Segregation and Recombination in Inter-Gene Pool Crosses of *Phaseolus vulgaris* L.

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Variation for segregation ratios and recombination rates was analyzed in crosses involving 13 common bean (*Phaseolus vulgaris* L.) genotypes from different gene pools and races. The segregation of RFLP, isozyme, and phaseolin markers belonging mostly to linkage groups D5 and D7 was analyzed in 11 F_2 populations derived from Middle American × Andean, and Andean × Andean crosses. An average level of 10.4% segregation distortion for RFLPs was observed compared with 19.2% for isozyme markers. Preferential transmission of Middle American alleles was detected in crosses between the two gene pools. Levels of recombination were highly variable among a subset of four of the 11 F_2 populations analyzed. These data, and comparisons with data published previously, suggest that recombination in common bean is under genetic control, although environmental factors may also affect it.

The accuracy of predictions regarding gene transfer in sexual crosses depends in part on information on segregation and recombination. An analysis of these two factors in various areas of the genome depends on the availability of loci marking these areas. Molecular markers identifying such loci are particularly useful for this type of study because they are more numerous and offer a better genome coverage than phenotypic markers. In addition, they segregate in a predictable way (i.e., in a dominant or co-dominant, single-gene fashion) and are usually devoid of deleterious or epistatic effects. Hence, deviations from expected segregation ratios can potentially be attributed to linkage to factors affecting gene transmission (in addition to deviations due to chance).

Various types of molecular markers have been developed in common bean (*Phaseolus vulgaris* L.). These include isozymes (Koenig and Gepts 1989a,b; Weeden 1984, 1986), seed proteins (Brown et al. 1981, 1982; Gepts et al. 1986; Romero-Andreas et al. 1986), RFLPs (Nodari et al. 1992), and RAPDs (Haley et al., in press; Miklas et al. 1993; Skroch et al. 1992). Low-density linkage maps of common bean have been constructed using these markers (e.g., Gepts et al. 1993; Nodari et al. 1993a; Vallejos et al. 1992). Availability of these markers has also allowed a better understanding of the organization of genetic diversity in *P. vulgaris* (reviewed in Gepts 1990, 1993; Gepts and Debouck 1991). Before its domestication, the species had diverged into two geographical gene pools in Middle America and the Andes. This divergence is most clearly suggested by the existence of reproductive isolation between these two gene pools (Koinange and Gepts 1992). Separate domestications in the two areas led to two cultivated gene pools. As a consequence of multiple domestications within the two areas or because of divergence subsequent to domestication, at least three races have been identified in each of the two cultivated gene pools (Singh et al. 1991a).

In the work reported here, we have analyzed segregation and linkage for a group of molecular markers located mainly on two linkage groups in inter-gene pool and interracial crosses of common bean. Our main findings were the existence of segregation distortion favoring Middle American alleles over Andean alleles and high levels of variation for recombination.

Materials and Methods

Plant Material

We carried out a crossing program involving two Chilean common bean landraces (Coscorrón and Tórtola) and 11 other common bean genotypes in a greenhouse in Davis during 1990 and 1991. In fall 1991, the F_1 generations were grown in close proximity in the greenhouse under con-

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Journal of Heredity 1995;86:98–106; 0022-1503/95/$5.00

trolled temperature conditions (20°C–25°C) and short days (<13 h of daylight). This was done to standardize as much as possible the growing condition of the F_1s during the reproductive (meiotic) phase and to avoid problems of photoperiod sensitivity that could be present in some of the genotypes. In 1992, the 12 F_2 populations were also grown under greenhouse conditions to study the type of segregation and levels of recombination in these populations using isozyme and DNA markers. The 12 F_2 populations, each comprising 75–80 individuals, were derived from the following crosses (with, in parentheses, their gene pool: M for Middle American; A for Andean): Brazil-2 (M) × Tórtola (A), Coscorrón (A) × Sal (M), Tórtola (A) × Pinto UI 114 (M), Coscorrón (A) × México 222 (M), Tórtola (A) × Rosa de Castilla (M), Coscorrón (A) × Flor de Mayo (M), Tórtola (A) × CDRK (A), Coscorrón (A) × Jalo EEP558 (A), Tórtola (A) × Coscorrón (A), Coscorrón (A) × Araucano (A), Tórtola (A) × Bola (A), and Coscorrón (A) × Bolón Bayo (A).

Phaseolin and Isozyme Analyses

Phaseolin seed protein and isozyme analyses were carried out following the procedures described by Gepts et al. (1992). We selected the eight isozyme systems used in this study based on the polymorphism reported in previous work (Koenig and Gepts 1989a,b; Singh et al. 1991b). These were diaphorase (DIAP), glucose phosphate isomerase (GPI), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), shikimate dehydrogenase (SKDH), the small subunit of ribulose bisphosphate carboxylase (RBCS), and aconitase (ACO). In each gel, the cultivars ICA-Pijao and California Dark Red Kidney (CDRK) were included as Middle American and Andean checks, respectively (Koenig and Gepts 1989a,b).

