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RAPD Analyses in Colonial and Ancestral Populations of *Capsella bursa-pastoris* (L.) Med. (*Brassicaceae*)

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Key Word Index—*Capsella*; RAPDs; colonization history; population structure; California; Spain; Germany.

Abstract—*Capsella bursa-pastoris* is a neophyte in the New World. It arrived in California not before 200 years ago. Thirteen populations from California, seven from Spain and one from Germany were analyzed with RAPDs in order to trace the introduction history and to study the structure of the colonial populations. The results not only supported earlier hypotheses on the colonization of *Capsella* which were based in isozyme studies (Hurka, 1993; Hurka *et al.*, 1989) and phenotypic traits (Hurka and Neuffer, 1991), but helped also to understand the colonization history in more detail. Overall similarity in RAPD markers was greater between Californian Central Valley and Spanish populations than it was between Central Valley and Californian mountain populations. It is concluded that genotypes preadapted to Mediterranean climate conditions were brought to California by Spaniards from Mexico. Their ancestral populations might be found in Spain which is strongly argued for by RAPD markers. They now occupy the Central Valley of California. The Californian mountains are colonized by other genotypes, the source of which is not yet clear, but unlikely to be of Mediterranean climate origin. In general, colonial Californian populations are genetically less variable than the European populations, but more variable than one would conclude by isozyme studies. Population structures of the colonial populations as revealed by RAPD studies are in full accordance with the expected organization of genetic variability within and between populations of a predominantly self colonizing plant. Copyright © 1996 Elsevier Science Ltd

Introduction

Capsella bursa-pastoris (L.) Med. is a very successful weed on arable soils and in ruderal habitats with world-wide distribution except for the hot and humid tropics. It is mostly annual (summer- or winter-annual), tetraploid and predominantly inbreeding. *Capsella bursa-pastoris* is probably a rather old polyploid (Hurka *et al.*, 990; Mummenhoff and Hurka, 1990) which originated in the Old World. From Eurasia, it extended its range into the New World and Australasia by following European colonists. *Capsella* reached North America in postcolumbian times and established itself first along the East Coast (Crosby, 1986). Less than 200 years ago, *Capsella* began to colonize California. First introductions could have followed the Spaniards, who, coming from Mexico, built 21 missions along the West Coast as outposts of their empire. The first and most southern mission was erected in 1769 (San Diego), the latest and most northern mission in 1823 (Sonoma). San Francisco was founded in 1776 as the sixth mission. The Spaniards only sporadically travelled east; they did not really settle further inland, but established rancheros along with the missions. This situation changed abruptly when gold was discovered near Sacramento in 1848. Enormous waves of settlement in the Central Valley and Sierra Nevada resulted from the gold rush, and hundreds of thousands of people entered California, by far the most entering via San Francisco. They came by ship from the East Coast, but also from Europe and Asia (for historical data see Hornbeck, 1983). In the light of the colonization history of California, it is reasonable to assume multiple introductions for *Capsella*. The source populations

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could be anywhere: Mexico, East Coast of North America, Europe, Asia. An intensive search for isozyme genotypes did indeed shed light on this problem (Hurka, 1993; Hurka *et al.*, 1989). A characteristic multilocus genotype which is nearly the only one found in the whole Central Valley, is also very frequent in the Mediterranean area of Europe, whereas a variety of other multilocus genotypes occurring in California outside the Central Valley are present in the eastern parts of the United States and in Middle Europe. It was argued that the ancestor population of the Central Valley *Capsella* was the Mediterraneis, introduced via Mexico by Spaniards (Neuffer and Hurka, in preparation).

The "Mediterranean" multilocus genotype of *Capsella* in the Central Valley of California displays early flowering in comparison to the later flowering traits of the Sierra Nevada and North Coastal Ranges. This lends further support to a Mediterranean origin of the Central Valley *Capsella* as the Californian Central Valley is of a typical Mediterranean climate type, and Mediterranean *Capsella*s are also early flowering. Judged by isozyme multilocus genotypes based on three different isozyme systems (AAT, LAP, GDH), there was virtually no variation detected throughout the whole Central Valley which, after all, stretches about 800 km from north to south and 100 km from east to west (Neuffer and Hurka, in preparation). This remarkable uniformity in the genotypic composition raises the question as to whether all *Capsella* populations in the Central Valley originate from a single successful introduction of just this one genotype. This question might be answered by using more variable molecular markers. The incorporation of highly sensitive markers will also help to trace the ancestral populations of the Californian *Capsella* and might provide insights into the population structures. Such markers are now available by RAPDs, which are molecular markers of high sensitivity (Random Amplified Polymorphic DNA, Mullis *et al.*, 1994; Welsh and McClelland, 1990; Williams *et al.*, 1990). This relatively new technique has already proven to be valuable in many cases and for many problems, from an individual basis up to the population and species level (Bachmann, 1992, 1994; Hu and Quiros, 1991; Weising *et al.*, 1995; Wolff and Peters-Van Rijn, 1993).

