

# ECOTYPIC AND ALLOZYME VARIATION OF *CAPSELLA BURSA-PASTORIS* AND *C. RUBELLA* (BRASSICACEAE) ALONG LATITUDE AND ALTITUDE GRADIENTS ON THE IBERIAN PENINSULA

by

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## Resumen

NEUFFER, B. & R. HOFFFROGGE (2000). Variación ecotípica y aloenzimática de *Capsella bursa-pastoris* y *C. rubella* (Brassicaceae) a lo largo de gradientes latitudinales y altitudinales en la Península Ibérica. *Anales Jard. Bot. Madrid* 57(2): 299-315 (en inglés).

Se han comparado diversos caracteres biológicos relacionados con la capacidad colonizadora (inicio de la floración, número de hojas, diámetro de la roseta, altura de la planta, número de ramas, dimensiones del fruto y número de semillas) de plantas de *Capsella* procedentes de la Península Ibérica mediante un experimento de bloques aleatorios en el campo. Los datos se evaluaron con un análisis de componentes principales. También se registraron el tipo de hojas y el perfil aloenzimático de las plantas. Las plantas de *Capsella bursa-pastoris* procedentes de altitudes altas y bajas de la zona climática Mediterránea de verano seco (Sierra Nevada) mostraron ser de floración temprana, mientras que las plantas de los Pirineos, con un clima alpino, presentaron una floración tardía. En *C. bursa-pastoris* el tipo de hoja "rhomboidea" resultó ser el más frecuente, en tanto que en *C. rubella* lo fue el tipo "heteris". Se observó un cambio en las frecuencias de los tipos de hojas a lo largo de una clina geográfica, lo que se explicaría por los componentes adaptativos que posee la forma de la hoja. Las aloenzimas presentaron un patrón de distribución geográfico y en *C. bursa-pastoris* un determinado genotipo multilocus podría ser un marcador molecular para el ecotipo de floración temprana.

Palabras clave: Aloenzimas, diferenciación ecotípica, dimensiones del fruto, forma de crecimiento, morfología foliar, inicio de floración, altura de la planta, *Capsella*, Brassicáceas, España.

## Abstract

NEUFFER, B. & R. HOFFFROGGE (2000). Ecotypic and allozyme variation of *Capsella bursa-pastoris* and *C. rubella* (Brassicaceae) along latitude and altitude gradients on the Iberian Peninsula. *Anales Jard. Bot. Madrid* 57(2): 299-315.

Life-history traits (onset of flowering, leaf number, rosette diameter, plant height, branching number, fruit dimensions, seed number) of *Capsella* species from the Iberian Peninsula associated with colonizing ability were compared in a random block field experiment. Data were evaluated by a principal component analysis. Allozymes (AAT, LAP, GDH) and leaf types were recorded. *C. bursa-pastoris* plants originating from low and high elevations of the summer dry Mediterranean climatic zone (Sierra Nevada) were early flowering, whereas those originating from the Pyrenees with an alpine climate were late. In *C. bursa-pastoris* the "rhomboidea" leaf type was very frequent, whereas in *C. rubella* it was the "heteris" leaf type. There was a change of leaf type frequencies along geographical clines which is explained by adaptive components of the leaf shape. The allozymes displayed a geographical distribution pattern and in *C. bursa-pastoris* a certain multilocus genotype appeared to be a molecular marker for an early flowering ecotype.

Key words: Allozymes, ecotypic differentiation, fruit dimensions, growth forms, leaf morphology, onset of flowering, plant height, *Capsella*, Brassicaceae, Spain.

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## INTRODUCTION

Colonizing species have received considerable attention during the past decades (BAKER, 1965; CLEGG & BROWN, 1983; BARRETT & RICHARDSON, 1986), and they share a number of common genetic features often including polyploidy (BROWN & MARSHALL, 1981). Their adaptation may be due to a "general purpose genotype" of high phenotypic plasticity (BAKER, 1965) or pronounced ecotypic variation. It has been shown that both concepts are not mutually exclusive.

In *C. bursa-pastoris* (L.) MEDIK., germination behaviour comes close to a "general purpose genotype" (NEUFFER & HURKA, 1988; HURKA & NEUFFER, 1991). On the other side, there is pronounced ecotypic variation in the time of flowering between "early" and "late" ecotypes on a macro-geographical scale (NEUFFER & HURKA, 1986a; NEUFFER, 1990; NEUFFER & ALBERS, 1996). Ecotypic variation patterns have also been demonstrated for growth form parameters (STEINMEYER & *al.*, 1985; NEUFFER & HURKA, 1986b; NEUFFER & BARTELHEIM, 1989; NEUFFER & ALBERS, 1996) and, although tentatively, for reproductive capacity (HURKA & NEUFFER, 1991). *C. bursa-pastoris* does also clearly display ecotypic variation in relation to man made habitats (NEUFFER & MEYER-WALF, 1996).