DNA Isolation and Digestion, Southern Blotting, and Hybridization

The DNA extraction, Southern blotting, and hybridization followed the procedure described by Gepts et al. (1992) with some modifications. We cross-linked membranes in an ultraviolet chamber (GS Gene Linker, Bio Rad), using the program C3 (Southern damp membrane, 150 mJoule). The hybridization protocol was modified to allow the use of a hybridization oven incubator (Robbins Scientific, Model 400). The prehybridization and hybridization solutions were the same and were prepared with 20% SDS (50%), 20% SSPE (25%), and Milli-Q water (25%). Membranes were prehybridized for 4 h in glass falcon tubes with 25 ml of prehybridization solution at 65°C. Probes labeled with ^{32}P (1–15 × 10^8 cpm) were added to 7–10 ml of hybridization solution, and the mixture was added to the falcon tube. Membranes were hybridized for at least 12 h at 65°C. After hybridization, the hybridization solution was recovered and reused for two or three more times. The first two washes were done by manually shaking three or four times the falcon tube containing a solution (100–150 ml) of 2 × SSC, 0.1% SDS at room temperature. The third wash was also done in the falcon tubes using 0.1 × SSC, 0.1% SDS at 65°C for 30 min. Finally, membranes were transferred to a plastic container for their fourth and final wash. This wash was carried out on an orbital shaker at 60°C for 30 min, using 0.1 × SSC, 0.1% SDS. X-Omat x-ray film was exposed to the washed membranes for 1–4 days.

Isolation of Plasmids, DNA Digestion, and Purification

We used a total of 21 genomic probes in this study. Sixteen of these probes originated in two *Pst*I libraries: one developed at Davis (D clones) and the other at Florida (prefixed Bng) (Nodari et al. 1992; Vallejos et al. 1992). These were D1031, D1080, D1107, D1157, D1198, D1251, D1301, D1390, D1461, D1492, D1861, Bng060, Bng153, Bng191, Bng199, and Bng211. Two clones, D0133 and D0190, originated in an *Eco*RI-*Bam*HI library developed at Davis (Nodari et al. 1992), and two, P1073 and P1091, originated in an *Mbo*I library developed at Paris (Adam et al. 1992). In addition, a chalcone isomerase (CHI) probe from Mehdy and Lamb (1987) was also used. Plasmids containing the DNA insert selected previously were isolated by the boiling method (Holmes and Quigley 1981). Isolated plasmids from *Escherichia coli* colonies were digested with *Eco*RI and *Bam*HI or *Pst*I restriction enzymes and resolved on a 0.8% low melting agarose gel. The isolated inserts were stored at 4°C. DNA from the 13 parents was purified with a cesium chloride gradient (Sambrook et al. 1989) to facilitate the scoring of polymorphisms. Genomic DNA samples (5–10 μg) from parents and F_2 plants were digested with *Eco*RI, *Eco*RV, or *Hin*dIII restriction enzymes, chosen because they revealed the highest level of polymorphism between the two gene pools (Nodari et al. 1992).

Statistical Analyses

Linkage analysis was performed with Mapmaker (Lander et al. 1987) and Linkage-1 (Suiter et al. 1983). We used tests of heterogeneity and standard error to detect statistical differences between recombination values across populations (Allard 1956).

Results

Most of the DNA clones selected to study segregation and recombination in common bean crosses mapped to linkage groups D5 and D7 (Gepts et al. 1993; Nodari et al. 1993a) (Figure 1). These linkage groups were chosen because preliminary results had shown polymorphism for seed protein, isozyme, and RFLP markers on these groups among the common bean genotypes used in this study. In addition, linkage group D7 carries genes controlling several traits of agronomic importance such as phaseolin seed protein (*Phs* locus), seed size, resistance to common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli*, and nodulation by *Rhizobium tropici* (Nodari et al. 1993b). All clones were hybridized to membranes carrying genomic DNA from the thirteen parents and digested with *Eco*RI, *Eco*RV, or *Hin*dIII. Only two clones (D1390 and P1073) did not show a clear banding pattern and were discarded from further evaluations. Furthermore, no allelic variation was observed for clones D0133, D1251, and D1861 for any of the parents included in this study.

We screened the 13 parental genotypes for eight isozyme systems, but only five systems (SKDH, ME, DIAP, ACO, MDH) showed a clear polymorphism between the parents and were selected for further evaluation. The five isozyme marker loci (*Skdh, Me, Diap-1, Aco-2,* and *Mdh-1*) mapped to four different regions of the common bean genome: *Skdh* mapped to linkage group D3, *Me* to D4, and *Diap-1* and *Aco-2* to D5 (Nodari et al. 1993a). *Mdh-1* was located on linkage group A by Vallejos et al. (1992), which corresponds to linkage group D7 (Gepts et al. 1993).

