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Insight on segregation distortions in two intraspecific crosses between annual species of *Medicago* (Leguminosae)

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Abstract About 40% ($\alpha = 0.05$) of the PCR-derived markers scored in a *Medicago truncatula* and *M. tornata* intraspecific cross departed from Mendelian expectations at $\alpha = 0.05$. This proportion is among the highest ever documented in the literature, notably for intraspecific crosses. Estimations of DNA amount were also implemented for the parental genotypes or parental lines, and significant variations were observed. Our results suggest that the parental genotypes have diverged for quite a while, and we propose that the level of distortion we documented is correlated with the genome size difference we measured.

Key words *Medicago truncatula* · *Medicago tornata* · RAPD · Segregation distortion · DNA content

Introduction

Classically, Mendelian heredity governs the fair segregation of characters. Prerequisites are an homogeneous distribution of each informational constituant among the gametes of a composite individual (fair segregation) and symetrical and equitable contributions of each parent to their composite offspring (fair syngamy). These factors depend on the location of the segregating genes in the chromosomes, the regular chromosome disjunction at anaphase of meiosis, the equal viability of different types of gametophytes or gametes, random fertilization and the equal viability of different classes of zygotes (Grant 1973). Departures from these conditions lead to departures from a simple Mendelian ratio. Apart from segregation distorter complexes, which have been thoroughly documented (Lyttle 1991), genetic mechanisms leading to segregation distortions are still poorly understood, notably in flowering plants. They have been frequently reported (Grant 1973, Landry et al. 1991), however, usually being attributed to the linkage between molecular markers and reproduction-regulating genes that operate in the pre- or post-zygotic phases of reproduction and whose coordination is disrupted following segregation (Zamir and Tadmor 1986). Unbalanced reproduction necessarily implies allelic variations between parental genotypes; distortions should thus correlate with their level of divergence. Likewise, meiotic irregularities are often liable for partial or even complete post-zygotic sterility of hybrids when parental individuals have diverged for a while (Radman and Wagner 1993).

Quantitative and evolutionary genetics assume a classical Mendelian segregation of characters that has been enacted as a law since De Vries, von Tschermark and Correns rediscovered Mendel's work in 1900. In mapping studies, however, recombination estimates are biased by departures of single-locus segregation ratios from Mendelian expectations (Säll and Nilsson 1994; Lorieux et al. 1995). These deviations may also have significant impact on the evolution of the genetic structure of populations because alleles favoured by distortions may tend to spread throughout a population. As we are involved in both types of studies for annual species of *Medicago* (Bonnin et al. 1996), it was a logical step to more closely examine potential departures from Mendelian expectations.

In this paper, we quantified the level of segregation distortions in a *Medicago truncatula* Gaertn. and a *Medicago tornata* (L.) Mill. intraspecific cross using

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polymerase chain reaction (PCR)-derived markers. These two diploid (2n = 16) autogamous species are native to the Mediterranean basin and have been spontaneously naturalized in Australia, where they are bred and used as fodder crop in the ley-farming system (cereal-legume rotations). This study focused on both random amplified polymorphic DNAs (RAPDs) (Williams et al. 1990) and a sequence-tagged-site (STS) marker resulting from the PCR amplification of the large intergenic spacer region (IGS) that separates transcription units from the 3′ end of the 25S gene to the 5′ end of the 18S gene. As genome size variations exist among *Medicago truncatula* ecotypes (Blondon et al. 1994), DNA amount estimations were determined for parental genotypes or parental lines. In addition to our own original data, we also present a review of the intraspecific and interspecific range of segregation distortion levels as documented in the literature.

Material and methods

Plant material

Two intraspecific crosses were studied. The two parental genotypes (F83.005-5 and DZA.045-5) used to develop the *M. truncatula* F_2 segregating population were derived from natural populations of France (F83.005) and Algeria (DZA.045). The *M. tornata* intraspecific cross involved 2 plants (ESP.050-4 and Tornafield-6) from the natural Spanish population ESP.050 and the Australian cultivated line Tornafield. Each effective parent plant stemmed from its original population by self-fertilization (S1). [F83.005-5 (female) × DZA.045-5 (male)] and [ESP.050-4 (female) × Tornafield-6 (male)] crossings were performed manually and produced a small number of offspring. In total, 122 *M. truncatula* and 80 *M. tornata* F_2 offspring resulted from the natural self-fertilization of a single F_1 plant for each particular cross (approx. 98% of the seeds germinated). Young leaves were sampled after 4 months and stored frozen (−20°C) until DNA preparation.

