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ORIGINAL PAPER

# Comparison of linkage maps from $F_2$ and three times intermated generations in two populations of European flint maize (*Zea mays* L.)

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**Abstract** Intermated mapping populations are expected to result in high mapping resolution for tightly linked loci. The objectives of our study were to (1) investigate the consequences of constructing linkage maps from intermated populations using mapping methods developed for $F_2$ populations, (2) compare linkage maps constructed from intermated populations ($F_2$Syn3) with maps generated from corresponding $F_2$ and $F_3$ base populations, and (3) investigate the advantages of intermated mapping populations for applications in plant breeding programs. We constructed linkage maps for two European flint maize populations (A × B, C × D) by mapping 105 SSR markers in generations $F_2$ and $F_2$Syn3 of population A × B, and 102 SSR markers in generations $F_3$ and $F_2$Syn3 of population C × D. Maps for $F_2$Syn3 were constructed with mapping methods for $F_2$ populations (Map A) as well as with those specifically developed for intermated populations (Map B). Both methods relate map distances to recombination frequencies in a single meiosis and, therefore, did not show a map expansion in $F_2$Syn3 compared with maps constructed from the respective $F_2$ or $F_3$ base populations. Map A and B differed considerably, presumably because of theoretical shortcomings of Map A. Since loosely linked markers could not unambiguously be mapped in the $F_2$Syn3 populations, they may hamper the construction of linkage maps from intermated populations.

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## Introduction

Genetic linkage maps are an important tool in genetic research and applied breeding programs. A linkage map attempts to reproduce the linear order of gene and/or marker loci on a chromosome and to quantify the degree of linkage between loci by their distance on the map. In construction of genetic maps for diploid species, linkage between loci is most commonly estimated from segregating populations obtained from biparental crosses of homozygous parents. The types of mapping populations differ in the number of meioses that the homologous chromosomes undergo and, in consequence, in the probability distribution of crossover events between adjacent loci on a homologous chromosome. To account for different number of meioses, the convention is employed that the genetic map distance between loci refers to the distribution of crossover events between these loci in a single meiosis (Haldane 1919; Kosambi 1944; Stam 1993; Weir 1996, p. 230).

For mapping tightly linked loci, it is advantageous to employ mapping populations where the individuals have undergone several meioses (cf. Allard 1956). This assures a sufficient frequency of individuals with recombination between tightly linked loci, resulting in a small standard error $\sigma_r$ of the estimated recombination frequency $r$ per meiosis. $F_2$ populations intermated randomly for $t$ generations were suggested as mapping populations in maize and *Arabidopsis thaliana* to

increase mapping accuracy of tightly linked loci (Beavis et al. 1992; Liu et al. 1996; Lee et al. 2002). The amount of information $i_r$ provided by a single individual of a mapping population can be used to compare the efficiency of mapping experiments (Mather 1936). Liu et al. (1996) derived $i_r$ for intermated $F_2$ populations and indicated situations where the amount of information from an individual in an intermated population was greater than in the corresponding $F_2$ population.

Construction of a linkage map involves four steps: (1) the genotypes of the individuals in a mapping population are assessed, (2) pairs of putatively linked loci are assigned to linkage groups, (3) the loci of a linkage group are ordered, (4) the map distance between loci is estimated. The well-established mapping theory for $F_2$, backcross, doubled haploid, or recombinant inbred line mapping populations is available and implemented in software (Lander et al. 1987; Holloway and Knapp 1993; Stam 1993, p. 305; Liu 1998; Van Ooijen and Voorrips 2001). However, theory and software for linkage mapping with intermated populations, have not been developed yet. As a consequence, studies on linkage mapping with intermated populations (Beavis et al. 1992; Liu et al. 1996; Lee et al. 2002) have so far employed mapping methods developed for $F_2$ populations. This approach yields in estimated recombination frequencies $R$ that result from the accumulated effect of all meioses a population underwent. The relation between $R$ and the recombination frequency $r$ that refers to a single meiosis is (Darvasi and Soller 1995; Liu et al. 1996)

$$R = \frac{1}{2}\left(1 - (1-2r)(1-r)^t\right), \quad (1)$$

where $t$ refers to the number of intermating generations.

The objectives of our study were to (1) investigate the consequences of generating linkage maps from intermated populations by using mapping methods developed for $F_2$ populations, (2) compare linkage maps generated from intermated populations with maps generated from the corresponding $F_2$ and $F_3$ base populations, and (3) investigate the advantages and disadvantages of intermated mapping populations for applications in plant breeding programs.