A strong correlation between the time to flowering and elevation above sea level was observed for *Capsella* populations from European alpine regions. Alpine populations comprise an array of genotypes with different susceptibility to low temperatures (NEUFFER & BARTELHEIM, 1989). It was shown that summer annual "early flowering" genotypes were replaced by winter annual "late flowering" genotypes along an altitudinal gradient. This topocline was paralleled by an ecocline expressed as a shortening of the period of vegetative growth. Furthermore with higher elevation the plants remain smaller and the frequency of to the midrib dissected leave lobes increased (NEUFFER &

BARTELHEIM, 1989). A similar pattern of variation was also observed for extra-European regions (e.g. Sierra Nevada California, USA, NEUFFER & HURKA, 1999). Differentiation along altitudinal gradients studied so far concentrated on temperate climates. Question arises whether in Mediterranean climate types variation patterns along altitudinal gradients are basically different from those in temperate climates.

The Iberian Peninsula is subdivided into two major vegetation zones: the cold-deciduous mesophytic broadleaved forest in the north and the sclerophyllous forest in the south (SCHMITHÜSEN, 1976). The Pyrenees in the northern part have an alpine climate, whereas the southern part of the central mountains and the Sierra Nevada in the south with an elevation above 3000 m show a summer-dry Mediterranean climate.

The present study highlights the genetic composition and adaptation strategy in populations of *C. bursa-pastoris* and *C. rubella* Reut. from different mountain, valley and coastal habitats on the Iberian Peninsula. *C. rubella* is a diploid species in contrast to *C. bursa-pastoris* which is tetraploid. Both species are closely related. The geographical range of *C. bursa-pastoris* is much wider than that of *C. rubella* which has been attributed to the greater genetic flexibility gained by the polyploidization (NEUFFER & ESCHNER, 1994). In the Mediterranean region, both species often occur together in the same sites. It will be demonstrated that ecotypic differentiation of *Capsella* in summerdry Mediterranean mountains is different from variation patterns observed in mountains with alpine climates.

## MATERIAL

*Provenances, sowing and growing conditions*

Seed samples of up to 10 individual plants of 25 populations of *Capsella* (Brassicaceae) were randomly taken from natural populations from different regions of the

Iberian Peninsula (Table 1, Fig. 1). Three of these populations (No. 1249, 1252, 1290, Table 1; ■ in Fig. 1) were "two species sites" (*C. bursa-pastoris* and *C. rubella* growing together), the others pure *C. bursa-pastoris* stands. Seeds were stored for at least six months at minus 20 °C. Progenies raised from the mother plants were grown in a random block design in an open field experiment at the Botanical Garden of the University of Osnabrück, Germany (52° 18' N, 8° 00' E, 90 m above sea level). Seeds were germinated in plastic pots (7 cm in diameter) on top of a substrate mixture containing TKS1 (Torfkultursubstrat) and crystal sand (2 : 1) in an unheated glasshouse on April 20, 1995. Young plants were planted into the experimental field on May 29, 1995. It was intended to analyze 10 families (= progenies) per population and 10 individuals per family. Different group sizes resulted from either low germination (fewer than 10 individuals per family), low survival of seedlings, or small population sizes (fewer than 10 plants per population).

## METHODS

### Quantitative parameters

The following parameters were recorded in the experimental field:

- onset of flowering: time from sowing to breaking the first flower bud (FI);
- rosette maximum diameter, recorded in most cases shortly after the onset of flowering (RD);
- number of rosette leaves at maximum rosette diameter (LN);
- total plant height of the inflorescence (PH) at the end of the growing season;
- branching number: number of rosette shoots (BB) and number of stem branches (SB);
- fruit dimensions: width (FW) and length (FL);
- number of fruits per 10 cm of one well developed branch (FN).

The number of stem branches was observed

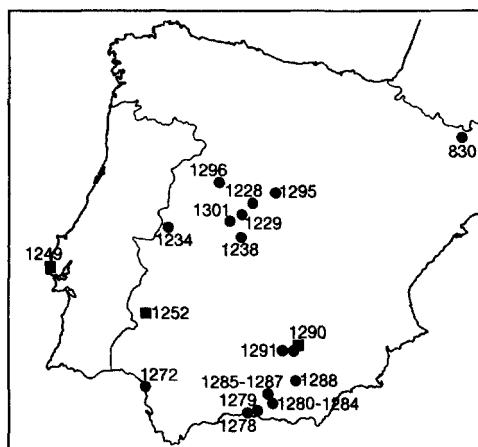


Fig. 1.—Map showing the locations of the different *Capsella* populations listed in Table 1: ■ populations including *C. bursa-pastoris* and *C. rubella* individuals; ● populations with *C. bursa-pastoris* individuals only.

in one individual from each family. The other parameters were observed in each individual.