Materials and Methods

Materials. Samples of eight *Capsella* populations from the Californian Central Valley and five from the Sierra Nevada (USA), seven from southwest Spain and one from Germany have been analysed for RAPDs. All populations were of known isozyme genotype composition. In this paper, we concentrate on six loci coding for AAT. Details are given in Table 1. In addition, the Californian populations had also been analysed for quantitative traits before (Neuffer and Hurka, in preparation). As is obvious from Table 1, the multilocus genotype M is characteristic of the Spanish and the Central Valley populations, but does also occasionally occur in population 735 and 742 from the North Coastal Ranges and the Sierra Nevada and in population 644 from Germany.

Method. DNA extraction. Fifty milligrams tissue of young fresh leaf-tips were stored at −80°C. Probes were placed in an Eppendorf cup (1.5 ml) with prewarmed (65°C) 2 × CTAB extraction buffer (500 µl, 5M NaCl, 1M Tris–HCl, 0.5 M EDTA) and 0.2% 2-mercaptoethanol added, grinded with a minipestle to a homogenate, mixed well and incubated for 45 min at 65°C. An equal volume (550 µl) chloroform/isoamylalcohol (24:1), gently mixed for 10 min, was added and centrifuged for 5 min at 14,000 rpm. The aqueous phase was transfered in an Eppendorf cup (1.5 ml) and the extraction step repeated using the solid phase. The solid phase was transfered in an Eppendorf cup, 275 µl precooled (−20°C) isopropanol added, and the DNA was precipitated at −20°C overnight. After centrifuging (5 min at 14,000 rpm) and removing all isopropanol, the DNA was redissolved in 100 µl sterile TE buffer (1 h at room temperature). A quantity of 7.5 µl 7.5 M NH_4Ac and 2 volumes of 96% ethanol (−20°C) were added, DNA precipitated for 20 min at −20°C, and after centrifuging (5 min, 14,000 rpm) the ethanol was removed. The pellet was vacuum-dried, dissolved in 100 µl sterile bidest and stored at 4°C (modified DNA extraction method after Doyle and Doyle, 1987).The template DNA for PCR was made up to a concentration of about 10 ng/µl. The puridity and the concentration of the DNA was controlled with a photometer at 260, 280 and 320 nm.

PCR protocol. RAPD-PCR reactions were performed in 50 µl in 0.5 ml Eppendorf cups containing: 5 µl of 10 × PCR reaction buffer (Eurogentec), 3 µl $MgCl_2$ (1.5 mM), 32 µl H_2O, 3 µl dNTP (150 µM each), 5 µl

TABLE 1. PROVENANCES OF THE ACCESSIONS

Pop. no	Provenance	Elevation	Genotype
644	Osnabrück (D)	85	O, M
690	Cadiz (E)	50	M
705	Davis (USA)	20	M
708	Dixon (USA)	20	M
709	Woodland (USA)	20	M
711	Nice (USA)	500	M
716	Mariposa (USA)	615	M
721	Shafter (USA)	50	M
725	Mohave (USA)	715	M
731	Redding (USA)	170	M
735	Eureka (USA)	3	O, M
740	Reno (USA)	1300	O
742	Carson City (USA)	1420	O, M
743	Lake Tahoe (USA)	1850	O
749	Sattley (USA)	1500	O
1236	Plasenzia (E)	400	M
1241	Guadalupe (E)	500	M
1245	Caceres (E)	600	M
1257	Cabeza del Buey (E)	300	M
1272	Matalascanas (E)	10	M
1274	Sevilla (E)	20	M

In brackets land of origin, D = Germany; E = Spain, USA = United States of America: California and Nevada. M = Mediterranean *Aat*-Isozyme multilocus genotype *1A 11, 1B 11, 2A 11, 2B 44, 3A 11, 3B 55*, Hurka *et al.*, 1989; O = other multilocus genotypes.

Goldstar polymerase (0.5 U, Eurogentec). Reaction mixes were overlaid with 40 µl oil to prevent evaporation.