Segregation of Isozyme and DNA Markers

Of the five polymorphic isozyme systems assayed, two isozyme systems, ACO and MDH, revealed loci with normal segregation (*Aco-2* and *Mdh-1*), whereas the three other systems, SKDH, ME, and DIAP, revealed loci with distorted segregation in some of the crosses (Table 1). Segregation

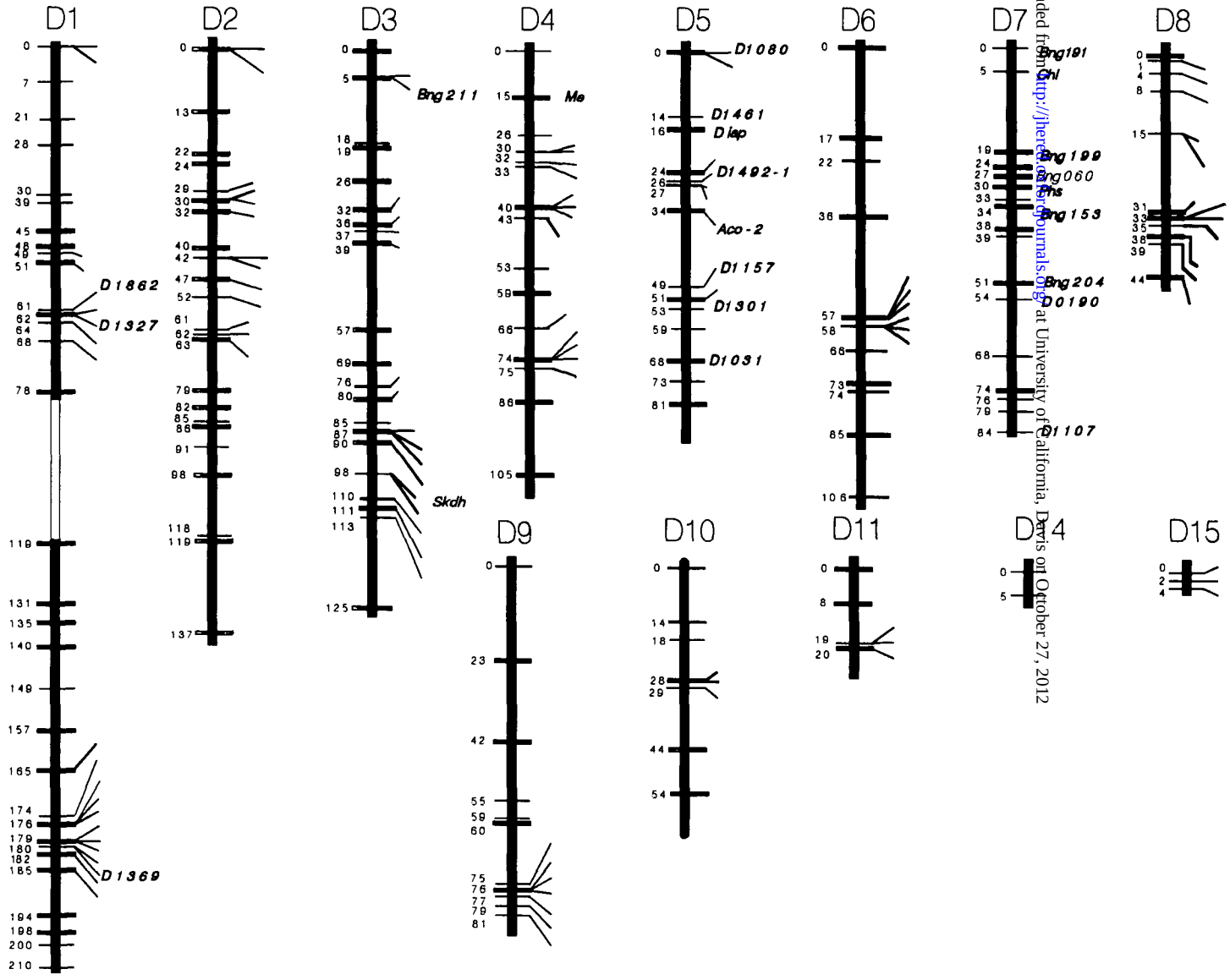

Figure 1. Linkage map of common bean (Gepts et al. 1993) showing the map distribution of markers analyzed in this study. Linkage groups D12 and D13 have been attached to linkage groups D6 and D7, respectively. Distances are in Kosambi map units.

Table 1. Segregation and chi-square goodness-of-fit tests for RFLP and isozyme markers in F_2 populations derived from crosses between and within gene pools