Template DNA preparation

Total DNA from the parents and each F_2 offspring was extracted according to the procedure described by Dellaporta et al. (1983) as modified by Tai and Tanksley (1990).

RAPD amplification

RAPD assays were performed in a final volume of 25 µl containing 10 m*M* TRIS-HCl, pH 9.0, 50 m*M* KCl, 0.01% (w/v) gelatin, 0.1% triton X-100, 1.5 m*M* $MgCl_2$, 200 µ*M* of each dNTP (Pharmacia), 50 µ*M* single Operon (Operon technology) primer, 0.4 U super Taq® (Stehelin & Cie AG) and approximately 30 ng total DNA template. Controls without DNA were also implemented. Each reaction mixture was overlaid with mineral oil (Sigma). Amplifications were carried out in a Techne PHC-3 thermal cycler programmed for 36 cycles with the following temperature profile: 1 min at 94°C, 1 min at 40°C and 1 min at 72°C. The initial and final steps were at 94°C for 4 min and 72°C for 6 min, respectively. RAPD products were resolved through a 2.4% agarose gel, run at 1.5 V/cm for 17 h in 1 × TRIS-acetate (0.04 *M*) EDTA (0.002 *M*), pH 8.0. A 1-kb DNA ladder (BRL) was run as a molecular size control. DNA was stained with ethidium bromide (3 mg/l) for 45 min and photographed in UV light ($\lambda = 260$ nm) with Polaroid 667 films. The presence or absence of bands was scored from negatives. A first screening step was performed on the parental genotypes only in order to identify polymorphic fragments. Three replicates were performed for each primer, and only those fragments that proved to be efficiently amplified each time were subsequently considered on the progenies.

IGS amplification

PCR amplifications were performed under the same conditions as for RAPD, with a few modifications. IGS amplifications were performed with 0.6 U super Taq® and specific primers anchored in the 25s (5′GCTGCCACGATCCACTGAGA3′) and 18s (5′CCAACTAGGACGGTCATCAG3′) rDNA genes, respectively. The cycling parameters (40 cycles) were 1 min at 93°C, 1 min at 55°C and 2 min 15 s at 72°C. Resulting products were loaded on a 1% agarose gel and run for 20 h at 50 V/cm.

DNA content measurement

DNA contents were determined using an EPICS V flow cytometer in the Service de Cytometrie, Institut des Sciences Végétales, CNRS, Gif-sur-Yvette, France. Measurements were assessed as described in Blondon et al. (1994) using ethidium bromide fluorescence and *Petunia hybrida* P × PC6 (2C = 2.85 pg) as the routine internal standard. Each genome size estimation resulted from five independent measurements for a single plant. As ESP.050-4 and Tornafield-6 genotypes were no longer available, we compared the average DNA content of both parental lines (5 plants for each).

Data scoring and analysis

RAPD markers were considered as discrete character states on a presence/absence alternative. When amplifications remained unefficient (at least three replicates were then performed), or when there were some doubts as to the presence or absence of a selected fragment, the sampled F_2 plant was not disregarded. Segregation analysis was checked against the expected 3:1 theoretical Mendelian ratio (F_2 population) using a χ^2 test (1 *df*). As multiple tests of significance were conducted, the Bonferroni sequential procedure (Holm 1979) was used to calculate table-wide significance levels, but no correction for continuity was implemented. This would have resulted in an excessivelly conservative test (Sokal et Rolhf 1995). IGS-amplified fragments corresponded to parent-specific alleles; their segregation was checked for goodness-of-fit to a 1:2:1 Mendelian expectation (χ^2 analysis; 2 *df*).

Analysis of variance (procedure GLM of SAS 1989) was performed among intraspecific and interspecific levels of distortions estimated at the 0.05 first-type error level. Pairwise comparisons were implemented to test whether the proportions of skewed loci documented in both *M. truncatula* and *M. tornata* were significantly different from those reported in the literature ($\alpha = 0.05$). For this purpose,

$$Z_{obs} = |Y_1 - Y_2| \Big/ \sqrt{\frac{1}{n_1} + \frac{1}{n_2}},$$

where $Y_i = 2\mathrm{Arc}\sin\sqrt{X_i/n_i}$, n_i and X_i represent the total number and the number of skewed loci of the study i, was estimated everytime and compared to the critical value of the normal law ($\alpha = 0.05$).

