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Somatic intrachromosomal homologous recombination events in populations of plant siblings

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Abstract

Intrachromosomal homologous recombination in whole tobacco plants was analyzed using β-glucuronidase as non-selectable marker. We found that recombination frequencies were additive for transgenes in allelic positions and could be enhanced by treatment of plants with DNA-damaging agents. We compared the patterns of distribution of recombination events of different transgenic lines of tobacco and *Arabidopsis* with the respective Poisson distributions. Some lines showed Poisson-like distributions, indicating that recombination at the transgene locus was occurring in a random fashion in the plant population. In other cases, however, the distributions deviated significantly from Poisson distributions indicating that for specific transgene loci and/or configurations recombination events are not randomly distributed in the population. This was due to overrepresentation of plants with especially many as well as especially few recombination events. Analysis of one tobacco line indicated furthermore that the distribution of recombination events could be influenced by treating the seedlings with external factors. Our results suggest that different plant individuals, or parts of them, might exhibit different transient 'states' of recombination competence. A possible model relating 'recombination silencing' and transcription silencing to heterochromatization of the transgene locus is discussed.

Introduction

Plants carry in their genomes large amounts of repeated DNA sequences consisting of non-coding elements, highly homologous ribosomal RNA genes and gene families [12]. These sequences provide targets for homologous recombination which result in continuous alteration of the genome. Mechanisms have to be postulated that balance the recombination behavior of a plant, which, on the one hand, needs flexibility in evolution and, on the other, relative stability to conserve evolutionary benefits. Somatic recombination in plants is of special biological significance, because plants, unlike animals, do not have a specialized cell line predetermined to produce the gametes. The germline is set aside only late during development. Hence, genetic changes occurring during vegetative growth can be incorporated in the progeny [46]. DNA changes due to

somatic intrachromosomal recombination have indeed been found transferred to the offspring of maize [9].

Intrachromosomal recombination has been studied in plant cells [2, 13, 31, 45; see for reviews 21, 35]. The strategies used mostly excluded the detection of recombination events in different organs and at different developmental stages of plants. Recently we set up a non-selective assay system which enabled us to visualize intrachromosomal homologous recombination events throughout the whole life-cycle and in all organs of the plant *Arabidopsis thaliana*. The assay system employed a disrupted chimeric β-glucuronidase (*uidA*) gene as the genomic recombination substrate. In cells in which recombination events occurred the *uidA* gene was restored [44]. Cells expressing β-glucuronidase, and their daughters, could be precisely localized by histochemical staining. We observed recombination in all examined organs of *Arabidopsis*, from the seed stage until the flowering stage. Interestingly, recombination frequencies were found to differ among different organs of particular transgenic lines (for details see [44]). In the present communication we use this system to analyze the recombination behavior of populations of transgenic tobacco and *Arabidopsis* siblings. The main question we ask here is whether somatic intrachromosmal recombination can be regarded as a stochastic process. For this we compared the frequencies of recombination in populations of different plant lines with the statistical expectations. For some transgenic lines we found that recombination events are distributed randomly, as described by a Poisson distribution. In other lines, however, recombination events are not randomly distributed between individuals. Thus, members of a plant population might differ from each other more than statistically expected.

Materials and methods

Construction of recombination substrates

Non-functional chimeric *uidA* genes containing different deletions (originating from plasmid pGUS 23) were made, as described [32], and inserted into the binary vector pGSC1704 (kindly provided by Plant Genetic Systems, Gent, Belgium). The fragment carrying the N-terminal deletion mutant of the *uidA* gene was inserted into the polylinker of pGSC1704, as described [44], resulting in the plasmid pVN1. A fragment carrying the C-terminal deletion construct C4 was cut out of pGUS 23 by digestion with *Eco* RI and *Msc* I. After filling in the ends, the fragments were inserted into the filled-in *Bst* XI site after the hygromycin phosphotransferase (*hpt*) gene of pVN1. The resulting recombination substrate plasmid pVN1IC4 carries the partially overlapping *uidA* sequences in inverted orientation on its T-DNA (see Fig. 1A). It was tested for its ability to restore a functional *uidA* gene by extrachromosomal recombination in plant cells, as described [34].

For obtaining plant lines that abundantly expressed β-glucuronidase, the plasmid pBG5 was constructed by inserting the *Eco* RI fragment of pGUS 23 containing the chimeric *uidA* gene into the *Eco* RI site of pBin19 [5].

Plant transformation, growth and molecular characterization of transgenic lines

The recombination substrate plasmids were transferred into *Agrobacterium tumefaciens* via electroporation as described [22]. The resulting strains were used to transform the inbred line SR1 of *Nicotiana tabacum*, as described [17]. The pattern of integration and the number of copies within the particular locus of each line were determined by Southern blot analysis. Our analysis indicated that lines 7 and 8 contained one copy of the recombination substrate, line 9 contained 4 copies in direct and the line 6 two copies in inverted orientation, line 3 contained at least two copies and line N1IC4 1 at least 4 copies in different orientations at a single locus each. Hemizygous and in some cases also homozygous seed material of these lines was further propagated for the analysis of recombination events. For outcrossing the same batch of SR1 plants was used as for transformation. Successive generations of