### Leaf morphology

The highly variable leaf morphology in *Capsella* can be assigned to four major phenotypes: "heteris", "rhomboidea", "tenuis", and "simplex" (Fig. 2; SHULL, 1918, 1923, 1929). Early inheritance studies of leaf shape characteristics indicated that a simple genetic system consisting of two Mendelian genes (*A* and *B*), each with two alleles (*Aa* and *Bb*), control the shape and lobing of rosette leaves in *Capsella* (SHULL, 1909,

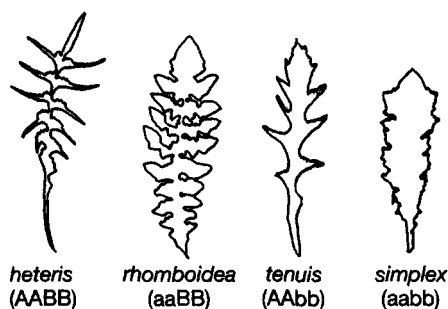


Fig. 2.—Shull's leaf forms of *Capsella* and their homozygotic gene formulas (from STEINMEYER & al., 1985).

TABLE 1  
PROVENANCES AND HABITAT CHARACTERIZATIONS OF INVESTIGATED *CAPELLA* POPULATIONS  
[Data on season of rain and temperature from WALTER & LIETH (1967)]

Pop. No.	Ploidy level	Provenance	Coordinates	Elevation (m)	Vegetation zone (SCHMITHÜSEN, 1976)	Season of rain	Temperature	Habitat
830	4X	Pyrenées, Benasque	42°40'N, 0°30'E	1650	montane cold deciduous broadleaved forest with coniferous trees	winter-spring 1330 mm	10 °C	roadside
1228	4X	Sierra de Gredos, Ávila	40°40'N, 4°41'W	680	submediterranean cold-deciduous broadleaved forest	winter-spring 1000 mm	10 °C	city
1229	4X	Sierra de Gredos	40°21'N, 5°07'W	1670	submediterranean cold deciduous broadleaved forest, dwarf shrubs	winter spring 1000 mm	8 °C	meadow
1234	4X	Coria, on Alagón	39°59'N, 6°32'W	270	submediterranean cold-deciduous broadleaved forest	autumn-winter 500 mm	16 °C	city, park
1238	4X	Talavera de la Reina	39°58'N, 4°49'W	400	sclerophyllous forest	autumn-winter 500 mm	15 °C	city, park
1249	2X, 4X	Sintra (Lisbon, Portugal) Atlantic coast	38°47'N, 9°23'W	150	submediterranean cold-deciduous broadleaved forest	autumn-winter 600 mm	16 °C	city, park
1252	2X, 4X	Jerez de los Caballeros	38°19'N, 6°46'W	150	sclerophyllous forest	autumn-winter 500 mm	16 °C	city
1272	4X	Sanlúcar de Barrameda	37°1'N, 6°34'W	30	coastal dune vegetation coniferous dry forest	winter 300 mm	18 °C	city
1278	4X	Nerja, Mediterranean Sea	36°46'N, 3°51'W	250	dwarf palm garrigue	winter 440 mm	18 °C	city, park
1279	4X	Vélez de Benaudalla, Mediterranean Sea	36°50'N, 3°31'W	300	dwarf palm garrigue	winter 440 mm	18 °C	city, park
1280	4X	Pampaneira, Sierra Nevada	36°55'N, 3°24'W	900	submediterranean cold deciduous broadleaved forest, <i>Quercus ilex</i>	winter 700 mm	14 °C	roadside

1281	4X	Bubión, Sierra Nevada	36°57'N, 3°21'W	1240	submediterranean cold deciduous broadleaved forest, <i>Q. ilex</i>	winter 700 mm	12 °C	city, beside compost
1282	4X	High mountain pass, Sierra Nevada	36°58'N, 3°21'W	2000	submediterranean cold deciduous broadleaved forest, <i>Q. pyrenaica</i>	winter 700 mm	10 °C	strawberry field
1283	4X	High mountain pass, Sierra Nevada	36°57'N, 3°21'W	1650	submediterranean cold deciduous broadleaved forest, <i>Q. ilex</i>	winter 700 mm	12 °C	roadside
1284	4X	Capileira, Sierra Nevada	36°58'N, 3°22'W	1400	submediterranean cold deciduous broadleaved forest, <i>Q. ilex</i>	winter 700 mm	12 °C	disturbed
1285	4X	High mountain pass, Sierra Nevada	37°9'N, 3°30'W	1000	open cold deciduous broadleaved forest, <i>Q. ilex</i>	winter-spring 450 mm	15 °C	roadside
1286	4X	High mountain pass, Sierra Nevada	37°8'N, 3°27'W	1250	open cold deciduous broadleaved forest, <i>Q. ilex</i>	winter-spring 600 mm	14 °C	wayside field
1287	4X	High mountain pass, Sierra Nevada	37°8'N, 3°27'W	1580	open cold deciduous broadleaved forest, <i>Q. pyrenaica</i>	winter-spring 800 mm	13 °C	wayside field
1288	4X	Baza, Sierra Cazorla	37°30'N, 2°10'W	1000	subtropical rain-green bunch-grass steppe	winter-spring 400 mm	14 °C	city
1289	4X	Mogón on Guadalquivir, Sierra Cazorla	38°4'N, 3°2'W	670	cold deciduous river meadow forest	spring 600 mm	15 °C	beanfield
1290	2X, 4X	Mogón, Sierra Cazorla	38°4'N, 2°56'W	1100	cold deciduous river-meadow forest	spring 600 mm	15 °C	city
1291	4X	Peal on Guadalquivir, Sierra Cazorla	37°58'N, 3°11'W	370	cold deciduous river meadow forest	spring 600 mm	15 °C	wayside river
1295	4X	Segovia, Sierra de Gredos	41°5'N, 4°10'W	1000	open cold deciduous broad leaved forest, <i>Q. pyrenaica</i>	winter-spring 550 mm	11 °C	city, wall
1296	4X	Salamanca	41°N, 5°45'W	800	sclerophyllous forest, <i>Q. ilex</i>	autumn 300 mm	12 °C	city, park
1301	4X	Sierra de Gredos	40°22'N, 5°25'W	1300	open cold deciduous broadleaved woodland, <i>Q. pyrenaica</i>	winter-spring 1000 mm	10 °C	<i>Nardus stricta</i> alpine meadow