Results

Level of polymorphism of parental genotypes

Eighty-five RAPD primers were individually used to screen for polymorphism between F83.005-5 and DZA.045-5. Of these, 25 turned out to be useless because they did not allow any efficient amplification nor did they lead to unambiguous and repeatable products. Furthermore, 8 efficient primers only showed monomorphic fragments between F83.005-5 and DZA.045-5. In the *M. truncatula* cross, only 52 primers uncovered 161 polymorphic markers (e.g. 3.0 polymorphic markers per primer) out of the 512 unambiguous and fully reproducible fragments (e.g. 9.8 fragments per primer). Twenty-five RAPD primers were tested between ESP.050-4 and Tornafield-6. Of these, 4 led to non-interpretable patterns, and 5 efficient primers only produced monomorphic fragments. In the *M. tornata* cross, only 16 primers yielded 44 polymorphic markers (e.g. 2.75 polymorphic markers per primer) among 158 unambiguous and fully reproducible fragments (9.8 fragments per primer).

IGS amplification results from a site-specific annealing of 2 primers in the flanking regions of the large intergenic spacer of the nuclear rDNA. In both the *M. truncatula* and *M. tornata* cross, it yielded a single fragment of 5.0 or 5.4 kbp, depending on the parental genotype. This difference in fragment length is in agreement with the mechanisms of subrepeat variation that have been previously reported (Yakura et al. 1984).

Segregation study

Of the 161 RAPD markers, 48 were selected to monitor their segregation among the F_2 progeny of the *M. truncatula* cross. While 19 (39.6%) of these did not fit the expected 3:1 ratio at the 0.05 first-type error level, only 6 (Table 1) still departed from theoretical expectations after the Bonferroni procedure ($\alpha = 0.001$). In the *M. tornata* cross, 32 out of 44 markers were used for the segregation analysis. Thirteen (40.6%) did not behave as dominant Mendelian characters at $\alpha = 0.05$, and 8 of these (Table 2) still exhibited skewed ratios after the Bonferroni procedure ($\alpha = 1.5.10^{-3}$). The segregation of alleles at the IGS locus provided a good fit to the Mendelian 1:2:1 expectation for *M. tornata*, but a departure from the theoretical ratio was detected for *M. truncatula* at $\alpha = 0.05$, (Table 3). Overall, 40.8% and 39.4% of the markers we scored in the progeny of [F83.005-5 × DZA.045-5] and [ESP.050-4 × Tornafield-6], respectively, did not fit the expected ratio at $\alpha = 0.05$, while according to the Bonferroni procedure, only 14.3% and 24.2% of these markers, respectively, significantly departed from Mendelian expectations. Moreover, the skewed alleles carried by DZA.045-5 were systematically favoured in the F_2 progeny (Table 1), but no preference towards either parent of the *M. tornata* cross could be observed (Table 2).

Comparisons with distortion values documented in the literature

As the Bonferroni procedure has never been implemented in the papers we reviewed, all the proportions of skewness we compared were estimated at $\alpha = 0.05$. On this basis, the analysis of variance proved that segregation distortions obviously have a stronger incidence in interspecific crosses than in intraspecific ones ($F = 5.39$; $P = 0.024$). Mean values of distortions were 18.4% ± 11.0 for intraspecific crosses and 28.7% ± 17.7 for interspecific ones. The proportion of skewed loci we observed in this study clearly exceeded most of the values reported in the literature for intraspecific crosses. Pairwise comparisons showed that only 6 out of the 33 intraspecific distortion levels we could review ranged over the same order of magnitude as 40% (Table 4a), 4 of these, however, were skewed by obvious methodological bias. Conversely, 9 out of 23 interspecific distortion levels were not significantly different from 40% (Table 4b).

DNA content measurement

DNA content measured on every plant always remained constant for five replicates so that genome size estimations appeared robust (Table 5). They were also consistent with the values previously reported for *M. truncatula* (Blondon et al. 1994). Significant variations were observed between F83.005-5 and DZA.045-5. They differ in their 2C DNA contents by 0.11 pg (9%) which approximated 0.55 10^8 bp per haploid genome. The average DNA content of both *M. tornata* parental lines were also different from each other (0.17 pg). Although Tornafield exhibited an unexpected range of genome size variation, the smallest difference in DNA content we could consider (5%) was still statistically significant (Table 5).