Cycling conditions: hot start at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 36°C for 30 s with a ramping phase of 0.4°C/s, elongation at 72°C for 1 min (44 cycles), final elongation at 72°C for 4 min and subsequent cooling down at 4°C.

Gel electrophoresis: 2 µl loading buffer (50% glycerin, 0.25% Bromphenol Blue, 0.25% xylencyanol, 0.5 × TBE buffer after Sambrook *et al.*, 1989) was added to 8 µl PCR reaction mix and applied to an 1.4% agarose gel. The gel run for about 3 h at 90 V in 1XTBE and stained for 10 min in ethidium bromide (1 µg/ml); DNA was visualized by an UV transilluminator.

Data analysis. PCR-amplification products (= bands) were identified by eye and their presence or absence scored with 1 or 0. Bands which were present only once between separate runs were not included in the further analysis. The 1/0 data matrix thus generated was the basis of a distance analysis (neighbor-joining method, program packages TREECON, Van de Peer and De Wachter, 1994; PHYLIP, Saitou and Nei, 1987). Bootstrap values result from 100 runs (program packages TREECON, PAUP 3.0, Swofford, 1990). The repeatability of the banding patterns was fully given tested by examples. In every PCR, we generally amplified an internal standard to obtain comparable results. The internal standard was one defined individual out of a Central Valley population.

Results

Neighbor joining analysis based on seven RAPD primers and 180 informative characters revealed a clear separation of Central Valley *Capsella* populations from those of the Sierra Nevada (indicated by long branches and high bootstrap values, Fig. 1). Population 690 from Spain was within the Central Valley cluster, pop. 644 from Germany within the mountain cluster, although rather distinct from it (Fig. 1). Individuals from the mountains sharing the same *Aat*-genotype with those from the Central Valley (735/8 and 742/7) were nevertheless distinguished by RAPDs (Fig. 4).

Californian Central Valley *Capsella* populations with an overall "Mediterranean" multilocus isoenzyme genotype were distinct from Spanish *Capsella* populations despite their similarity in isozyme patterns. However, there was a remarkable

exception: pop. 690 from Spain clustered within the Californian populations (neighbor joining, Fig. 1).

Populations of the Central Valley—all uniform by isozyme evidence—turned out to be polymorphic when screened with RAPDs. Some were rather uniform with low bootstrap values (<60%) and low number of informative bands (see pop. 711, 725, 731, Table 2); others, however, were rather variable with higher bootstrap values (>60%) and higher numbers of informative characters (see pop. 644, 716, 1236, 1241, Table 2).

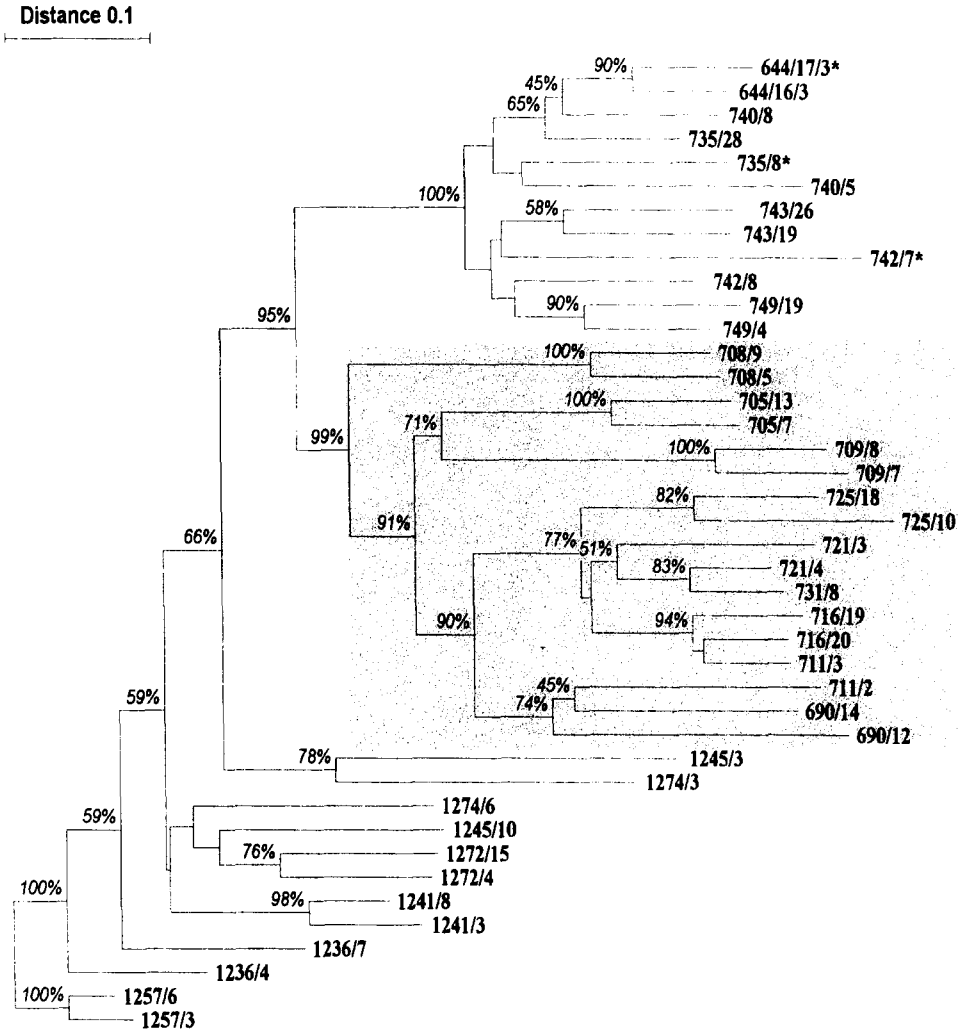
The detection of differences within an accession depends, of course, to a certain degree on sample size and on the number of RAPD primers employed. Taking this into account, it is obvious nevertheless that on the whole, European populations tend to be more variable than the Californian populations irrespective of their place of origin.