1911). One well-developed leaf of each individual was deposited in the Herbarium at the University of Osnabrück (OSBU).

#### Statistical evaluation

Mean value, standard deviation, range, median value (m) and the coefficient of variability (cV%) were computed. Since the data did not show a normal distribution (KOLMOGOROV-SMIRNOV Test) and variances were not equal, a non-parametric one-way analysis of variance for unbalanced group numbers was employed (H-test of KRUSKAL & WALLIS). The test assesses the phenotypic homogeneity (–) and heterogeneity (+) within and between populations. Plant-specific parameters including median values and coefficient of variability have been evaluated by a principle component analysis (PCA) and a regression analysis. The data were statistically evaluated with the SPSS program (Superior Performance Software System, Version 6.0, ANOVA).

#### Allozyme analyses

Allozyme analysis was carried out for one individual out of each progeny. The enzyme AAT (aspartate amino transferase; EC 2.6.1.1) is a dimeric enzyme. In *Capsella rubella* the AAT is encoded by three nucleic loci. The monomeric LAP (leucine aminopeptidase; EC 3.4.11.1) is also encoded by three nucleic loci, and the plastidic-expressed homohexameric GDH (glutamate dehydrogenase; EC 1.4.1.4) by two nucleic loci. Details as to the extraction, electrophoresis and visualization of the enzymes and as to the genetic interpretation of the zymograms are given in HURKA & *al.* (1989) for AAT, HURKA & DÜRING (1994) for GDH, and HURKA & NEUFFER (1997) for LAP. All loci are duplicated in the tetraploid *C. bursa-pastoris* (HURKA & NEUFFER, 1997). The allozyme patterns of the two species *Capsella bursa-pastoris* and *C. rubella* are different, and both species can easily be keyed out.

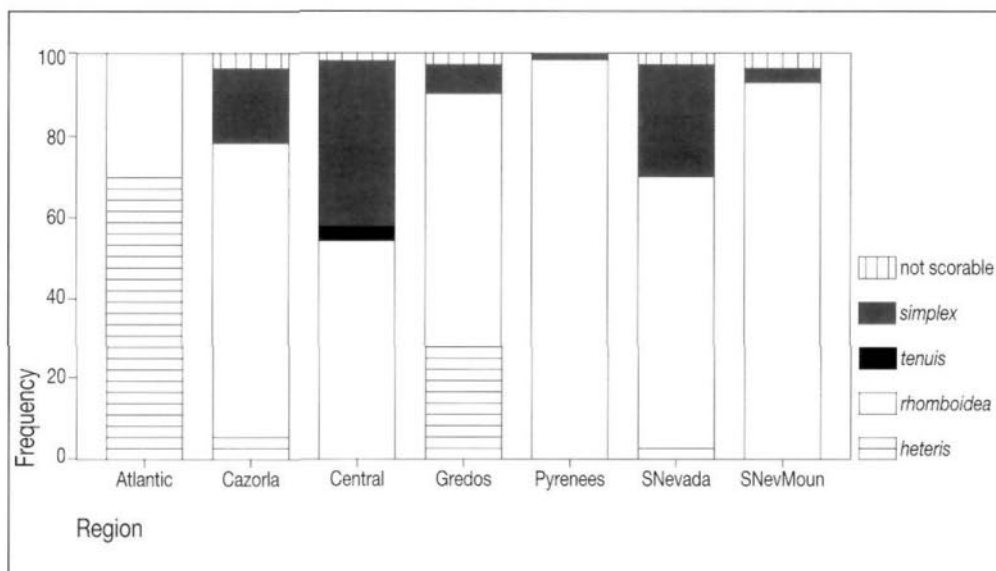


Fig. 3.—Distribution and frequencies of leaf types of *Capsella bursa-pastoris* on the Iberian Peninsula: Atlantic = pop. 1249 ( $n = 7$ ); Cazorla = pop. 1288-1291 ( $n = 330$ ); Central = pop. 1234, 1252, 1272, 1278, 1279 ( $n = 368$ ); Gredos = pop. 1228, 1229, 1238, 1295, 1296, 1301 ( $n = 539$ ); Pyrenees = pop. 830 ( $n = 75$ ); SNevada = pop. 1281, 1282, 1283-1287 ( $n = 611$ ); SNevMoun = pop. 1282 ( $n = 100$ ). The leaf type "tenuis" occurred only in the pop. 1272 ( $n = 1$ ), 1287 ( $n = 3$ ), 1290 ( $n = 2$ ).