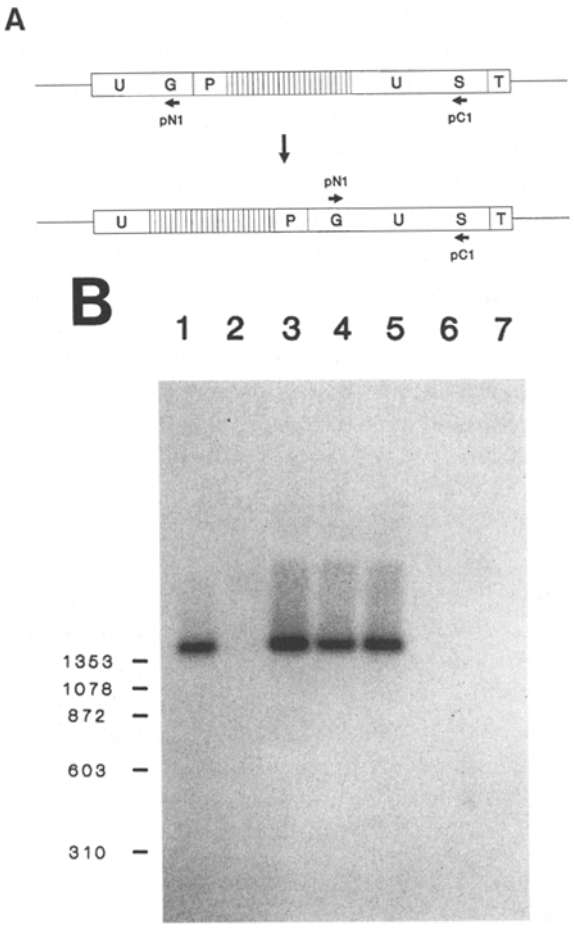

Fig. 1. A. Schematic representation of a recombination substrate of the pVN1IC4 type, integrated in the plant genome. The binding sites of the two primers (pC1 and pN1) used for PCR to detect the recombined *uidA* gene are depicted. As shown, only after homologous recombination can a PCR product be formed. P, 35S promoter of cauliflower mosaic virus; GUS, *uidA* gene; T, nopaline synthase terminator; hatched box, hygromycin phosphotransferase gene. B. Detection of the recombined *uidA* gene by PCR. Autoradiogram of a Southern blot hybridized with an *uidA*-specific probe. Fragment sizes are given in bp. Lane 1, positive control, plasmid pGUS 23, carrying the functional *uidA* gene; lane 2, DNA extracted from 'white callus' material of the tobacco line N1IC4 9 (cf. Fig. 2F); lanes 3 and 4, DNA extracted from two different 'blue calli' regenerated from the line N1IC4 9 (cf. Fig. 2F); lane 5, DNA of 'blue callus' material from a positive control plant line harboring a complete *uidA* gene; lane 6, DNA from original plant material of the line N1IC4 9; lane 7, DNA of wild-type tobacco SR1.

progeny plants were usually germinated on MS medium [28] supplemented with 50 mg/l hygromycin, and kept in growth chambers at 25 °C with a 16 h light 8 h dark regime.

Transformation of *Arabidopsis thaliana*, maintenance of transgenic lines and their molecular characterization were previously described [44]. The transgenic line N1DC1 11 contains 3 copies of the recombination substrate, whereas line N1IC4 651 contains 1 copy, at a single transgene locus each.

Detection of recombination events and calculation of recombination frequencies in tobacco

Seedlings were germinated on MS medium supplemented with 50 mg/l hygromycin. Two weeks after germination, plants were transferred to MS medium and in some cases treated as described below. Ten plant siblings were grown per culture vessel. Vessels containing different lines or differently treated plants were randomly distributed in the growth chambers and positions of the vessels were changed daily. Experiments were performed in polyethylene culture vessels (Semadeni, Ostermundigen, Switzerland) in growth chambers 500/ + 10–40 JU (Weiss Technik, Reiskirchen, Germany). Histochemical staining, as described [18], was usually done with seedlings 6 weeks after germination under sterile growth conditions. Conditions for destructive and non-destructive staining were as described [44]. Regeneration of tissue after staining was performed by using the media described above.

Total DNA of the respective transgenic lines was isolated from whole seedlings, as described [44]. The yield of total DNA (in micrograms per seedling) was compared with the mean DNA content (in picograms) of a tobacco cell [4], to give an estimate of the number of genomes present in a seedling [43, 44].

Induction of recombination by UV and MMS

Two week old tobacco seedlings were put on MS medium containing 20 ppm (MMS) (Fluka, Buchs, Switzerland) or irradiated with UV light

(254 nm; 1000 J/m^2 for 30 s) using a UV Stratalinker 1800 (Stratagene, La Jolla, CA). Neither treatment created visible damage on the plants. Plants were used for histochemical analysis 3 to 4 weeks later.

PCR and Southern blot analysis

Total DNA was isolated from plants as described [44] and purified by CsCl density gradient centrifugation. PCR reactions with the primers pC1 and pN1 [32] were performed as described [33]. Southern blot analysis was carried out essentially as described [34].

Statistical analysis

The expected Poisson distributions were calculated for the different transgenic lines using the mean numbers of recombination events in a population of plant siblings according to Simpson *et al.* [40]. The observed distributions were then compared to the calculated distributions with a χ^2 goodness-of-fit test [42]. Classes of size <5, mostly containing large numbers of recombination events, were pooled according to Cochran [8], and the degrees of freedom were modified after pooling (Table 2).

After a χ^2 homogeneity test [42] which indicated no significant deviations, the results of four independent experiments with the homozygous line N1IC4 9 and two independent experiments with the homozygous line N1IC4 9 after MMS treatment were pooled. The pooled data were then compared to the respective Poisson distributions.