Locus	Observed segregation numbers	Expected segregation ratio	χ^2	P
Middle American × Andean				
Cross: Tórtola (A)[a] × Brazil 2 (M)[a]				
Linkage group D3				
Skdh	17:33:22	1:2:1	1.94	.55
Bng211	16:29:15	1:2:1	0.84	.66
Linkage group D4				
Me	52:20	3:1	0.30	.59
Linkage group D5				
D1080	54:17	3:1	0.04	.83
D1461	15:33:17	1:2:1	0.13	.93
Diap-1	21:33:15	1:2:1	0.17	.56
D1492	8:34:21	1:2:1	5.76	.06
Aco-2	18:37:17	1:2:1	0.08	.96
D1157	10:40:15	1:2:1	4.23	.12
D1031	20:33:16	1:2:1	0.59	.74
Linkage group D7				
Bng191	16:31:22	1:2:1	1.75	.42
Chl	41:29	3:1	10.07	.00
Bng199	17:33:17	1:2:1	0.01	.99
Bng060	12:41:19	1:2:1	2.75	.25
Phs	16:30:25	1:2:1	3.99	.14
Bng153	14:42:14	1:2:1	2.80	.25
D0190	43:21	3:1	2.08	.15
D1107	19:35:8	1:2:1	5.42	.07
Cross: Sal (M) × Coscorrón (A)				
Linkage group D3				
Skdh	19:32:16	1:2:1	0.40	.81
Linkage group D4				
Me	38:32	3:1	32.01	.00
Linkage group D5				
D1080	38:33	3:1	17.48	.00
Diap-1	28:34:8	1:2:1	11.49	.00
D1492	28:25:8	1:2:1	15.09	.00
Aco-2	16:33:20	1:2:1	0.59	.74
D1157	24:32:11	1:2:1	5.18	.75
D1301	22:34:12	1:2:1	0.74	.69
D1031	20:34:15	1:2:1	2.94	.23
Linkage group D7				
Bng191	17:30:18	1:2:1	0.41	.81
Chl	51:17	3:1	0.00	1.00
Bng199	16:33:14	1:2:1	0.26	.87
Phs	18:35:15	1:2:1	0.32	.85
Bng153	12:38:18	1:2:1	2.00	.37
Cross: Pinto UI 114 (M) × Tórtola (A)				
Linkage group D3				
Skdh	15:36:15	1:2:1	0.54	.76
Bng211	20:39:11	1:2:1	3.22	.20
Linkage group D4				
Me	29:27:14	1:2:1	10.10	.00
Linkage group D5				
D1080	47:23	3:1	2.30	.12
D1461	14:27:18	1:2:1	0.96	.61
Diap-1	19:35:15	1:2:1	0.47	.79
Aco-2	17:37:15	1:2:1	0.47	.79
D1157	26:26:14	1:2:1	7.33	.26
D1301	23:30:11	1:2:1	4.75	.09
D1031	19:31:17	1:2:1	0.49	.78
Linkage group D7				
Bng191	19:29:19	1:2:1	1.21	.55
Chl	52:18	3:1	0.01	.89
Bng060	16:33:18	1:2:1	0.13	.93
Phs	19:32:16	1:2:1	0.40	.82
Bng153	16:36:14	1:2:1	0.67	.72
Bng204	18:38:13	1:2:1	0.88	.62
D0190	44:18	3:1	0.53	.46
Cross: México 222 (M) × Coscorrón (A)				
Linkage group D3				
Skdh	13:27:10	1:2:1	0.68	.71
Bng211	9:28:8	1:2:1	2.73	.25
Linkage group D4				
Me	13:24:14	1:2:1	0.22	.90
Linkage group D5				
D1080	38:12	3:1	0.06	.79
D1461	18:22:10	1:2:1	3.28	.19
Diap-1	18:19:14	1:2:1	3.94	.14
D1198	14:15:16	1:2:1	5.20	.08
D1157	14:22:9	1:2:1	1.13	.57
D1301	12:24:12	1:2:1	0.00	1.00
Linkage group D7				
Bng191	17:28:4	1:2:1	7.90	.02
Chl	40:11	3:1	0.32	.57
Bng199	14:28:7	1:2:1	3.00	.22
Bng060	13:24:14	1:2:1	0.22	.90
Phs	33:10	3:1	0.06	.79
Bng153	6:34:10	1:2:1	7.12	.03
Bng204	15:19:13	1:2:1	1.89	.39
D0190	33:16	3:1	1.53	.22
Cross: Tórtola (A) × Rosa de Castilla (M)				
Linkage group D3				
Skdh	7:49:16	1:2:1	11.63	.00
Linkage group D5				
D1080	38:31	3:1	14.61	.00
Diap-1	4:32:36	1:2:1	20.33	.00
Aco-2	18:36:18	1:2:1	0.00	1.00
D1157	12:35:15	1:2:1	1.32	.52
Linkage group D7				
Chl	50:21	3:1	0.79	.37
Bng153	11:39:15	1:2:1	3.10	.21
Andean × Andean				
Cross: CDRK × Tórtola				
Linkage group D3				
Bng211	17:35:18	1:2:1	0.03	.99
Linkage group D5				
D1080	47:21	3:1	1.25	.26
D1031	17:31:19	1:2:1	0.49	.78
Linkage group D7				
Chl	19:31:18	1:2:1	0.56	.76
Cross: Coscorrón × Jalo				
Linkage group D5				
Aco-2	18:34:20	1:2:1	0.33	.85
D1031	39:19	3:1	1.86	.17
Linkage group D7				
Phs	49:19	3:1	0.31	.57
Cross: Tórtola × Coscorrón				
Linkage group D5				
Aco-2	19:35:18	1:2:1	0.08	.96
Cross: Coscorrón × Araucano				
Linkage group D7				
Chl	16:30:14	1:2:1	1.32	.52
D0190	47:19	3:1	0.51	.48
Cross: Tórtola × Bola				
Linkage group D5				
D1031	16:37:13	1:2:1	1.2	.54
Linkage group D7				
Phs	12:33:22	1:2:1	3.00	.22
D0190	42:19	3:1	1.22	.27
Cross: Bolón Bayo × Coscorrón				
Linkage group D7				
Phs	36:34	3:1	20.74	.00
Mdh-1	22:31:15	1:2:1	1.97	.37

[a] A = Andean; M = Middle American.

distortion in the F_2 populations resulted mostly from an overrepresentation of the Middle American alleles, whether they originated from the paternal or maternal parents (e.g., *Diap-1* in the crosses Sal × Coscorrón and Tórtola × Rosa de Castilla). In some cases, segregation distortion resulted from an excess of heterozygotes (e.g., *Skdh* in the cross Tórtola × Rosa de Castilla).