## RESULTS

*Quantitative parameters*

All populations were heterogeneous for at least one character (Table 2). One population was homogenous with only one exception (fruit length, pop. 1283, Sierra Nevada), other populations were variable in most characters (pop. 830, 1229, 1238, 1281, 1289, 1291, 1295, and *Capsella rubella* plants of 1249). For all *C. bursa-pastoris* plants the rosette diameter was the most homogeneous (Table 2, Total, cV% 14.6) and the rosette leaf number the most variable character (cV%

43.4). For *C. rubella* onset of flowering was the most homogeneous (cV% 9.4) and plant height the most variable character (cV% 47.7). The populations 1252, 1278 and 1283 showed no significant intrapopulation differences for onset of flowering which in general was the most variable character (Table 2). 14 from 25 populations were homogenous in fruit number.

In comparison with *C. bursa-pastoris*, *C. rubella* plants were generally flowering later, developed more rosette leaves, and the fruits were smaller. A regression analysis (Table 3, Fig. 4) demonstrated for both species a high positive correlation between

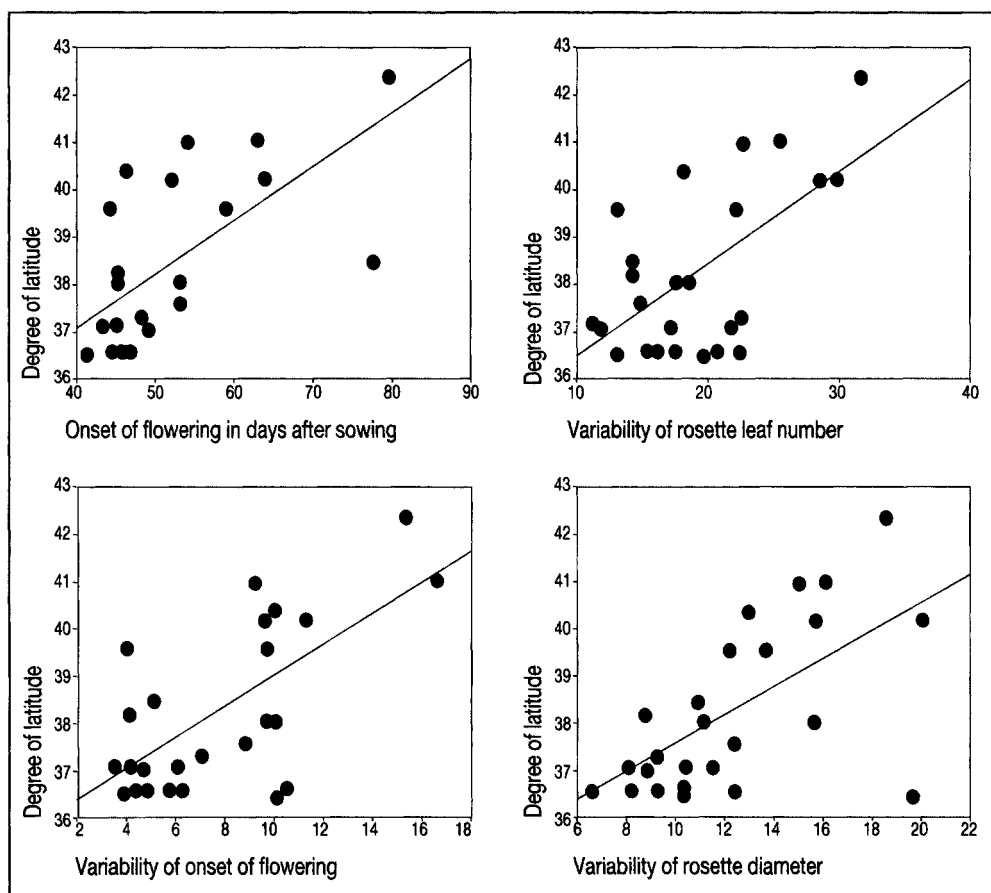


Fig. 4.—Scatter diagrams for onset of flowering ( $r = 0.675$ ), variability of onset of flowering ( $r = 0.664$ ), leaf number ( $r = 0.612$ ), and rosette diameter ( $r = 0.629$ ) with degree of latitude. Linear regression analysis, probability value  $P < 0.05$ . *Capsella rubella* not included.