Results

Somatic recombination events during the development of transgenic tobacco plants harboring recombination substrates

Tobacco SR1 plants were obtained that carried as transgene overlapping sequences of the 5′ and 3′ parts of the *uidA* gene (length of overlap 566 bp)

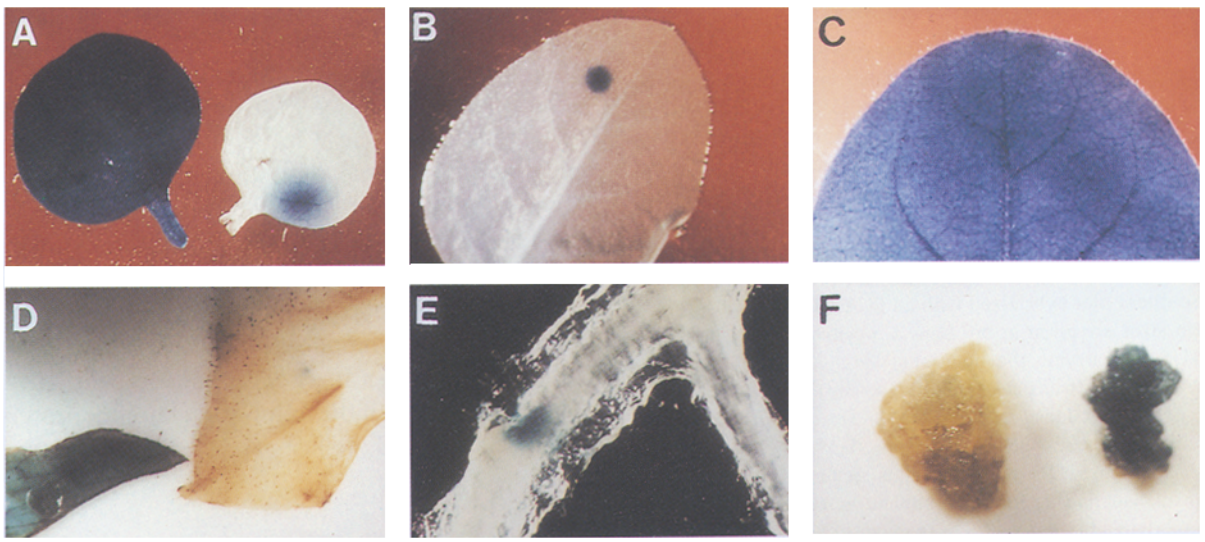

Fig. 2. Visualization by histochemical staining of recombination events in whole tobacco plants. A. Cotyledon of a plant containing a functional *uidA* gene on the left and cotyledon of a plant (line N1IC4 9) revealing a recombination event on the right. B. Leaf of a plant of the line NIC4 9. C. Leaf of a positive control plant. D. Petals of the line N1IC4 9 (right) and a positive control plant (left). E. Root of plant line N1IC4 9 revealing one recombination event. F. Callus material regenerated from tissues of one plant individual which had recombined before ('blue callus') or which had not recombined ('white callus').

in inverted orientation, separated by a hygromycin phosphotransferase gene (Fig. 1A). The progeny of the original transformed plants were selfed and/or outcrossed with wild-type SR1 plants. Single-locus transgenic lines, as judged by a 3:1 segregation for hygromycin resistance in the T1 generation, were identified. The single-locus lines N1IC4 1, 3, 6, 7, 8 and 9 were chosen for further experiments. As control for the expression of the marker gene, transgenic plants were produced that contained the functional chimeric *uidA* gene.

Histochemical staining of whole plants revealed blue sectors. To verify that blue sectors are the result of homologous recombination events, seedlings of the line N1IC4 9 were stained, blue and colorless tissues of the same plant were excised and propagated to yield callus material (Fig. 2F). Total callus DNA was isolated and used for PCR analysis with the primers pC1 and pN1. Prior to a recombination event, the sequences recognized by the primers point in the same direction and thus do not form a PCR product (see Fig. 1A). In contrast, if a recombination event leads to the restoration of the *uidA* gene, the 3′ ends of both primers face each other and PCR results in a 1.4 kb band. An autoradiogram of PCR products probed with an *uidA*-specific DNA fragment is shown in Fig. 1B. The band specific for the restored functional *uidA* gene (lane 1) was detected in DNA samples of two different, independent 'blue calli' regenerated from N1IC4 9 (lanes 3, 4). This band was not detected with DNA of 'white callus' material (lane 2) or DNA from the original plant line (lane 6). Thus, as in *Arabidopsis* [44], a blue sector on the tobacco plant represents a homologous recombination event.

The *uidA* gene used was strongly and abundantly expressed in positive control plant lines (Fig. 2A, C, D). This allowed quantitative analysis of recombination events in whole plants. Staining of seedlings of the line N1IC4 9 at different developmental stages revealed recombination events in various tobacco organs tested, such as cotyledons (Fig. 2A), leaves (Fig. 2B), roots (Fig. 2E) or petals (Fig. 2D).

Recombination frequencies in tobacco seedlings are dependent on the position of the transgene and the allelic state, and can be induced by DNA damaging agents

We quantified recombination events in 6 week old seedlings (6–8 leaves stage) in different transgenic lines. The total DNA content of three seedlings of each particular line was determined to allow estimation of recombination frequencies on a 'per genome' basis. We tested at least 30 individuals of all described lines in their hemizygous state. We found between 9 and 138 recombination events per population in these lines (Table 1), which corresponds to one recombination event in about 10^6 to 10^7 genomes. There was no correlation between copy numbers or configurations of the recombination substrates and recombination frequencies; for example, plant line N1IC4 1 containing at least 4 copies of the recombination substrate exhibited a lower recombination frequency than plant line N1IC4 7 containing only one re-

Table 1. Recombination frequencies in different transgenic tobacco lines, in different allelic states and in presence of DNA-damaging agents.