All Californian Central Valley populations had characteristic RAPD bands (OPB01/2, OPR03/1, OPR04/2, OPR06/2) as did the Californian mountain populations (OPB01/1, OPB01/3, OPR04/1, OPR06/1, see Figs 2 and 3). Marker bands OPR03/1 and OPR04/2 are shared between Central Valley plants and the Spanish pop. 690, characters OPB01/3 and OPR04/1 between Californian mountain plants and pop. 644 from Germany (Figs 2 and 3).

This is further outlined in Table 3. Plants from within Spain shared less common bands than did the Central Valley plants although they were all of the same *Aat* genotypes (Table 1). Mountain populations of California also had many bands in common (indeed the highest number recorded, see Table 3) despite the fact that they were polymorphic for isozyme loci and quantitative traits (Table 2; Hurka and Neuffer, in preparation). These RAPD markers characteristic for the Californian mountain populations were also nearly completely found (27 out of 28) in pop. 644 from Germany. The similarity between the Central Valley and Spain was less pronounced. This picture changed, however, when only pop. 690 (Spain) was compared with the Central Valley populations: the similarity between the latter two

TABLE 2. RAPD DATA OF ANALYSED POPULATIONS

Pop. no.	Number of individuals tested	Number of bands shared within an accession	Number of variable markers per accession	Number of primers tested	Number of bootstrap values above 60%
644	6	40	24	7	3
690	8	89	23	11	1
705	21	144	222	20	3
708	12	85	54	12	0
709	18	64	67	9	3
711	8	89	27	11	2
716	8	90	40	11	5
721	8	78	38	11	1
725	8	92	14	11	1
731	8	97	7	11	0
735	6	35	31	7	0
740	6	24	52	7	2
742	6	27	44	7	1
743	6	40	17	7	1
749	6	30	33	7	0
1236	6	39	27	7	3
1241	6	28	36	7	3
1245	6	12	59	7	2
1257	6	48	6	7	2
1272	6	34	35	7	2
1274	6	24	49	7	2



Neighbor-Joining Analysis
41 individuals, 21 populations, 180 informative markers
shaded cluster: Central Valley and pop. 690 from Spain
cluster below: Spanish populations
cluster above: mountains of California, * means mediterranean genotype

FIG. 1. NEIGHBOR JOINING ANALYSIS (TREECON) OF 41 INDIVIDUALS OUT OF 21 POPULATIONS, 180 INFORMATIVE MARKERS, SEVEN PRIMERS. Shaded cluster = Central Valley populations, cluster below = Spanish populations, cluster above = populations from the mountains of California and Germany (644). Spanish and Central Valley individuals share the same *Aat* multilocus genotype M (see Table 1), Californian mountain and population 644 individuals have various genotypes. Individuals marked with an asterisk (*) genotype M, too.

groups was high (Table 3). Nine RAPD markers were common to all *Capsella* plants analysed.

Although neighbor-joining distance analyses separated Californian Central Valley populations from Spanish populations with the exception of pop. 690 (Fig. 1), some RAPD markers were nevertheless clearly shared by the two geographic areas (Fig. 4). In Fig. 5, typical banding patterns for Spain and the Central Valley of California are displayed.