Most of the DNA markers displayed simple hybridization patterns and showed fewer than two bands. Only a few of those markers showed more than three bands. In all cases, one segregating locus could be scored. The examination of the RFLP data revealed that only a small number of loci in the F_2 populations deviated significantly from the expected Mendelian ratios. The average proportion of distorted segregation in the two linkage groups in the 11 F_2 populations analyzed was 10.4% for RFLP data and 19.2% for isozyme data (Table 2). Segregation distortion in populations derived from crosses between Middle American and Andean genotypes was 12.2% for RFLP data and 22.7% for isozyme data. In F_2 populations derived from Andean × Andean crosses, segregation distortion for RFLP data was 5.6% and for isozyme data could not be detected (Table 2). However, the latter percentages could be biased due to the small number of polymorphic markers detected in these populations.

Analysis of allele frequencies in all populations derived from crosses between genotypes from Andean and Middle American gene pools showed a higher than expected frequency of alleles from the Middle American gene pool (Table 3). The magnitude of the excess of Middle American alleles varied among loci. In general, loci on linkage group D5 were more affected than those on linkage group D7 (Table 3). The consistency of these results across populations, however, could indicate the presence of a common mechanism that could be present in many, but not all, Mid-

Table 2. Total polymorphic markers and number of distorted isozyme and DNA markers in F_2 populations derived from crosses between genotypes from different gene pools

	Polymorphic markers		Distorted markers	
	RFLP	Isozymes and phaseolin	RFLP	Isozymes and phaseolin
Andean × Middle American				
Race Chile × Race Mesoamerica				
Tórtola × Brazil 2	12	5	1	0
Sal × Coscorrón	9	5	2	2
Total	21	10	3	2
Race Durango × Race Chile				
Pinto UI 114 × Tórtola	11	5	0	1
México 222 × Coscorrón	13	4	2	0
Total	24	9	2	1
Race Chile × Race Jalisco				
Tórtola × R. de Castilla	4	3	1	2
Total Andean × Middle American	*49*	*22*	*6*	*5*
%			*12.2*	*22.7*
Andean × Andean				
Race Nueva Granada × Race Chile				
CDRK × Tórtola	4	0	0	0
Coscorrón × Jalo	1	2	0	0
Total	5	2	0	0
Race Chile × Race Perú				
Tórtola × Bola Bolón Bayo	5	1	0	0
× Coscorrón	2	0	1	0
Total	7	1	1	0
Race Chile × Race Chile				
Tórtola × Coscorrón	0	1	0	0
Coscorrón × Araucano	6	0	0	0
Total	6	1	0	0
Total Andean × Andean	*18*	*4*	*1*	*0*
%			*5.6*	*0*
General average	*67*	*26*	*7*	*5*
%			*10.4*	*19.2*

Table 3. Proportion of Middle American (M) and Andean (A) alleles in F_2 population derived from crosses between genotypes from different gene pools

Cross	No. of loci	No. of alleles[a]	Proportion of Middle American alleles (%)	χ^2	df	Significance[b]
Linkage group D5						
Tórtola (A) × Brazil 2 (M)	6	806	51.1	0.402	1	ns
Sal (M) × Coscorrón (A)	6	808	57.9	20.28	1	***
Pinto UI114 (M) × Tórtola (A)	6	788	53.6	3.98	1	*
México 222 (M) × Coscorrón (A)	5	478	53.1	1.88	1	ns
Tórtola (A) × Rosa de Castilla (M)	3	412	58.5	11.89	1	***
Pooled data	26	3292	54.6	27.71	1	***
Homogeneity				10.72	4	*
Linkage group D7						
Tórtola (A) × Brazil 2 (M)	5	698	53.2	2.774	1	ns
Sal (M) × Coscorrón (A)	4	528	49.6	0.03	1	ns
Pinto UI 114 (M) × Tórtola (A)	4	534	50.6	0.067	1	ns
México 222 (M) × Coscorrón (A)	5	492	53.5	2.35	1	ns
Tórtola (A) × Rosa de Castilla (M)	1	130	53.1	0.492	1	ns
Pooled data	19	2382	51.8	3.251	1	ns
Homogeneity				2.462	4	ns

[a] Number of loci × number of individuals in the population × 2.
[b] ns = not significant.
* Significant at $P = .05$.
*** Significant at $P = .001$.

dle American × Andean genotype crosses. A previous study in beans did not detect any difference in allele frequency in a cross between a Middle American and an Andean genotype (Nodari et al. 1993a). Distorted segregation for molecular markers has been reported for several regions of the common bean linkage map (Koinange 1992; Nodari et al. 1993a; Vallejos et al. 1992). It has been detected in clusters in specific regions of the different linkage groups as well as for isolated markers (Koenig and Gepts 1989a; Koinange 1992; Nodari et al. 1992; Vallejos et al. 1992).

An analysis of additional markers that had shown segregation distortion in other studies (Nodari et al. 1993a) confirmed that segregation distortion is related to the genetic background of the population studied. Markers D1107 (located on linkage group D7), D1862, D1327, and D1369 (located on linkage group D1) had shown segregation distortion in the cross BAT93 (Middle America) × Jalo EEP558 (Andes) (Nodari et al. 1993a). Markers D1107 and D1862 did not show segregation distortion in the populations evaluated here, whereas markers D1327 and D1369 showed a skewed segregation. Marker D1369 did not exhibit a consistent behavior, in that it showed segregation distortion in one population (Pinto UI 114 × Tórtola) but not in the other (Sal × Coscorrón) (data not shown).