## Materials and methods

### Plant materials

The plant materials used in this study were partly identical to those employed in previous QTL studies on grain traits (Schön et al. 1994; Melchinger et al. 1998; Utz et al. 2000; Mihaljevic et al. 2005) and forage traits (Lübberstedt et al. 1997) in maize. Briefly, four early maturing European flint inbred lines KW1265, D146, D145, and KW1292, further referred to as A, B, C, and D, respectively, were used as parents. Randomly chosen $F_2$ plants were selfed to produce 380 $F_3$ lines of cross A × B, and 140 $F_4$ lines of cross C × D. Additionally, with each $F_2$ population three generations of intermating were performed by chain crossing of 240 unselected plants (i.e., 1 × 2, 2 × 3, ..., 240 × 1) to produce generation $F_2$Syn3. In the present study, only random subsets of 146 $F_3$ lines of cross A × B, 110 $F_4$ lines of cross C × D, and 148 plants of the $F_2$Syn3 generation of each cross were analyzed.

### Leaf collection and DNA extraction

Leaves were harvested from 10 to 20 plants of each $F_3$ line of cross A × B and each $F_4$ line of cross C × D. Equal amounts of leaf material from each plant per line were bulked for DNA extraction to determine the marker genotypes of the parental $F_2$ and $F_3$ plants. In the intermated generations, leaves were harvested from individual plants. Harvested leaves were freeze-dried and ground to powder. DNA extraction was performed according to the CTAB method (Hoisington et al. 1994).

### SSR analyses

Parental lines A, B, C, and D were screened with 860 public SSR markers from the MaizeGDB (http://www.maizegdb.org/ssr.php). Out of the 319 (A × B) and 354 (C × D) polymorphic markers per cross, we assayed 105 and 102 SSRs with a uniform distribution over the maize genome. Primer pairs were synthesized by Sigma–Genosys (Steinheim, Germany), with one primer of each pair being tagged at the 5′ end with a fluorescent label (Indodicarbocyanine (Cy5) phosphoramidite). The PCR reactions were conducted in a volume of 15 µl containing 25 ng template DNA, 0.15 mM of each dNTP, 2.5 mM $MgCl_2$, 0.25 µM of each primer, 1× *Taq* DNA polymerase buffer, and 0.5 U of *Taq* DNA polymerase (Invitrogen GmbH, Karlsruhe, Germany). Amplifications were performed using a Primus HT thermal cycler (MWG BIOTECH, Ebersberg, Germany). The PCR cycling conditions that yielded the strongest amplification product were considered optimum and used for analyses. The resulting PCR products were separated by using polyacrylamid gels (ultra pure SequaGel-XR, National Diagnostics, Atlanta, GA) run on an ALF Express

(Amersham Pharmacia Biotech, Freiburg, Germany) automated sequencer and transferred to a 1/0 matrix.

### Linkage analyses

Observed genotype frequencies at each marker locus were checked for deviations from Mendelian segregation ratios (1:2:1 in $F_2$ or $F_2$Syn3, and 3:2:3 in $F_3$ populations) and allele frequency of 0.5 by $\chi^2$ tests, with adjustment for multiple tests according to Sidak with an experiment wise error of $\alpha = 0.05$.

We assumed no interference in crossover formation, such that the relationship between the map distance $d$ and the recombination frequency $r$ is described by Haldane's (1919) mapping function:

$$r = \frac{1}{2}\left(1 - e^{-2d}\right), \tag{2}$$

where $d$ denotes here the map distance in Morgan units.

For population $F_2$ of cross A × B and for population $F_3$ of cross C × D, linkage maps were constructed with the algorithm described by Stam (1993), employing software JoinMap Version 3.0 (Van Ooijen and Voorrips 2001).

Briefly, the underlying computational steps were: (1) pair wise recombination frequencies $r$ for all locus pairs were estimated from the observed data using maximum likelihood (Fisher 1946; Bailey 1961 p. 38; Stam 1993), and the corresponding map distances $d_o$ were calculated using Haldane's mapping function, (2) loci were assigned to linkage groups and ordered, (3) a least squares procedure was used to estimate the locus distances $d_m$ on the final linkage map by minimizing

$$\sum_{\text{locus pairs}} w(d_o - d_m)^2 \rightarrow \min, \tag{3}$$

where $w$ are weights obtained from a test of linkage between pair of loci.

For both $F_2$Syn3 populations, linkage maps were generated with the method employed in earlier studies on mapping with intermated populations (Beavis et al. 1992; Liu et al. 1996; Lee et al. 2002). This approach consists of the following steps: (1) the maximum likelihood equations for estimating recombination frequencies in $F_2$ populations are applied to intermated populations, (2) recombination frequencies between adjacent loci on the resulting map are interpreted as the recombination frequencies $R$, which refer to all meioses events during intermating, (3) Eq. 1 is employed to derive the recombination frequencies $r$ that refer to one single meiosis from the values of $R$. Map distances $d$ between adjacent loci on the final map were calculated using $r$. We refer to the resulting linkage map as Map A.