TABLE 2. STATISTICAL DATA ON BIOLOGICAL TRAITS

Population		830	1228	1229	1234	1238	1249	1252		1272	1278	1279	1280	1281	
elevation (m)		1650	680	1670	270	400	150	150		30	250	300	900	1240	
latitude °N		42.4	40.4	40.21	39.59	39.58	38.47	38.19		37.01	36.46	36.5	36.55	36.57	
Ploidy level		4X	4X	4X	4X	4X	4X	2X	4X	2X	4X	4X	4X	4X	
Fl (days)	m	80	46	52	44	59	78	72	45	61	49	41	41	46	44
	cV%	15.5	10.1	9.7	4.0	9.8	5.1	6.6	4.1	4.0	4.6	10.3	4.0	10.5	6.3
	H-test	+	+	+	+	+	/	+	-	-	+	-	+	+	+
	n	74	93	92	100	100	7	88	60	40	100	30	80	98	99
LN	m	38	12	16	12	18	35	30	12	17	16	11	11	10	11
	cV%	32.1	18.3	28.9	13.1	22.4	14.3	14.4	14.3	14.8	11.8	19.8	13.1	22.6	17.6
	H-test	+	+	+	+	+	/	+	+	-	-	-	-	+	+
	n	76	91	91	100	100	10	88	60	40	100	30	80	98	99
RD (cm)	m	27	23	26.5	24.5	27	16.8	19	26.8	25.5	29.5	21	24.5	25.5	26
	cV%	18.8	13.1	15.9	12.3	13.8	11	13.6	8.8	10.5	8.9	19.9	10.4	12.5	10.4
	H-test	+	-	+	+	+	/	+	-	+	-	+	+	+	+
	n	76	92	91	100	100	10	88	60	40	100	30	80	98	99
PH (cm)	m	29	52	56	52	60	22	31	54	74	67	31.5	45	56	54
	cV%	29.2	12.4	17.1	14	15.6	44.2	30.5	15.9	12.1	10.8	27.5	10.6	11	14.7
	H-test	+	-	+	-	+	+	+	+	-	-	+	-	-	+
	n	54	93	89	99	99	5	85	60	40	99	30	80	98	99
BB	m	9	7	10	9	9	6	9	8	15	8	9	7	7	7
	cV%	42.0	18.5	22.9	22.1	24.4	42.5	23.1	19.5	34.1	18.6	18.5	18.6	18.4	19.4
	H-test	+	+	-	+	+	/	-	-	+	-	-	-	-	+
	n	53	93	89	99	99	5	83	60	40	99	30	80	98	99
SB	m	71	126	134	103	126	105	69	141	138	146	85	196	121	132
	cV%	34.8	34.5	51.7	38.2	41.9	/	28.8	35.3	21.2	34.3	29.9	38.6	33.5	31.7
	H-test	-	-	-	-	-	/	-	-	-	-	-	-	-	-
	n	12	19	20	20	20	1	14	12	8	20	6	15	20	19
FL (mm)	m	7.5	8	7	8.5	8.5	5.8	6	8.5	8	8	7.5	7.5	8.5	8
	cV%	9.8	9.8	12.8	7.1	10.9	16.4	13.9	8.4	7.2	5.8	7.3	8.6	7.5	9.9
	H-test	-	-	+	+	+	/	+	+	+	-	+	-	-	+
FW (mm)	m	4.5	5.5	4.5	6	5.5	4.5	5	5.5	6	5.5	5	5	5.5	5
	cV%	10.4	9.1	13.6	8.8	9.8	15.8	12.2	11.6	6.3	7.8	7	8.5	9.2	10.3
	H-test	+	+	+	+	+	/	+	+	-	+	-	-	+	+
(FL + FW) n		60	93	91	100	99	6	87	60	40	100	30	80	98	99
FN	m	44	34	37	30	36	49	41	37	30	37	35	33	38	39
	cV%	23.6	17.1	17.8	17.5	18.7	30.2	19.2	16.7	15.4	15.5	17.2	18.3	15.1	15.6
	H-test	+	-	+	+	+	/	+	-	-	-	-	-	-	+
	n	51	92	88	100	99	4	81	60	40	100	30	80	98	99

m = median value over all individuals; cV% = coefficient of variability; n = number of individuals

Fl = onset of flowering; LN = rosette leaf number; RD = rosette diameter; PH = plant height; BB = number of basal branches; SB = number of stem branches; FL = fruit length; FW = fruit width; FN = number of fruits. Results of H-test within and between populations (ANOVA,  $\alpha < 0.05$ ), + = significant differences, - = no significant differences