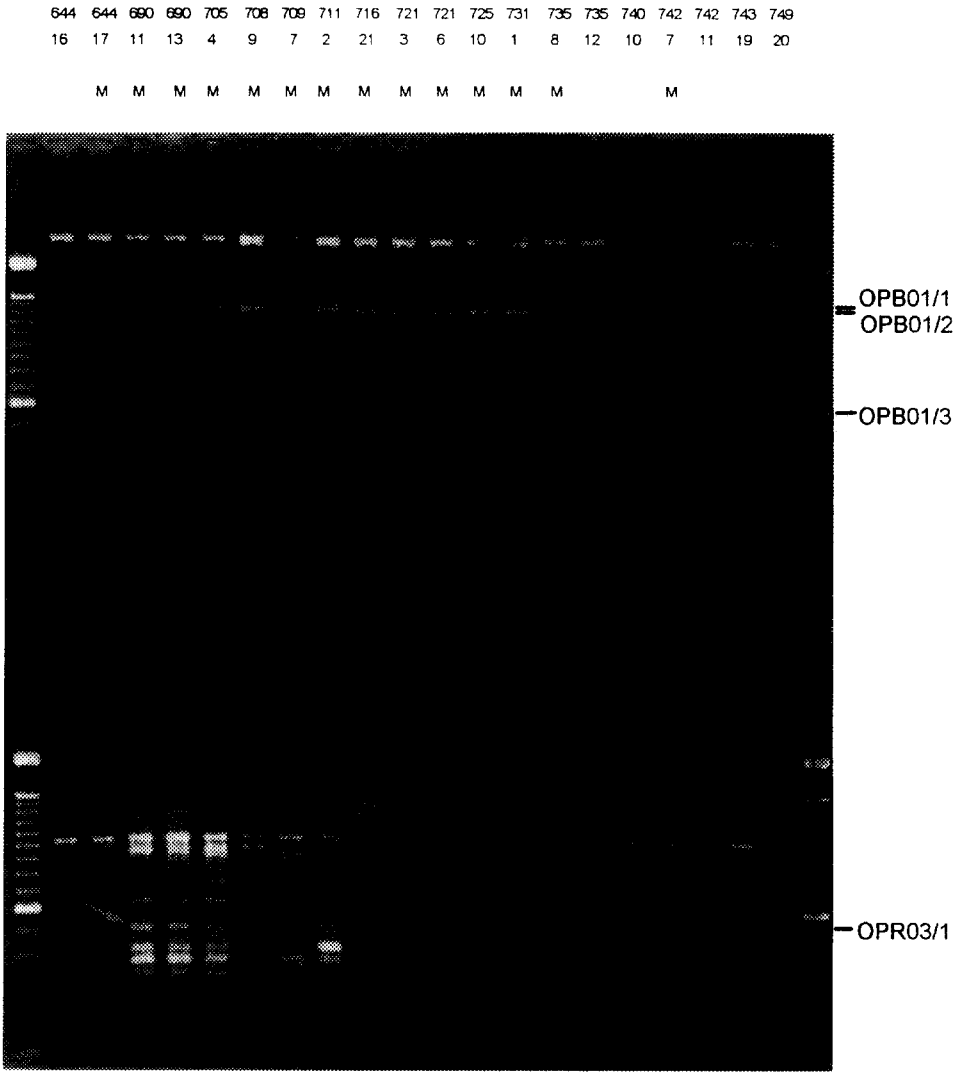


FIG. 2. CALIFORNIAN POPULATIONS—CENTRAL VALLEY AND MOUNTAIN POPULATIONS—COMPARED WITH THE MIDDLE EUROPEAN LOWLAND (644) AND THE SPANISH POPULATION (690). PRIMER OBP 01 ABOVE AND OPR 03 BELOW. First line = population number, second line = individual identity number, M stands for Mediterranean genotype (see Table 1). White arrows mark bands corresponding to the denominations used in the text and repeated on the right margin of the figure.

Discussion

The RAPD studies provide convincing evidence for genetic differences between those *Capsella* plants which, based on a multilocus *Aat*-genotype, appeared to be uniform. Not only does this prove the high sensitivity of RAPDs and their value for studies at the population level in general, but in the context of the overall topic of the present paper, the colonization history of *Capsella*, RAPD analyses not only supported earlier hypotheses but also helped to understand the colonization history in more detail.