For both intermated populations, a second linkage map (further referred to as Map B) was constructed, using the approach described for $F_2$ and $F_3$ populations. For estimating pair wise recombination frequencies, the expected phenotype frequencies in the intermated populations are required. The expected two-locus phenotype frequencies in an intermated population can be determined as follows: (1) Eq. 1 yields directly the frequencies of recombinant gametes, (2) due to symmetry reasons, the frequency of recombinant gametes can be used to calculate the frequency of all four possible gametes, (3) due to random mating, the frequencies of the 16 possible genotypes can be obtained by multiplying the corresponding gamete frequencies, (4) the phenotype frequencies are obtained from the genotype frequencies by summing up genotype classes resulting in the same phenotype. Calculations were performed with an extension of software JoinMap Version 3.0 (Van Ooijen, unpublished).

For all linkage maps we used the locus orders published in MaizeGDB (http://www.maizegdb.org). We employed the ''fixed order'' command of JoinMap Version 3.0 using an LOD threshold of 0.01 (partially 0.001) and a recombination threshold of 0.499 for marker pairs. The low stringency mapping procedure was chosen to construct maps with as many markers as possible. For comparing the two mapping methods and the two populations, the maps in both crosses included only SSR markers that could be mapped in both populations.

## Results

Significant ($P < 0.05$) deviations from the expected segregation ratios (1:2:1 and 3:2:3) were observed in zero (A × B, $F_2$), 11 (C × D, $F_3$), and six cases (A × B, $F_2$Syn3; C × D, $F_2$Syn3). Allele frequencies deviated significantly ($P < 0.05$) from 0.5 at none (A × B, $F_2$ and $F_2$Syn3), four (C × D, $F_3$), and two (C × D, $F_2$Syn3) marker loci. In population $F_2$ of cross A × B and $F_3$ of cross C × D, the total map distances covered by all SSR markers spanned 1,803 and 1,608 cM, with an average interval length of 19 and 17 cM, respectively (Figs. 1, 2). In population $F_2$Syn3 of cross A × B, only 89% (Map A) and 92% (Map B) of all SSRs could be mapped. In the same population of cross C × D,

94% (Map A) and 93% (Map B) of all SSRs could be mapped. The total map distances of cross A × B spanned 1,371 cM in Map A and 1,518 cM in Map B, with an average interval length of 17 cM (Fig. 1). The maps obtained for cross C × D spanned 1,336 cM in Map A and 1,406 cM in Map B, with an average interval length of 16 and 17 cM, respectively (Fig. 2).

In cross A × B, the number of SSRs that could be mapped in population $F_2$Syn3 was lower for Map A (93) than for Map B (97) (Table 1). The 93 common loci defined 83 intervals on Map A and Map B. Although at 84% of the intervals the estimated *d* values were smaller with Map A than with Map B, we could observe major differences (> 10 cM) only at four intervals. Differences between the two mapping methods became evident with increasing marker intervals (Table 2). Correlations $\rho$ of estimated *d* values between $F_2$Syn3 and the corresponding $F_2$ varied widely among chromosomes, ranging from 0.02 at chromosome 3 to 0.90 at chromosome 4 (Map A), and from –0.55 at chromosome 5 to 0.91 at chromosome 4 (Map B). The average *d* ratios between population $F_2$Syn3 and $F_2$ were smaller for Map A (0.86) than for Map B (0.91). Comparing population $F_2$Syn3 with the corresponding $F_2$ of cross A × B, we observed a shrinkage of the total map length of 0.86 using Map B (Table 1). The shrinkage amounted 0.56 on chromosome 5, while a 1.32-fold expansion was observed on chromosome 10. The 97 common loci defined 87 intervals on the maps of population $F_2$ and $F_2$Syn3 (Map B). For $F_2$, estimates of *d* were larger (> 10 cM) at 12 and for $F_2$Syn3 at nine intervals.

In population $F_2$Syn3 of cross C × D, the linkage map of Map A was smaller than that of Map B, although almost the same number of SSRs was mapped (Table 1). At 16% of the 83 common intervals, estimated *d* values were larger in Map A than in Map B, but differences greater than 10 cM were observed only for three intervals. Differences between Map A and B became apparent with increasing marker intervals (Table 2). Significant ($P < 0.01$) correlations between estimates of *d* from the $F_2$Syn3 and the corresponding $F_3$ were observed at chromosomes 2, 3, 5, and 10 of Map B, and only at chromosomes 2, 3, and 10 of Map A. The *d* ratio averaged 0.92 (Map A) and 0.96 (Map B). We observed a marginal shrinkage of the total map length (0.95) between population $F_2$Syn3 and the corresponding $F_3$ with Map B, ranging from 0.65 at chromosome 4 to 1.20 at chromosome 10. Of 85 common marker intervals, 12% showed larger map distances *d* (> 10 cM) for the base population and 7% larger *d* values for $F_2$Syn3.