Variability in Recombination Values Among F_2 Populations

The analysis of highly polymorphic populations derived from crosses between genotypes from the Middle American and Andean gene pools showed a wide range of estimated recombination values (r) among populations in linkage groups D5 and D7 (Tables 4 and 5). The distribution of recombination values across populations varied depending on the markers analyzed. For instance, independent assortment between loci *D1157* and *Aco-2*, included in linkage group D5, was observed across the three populations that presented polymorphism for these markers (Tórtola × Brazil2, $r = .44$; Sal × Coscorrón, $r = .47$; Pinto UI 114 × Tórtola, $r = .49$). Markers *D1157* and *Aco-2* had previously been shown to be linked with a recombination value of $r = .16$ (Nodari et al. 1993a). The recombination values between markers *Phs* and *Bng060* of linkage group D7 were smaller across the polymorphic populations evaluated (Tórtola × Brazil2, $r = .07$; Pinto UI114 × Tórtola, $r = .10$; México × Coscorrón, $r = .18$) (Table 5). A recombination value of .015 had been obtained earlier between these two loci in population BAT93 × Jalo EEP558 (Gepts et al. 1993; Nodari et al. 1993a). On linkage group D5, marker *D1080* was linked to *D1461* in the cross Pinto UI 114 × Tórtola ($r = .20$) and in the cross México 222 × Coscorrón ($r = .19$) but was not linked in the cross Tórtola × Brazil 2 ($r = .46$). A recombination value of $r = .14$ had been obtained previously for this interval (Nodari et al. 1993a). On linkage group D7, marker *Bng199* was not linked to *Chl* in the cross Tórtola × Brazil 2 ($r = .37$), but it was in the crosses Sal × Coscorrón ($r = .23$) and México 222 × Coscorrón ($r = .10$). In the cross BAT93 × Jalo EEP558, a recombination value of $r = .14$ had previously been obtained (Nodari et al. 1993a).

In general, the gene order in the different populations was maintained. However, there were a few cases in which the order was modified. These cases involved closely adjacent markers such as markers *Diap-1* and *D1461* on linkage group D5 in population México 222 × Coscorrón and markers *Bng060, Phs,* and *Bng153* on linkage group D7 in population México 222 × Coscorrón (Tables 4 and 5).

Discussion

Differential Transmission of Alleles

The presence of a small percentage of markers that showed segregation distor-

Table 4. Recombination frequency differences in linkage group D5 in five F_2 populations of common bean

Locus[a]	F_2 population[b] BaJ (n = 75)	TBr (n = 72)	SC (n = 72)	PT (n = 70)	MC (n = 51)
D1080			—[d]		*D1080*
	0.14 (0.05)[c]	>0.40 (0.07)		0.20 (0.05)	0.19 (0.06)
	6.28	<0.5		4.09	3.82
D1461					*Diap-1*
	0.02 (0.01)	>0.40 (0.06)	—	0.18 (0.04)	0.25 (0.05)
	24.83	<0.5		6.06	4.04
Diap-1					*D1461*
	0.08 (0.02)				0.37 (0.06)
	16.67	—	—	—	1.69
D1198					*D1198*
	0.02 (0.01)		—	—	—
	12.76	—			
D1492					
	0.05 (0.03)	>0.40 (0.06)	>0.40 (0.06)	—	—
	10.24	<0.5	<0.5		
Aco-2					
	0.16 (0.04)	>0.40 (0.06)	>0.40 (0.06)	>0.40 (0.06)	—
	7.66	<0.5	<0.5	<0.5	
D1157			*D1301*		
	0.04 (0.02)	—	0.18 (0.04)	0.19 (0.04)	0.29 (0.05)
	18.95		7.61	7.63	2.00
D1301			*D1157*		
	0.09 (0.03)	—	0.16 (0.04)	0.09 (0.03)	—
	13.15		9.04	13.93	
D1031			*D1031*		

[a] Loci are listed in the order defined in population BJ (Gepts et al. 1993). The order of loci in the other populations is the same unless otherwise noted.

[b] BaJ = BAT93 × Jao EEP558 (Nodari et al. 1993); TBr = Tórtola × Brazil 2; SC = Sal × Coscorrón; PT = Pinto UI114 × Tórtola; MC = México 222 × Coscorrón.

[c] Top line is the recombination frequency (SE); bottom line is the LOD score.

[d] Recombination could not be measured because of monomorphism at one or both loci of the interval.