Our results definitely support the view that the geographic distribution pattern of isozyme genotypes in California reflects the colonization history of *Capsella*

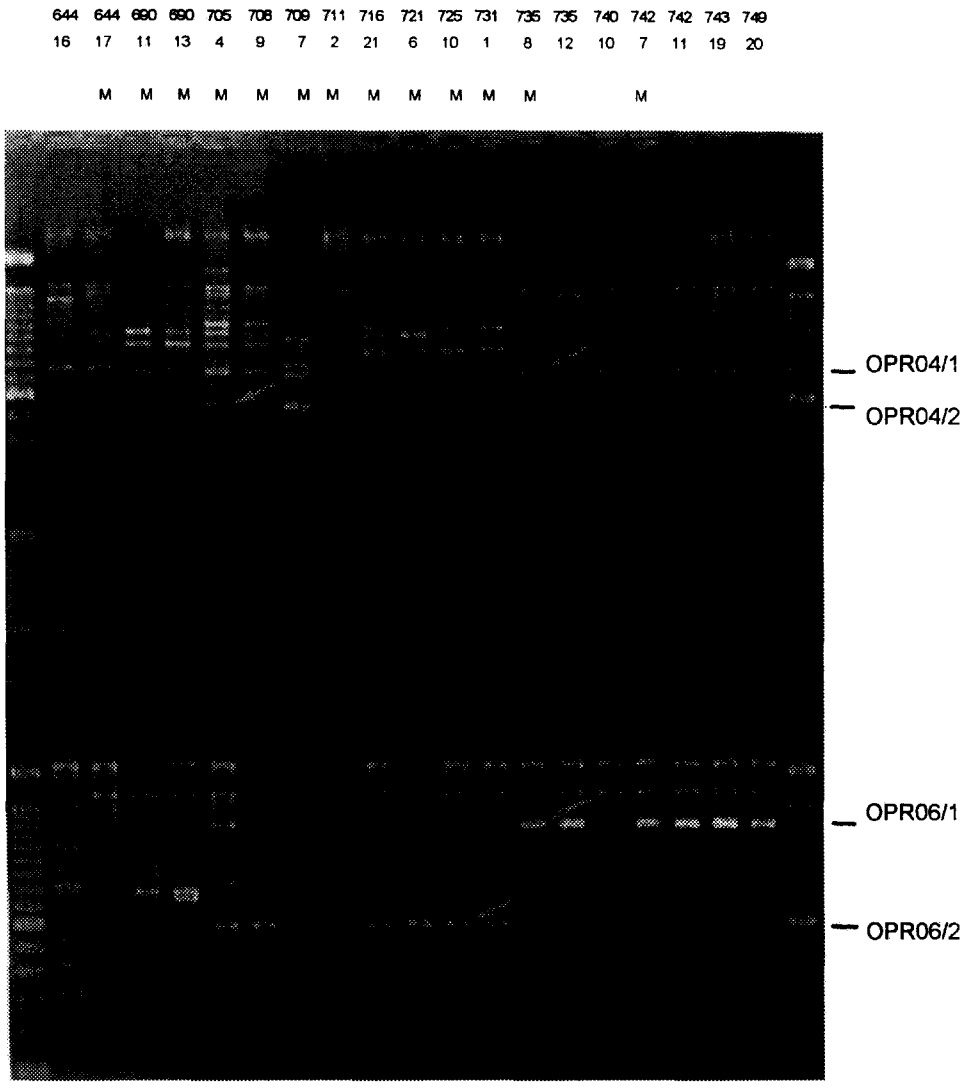


FIG. 3. CALIFORNIAN POPULATIONS—CENTRAL VALLEY AND MOUNTAIN POPULATIONS—COMPARED WITH THE MIDDLE EUROPEAN LOWLAND (644) AND THE SPANISH POPULATION (690). PRIMER OPR 04 ABOVE AND OPR 06 BELOW. M stands for Mediterranean genotype (see Table 1; for further explanations see Fig. 2)

(Neuffer and Hurka, in preparation). The Central Valley populations of California are more similar to Spanish populations than they are to the adjacent populations of the Californian Sierra Nevada populations (Figs 1 and 5). Nevertheless, clear differences between Spanish and Californian Central Valley populations are also evident (Fig. 1), differences which were not obvious in isozyme studies. However, one Spanish population from the vicinity of Cadiz (690) grouped together with the Californian Central Valley populations. It is reasonable to discuss whether this particular Spanish *Capsella* population comes close to an ancestral gene pool from which colonial Californian populations might have been derived. On the other side, one has to consider evolutionary changes accompanied by the colonization process which might result in new combinations of the genetic material not found

TABLE 3. NUMBER OF RAPD MARKERS (=BANDS) SHARED BETWEEN GROUPS OF POPULATIONS

Geographical region (Pop. no., see Table 1)	Number of bands shared
Central Valley (705–731)	24
Spain (690, 1241–1274)	16
Central Valley + Spain (690, 705–731, 1241–1274)	10
Central Valley + 690 (690, 705–731)	20
Mountains of California (735–749)	28
Mountains of California and Middle European lowland (644, 735–749)	27
Central Valley and mountains of California (705–749)	13
All	9

RAPD—Analysis of 41 individuals out of 21 populations, 7 primers, 180 informative markers (see Fig. 1).