## Discussion

### Construction of linkage maps

Earlier studies on linkage mapping with intermated populations (Beavis et al. 1992; Liu et al. 1996; Lee et al. 2002) employed mapping methods developed for $F_2$ populations, similar to our linkage map construction in Map A. From a theoretical point of view, this method is questionable because construction of multilocus linkage maps includes minimizing or maximizing a target function, which fits the estimated map to the observed data. In the approach of Stam (1993), the sum of squared deviations of the estimated map distances from the observed map distances is minimized. In the maximum likelihood approach (Lander et al. 1987) for estimation of map distances, the likelihood for the observed data is maximized. In general, the extremes of the sum or product of a set of functions are not equal to the extremes of the sum or product after nonlinear transformations of the original functions. Therefore, the map that fits best the values *R* is not guaranteed to fit best the nonlinear transformations *r*. In consequence, linkage maps constructed with this approach should be regarded as an approximation of the maps constructed with an approach where accounting for the number of meioses is performed before minimization or maximization step, such as in Map B.

In our experimental data, the differences between the two mapping methods (Map A and B) became apparent with increasing marker intervals (Table 2), and in particular, four (A × B) and three (C × D) marker intervals differed by more than 10 cM (Figs. 1, 2). Moreover, the chromosome lengths differed considerably (~ 10–30 cM), as did the genome length. The reason for these differences is presumably the theoretical shortcoming of Map A. Hence, for obtaining precise linkage maps, it seems prudent to use algorithms that correct for the number of meioses before finding extremes of the underlying target function.

### Map expansion

When compared with linkage maps constructed from $F_2$ populations, genetic maps constructed with intermated populations or populations of recombinant inbred lines developed from intermated populations were reported to show a map expansion (Beavis et al. 1992; Liu et al. 1996; Lee et al. 2002; Winkler et al. 2003; Teuscher et al. 2005). The maps in these studies were constructed by employing methods developed for $F_2$ populations to data from intermated populations. In

