprocal crosses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green x green (Ettinger x Tova)</td>
<td>37</td>
<td>1</td>
<td>1</td>
<td>37.0</td>
</tr>
<tr>
<td>Green x green (Tova x Ettinger)</td>
<td>102</td>
<td>3</td>
<td>1</td>
<td>34.0</td>
</tr>
<tr>
<td>Green x purple (Ettinger x Rosh-Hanikra II)</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Purple x green (Rosh-Hanikra II x Ettinger)</td>
<td>13</td>
<td>12</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### References

Bergh (1975) reported several studies aimed at understanding the genetics of several traits. Skin color was concluded to be inherited as a typical polygenic character, and flowering group was affected by segregation at a number of loci. Data concerning the genetics of leaf anise scent, skin color, or flowering group traits were not reported. This article reports the genetics of these traits.