Discussion

Our segregation analysis of PCR-derived markers revealed a high level of segregation distortions in a *M. truncatula* and a *M. tornata* intraspecific cross. In each cross, about 40% ($\alpha = 0.05$) of the markers did not fit the expected ratios (Table 1–3). According to a review of the abundant data available in the literature, these ratios are unexpectedly high for intraspecific crosses. Based on this review and the differences in

Table 1 Origin and segregation of each scored RAPD marker in the *M. truncatula* intraspecific cross

Locus[a]	Origin		Observed		Probability
	F83.005-5	DZA.045-5	AA + Aa[b]	aa	
A17-1500	0	1	71	27	0.559
A17-1300	0	1	98	8	0.006*
A17-950	0	1	95	27	0.462
A17-800	1	0	87	32	0.063
A17-480	0	1	86	35	0.319
B7-900	0	1	92	30	0.920
B7-600	1	0	85	37	0.173
D9-1400	0	1	97	22	0.100
D9-1100	0	1	100	19	0.022*
D9-550	0	1	93	26	0.334
D9-490	0	1	99	20	0.039*
D9-400	0	1	98	21	0.064
D20-1650	0	1	82	35	0.219
D20-1500	1	0	68	41	0.002*
D20-950	0	1	103	18	0.010*
D20-700	1	0	85	36	0.227
L2-1500	0	1	99	22	0.083
L2-1200	1	0	82	38	0.092
L2-950	1	0	95	26	0.371
L2-600	0	1	84	37	0.156
L2-350	1	0	81	40	0.040*
L2-320	0	1	84	35	0.265
L4-1200	0	1	113	9	0.000***
L4-1050	0	1	97	25	0.250
L4-550	1	0	78	44	0.005*
L4-400	0	1	107	15	0.002*
L4-370	1	0	31	91	0.000***
L12-900	0	1	102	20	0.028*
L12-540	1	0	80	42	0.016*
L12-520	0	1	90	32	0.752
L12-400	1	0	75	47	0.000***
M4-2900	0	1	95	27	0.462
M4-1500	1	0	59	63	0.000***
M4-900	1	0	99	23	0.117
M10-1050	0	1	91	30	0.999
M10-1000	1	0	75	46	0.000***
M10-950	0	1	92	27	0.559
M10-930	0	1	95	21	0.086
M10-550	1	0	84	38	0.116
M10-350	0	1	85	37	0.173
M10-180	1	0	77	45	0.002*
M11-1050	1	0	86	35	0.319
M11-800	0	1	85	36	0.227
M11-300	1	0	85	35	0.292
M11-210	1	0	81	40	0.040*
M12-1000	0	1	107	10	0.000***
M12-300	1	0	86	35	0.319
M12-200	1	0	77	45	0.002*

* and *** indicate significant departure from Mendelian expectations at $P < 0.05$ and after the Bonferroni procedure $P < 0.001$, respectively

[a] Nomenclature for each individual RAPD fragment describes the primer used and its fragment's size (bp)

[b] Heterozygote (Aa) and dominant homozygote (AA) classes are pooled since they are indistinguishable

DNA content that were measured, we will now discuss several distorting mechanisms.

RAPD and segregation distortion

Few mechanisms bringing distortion about have been thoroughly documented. Since RAPD markers have been suspected not to be fully repeatable, misclassification (Säll and Nilsson 1994) should obviously be considered first. Profiles often present 'minor bands' whose amplifications are prone to variations. Heun and Helentjaris (1993) emphasized that these fragments always present a higher propensity for irreproducibility and remain consequently hazardous to interpret (see

Table 2 Origin and segregation of each scored RAPD marker in the *M. truncatula* cross

Locus[a]	Origin		Observed		Probability
	ESP.050-4	Tornafield-6	AA + Aa[b]	aa	
A17-800	0	1	64	14	0.150
A19-940	1	0	29	30	0.000***
A19-880	0	1	38	22	0.036*
B1-750	0	1	68	11	0.023*
B1-600	1	0	60	19	0.842
B5-2500	0	1	47	32	0.001***
B5-1000	0	1	52	28	0.039*
B5-350	1	0	45	22	0.139
B6-1450	0	1	60	20	1.000
B6-1400	1	0	54	26	0.121
B11-1550	1	0	55	16	0.631
B11-1400	1	0	59	11	0.073
B11-150	0	1	60	20	1.000
C8-680	1	0	49	26	0.053
C8-430	1	0	63	12	0.072
J9-980	0	1	58	13	0.193
J9-960	0	1	60	9	0.022*
K2-1450	0	1	21	43	0.000***
K2-1350	1	0	49	15	0.777
K2-1250	0	1	20	43	0.000***
K2-1050	1	0	47	17	0.777
K4-520	0	1	63	17	0.438
K4-420	1	0	24	33	0.000***
K5-900	1	0	47	34	0.000***
K5-880	0	1	55	26	0.139
K5-540	0	1	65	16	0.275
K7-1100	1	0	35	35	0.000***
K7-800	0	1	38	27	0.002*
K7-490	0	1	20	59	0.000***
K7-350	1	0	62	15	0.260
K12-480	0	1	59	20	0.999
K12-460	1	0	66	13	0.079

*,*** As in Table 1
[a,b] As in Table 1

Table 3 Segregation of IGS alleles among the F_2 populations of *M. truncatula* and *M. tornata*

F_2 population	Observed			Probability
	A1A1	A1A2	A2A2	
M. truncatula	15	70	36	0.005*
M. tornata	13	37	20	0.442

* indicates significant departure from the 1:2:1 expected ratio at $P < 0.05$

also Kesseli et al. 1994). As we were aware of such risks, we took particular care to only score fragments that proved to be efficiently amplified and exhibited unambigous polymorphism. When such precautions are taken, RAPD does not induce higher levels of distortion than restriction fragment length polymorphism (RFLP) (Table 4a, b).