Table 5. Recombination frequency differences in linkage group D7 in five F_2 populations of common bean

Locus[a]	F_2 population[b] BaJ (n = 75)	TBr (n = 72)	SC (n = 72)	PT (n = 70)	MC (n = 51)
Bng191				*Chl*	
	0.05 (0.03)[c]	>0.04 (0.07)	>0.40 (0.07)		>0.40 (0.07)
	13.24	<0.5	<0.5		<0.5
Chl				*F191*	
	0.18 (0.05)	>0.40 (0.07)	0.23 (0.06)		0.10 (0.04)
	5.12	<0.5	3.07		5.39
Bng199				—[d] 29.4 (0.07)	
	0.10 (0.03)	0.11 (0.03)	—[e]	3.75	0.24 (0.06)
	13.06	12.31			2.61
Bng060				*Bng060*	*Bng153*
	0.02 (0.01)	0.07 (0.02)	—	0.10 (0.03)	0.33 (0.08)
	24.99	18.08		13.67	0.88
Phs					*Phs*
	0.03 (0.02)	0.13 (0.03)	0.12 (0.03)	0.06 (0.02)	0.18 (0.06)
	18.83	11.83	11.60	17.65	3.16
Bng153					*Bng060*
	0.15 (0.04)	—	—	0.08 (0.02)	0.22 (0.05)
	7.80			14.82	4.79
Bng204					
	0.05 (0.03)	—	—	>0.40 (0.08)	0.35 (0.08)
	9.01			<0.50	1.20
D0190					

[a] Loci are listed in the order defined in population BJ (Gepts et al. 1993). The order of loci in the other populations is the same unless otherwise noted.

[b] BaJ = BAT93 × Jao EEP558 (Nodari et al. 1993); TBr = Tórtola × Brazil 2; SC = Sal × Coscorrón; PT = Pinto UI114 × Tórtola; MC = México 222 × Coscorrón.

[c] Top line is the recombination frequency (SE); bottom line is the LOD score.

[d] Marker *Bng199* was not polymorphic in population SC.

[e] — = Recombination could not be measured because of monomorphism at one or both loci of the interval.

tion in crosses between Andean genotypes indicates that this phenomenon can also be present among more closely related common bean genotypes. Different proportions of distorted segregation have been reported in the literature, depending on the genetic diversity of the parents involved in the cross. For instance, in interspecific crosses of lentil, pepper, and tomato, Zamir and Tadmor (1986) observed segregation distortion in 54% of the loci compared to 13% in intraspecific crosses. Results from intraspecific crosses in other crops show frequencies of distorted segregation of 13% in soybean (Keim et al. 1990), 9–22% in common bean (Koinange 1992; Nodari et al. 1993a), 17% in lettuce (Landry et al. 1987), 10% in barley (Heun et al. 1991), 19% in rice (McCouch et al. 1988), and 26% in diploid *Solanum tuberosum* (Gebhardt et al. 1988).

Allelic frequencies for loci segregating according to a 1:2:1 ratio in F_2 populations showed an excess of the Middle American allele in most populations, especially on linkage group D5 (Table 3). On this linkage group, the overrepresentation of the Middle American genotypes ranged from 1.1% to 8.5% depending on the population (Table 3). On the other hand, the excess of Middle American alleles occurred whether they originated in the paternal or maternal parent (Table 3). Similar results were reported in common bean by Koenig and Gepts (1989a), in soybean by Keim et al. (1990), and in rice by McCouch et al. (1988). This argues against a role of nuclear–cytoplasmic interactions on the distortion mechanism.

These results also suggest that the markers located on linkage groups D5 and D7 are differentially affected by this phenomenon. Whereas markers on both linkage groups showed an excess of Middle American alleles, only the segregations on linkage group D5 showed statistically significant distortions. It is possible, however, that the segregation distortion is cumulative because the underlying mechanism of the distortion is expected to operate beyond the F_1 generation. The magnitude of the distortion may therefore become larger in advanced generations. Loci on linkage groups D5 and D7 mark genomic regions carrying genes involved in characters of economic, nutritional, and agronomic importance such as nitrogen fixation and bacterial blight resistance, phaseolin, dormancy, pod length and width, and seed weight (Brown et al. 1981; Koinange 1992; Nodari et al. 1993a,b; Vallejos and Chase 1991a,b). These results

could explain why certain traits are more difficult to recover from crosses between members of the Middle American and Andean gene pools.

Levels of Recombination

Our most striking result was that recombination values in the F_2 populations analyzed were highly variable within each linkage group (Tables 4 and 5). Differences in linkage relationships in common bean have been reported previously. For example, in common bean, linkage differences between *Rbcs* and *Me* have been reported in different populations (Koenig and Gepts 1989a; Koinange 1992; Nodari et al. 1993a; Weeden 1984, 1986). Similar results have been reported in studies of morphological markers in bean (Allard 1963), tomato (Butler 1968), and barley (Säll 1990; Säll et al. 1990); and of molecular markers in pea (Ellis et al. 1992) and maize (Beavis and Grant 1991; Tulsieram et al. 1992). Other reports have shown that differences in recombination rate can be detected among plants of the same genotype (Allard 1963; Stadler 1926) and between F_2-derived families (Tulsieram et al. 1992). Genetic differences in estimated recombination frequencies between genotypes can be associated with heterochromatic regions (Rhoades 1978), genes controlling chiasma formation and distribution (Butler 1968; Jones 1967, 1974; Lelley 1978), specific genes controlling recombination in certain areas of the genome (Tulsieram et al. 1992), and environmental effects (Allard 1963; Jensen 1981; Powell and Nilan 1963).

Because our F_1 and F_2 populations were grown simultaneously and adjacently under environmentally controlled conditions (greenhouse), the differences in recombination ratios found between populations for the same markers probably have a genetic component. However, we cannot discard at this point the influence of the environment on recombination levels. Allard (1963) indicated that the environmental conditions prevailing during meioses are critical factors influencing recombination in lima bean. In the same study, he also showed an interaction between different genotypes and environmental conditions. However, in order to estimate the genetic, environmental, and the genetic × environment component of variation, the segregating populations should be evaluated in replicated trials in different environments. These are very cumbersome experiments that call for a simplification in the current marker technology.