Plant line	*z* (*n*)	*r*
N1IC4 6 hemi	9 (30)	
N1IC4 8 hemi	23 (30)	
N1IC4 1 hemi	31 (30)	
N1IC4 7 hemi	138 (30)	
N1IC4 3 hemi	48 (31)	0.6
N1IC4 3 homo	82 (30)	1
N1IC4 9 hemi	242 (124)	0.5
N1IC4 9 homo*	899 (214)	1
N1IC4 9 homo MMS$^+$	770 (100)	1.8
N1IC4 9 homo UV	308 (30)	2.5

The number of recombination events (*z*) per plant population in 6-week old tobacco plants of single-locus transgenic lines in their hemizygous (hemi) or/and homozygous (homo) state are given. The number of plant individuals (*n*) per population is indicated. *r*, relative number of recombination events per plant in the specific transgene line in relationship to this number in the untreated homozygous state; * data pooled from four independent experiments; $^+$ data pooled from two independent experiments.

combination substrate at a different chromosomal locus. The finding that the number of recombination events in the populations of the lines N1IC4 7 and N1IC4 8, both containing a single copy of the recombination substrate, differs by a factor of 6 indicates that the genomic position itself seems

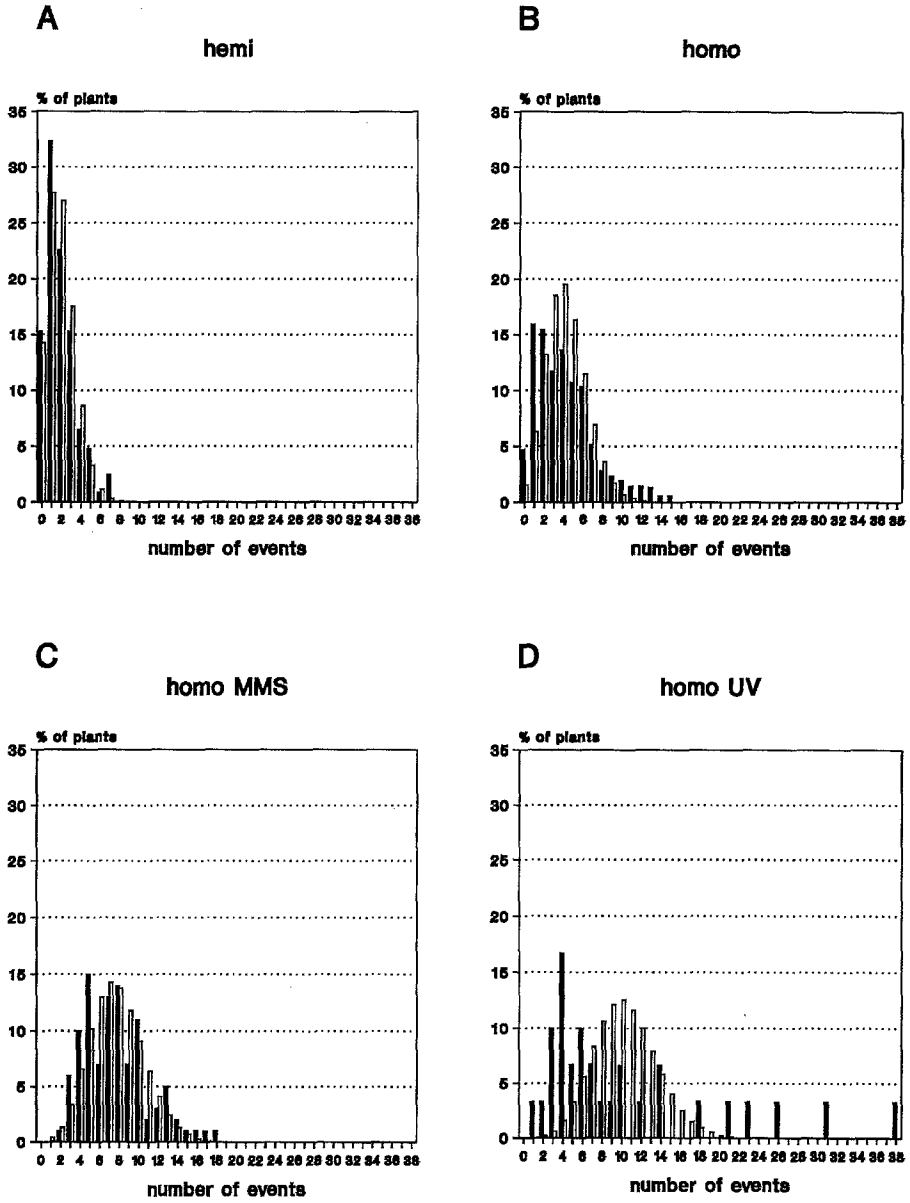

Fig. 3. Detection of recombination events in siblings of the line N1IC4 9 (cf. Tables 1 and 2). Recombination events were scored 5 weeks after germination. A. Distribution of 124 hemizygous plants. B. distribution of 214 homozygous plants. C. Distribution of 100 homozygous, MMS-treated plants (20 ppm in the medium, starting 2 weeks after germination). D. Distribution of 30 homozygous, UV-irradiated plants (pulse with 254 nm, 1000 J/m^2, 2 weeks after germination). White boxes represent the expected Poisson distributions and black boxes the actual distributions found (note that in the case of the UV treated plants due to the small numbers both distributions can not be compared statistically using the Cochran rules as done for the other distributions in Table 2).

to have a strong influence on recombination frequencies (see also [2, 43]). Plants of the lines N1IC4 3 and 9 were also analyzed in their homozygous state. Data from four independent experiments with line 9 were pooled after a test for homogeneity. The mean recombination frequency per plant in these lines was about twice as high in the respective homozygous populations (Table 1). This finding is in line with results obtained with other approaches [20, 43] and indicates that there is no significant allelic interaction between the recombination substrates.