Each polymorphic RAPD fragment was assumed to descend from a specific locus and to be expressed as a dominant marker. Dominance was a posteriori confirmed since no heteroduplex fragment was observed (Davis et al. 1995), neither did we find evidence for the existence of a single pair of fragments that simultaneously stemmed from the same primer and fit a 1:2:1 ratio (data not shown). Moreover, none of the skewed loci displayed uniparental inheritance; this ascertained that amplified fragments were generated from neither mitochondrial nor chloroplastic DNA. In the F_2 populations, the simultaneous presence of both alleles at each locus proved that F_1 plants were heterozygous. These results check out with the chi-square null hypothesis we tested (e.g. expected 3:1 segregation). However, homoplasy, which is the amplification of 2 fragments of the same length from non-allelic regions, could be suspected for A17-1300, L4-1200 and M12-1000 in *M. truncatula*, because their segregation fit the corresponding $15(9 + 3 + 3):1$ expected ratio at $\alpha = 0.05$ (χ^2 values are 0.3, 0.26 and 1.05, respectively). The comigration of 2 different fragments from a single individual may have resulted from amplifications at paralogous loci (repetitive DNA with regularly distributed primers site). But even under this homoplasy

Table 4a Proportions of skewed markers documented in intraspecific crosses

Species	Segregating population		Main Markers		Percentage of skewed loci[b]		References
	Nature[a]	Nb	Nature	Total Nb			
Vicia faba[b]	F_2	20	RFLP		ns	nc	Torres et al. (1993)
	F_2	44	RAPD				
Pinus pinaster	Mgam.	62	RAPD	263	2.6		Plomion et al. (1995)
Brassica rapa pekinensis × *B. rapa italica*	F_2	95	RFLP	273	3.0	nc	Song et al. (1991)
Brassica oleracea italica × *B. oleracea capitata*	F_2	96	RFLP	258	6.5	nc	Slocum et al. (1990)
Phaseolus vulgaris L.	BC	?	RFLP	224	8.4	C (3)	Vallejos et al. (1992)
Phaseolus vulgaris L.	F_2	75	RFLP	152	9.0	C (4)	Nodari et al. (1993)
Lactuca sativa	F_2	66	RFLP,				
			RAPD	319	9.0	C (3)	Kesseli et al. (1994)
Hordeum vulgare (L.)	DH	113	RFLP	157	9.5	C (5)	Heun et al. (1991)
Helianthus annuus	F_2	289	RFLP	234	9.8	C (4)	Berry et al. (1995)
Brassica oleracea (collard × ssp. *botrytis*)	F_2	60	RFFL	41	9.7	nc	Kianian and Quiros
Brassica oleracea (collard × ssp. *italica*)	F_2	60	Isozyme	57	5.2	nc	1992
Brassica oleracea (wild kale × ssp. *botrytis*)	F_2	60		58	12.0	C (3)	
Brassica oleracea L.	$F_2(\times 2)$	$90 + F_3$	RFLP	201	12.0	C (2)	Landry et al. (1992)
Zea mays L.	F_2	50	RFLP	50	12.0		Helentjaris et al. (1986)
Helianthus anomalus	F_1	56	RAPD	161	13.6	C	Rieseberg et al. (1993)
Hordeum vulgare (L.)	DH	150	RFLP	295	14.0	C (4)	Kleinhofs et al. (1993)
Beta vulgaris vulgaris	F_2	96	RFLP	117	15.4	C (5)	Pillen et al. (1992)
Lactuca sativa L.	F_2	66	RFLP	53	17.0		Landry et al. (1987)
Prunus persica (L.) Batsch	F_2	96	RAPD	99	17.2		Chaparro et al. (1994)
Beta vulgaris L. ssp. *vulgaris* var	$F_1 \times F_1$	49	RAPD	50	18.0	C(4)	Barzen et al. (1995)
altissima[c]			RFLP	248	19.0		
Oryza sativa indica × *O. sativa javanica*	F_2	50	RFLP	135	18.5	C	McCough et al. (1988)
Brassica napus ssp. *oleifera*	F_2	90	RFLP	120	21.0	C(3)	Landry et al. (1991)
Brassica rapa	F_2	104	RFLP	360	23.6	C(6)	Chyi et al. (1992)
Cryptomeria japonica D. Don[c]	F_2	73	RAPD	33	21.0	C(4)	Mukai et al. (1995)
			RFLP	128	24.0		
Saccharum spontaneum L.	F_1	88	RAPD	279	25.0	C	Al-Janabi et al. (1993)
Solanum tuberosum	BC	67	RFLP	263	25.5		Gebhardt et al. (1989)
Capsicum annuum L.	DH	18	RFLP	57	17.0	C	Lefebvre et al. (1994)
	DH	94		85	22.0		
	DH	44	RAPD	61	39.0[d]		
Musa acuminata[c]	F_2	92	RFLP	58	38.0[d]	C(3)	Fauré et al. (1993)
			RAPD	28	28.5		
Medicago sativa (CADL)[c]	BC		RFLP	102	34.3[d]	C(4)	Echt et al. (1993)
			RAPD	80	36.2[d]		
Secale cereale L.	$S_1(\times 5)$	25–48	RFLP	60	34.3[d]	C	Philipp et al. (1994)
		each	RAPD				
Arabidopsis thaliana	RI (F8)	150	RAPD	292	43.0[d]		Reiter et al. (1992)
Hordeum vulgare L.	DH	71	RFLP		44.0[d]	C(4)	Graner et al. (1991)