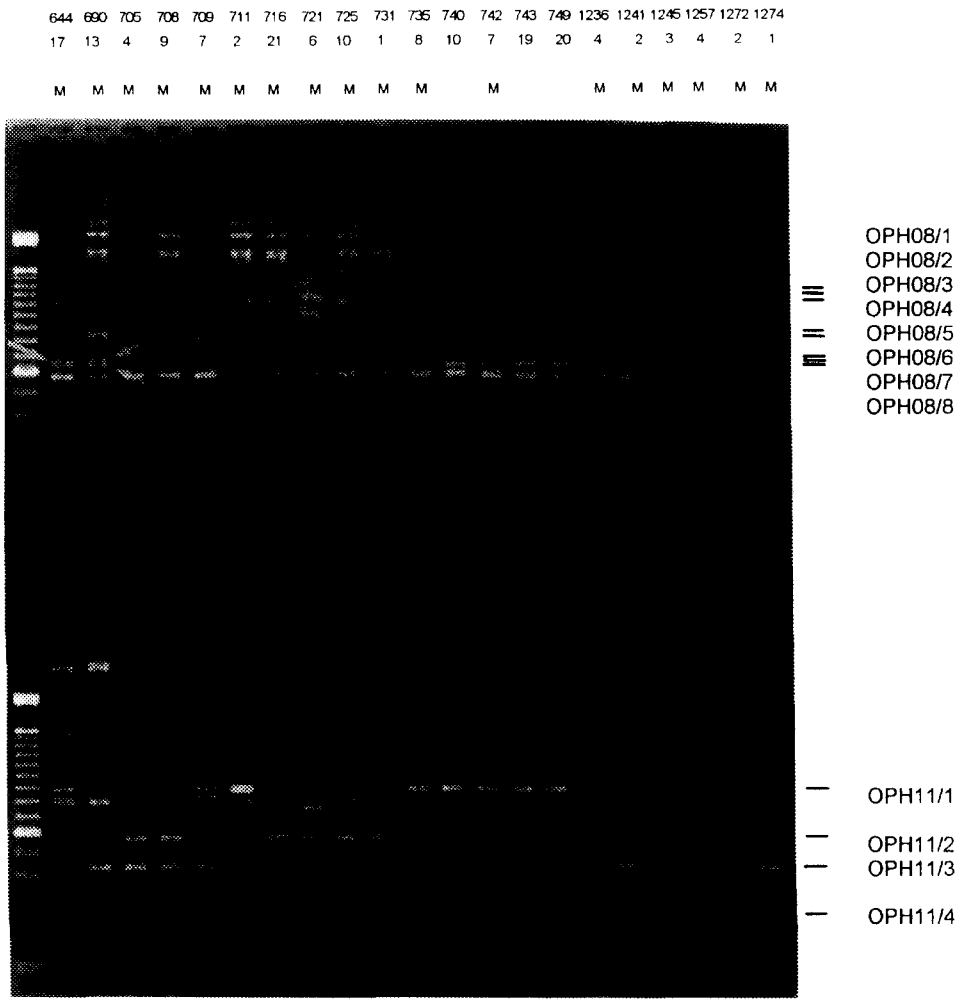


FIG. 4. INDIVIDUALS OUT OF ALL POPULATIONS: PRIMER OPH 08 ABOVE AND OPH 11 BELOW. M stands for Mediterranean genotype (see Table 1; for further explanations see Fig. 2).

in the ancestral genepool. This was—based on isozymes—argued for *Avena barbata*, a Mediterranean weedy grass introduced to California (Allard *et al.*, 1993; Garcia *et al.*, 1989, 1991; Perez de la Vega *et al.*, 1991). Since RAPD banding patterns cannot be easily interpreted in terms of genes and their alleles in contrast