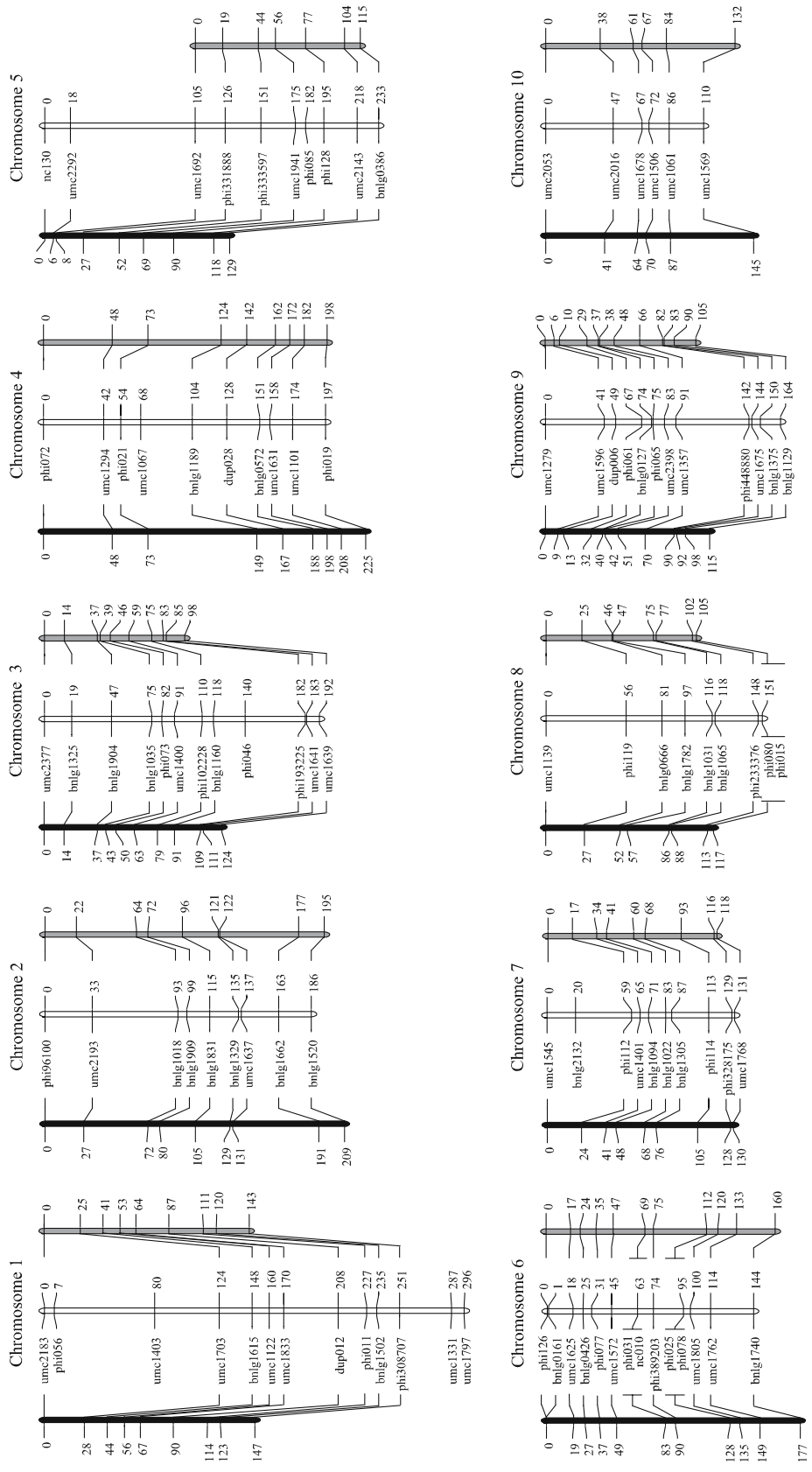


**Fig. 1** Comparison of linkage maps of population $F_2$ (*white*) with population $F_2$Syn3 Map A (*grey*) and population $F_2$Syn3 Map B (*black*) of cross A × B

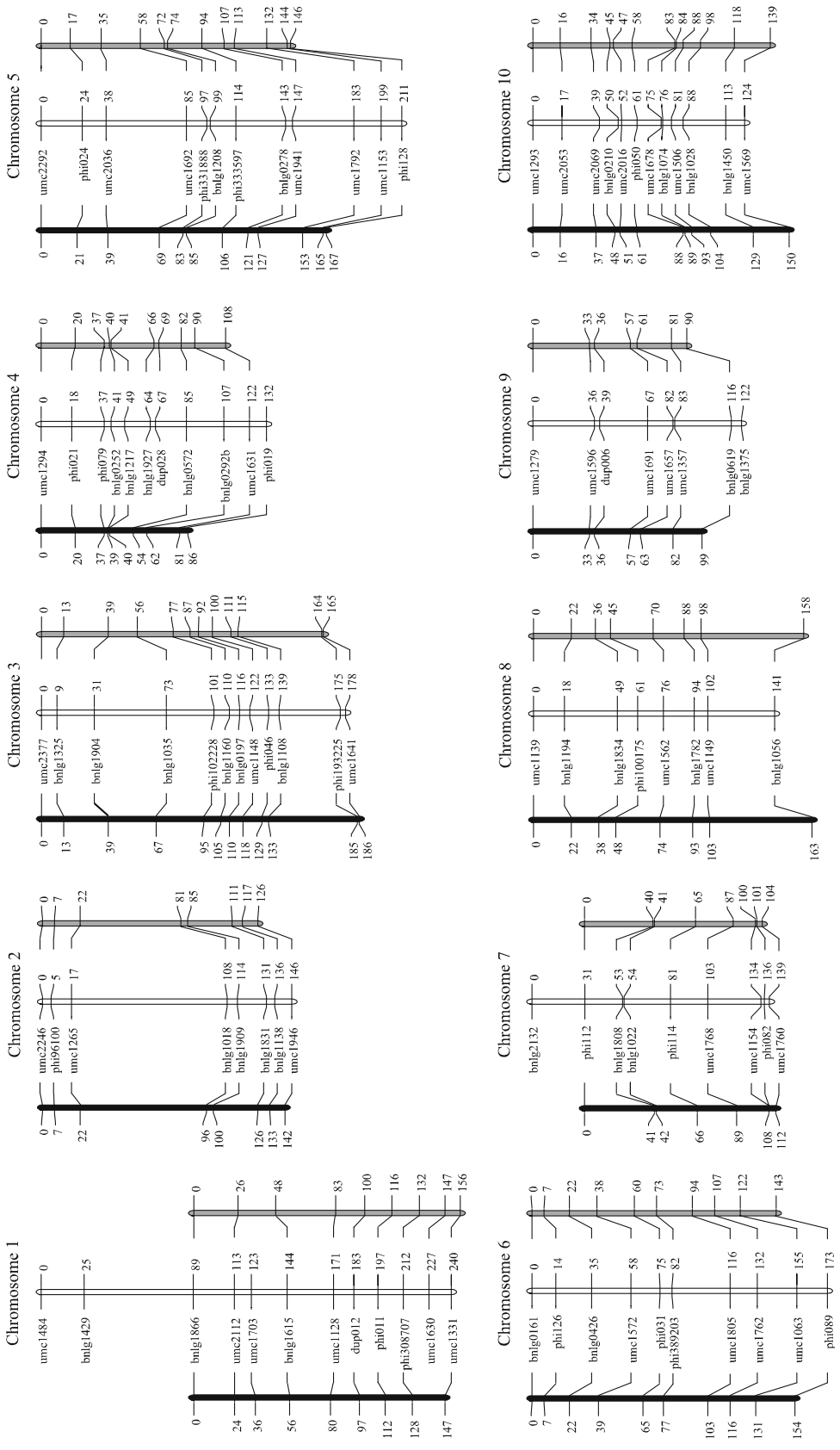


**Fig. 2** Comparison of linkage maps of population $F_3$ (*white*) with population $F_2$Syn3 Map A (*grey*) and population $F_2$Syn3 Map B (*black*) of cross C × D