[a] Mgam., Megagametophyte; DH, double haploid lines; RI, recombinant inbred lines
[b] C (nc) indicates that skewed marked are (are not) clustered. When documented, the number of clusters is reported between brackets
[c] When documented in the corresponding paper, RFLP and RAPD levels of distortion are specified
[d] The proportion of skewed loci is not significantly different from the values estimated in *M. truncatula* and *M. tornata* ($\alpha = 0.05$)

hypothesis for 3 markers, 16 markers (33.3%; $\alpha = 0.05$) would keep on skewing, and this ratio does not correspond to values generally observed in intraspecific crosses.

Range of variation of segregation distortions in intraspecific crosses

When estimated at $\alpha = 0.05$, the proportions of skewed markers we documented were in the range of the highest values reported for higher plants, with most of the intraspecific crosses reported previously usually exhibiting significantly lower values of distortion than 40% (Table 4a, b). Similar ratios were also reported at the within-species level (Table 4a). But, apart from *Musa acuminata* (Fauré et al. 1993) and *Arabidopsis thaliana* (Reiter et al. 1992), particular methodology biases were documented, and skewness may have resulted from either selection pressures induced by androgenetic in vitro processes (Graner et al. 1991; Lefebvre et al. 1995), the artificial accumulation of deleterious recessive