1282	1283	1284	1285	1286	1287	1288	1289	1290		1291	1295	1296	1301	Total	
2000	1650	1400	1000	1250	1580	1000	670	1100		370	1000	800	1300		
36.58	36.57	36.58	37.09	37.08	37.08	37.3	38.04	38.04		37.58	41.05	41	40.22		
4X	4X	4X	4X	4X	4X	4X	4X	4X	2X	4X	4X	4X	4X	4X	2X
44	44	45	43	43	45	48	45	53	68	53	63	54	64	46	69
4.4	4.8	5.8	4.2	6.1	3.5	7.1	10.1	9.8	4.3	8.9	16.8	9.3	11.4	18.5	9.4
+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
100	30	99	100	92	100	40	100	77	20	98	100	100	61	2002	148
13	14	14	12	11	14	15	13	18	28	17	24	18	15	14	28
16.2	15.5	20.9	17.3	22	11.4	22.7	18.7	17.7	19.3	14.9	25.8	22.9	30.2	43.4	26.3
+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
100	30	100	100	92	100	40	100	78	20	98	100	100	60	2005	148
22	25.5	26.5	27.3	25.5	26	25.5	25.5	26	23.5	27.3	27.5	28.3	27.8	26	21.5
8.2	6.6	9.3	11.6	10.5	8.1	9.3	11.2	15.8	12.3	12.5	16.3	15.2	20.3	14.6	17.8
+	-	+	+	+	+	-	+	+	-	+	+	+	+	+	+
100	30	100	100	92	100	40	100	78	20	98	100	100	60	2006	148
33	52	55	58	52	57	47.5	59	56	69	55	50.5	61	52	52.8	47.2
14.5	9.9	11	15.4	9.2	9.4	13.1	14.9	14.6	14.7	12	23.7	14.4	22.7	20.9	47.7
-	-	-	+	-	-	-	+	+	-	+	+	+	+	+	+
100	29	99	100	92	99	40	99	77	20	92	92	99	55	1952	145
10	7	8	7	7	8	8	8	7	13	9	9	8	8	8	10
22.1	27.5	19.6	17.4	21.6	14.7	22.3	21.4	19.3	18.6	29.4	20.8	21.7	31.1	26.5	37.4
+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	+
100	30	100	100	92	99	40	99	77	20	93	92	100	50	1953	143
52	83	112	131	126	96	93	116	102	120	126	133	131	52	118	89
20	33.1	21.2	26.6	32.3	25.8	29	41.9	33.4	34.8	24.8	32	36.4	44.3	41.7	36.7
-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
19	5	19	20	20	20	8	20	16	4	20	20	20	13	397	26
8,5	8,5	8,5	9	8,5	8	8,5	9	8,5	7,5	9	8	8,5	7,5	8	7
7.3	7.2	8.1	9.2	7.8	7.3	7.1	9	8.5	8.9	8.3	9.6	9.5	8.6	10.3	17
-	+	+	+	-	-	+	+	+	-	-	+	+	-	+	+
5.5	5,5	5,5	5,5	5	5	5,5	6	5,5	5	5,5	5	5,5	4,5	5,5	5
7.7	6.9	9	9.7	7.9	8	7.2	8.1	10.4	11.7	10	12.8	8.4	11	10.9	15.4
-	-	+	+	-	-	-	+	+	-	-	+	-	-	+	+
100	30	100	100	92	99	40	100	77	20	94	91	99	58	1971	147
41	39	37	33	34	36	35	31	33	28	38	36	36	32	35	35
16.3	14	14.4	14.3	17.3	13.6	15.2	16.7	21.9	17.9	18.2	23	16.3	19.5	18.8	24.9
-	-	+	-	+	-	-	+	-	-	+	+	-	-	+	+
100	30	99	100	92	100	40	100	77	20	94	93	98	57	1956	141

leaf number and onset of flowering. The low correlation between quantitative trait parameters within the *C. bursa-pastoris* plants and higher correlation within the *C. rubella* plants is in concordance with our earlier findings (NEUFFER & ALBERS, 1996). Populations from the northern part of Spain were slightly later flowering and more variable (Fig. 4).

Figure 5 demonstrates the relationships between populations and the three factors

given by PCA analysis. The Atlantic population 1249 and the Pyrenean population 830 are separated from the other populations by factor 1 but pulled apart by factor 2. The populations from the different mountain regions form subgroups. The Sierra de Gredos populations and the Sierra Nevada populations are connected by the overlapping group of the Sierra Cazorla. The Sierra de Gredos populations are more similar to the Pyrenean populations than to the more