The analysis of linkage relationship between markers showed several differences with the bean genetic linkage map (Nodari et al. 1993a). These differences could be attributed to genetic differences in recombination values detected between genotypes, environmental influences, and differences in population size in which markers were scored. Because common bean chromosomes are small (Smartt 1990), the genetic structure of the chromosomes is unknown, therefore, we cannot discard a priori the potential presence of some kind of chromosomal rearrangements that could also be influencing the linkage relationships between markers in different populations. Similar results have been reported in maize (Beavis and Grant 1991; Tulsieram et al. 1992), pea (Ellis et al. 1992), and Drosophila (Bridges 1915).

The evolutionary significance of such variation for recombination levels sensu stricto (i.e., crossing over at meiosis) remains to be determined. It is but one of the factors involved in recombination sensu lato as defined by Grant (1975), which include also among others the reproductive system, population size, and the pollination system. High levels of recombination may be important in a species like *P. vulgaris* that is predominantly self-pollinating. Recombination after occasional outcrosses may release new allele combinations that provide adaptations to new environments. Empirical studies relating the various factors of the recombination system, including crossing-over frequency, could shed light on the relationships among these various factors.

Implications for Bean Breeding

The presence of different levels of recombination between common bean genotypes has implications in common bean breeding programs. For example, it could influence the process of selection of parental genotypes. Higher recombination values in crosses between Middle American and Andean genotypes could increase the probability of finding favorable recombinants and facilitate the improvement of a desirable character(s). Unfortunately, due to the low level of polymorphism detected within the Andean gene pool, we were not able to compare levels of recombination in crosses between genotypes from the Middle American and Andean gene pools with those in crosses within the Andean gene pool. On the other hand, we found no clear association between levels of recombination and membership in any particular race included in this study. An association between sources of variability for recombination and types of germplasm could help locate parental genotypes that have high recombination values or specific recombination values for certain regions of the genome of interest to breeders. Alternatively, specific environmental conditions could be identified that increase recombination in the F_1 generation in order to break up undesirable linkages. The presence of different recombination values between populations could also present some problems to breeders performing marker-assisted selection. For example, it could lead to different levels of tightness of linkage in different maps and could change the relationships between markers and some traits; that is, a characteristic significantly associated with a marker in one population could be unlinked or loosely linked with the same marker in another population.

Implications for Genome Mapping and Map-Based Cloning

The frequency of segregation distortion was low, especially among RFLP markers (<12%), and should therefore not have a major effect on the accuracy of the recombination estimates. Only estimates in which both members of a pair of markers show distorted segregation become unreliable using the distorted maximum likelihood estimation equations (Bailey 1961). This was not the case for any of the intervals with a statistically significant linkage value in our experiments.

The variation in recombination was such that for several intervals no statistically significant linkage could be observed in contrast to prior results of Nodari et al. (1993a) and Gepts et al. (1993). This lack of linkage could be attributed to at least two major causes, chromosome rearrangements and inherent variation for recombination in the absence of rearrangements. Little information is available from cytogenetic studies on the existence of translocations or inversions in *P. vulgaris*. However, mapping experiments showed that most RFLP markers map consistently to the same linkage group. For example, seven *Pst*I markers (of seven tested) used by Vallejos et al. (1992) mapped to the corresponding linkage groups of the map developed by Nodari et al. (1993a) and Gepts et al. (1993). Three markers that were unlinked but were located on linkage group D1 of the Davis map (Gepts et al. 1993) showed significant linkage in the same order on linkage group P1 of the Paris map (Adam and Dron, personal communica-

tion). Mapping of 82 markers from the BAT93 × Jalo EEP558 map (Nodari et al. 1993a) in the Midas × G12873 population showed that only three mapped on a different linkage group (Koinange 1992). These observations suggest that the absence of significant linkage can be attributed primarily to inherent variation in recombination frequency with interchromosomal rearrangements playing only a minor role.

Within linkage groups, we observed several differences in gene order, which can be attributed to local chromosomal rearrangements, or sampling effects during mapping experiments. At this stage, it is difficult to distinguish between these two possibilities, although these differences only appear to affect adjacent markers on relatively small intervals. Both the uncertainty about local gene orders and the high level of variation in recombination frequency suggest that special precautions should be taken when using this information for map-based cloning. Estimates of recombination should be obtained from several populations in order to get an average estimate and should be related to the physical distance if possible. It is not known whether differences in recombination levels are related to nuclear DNA content. Although inter- and intraspecies differences for nuclear DNA amount have been documented (e.g., Aramuganathan and Earle 1991; Mowforth and Grime 1989), we are not aware of any reports on the relationship within a species between nuclear DNA amount and recombination levels over fairly large intervals (>5 cM). In addition, population sizes should be large enough to minimize sampling effects on gene order.

In conclusion, our results show substantial variation for recombination levels within *P. vulgaris,* which appears to be at least partially under genetic control. The actual number of genes and the magnitude of their effect involved in this genetic control remain to be determined, as do the specific environmental factors that can influence recombination.

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Received March 8, 1994
Accepted September 28, 1994