**Table 1** Map distances ($d$ in cM), correlation coefficients ($\rho$) and ratios of $d$ between population $F_2$Syn3 and $F_2$ ($F_3$) of cross A × B and C × D

| | Chromosomes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total[a] Mean[b] |
| Cross A × B | | | | | | | | | | | |
| Population $F_2$ | | | | | | | | | | | |
| No. of loci | 13 | 9 | 12 | 10 | 10 | 14 | 10 | 9 | 12 | 6 | 105[a] |
| $d$ | 296 | 186 | 192 | 197 | 233 | 144 | 131 | 151 | 164 | 110 | 1803[a] |
| Comparison of population $F_2$ vs. $F_2$Syn3 with Map A | | | | | | | | | | | |
| No. of loci | 9 | 9 | 10 | 9 | 7 | 13 | 9 | 9 | 12 | 6 | 93[a] |
| $d$ | | | | | | | | | | | |
| $F_2$ | 251 | 186 | 192 | 197 | 128 | 144 | 131 | 151 | 164 | 110 | 1653[a] |
| $F_2$Syn3 | 143 | 195 | 98 | 198 | 115 | 160 | 118 | 105 | 105 | 132 | 1371[a] |
| ratio | 0.57 | 1.05 | 0.51 | 1.01 | 0.90 | 1.11 | 0.90 | 0.70 | 0.64 | 1.20 | 0.86[b] |
| $\rho$ | 0.58 | 0.69 | 0.02 | 0.90** | 0.52 | 0.88** | 0.44 | 0.75* | 0.38 | 0.73 | 0.59[b] |
| Comparison of population $F_2$ vs. $F_2$Syn3 with Map B | | | | | | | | | | | |
| No. of loci | 9 | 9 | 11 | 9 | 9 | 14 | 9 | 9 | 12 | 6 | 97[a] |
| $d$ | | | | | | | | | | | |
| $F_2$ | 251 | 186 | 192 | 197 | 233 | 144 | 131 | 151 | 164 | 110 | 1758[a] |
| $F_2$Syn3 | 147 | 209 | 124 | 225 | 129 | 177 | 130 | 117 | 115 | 145 | 1518[a] |
| ratio | 0.58 | 1.13 | 0.65 | 1.14 | 0.56 | 1.23 | 0.99 | 0.77 | 0.70 | 1.32 | 0.91[b] |
| $\rho$ | 0.67 | 0.70 | 0.53 | 0.91** | –0.55 | 0.88** | 0.64 | 0.79* | 0.54 | 0.65 | 0.58[b] |
| Cross C × D | | | | | | | | | | | |
| Population $F_3$ | | | | | | | | | | | |
| No. of loci | 12 | 8 | 12 | 11 | 12 | 10 | 9 | 8 | 8 | 12 | 102[a] |
| $d$ | 240 | 146 | 178 | 132 | 211 | 173 | 139 | 141 | 122 | 124 | 1608[a] |
| Comparison of population $F_3$ vs. $F_2$Syn3 with Map A | | | | | | | | | | | |
| No. of loci | 9 | 8 | 12 | 10 | 12 | 10 | 8 | 8 | 7 | 12 | 96[a] |
| $d$ | | | | | | | | | | | |
| $F_3$ | 151 | 146 | 178 | 122 | 211 | 173 | 109 | 141 | 116 | 124 | 1471[a] |
| $F_2$Syn3 | 156 | 126 | 165 | 108 | 146 | 143 | 104 | 158 | 90 | 139 | 1336[a] |
| ratio | 1.03 | 0.87 | 0.93 | 0.88 | 0.69 | 0.83 | 0.96 | 1.12 | 0.78 | 1.12 | 0.92[b] |
| $\rho$ | 0.78* | 0.97** | 0.76** | 0.66 | 0.72* | 0.56 | 0.72 | 0.74 | 0.48 | 0.80** | 0.72[b] |
| Comparison of population $F_3$ vs. $F_2$Syn3 with Map B | | | | | | | | | | | |
| No. of loci | 9 | 8 | 12 | 9 | 12 | 10 | 8 | 8 | 7 | 12 | 95[a] |
| $d$ | | | | | | | | | | | |
| $F_3$ | 151 | 146 | 178 | 132 | 211 | 173 | 109 | 141 | 116 | 124 | 1481[a] |
| $F_2$Syn3 | 147 | 142 | 186 | 86 | 167 | 154 | 112 | 163 | 99 | 150 | 1406[a] |
| ratio | 0.97 | 0.97 | 1.05 | 0.65 | 0.79 | 0.89 | 1.03 | 1.16 | 0.85 | 1.20 | 0.96[b] |
| $\rho$ | 0.82* | 0.99** | 0.87** | 0.56 | 0.84** | 0.54 | 0.80* | 0.77* | 0.62 | 0.86** | 0.77[b] |