Table 4b Proportions of skewed markers documented in intraspecific crosses

Species	Segregating population		Main Markers		Percentage of skewed loci[b]		References
	Nature[a]	Nb	Nature	Total Nb			
(Vaccinium darrowi Camp. × *V. elliottii* Chapm.) × *V. elliottii* Chapm.	Test cross	38	RAPD	89	1.0	nc	Rowland and Levy (1994)
Eucalyptus grandis x *E. urophyla*	F_1	62	RAPD	272 286	3.6 3.5	nc	Grattapaglia and Sederoff (1994)
Hordeum vulgare L. × *H. spontaneum* L.	F_2	135	RFLP	160	10.0		Graner et al. (1991)
Apium graveolens L.var *rapaceum x*			RFLP	33	9.0		Yang and Quiros (1995)
A. graveolens L. var *secalinum*[c]	F_2		RAPD	128	10.0		
Glycine max. (L.) Merr. *x G. soja* (Seib et Zucc.)	F_2	60	RFLP	150	13.3		Keim et al. (1990)
Oryza sativa [*O. sativa* x *O. longistamina*]	BC	113	RFLP	588	16.0	C	Causse et al. (1994)
Avena atlantica × *Avena hirtula*	F_3	194	RFLP	194	19.0	C(2)	O'Donoughue et al. (1992)
Helianthus annuus × [*H.argophyllus* × *H. annuus*]	BC	133	RAPD	48	23.0	C(2)	Quillet et al. (1995)
Solanum phureja (Juz. et Buz.) × *S. tuberosum*	F_1	65	RFLP	134	24.0	C(6)	Bonierbale et al. (1988)
{*Citrus paradisi* × *Poncirus trifoliata*} × {*Citrus sinensis* × *Poncirus trifoliata*}	F_2	60	RFLP	37	24.3	C(3)	Jarrell et al. (1992)
Stylosanthes scraba × *S. hamata*	F_2	60	RAPD	73	24.6		Kazan et al. (1992)
Arachis stenoderma × *Arachis cardenasii*	F_2	87	RFLP	132	25.0		Halward et al. (1993)
Lens culinaris M. × *Lens orientalis*	F_2	66	RFLP	34	26.5	C	Havey and Muehbauer (1989)
Lycopersicon hirsutum L. *glabratum* f. × *Lycopersicon esculentum*	F_2	50	RFLP	50	34.0[d]		Helentjaris et al. (1986)
Citrus paradisi × [*C. reticulata* × *C. paradisi*]	BC		RFLP	42	28.5		Durham et al. (1992)
Poncirus trifoliata × [*C. grandis* × *P. trifoliata*]	BC		RFLP	57	36.8[d]		
Citrus grandis × [*C. reshni* × *Poncirus trifoliata*]	F_1	52	RFLP RAPD	51 46	39.2[d] 37.0[d]	C	Luro et al. (1994)
Lens culinaris × *Lens odemensis*	F_2		isoz.	13	46.1[d]	C	Tadmor et al. (1987)
Lens culinaris × *Lens ervoides*	F_2	107	isoz	19	47.4[d]		
Medicago sativa (CADL) × *M. coerulea*	F_2	86	RFLP	108	47.2[d]	C	Brummer et al. (1993)
Medicago coerulea × *M. quasifalcata*	F_2	138	RFLP	89	49.4[d]	C	Kiss et al. (1993)
Brassica oleracea × *B. insularis*	F_2	52	RFLP	71	59.1	C(4)	Kianian and Quiros (1992)
Lycopersicon esculentum × *L. chmielewskii*	BC	237	RFLP	70	68.5		Paterson et al. (1988)

[a–d] As in Table 4a

Table 5 DNA contents of *M. truncatula* parental genotypes and *M. tornata* parental lines

		Mean DNA content	
		Mean (pg/2C)	± SD
M. truncatula (parental genotypes)	F83.005-5	1.12	0.010
	DZA.045-5	1.23	0.001
	ESP.050[a]	1.09	0.007
		1.12	0.005
		1.13	0.008
		1.12	0.008
		1.12	0.008
M. tornata (parental lines)	Mean value	1.11	0.015
	Tornafield[a]	1.36	0.024
		1.36	0.023
		1.29	0.041
		1.23	0.007
		1.19	0.009
	Mean value	1.29	0.076

[a] Measurements were implemented on 5 plants/population and 5 replicates/plant

alleles after a diploidization step (Echt et al. 1993) or the unmasking of self-incompatibility loci following the self-fertilization of self-incompatible plants using pseudo-compatibility at high temperature (Philipp et al. 1994). None of these processes appeared to interfere with our data. On the other hand, the occurrence of chromosomal rearrangements, which have been demonstrated in *Musa acuminata*, can not be excluded (see below).

Relationship between segregation distortions and genetic divergence

It has been previously suggested that deviations should increase with the level of divergence among the parents (Zamir and Tadmor 1986). A low level of distortions in interspecific crosses is often correlated with a tight genetic proximity between the plants they originated from (Table 4b). However, it is essential to realize that

mating systems can have an important part to play in divergence within a same species. Selfing species usually exhibit a strong spatial differentiation (Hamrick and Godt 1990), as confirmed among *M. truncatula* populations by Bonnin et al. (1996). In this study, the original parents of each F_2 segregating population came from very distant populations (Southern France and Algeria for *M. truncatula* ; Australia and Spain for *M. tornata*), so that they are likely to have been diverging for a while. Statistically important differences in genome size between plants originating from each original population supports this suggestion and ascertains intraspecific diversity with distinctive genetic pools (Table 5).

Distorting factors

Departures from expected Mendelian ratios often indicate the linkage between molecular markers and distorting factors (Zamir and Tadmor 1986): the tighter the linkage, usually the similar their segregation and, thus, the more extreme the marker distortion. Skewed molecular markers will thus appear to be clustered as far as they mirror the distortion of the same distorter (Table 4a, b). Distorting factors have been described as deletorious recessive alleles (Echt et al. 1993; Berry et al. 1995), pre- or post-syngamic selected allelic combinations (Helentjaris et al. 1986; McCough et al. 1988; Causse et al. 1994; Vallejos et al. 1992; O'Donoughue et al. 1992; Pillen et al. 1992; Mukai et al. 1995), self-incompatibility alleles (Philipp et al. 1994; Barzen et al. 1995) or even structural rearrangement (Tadmor et al. 1987; Jarrell et al. 1992; Kianian and Quiros 1992; Fauré et al. 1993; Barzen et al. 1995; Quillet et al. 1995).