We chose the line N1IC4 9 to analyze its recombination behavior under different experimental conditions. We applied two factors which we found to enhance homologous recombination in plants [36]: UV irradiation and MMS treatment. Treatment of 2-week old homozygous seedlings resulted in enhancement of the number of recombination events per plant by a factor of 2 to 3 for each agent (Table 1; the data of the MMS treatment are pooled from two independent experiments).

The distribution of recombination events in differently treated populations of plant siblings of the line N1IC4 9 is shown in Fig. 3 (black bars). The mean number of recombination events per plant in the population was used to calculate the respective Poisson distribution (Table 2). This distribution is expected for events which are distributed randomly among individuals in the population (white bars). The sample size of the UV treated plants was too small for a statistical analysis (Table 2); nevertheless the data indicate that after UV treatment several plants displayed enormous numbers of blue sectors (Fig. 3D).

Statistical analysis of the distribution of recombination events in siblings of tobacco and Arabidopsis thaliana

To test whether the distribution of somatic intrachromosomal recombination events was stochastic, it was compared to the expected data generated by a Poisson distribution by means of a goodness-of-fit test. This could be done for populations of 100 individuals or more (see also Materials and methods), i.e. for the tobacco line N1IC4 9 in its hemizygous, homozygous and MMS-treated state (Table 2 and Fig. 3A–C). Results were reproducible as revealed by a χ^2 homogeneity test [42] and data for the homozygous and the MMS treated seedlings were pooled from

Table 2. Statistical analysis of the distribution of recombination events in populations of transgenic tobacco and *Arabidopsis* seedlings.

Plant line	ex	x	s	n	χ^2	df	p	Poisson distribution
Tobacco								
N1IC4 9 hemi		1.95	1.58	124	3	6	0.8507	accepted
N1IC4 9 homo*		4.20	3.11	214	33	11	0.0006	rejected
N1IC4 9 homo MMS$^+$		7.70	3.35	100	10	10	0.4413	accepted
Arabidopsis								
N1IC4 651 homo	1	1.71	1.35	121	2	5	0.8358	accepted
	2	2.21	1.48	110	3	6	0.8263	accepted
N1DC1 11 homo	1	3.36	4.19	165	77	9	0.0001	rejected
	2	2.69	3.63	110	172	7	0.0001	rejected

The average numbers of recombination events per plant (x) in 6-week old tobacco or 5-week old *Arabidopsis* seedlings of single locus transgenic lines in their hemizygous (hemi) or homozygous (homo) states are indicated. The standard deviation (s) and the number of plants tested (n) are given. The results of the homogeneity test are given as χ^2 values with degrees of freedom (df) and the probabilities (p) that the detected distribution reflects a Poisson distribution ($p < 0.05$ is significant for the rejection of the Poisson distribution); ex, experiment. * data pooled from four independent experiments; $^+$ data pooled from two independent experiments.

four and two independent experiments, respectively. The null hypothesis that the observed distributions followed a Poisson distribution was accepted for the hemizygous ($p = 0.8507$) and the homozygous state after MMS treatment ($p = 0.4417$) but rejected for the homozygous line ($p = 0.0006$).

To extend our analysis to another plant species we also analyzed *Arabidopsis* plants carrying the *uidA* gene-derived recombination substrates N1DC1 and N1IC4 at a single locus in their genomes [44]. We analyzed 5-week old homozygous plants of two different lines at the full rosette stage (see Materials and methods). Interestingly, the distribution pattern found in a homozygous population of the line N1IC4 651 (black bars in Fig. 4A) was in accordance with a Poisson distribution ($p = 0.8358$) whereas the pattern detected in a homozygous population of the line N1DC1 11 (black bars in Fig. 4B) differed drastically from a Poisson distribution ($p = 0.0001$). The observed distribution in line N1DC1 11 is more bimodular with an overrepresentation of plants with especially many and especially few (or no) recombination events (compare black and white bars in Fig. 4B). A similar pattern was found for the homozygous tobacco line N1IC4 9 (Fig. 3B). Another experiment with 110 homozygous plants of both *Arabidopsis* lines sustained these results (Table 2). Whereas the distribution pattern of the line N1IC4 651 was in accordance with a Poisson distribution ($p = 0.8263$), the pattern of line N1DC1 11 was not ($p = 0.0001$).