*,**Significant at the 0.05 and 0.01 probability levels, respectively

[a]Total

[b]Mean

consequence, the resulting recombination frequencies $R$ refer to the accumulated recombination events occurring with more than one meiosis. Visualizing such $R$ values on a linkage map is misleading. Commonly, a map visualizes additive map distances, which are related via a mapping function to recombination frequencies $r$ referring to a single meiosis. This convention is adhered to in all mapping studies and linkage mapping software we are aware of, the only exception being the above studies on mapping in intermated populations. We therefore conclude that the expansion of linkage maps, reported in the above studies, is simply a consequence of the fact that the amount of linkage visualized on the genetic map does not refer to a single meiosis, but to accumulated effects of all meioses occurring during the development of the mapping population.

For constructing Map A and B, we followed the convention to visualize map distances calculated from recombination frequencies referring to a single meiosis. Therefore, neither systematic expansion nor shrinkage of maps is expected when comparing maps from $F_2$ or $F_3$ populations with maps from intermated populations. In our experimental data, we observed considerable differences in the length of individual chromosomes depending on the type of mapping population (Figs. 1, 2). Some chromosomes expanded substantially in the intermated populations, but others

**Table 2** Differences in marker intervals ($d$ in cM) in population $F_2$Syn3 of cross A × B and C × D between Map A and B, resulting from two different mapping methods

| Population | $d_{F2}$ (cM) | No. of intervals | $\lvert d_{\text{Map A}} - d_{\text{Map B}} \rvert$[a] (cM) |
|---|---|---|---|
| A × B | 0 | 3 | 0.0 |
| | 0–5 | 10 | 0.0 |
| | 5–10 | 16 | 0.1 |
| | 10–20 | 23 | 1.0 |
| | 20–50 | 24 | 2.0 |
| | > 50 | 7 | 9.5 |
| Σ | | 83 | |
| C × D | 0 | 0 | 0.0 |
| | 0–5 | 14 | 0.0 |
| | 5–10 | 11 | 0.1 |
| | 10–20 | 28 | 0.5 |
| | 20–50 | 29 | 4.4 |
| | > 50 | 1 | 15.4 |
| Σ | | 83 | |

[a] $\lvert d_{\text{Map A}} - d_{\text{Map B}} \rvert$ refers to means of absolute values

shrunk. The total map lengths of both intermated populations were smaller than in the base population. This marginal shrinkage is most likely attributable to the effects of drift occurring through the intermating. However, in accordance with theory, a systematic expansion of the entire linkage maps was not observed in our study. In conclusion, an expansion of linkage maps through intermating is neither expected nor observed in our data, under the above-mentioned convention.

## High mapping resolution

The expected constant length of linkage maps is not related to the fact that intermating may enhance mapping resolution. High mapping resolution is a consequence of precise estimation of small recombination frequencies. Commonly, the precision of linkage mapping is measured by the standard error of the estimated recombination frequency (Allard 1956)

$$\sigma_r = \sqrt{\frac{1}{N\, i_r}}, \tag{4}$$

where $N$ is the sample size and $i_r$ is the mean amount of information from an individual, depending on the type of the mapping population and the (true unobservable) linkage between loci. The theoretical advantage of intermated populations for high-resolution mapping is that the value of $i_r$ is greater than that of $F_2$ populations if map distances are small, as demonstrated numerically by Liu et al. (1996). This results in a more precise estimation of small recombination frequencies.

For the linkage map of cross A × B constructed with $F_2$ individuals, we have (Mather 1936)

$$i_r = \frac{2\left(1 - 3r + 3r^2\right)}{r\,(1-r)\,(1 - 2r + 2r^2)}, \tag{5}$$

for the map of cross C × D constructed with $F_3$ individuals (Allard 1956)

$$i_r = \frac{4\left(2 - 6r + 3r^2 + 4r^3\right)}{r\,(1-r)\,(2 + r^2)^2 (1 - 2r + 2r^2)}, \tag{6}$$

and for the maps constructed with $F_2$Syn $t$ individuals (Liu et al. 1996)

$$i_r = \frac{(1-r)^{2t-2}\left[2(1-r) + t(1-2r)\right]^2 \left[1 + 3(1-2r)^2 (1-r)^{2t}\right]}{\left[1 - (1-2r)^4 (1-r)^{4t}\right]}. \tag{7}$$

It follows that for cross A × B a smaller standard error $\sigma_r$ in the intermated population is expected than in $F_2$ if the true value $r \leq 0.1419$. For C × D, the threshold is $r \leq 0.1966$ (Fig. 3). In total, 49% of the marker intervals were estimated to be larger than the threshold in cross A × B, and 19% in cross C × D.