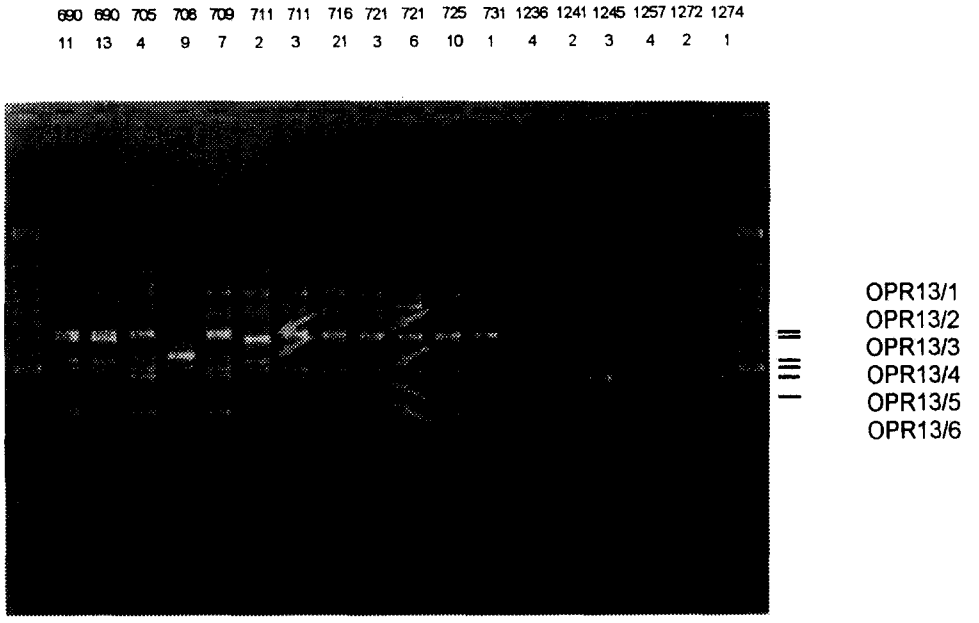


FIG. 5. POPULATIONS OUT OF THE MEDITERRANEAN CLIMATE—CENTRAL VALLEY AND SPANISH INDIVIDUALS. All individuals with Mediterranean *Aat* multilocus genotype M. Lane 708/9: unsuccessful run.

to isozyme banding patterns, which reflect clearly defined genes, there is, at the moment no information about possible RAPD multilocus associations of colonial *Capsella* populations. The advantage of RAPD studies is the generation of any number of randomly primed DNA sections which greatly helps in assessing overall similarities at the DNA level.

It turned out that the Central Valley populations of *Capsella* which appeared genetically uniform with respect to isozyme multilocus genotypes and also with respect to some phenotypic traits (Neuffer and Hurka, in preparation) are genetically distinct when screened with RAPDs (Fig. 1, Table 2). RAPDs are a much more variable marker than isozymes. The RAPD data show a similar degree of variability within Spanish, Central Valley and Californian mountain populations (Table 2). This could be explained by the other power of statement shown by different markers. However, the bands, especially of mountain populations and of "Mediterranean" genotype populations, are clearly distinct (Figs 2–4, Table 3). Furthermore "Mediterranean" genotypes that are not from Mediterranean regions of Europe and from the mountains of California are more similar to their sister individuals as shown in Figs 1–4 (individuals 644/17/3, 735/8, 742/7). The origin of Central Valley populations out of rare "Mediterranean" genotypes from the Central or Northern part of Europe can be excluded. This statement was not reflected in the isozyme data. However, at the moment, the question of a possible multiple introduction of these uniform isozyme types cannot be answered with certainty. More information is needed on the RAPD variation of Mediterranean and of Californian populations.

Within-population variability was low in some populations (e.g. 711, 725), and higher in others (e.g. 705). This is certainly partly due to sample size *n* as those populations with a higher *n* were more variable (Table 2). However, there were undoubtedly differences between and within samples of equal size (Table 2) indicating different population structures (low distances and bootstrap values below 40%, which are not demonstrated in Fig. 1, are regarded as random

"noise"). The non-random distribution of genetic variation between and within a population may be the result of the breeding system, of gene flow events by seeds, and of chance effects like genetic drift and founder events. All of these factors are in full accordance with the biology of *Capsella* (Hurka *et al.*, 1989; Hurka, 1990; Hurka and Neuffer, 1991; Neuffer, 1990); thus the RAPD analyses confirm the dynamics of the organization of the genetic variability in this colonizing plant species. It might well be that also new and recent mutations contribute to the observed variation pattern as well as microhabitat adaptations. This, however, will be difficult to demonstrate. Cosegregation analyses between RAPD markers and QTLs (quantitative trait loci) may improve our understanding.

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