Nine pairs of skewed loci in *M. truncatula* and 3 pairs in *M. tornata* exhibited a non-independant segregation pattern through a χ^2 contingency analysis ($\alpha = 2.5\,10^{-4}$ or $\alpha = 3.8\,10^{-3}$ after a Bonferroni procedure) (data not shown). However, caution is advised because statistical dependance do not necessarilly imply genetic linkage since linkage disequilibrium may also be suspected. The estimates of recombination rates between 2 skewed dominant markers in F_2 populations suppose that they are affected by two selections of a different biological source (Lorieux et al. 1995). Unfortunately, it is not straightforward to check this hypothesis and, to our knowledge, no alternative method has been published so far.

Hypothetical mechanisms leading to segregation distortion in *M. truncatula* and *M. tornata*

The most classical ways to explain the high level of segregation distortions documented in this study would be either certation or the occurrence of chromosomal rearrangements between parental karyotypes. Simon and Millington (1967) have already underlined that intraspecific hybrids of *M. truncatula* or *M. littoralis* are fully fertile as far as parental strains present similar chromosome structure. Otherwise, and due to the very high selfing rate of *M. truncatula* and *M. tornata* (Heyn 1963), deleterious recessive alleles should have been purged so that they may not be responsible for any of the observed deviations. Conversely, the inbreeding system contributes to the whole genome being organized into a co-adapted gene complex with pleiotropic and epistatic relationships. One may thus assume that controlled hybridizations, which were performed to produce F_1, have promoted a burst of recombinations and dismantled this integrated system into new allelic combinations with partial or complete lethality. This hypothesis has already been suggested (Heyn 1963) for natural populations of *M. truncatula* and *M. tornata* where outcrossing events were occasional. Under this assumption, both parental forms should be predominantly favoured over recombinant ones. This is not the case for *M. truncatula*, since only the skewed male parent alleles are promoted. However, subsequent codominant markers would be necessary to definitely test this hypothesis.

Segregation distortions could also be related to the significantly different sizes of the parental genomes. Numerous studies have suggested that heterochromatin and repeated sequences may often be involved in variations in genome size (Tito et al. 1991; Smyth 1991). Regarding the nature and amounts of dispersed repeat uncovered so far, it looks as if retroelements, and notably retrotransposons, may be considered to be the best candidates for genome size expansion (Smyth 1991). But these sequences can also contribute to the ectopic pairing of chromosomes (Montgomery et al. 1991) that may generate abnormal chromosomes after recombination (Montgomery et al. 1991; Radman and Wagner 1993) and, consequently, segregation distortions.

Whatever the nature of these repeated sequences, this last hypothesis is very tempting, as a similar cross between 2 *M. truncatula* plants, stemming from an Australian and an Algerian population but presenting amounts of DNA of the same magnitude (1.15 and 1.17pg), led to less than 10% of segregation distortions (unpublished results). This hypothesis is also consistent with the promotion of the male alleles in the *M. truncatula* cross. When genomes are brought together in a hybrid, the overall genetic background may promote the release, amplification and/or redistribution of retroelements (Price et al. 1983). These rearrangements should have a major deleterious impact on the smallest genome (in this study, F83.005-5), since random insertions have a greater chance to disrupt necessary genes (Smyth 1991). On the other hand, Price et al. (1983) have pointed out that the F_2 DNA contents of an intraspecific cross of *Microseris douglasii* were distributed below the midparent value. In maize, female

heterozygotes which contain both a standard and a knobbed chromosome 10 (due to a large heterochromatic segment) provide a preferential maternal transmission of the knobbed chromosome (Rhoades 1952). If this is a general result, then the predominance of male alleles in the *M. truncatula* cross could be explained by the large genome size of DZA.045-5. Notice, however, that the preferential transmission of small genomes have also been reported (Hutchinson et al. 1979; Price et al. 1983).

To our knowledge, although some select papers have attempted to analyse the inheritance of DNA contents (Hutchinson et al. 1979; Price et al. 1983), the relationship between genome size and segregation distortions has never been studied so far. This would be an exciting task, whose first steps will concern a fine mapping of distorted segments, a cytological study of parental genotypes and an analysis of the segregation of DNA amounts. Our study has also stressed that intraspecific crosses between almost totally self-fertilizing plants require special consideration, since divergence may take place sooner. If so, segregation distortions are likely to occur, and subsequent genetic studies could be strongly biased.

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