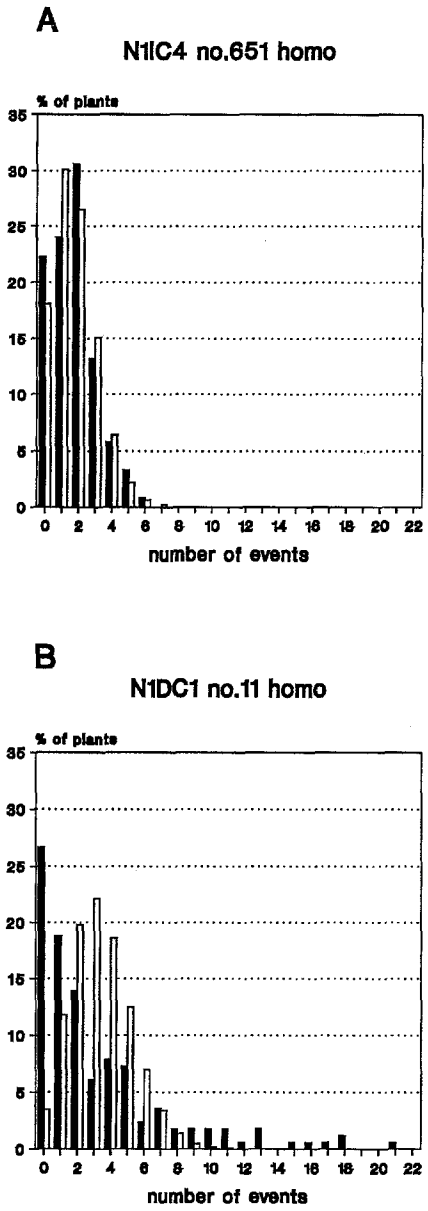

Fig. 4. Detection of recombination events in *Arabidopsis* siblings 5 weeks after germination. A. distribution of 121 homozygous plants of the line N1IC4 651 (experiment 1, Table 2). B. Distribution of 165 homozygous plants of the line N1DC1 11 (experiment 1, Table 2). White boxes represent the expected Poisson distributions and black boxes the actual distributions found.

Discussion

The phenotype of an organism is the result of interaction between genotype and the environment. The variations in phenotype therefore are dependent on the variations in genotype and environment [39]. Under constant environmental conditions the genotype determines the phenotype of individuals of a plant population. In seed families a random distribution of particular phenotypes is expected [47]. Therefore, in principle, also recombination should be a random process and no association of individual recombination events should be seen in individual plants. However, there are multiple recombination phenomena, such as negative interference [6] or recom-

binational hotspots [41] in which recombination events are not occurring randomly along chromosomes. The aim of our analysis was to find out whether somatic intrachromosomal recombination is occurring randomly in populations of plant siblings.

Somatic recombination is random in some but not in all lines

The statistical interpretation of our data strongly indicates that for the hemizygous tobacco line N1IC4 9 and the homozygous *Arabidopsis* line N1IC4 651 the distribution of stained sectors follows a Poisson distribution (in both cases $p > 0.8$), i.e. somatic homologous recombination can be regarded as random process in these plant lines. However, in the homozygous tobacco line N1IC4 9 ($p = 0.0006$) and in the homozygous *Arabidopsis* line N1DC1 11 ($p = 0.0001$) the number of stained sectors was distributed in a non-random fashion and exhibited a positive association in its incidence. The variation was reproducible (Table 2). Our experiments with the line N1IC4 9 demonstrated that intrachromosomal recombination in tobacco can be enhanced by UV irradiation and MMS treatment. Moreover, MMS treatment led to a change in the distribution of stained sectors from a pattern that was not in accordance with a Poisson distribution ($p = 0.0006$) to one that was ($p = 0.4433$).

An interesting question is, of course, whether the detected variation in the number of stained sectors actually reflects the variation in the number of recombination events. A trivial explanation for the variation in the distribution of recombination events could be variations in the ploidy level of individual plants of certain transgenic lines. This explanation is unlikely, as in several experiments in which we extracted DNA from a number of plant individuals we never found differences of more than 10–20% in the DNA content between individual plant siblings (data not shown). Moreover, the effect of MMS on the distribution of recombination events in the line N1IC4 9 argues, at least for this line, against a major influence of polyploidy on the distribution of recombination.

Alternatively, the non-random pattern of distribution could be the consequence of differences in stainability of plant individuals of a population. Differences in stainability in plant organs should be due to physiological differences. However, physiological parameters should be identical for all plants of the tobacco line N1IC4 9, irrespective of whether they are hemi- or homozygous for the recombination substrate. In addition, the two *Arabidopsis* lines exhibiting different recombination behaviors were generated by transformation of the same batch of *Arabidopsis* leaves. These lines therefore differ only in their recombination substrates. These considerations exclude the possibility that a general property such as stainability is different in the two lines and point towards the transgenes themselves and their genomic environment as the cause of random/non-random recombination behavior.

With our assay system the detection of recombination events is dependent on the expression of the recombined gene. Non-random variations in GUS expression might therefore also lead to non-random distributions of blue sectors in certain plant populations (see discussion below). However, under this assumption it would be difficult to explain why MMS, an agent known to enhance recombination, is able to change the distribution of visible recombination events from a non-random to a random fashion, as it does for the tobacco line N1IC4 9.

Nevertheless, we believe that as a rule, somatic homologous recombination in plants is a stochastic process. However, in some cases such as in the homozygous tobacco line N1IC4 9 and in the *Arabidopsis* line N1DC1 11 the pattern of distribution is changed.

Why do some lines exhibit a non-random distribution of recombination events?

To explain the observed non-random patterns in the distribution of recombination events we favor the theory that a plant can exhibit transient 'states'

of recombination competence. The boundaries of these 'states' might be fluctuating, some plants being in a low recombination 'state' (0 to few sectors), some in a high recombination 'state' (many sectors).

The term 'states' needs further clarification. On the one hand, parameters important for recombination include an accessible chromosomal organization at the recombination locus. On the other hand, the availability of the enzymatic recombination machinery might be rate-limiting in the cells ('competence' of recombination of certain subpopulations of cells [e.g. 15]). The supply of the necessary enzymes is a factor external to the recombination target site (*trans*-acting) and is unlikely to vary within populations of siblings, as other transgenic lines show random distribution of recombination events. However, the susceptibility for the action of the recombination apparatus is intrinsic to the recombination locus (in *cis*) and it can, according to our working hypothesis, be subject to change. Only a genetic approach, such as the use of two different recombination markers at different transgene loci, will allow a final distinction between effects in *cis* (the markers would act independently) or in *trans* (both markers would behave the same [e.g. 15]).