The main purpose of the linkage maps constructed for crosses A × B and C × D is QTL mapping and monitoring allele frequency changes in recurrent selection programs. In both applications, the focus lies on marker loci with tight linkage to gene loci. We expect that the increased accuracy in estimating small map distances in these studies is worth the additional effort in establishing a linkage map from the intermated populations.

## Mapping of loosely linked markers

While linkage between closely linked markers is expected to be estimated more accurately from intermated populations than from corresponding $F_2$ or $F_3$ populations, the reverse is true for loosely linked markers (Fig. 3). Assignment of loci to linkage groups was difficult with our mapping data from the intermated populations, and several loci could not even be assigned to their linkage groups (Figs. 1, 2). We attribute this problem to (1) the large standard errors of estimated map distances between more distant markers when using intermated mapping populations, and (2) the fact that for a given map distance $d$, the value $r < R < 0.5$ and, therefore, a test on linkage ($H_0$: $r = 0.5$, $H_0$: $R = 0.5$) is expected to have greater power

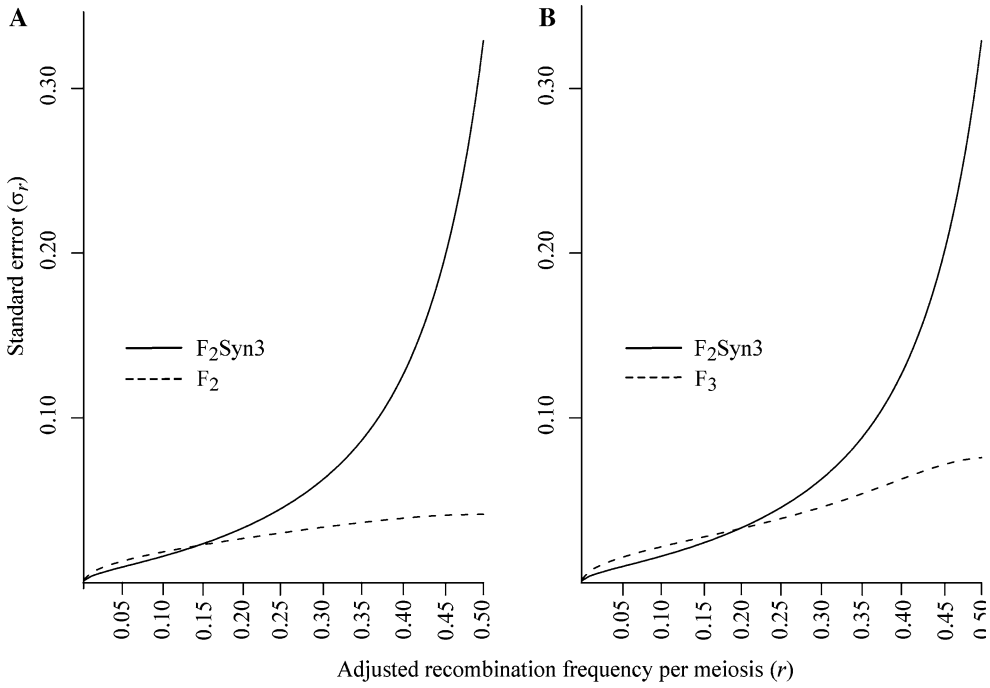


**Fig. 3** Standard error $\sigma_r$ of the adjusted recombination frequencies $r$ calculated with Eq. 4 for (**a**) cross A × B population $F_2$ ($N$ = 146) and $F_2$Syn3 ($N$ = 148) ($\sigma_{r\ F2Syn3} < \sigma_{r\ F2}$, when $r < 0.1419$), and (**b**) cross C × D population $F_3$ ($N$ = 110) and $F_2$Syn3 ($N$ = 148) ($\sigma_{r\ F2Syn3} < \sigma_{r\ F3}$, when $r < 0.1966$)

for $r$ than for $R$. Furthermore, gaps on the linkage map larger than 20 cM hamper severely the construction of linkage maps from intermated populations.

## Sampling error caused by drift

The theoretical derivations of the amount of information and, consequently, the standard deviation of estimated recombination frequencies (Liu et al. 1996), are based on the assumption that intermating is conducted with infinitely large populations. In practice, however, finite samples from the population are used as parents for the next generation. As a consequence, random genetic drift occurs. Drift is increasing the standard error $\sigma_r$, and because the advantage of intermating is relatively small (Fig. 3), the increase in $\sigma_r$ due to drift may even overrule the positive effects of intermating.

In our experiment, we attempted to minimize drift by using the chain crossing procedure for intermating, but this effect could not be quantified. Therefore, a final assessment of whether intermating actually increased the mapping accuracy remains open. As intermated populations are expected to play an increasingly important role in fine-mapping and map-based cloning of genes, we plan to conduct simulation studies for quantifying the effects of drift and obtaining an indication on the minimum effective population size required during the intermating generations.

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