A 'state' of a locus could be defined by a particular chromatin structure. Information on influences of nucleosomal and higher order chromatin structures on the frequency of homologous recombination is not abundant [14]. However, relationships between chromatin and change in gene activity have been found. For instance, the phenomenon of position-effect variegation documents the mosaic expression of genes located adjacent to euchromatin-heterochromatin boundaries [1, 16].

Can the phenomenon be explained by gene silencing and/or 'recombination silencing'?

Models which take into account changes in higher order chromatin structure have also been proposed to explain the different behavior of plant siblings in gene silencing [3, 19, 23], a phenomenon that requires sequence homology. A similar phenomenon has been described recently for *Drosophila*. Here repeated transgene sequences led to position-effect variegation-like phenotypes, which was explained with induction of heterochromatin formation [11]. The fact that the transgenic *Arabidopsis* line N1DC1 11, containing 3 copies of the transgene at a single locus, behaves non-randomly in recombination in contrast to the single-copy line N1IC4 651 is reminiscent of silencing phenomena of multiple-copy transgenes. Our finding that the tobacco line N1IC4 9 in its hemi- but not in its homozygous state behaves in accordance to a Poisson distribution is also indicative of a connection of some silencing phenomena and recombination; in a number of homozygous transgenic lines more pronounced gene silencing effects were found than in their hemizygous counterparts [3, 10, 25, 26, 29, 30]. One is tempted to speculate that a possible 'heterochromatization' of the locus carrying the recombination substrate would result in a reduced recombination frequency at the specific locus in certain plant individuals. This would be in line with the fact that recombination frequencies in eukaryotes are generally lower in heterochromatin than in euchromatin [7, 38]. However, since our assay system is based on gene expression, we might fail to detect genes, which are physically restored after recombination, yet are transcriptionally silent. This phenomenon might also result in non-random patterns of blue sectors in the plant population. However, as the repetitive structure of the transgene locus is reduced upon recombination, the putative 'heterochromatization' may be reversed as a consequence of the recombination reaction, now allowing gene expression. Consistent with this model are recent findings that gene silencing in plants can be overcome by recombination within the repetitive transgene locus [24, 27]. The observed pattern in the *Arabidopsis* line 11 and the homozygous tobacco line 9 might be even due to a combination of 'recombination silencing' of the recombination substrate and gene silencing of the recombined gene. The fact that MMS treatment of the tobacco line N1IC4 9 changed the recombination pattern sig-

nificantly to a Poisson distribution indicates that at least for this line possible changes in chromatin structure of the recombination locus can be influenced by external factors. Repair processes in yeast have been shown to be correlated with changes in chromatin structure [37]. A 'heterochromatization' of the recombination locus might be hindered by the constant application of the DNA-damaging agent.

What are the consequences of non-random distributions of recombination events in certain plant populations? It seems that these plant populations as a whole have a broader repertoire to deal with their environments. Single individuals that recombine more frequently might have a better fitness under changing environmental conditions. In the case of somatic selection, the new rearrangement might even be transferred to the next generation [9]. Plants with rare recombination events, on the other hand, might do better under constant growth conditions.

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References

1. Allshire RC, Javerzat J-P, Redhead NJ, Cranston G: Position effect variegation at fission yeast centromers. Cell 76: 157–169 (1994).
2. Assaad FA, Signer ER: Somatic and germinal recombination of a direct repeat in *Arabidopsis*. Genetics 132: 553–566 (1992).
3. Assaad FA, Tucker KL, Signer ER: Epigenetic repeat-induced gene silencing (RIGS) in *Arabidopsis*. Plant Mol Biol 22: 1067–1085 (1993).
4. Bennett MD, Smith JB: Nuclear DNA amounts in angiosperms. Phil Trans R Soc Lond B274: 227–274 (1976).
5. Bevan M: Binary *Agrobacterium* vectors for plant transformation. Nucl Acids Res 12: 8711–8721 (1984).
6. Brooks Low K: Genetic recombination: a brief overview. In: Brooks Low K (ed) The Recombination of Genetic Material, pp. 1–18. Academic Press, San Diego (1988).
7. Charlesworth B, Sniegowski P, Stephan W: The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371: 215–220 (1994).
8. Cochran WG: Some methods for strengthening the common chi-square tests. Biometrics 10: 417–451 (1954).
9. Das OP, Levi-Minzi S, Koury M, Benner M, Messing J: A somatic gene rearrangement contribution to genetic diversity in maize. Proc Natl Acad Sci USA 87: 7809–7813 (1990).
10. de Carvalho F, Geysen G, Kushnir S, Van Montagu M, Inze D, Castresana C: Suppression of β-1,3-glucanase transgene expression in homozygous plants. EMBO J 11: 2595–2602 (1992).
11. Dorer DR, Henikoff S: Expansions of transgene repeats cause heterochromatin formation and gene silencing in *Drosophila*. Cell 77: 993–1002 (1994).
12. Flavell RB: Repeated sequences and genome change. In: Hohn B, Dennis E (eds) Plant Gene Research, vol. 2. Genetic Flux in Plants, pp. 139–156. Springer-Verlag, Wien/New York (1985).
13. Gal S, Pisan B, Hohn T, Grimsley N, Hohn B: Genomic homologous recombination *in planta*. EMBO J 10: 1571–1578 (1991).
14. Gangloff S, Lieber MR, Rothstein R: Transcription, topoisomerases and recombination. Experientia 50: 261–269 (1994).
15. Grossenbacher-Grunder AM: Spontaneous mitotic recombination in *Schizoaccharomyces pombe*. Curr Genet 10: 95–101 (1985).
16. Henikoff S: Position-effect variegation after 60 years. Trends Genet 6: 422–426 (1990).
17. Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, Rogers SG, Fraley RT: A simple and general method for transferring genes into plants. Science 227: 1229–1231 (1985).
18. Jefferson RA: Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep 5: 387–405 (1987).
19. Jorgenson R: Altered gene expression in plants due to *trans* interactions between homologous genes. Trends Biotechnol 8: 340–344 (1990).
20. Lebel EG, Masson J, Bogucki A, Paszkowski J: Stress-induced intrachromosomal recombination in plant

somatic cells. Proc Natl Acad Sci USA 90: 422–426 (1993).
21. Lichtenstein C, Paszkowski J, Hohn B: Intrachromosomal recombination between genomic repeats. In: Paszkowski J (ed) Homologous Recombination and Gene Silencing in Plants, pp. 95–122. Kluwer Academic Publishers, Dordrecht (1994).
22. Mattanovich D, Rüker F, de Camara Machado A, Laimer M, Regner F, Steinkellner H, Himmler G, Katinger H: Efficient transformation of *Agrobacterium* ssp. by electroporation. Nucl Acids Res 17: 6747 (1989).
23. Matzke M, Matzke AJM, Mittelsten Scheid O: Inactivation of repeated genes: DNA-DNA interaction. In: Paszkowski J (ed) Homologous Recombination and Gene Silencing in Plants, pp. 271–307. Kluwer Academic Publishers, Dordrecht (1994).
24. Matzke AJM, Neuhuber F, Park Y-D, Ambros PF, Matzke MA: Homology-dependent gene silencing in transgenic plants: epistatic silencing loci contain multiple copies of methylated transgenes. Mol Gen Genet 244: 219–229 (1994).
25. Meins F, Kunz C: Silencing of chitinase expression in transgenic plants: an autoregulatory model. In: Paszkowski J (ed) Homologous Recombination and Gene Silencing in Plants, pp. 335–348. Kluwer Academic Publishers, Dordrecht (1994).
26. Meyer P, Heidmann I, Niedenhof I: Differences in DNA-methylation are associated with a paramutation phenomenon in transgenic petunia. Plant J 4: 89–100 (1993).
27. Mittelsten Scheid O, Afsar K, Paszkowski J: Gene inactivation in *Arabidopsis thaliana* is not accompanied by an accumulation of repeat-induced point mutations. Mol Gen Genet 244: 325–330 (1994).
28. Murashige T, Skoog F: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473–497 (1962).
29. Neuhaus J-M, Ahl-Goy P, Hinz U, Flores S, Meins F: High-level expression of a tobacco chitinase gene in *Nicotiana sylvestris*. Susceptibility of transgenic plants to *Cercospora nicotianae* infection. Plant Mol Biol 16: 141–151 (1991).
30. Neuhuber F, Park Y-D, Matzke AJM, Matzke MA: Susceptibility of transgene loci to homology-dependent gene silencing. Mol Gen Genet 244: 230–241 (1994).
31. Peterhans A, Schlüpmann H, Basse C, Paszkowski J: Intrachromosomal recombination in plants. EMBO J 9: 3437–3445 (1990).
32. Puchta H, Hohn B: A transient assay in plant cells reveals a positive correlation between extrachromosomal recombination rates and length of homologous overlap. Nucl Acids Res 19: 2693–2700 (1991).
33. Puchta H, Kocher S, Hohn B: Extrachromosomal homologous DNA recombination in plant cells is fast and is not affected by CpG methylation. Mol Cell Biol 12: 3372–3379 (1992).
34. Puchta H, Dujon B, Hohn B: Homologous recombination in plant cells is enhanced by *in vivo* induction of double strand breaks into DNA by a site-specific endonuclease. Nucl Acids Res 21: 5034–5040 (1993).
35. Puchta H, Swoboda P, Hohn B: Homologous recombination in plants. Experientia 50: 277–284 (1994).
36. Puchta H, Swoboda P, Hohn B: Induction of intrachromosomal homologous recombination in whole plants. Plant J 7, 203–210 (1995).
37. Ramanathan B, Smerdon MJ: Enhanced DNA repair synthesis in hyperacetylated nucleosomes. J Biol Chem 264: 11026–11034 (1989).
38. Säll T, Nilsson N-O, Bengtsson BO: When everyone's map is different. Curr Biol 3: 631–633 (1993).
39. Schmid B: Phenotypic variation in plants. Evol Trends Plants 6: 45–60 (1992).
40. Simpson GG, Roe A, Lewontin RC: Quantitative Zoology. Harcort, Brace and World, New York (1960).
41. Smith GR: Hotspots of homologous recombination. Experientia 50: 234–241 (1994).
42. Sokal RR, Rohlf FJ: Biometry. Freeman, San Francisco (1973).
43. Swoboda P, Hohn B, Gal S: Somatic homologous recombination *in planta*: the recombination frequency is dependent on the allelic state of the recombining sequences and may be influenced by genomic position effects. Mol Gen Genet 237: 33–40 (1993).
44. Swoboda P, Gal S, Hohn B, Puchta H: Intrachromosomal homologous recombination in whole plants. EMBO J 13: 484–489 (1994).
45. Tovar J, Lichtenstein C: Somatic and meiotic chromosomal recombination between inverted duplications in transgenic tobacco plants. Plant Cell 4: 319–332 (1992).
46. Walbot R: On life strategies of plants and animals. Trends Genet 1: 165–169 (1985).
47. Walter V: Grundlagen der Pflanzenkunde. Das Verhalten erbgleicher Pflanzen; die Zufallskurve. Ulmer Verlag, Stuttgart, pp. 401–407 (1962).