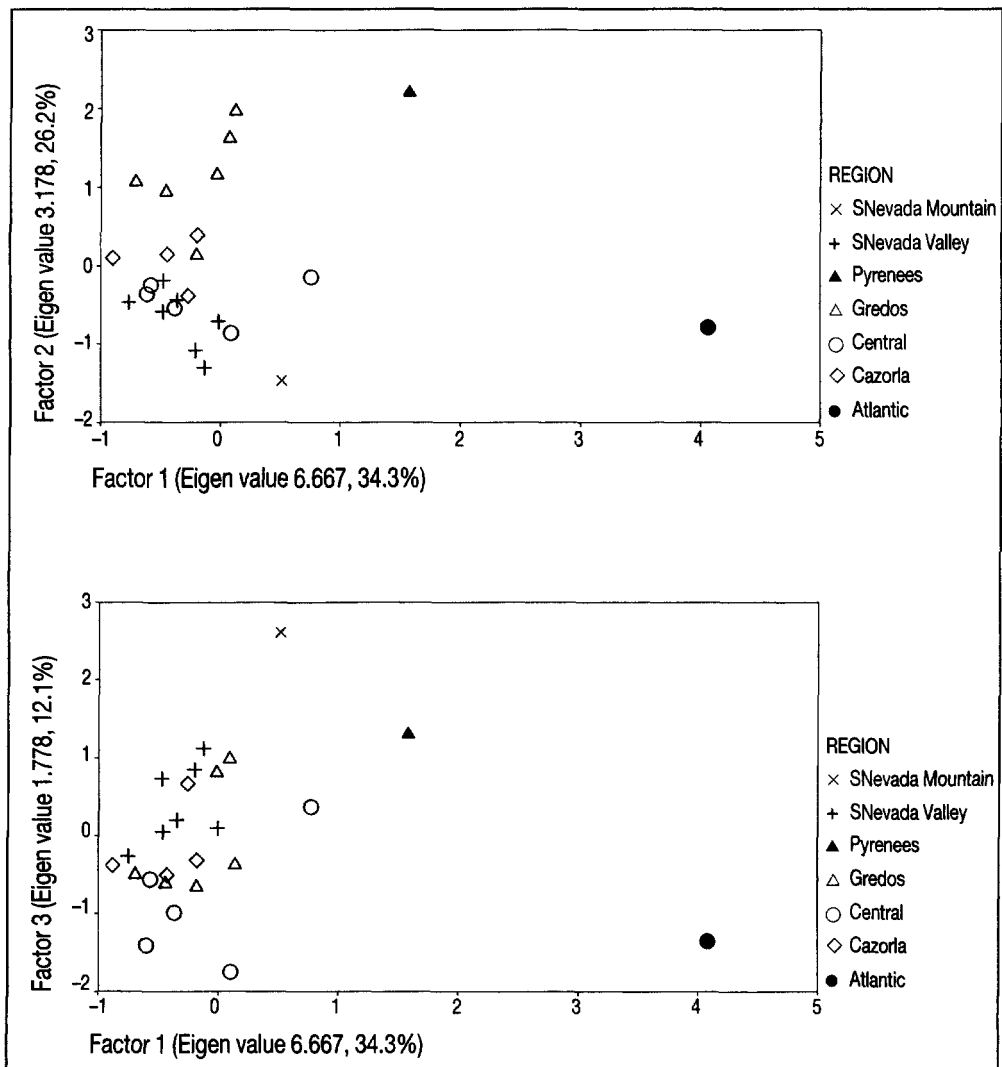


Fig. 5.—Principal component analysis of *Capsella bursa-pastoris* on the Iberian Peninsula.

southern populations. The populations from the mesetas (named "Central") are in between. The Sierra Nevada population 1282 from the highest elevation (SNevada Mountain, Fig. 5) is different from the other populations of the Sierra Nevada region.

### Leaf morphology

*Capsella rubella* plants were predominantly "heteris" (110 individuals "heteris", 40 individuals "rhomboidea"), whereas within *C. bursa-pastoris* the leaf type "rhomboidea" was prevalent (1235 out of 1944 individuals). The "tenuis" type occurred in very low frequency (only 6 individuals within *C. bursa-pastoris*, none within *C. rubella*). The frequency of "rhomboidea" type increased in the mountainous regions (Pyrenees, Sierra Cazorla, Sierra de Gredos, Sierra Nevada, Fig. 3). The "heteris" type was frequent in Atlantic and central regions and in the Sierra de Gredos (Fig. 3). The "simplex" type predominated at the Sierra Nevada, Cazorla and central regions, and disappeared completely at the Atlantic coast in favour of the "heteris" type (Fig. 3). It was also rare in the Pyrenees and in high mountain regions of the Sierra Nevada. A geographic differentiation pattern was obvious (Fig. 3).

### Allozyme analyses

The allelic multilocus structure in the *C. bursa-pastoris* populations from Spain exhibited low variability (Table 4). Only the *Aat1B* locus, the *Lap3B* locus and the *Gdh2A* locus varied considerably between the populations. Some rare alleles seemed to be restricted to the central and northern part of Spain (e.g. *Aat3A-3*, *Gdh2B-3*, Table 4).

In the populations studied in this paper *C. bursa-pastoris* was fixed for *Aat2B-4*, whereas *C. rubella* was fixed at all *Aat*-loci, but not at the *Lap*- and *Gdh*-loci (Table 4). In *C. bursa-pastoris* we detected six heterozygotes (pop. 1229 *Aat3B-35*, pop. 1234 *Aat2A-14*, pop. 1238 two individuals *Lap3B-56* and 57, pop. 1296 *Aat1A-14*), in *C. rubella* one (pop. 1252 *Gdh2-12*). The low frequency of heterozygotes was in

concordance with the predominantly selfing mating system (HURKA & *al.*, 1989).

### Geographical distribution and characterization of the populations

The populations observed in this study originated from different vegetation zones, climatic regions, and habitats as shown in Table 1. Population 830 from the Pyrenees was late flowering with large rosette diameter and predominantly "rhomboidea" leaf type (Fig. 3), but remained small with few branches and many small fruits (Table 2). Some allozymes occurring in the Pyrenees were not observed further south except for the Sierra de Gredos (*Aat3B-2*, *Gdh2B-3*, 1229, 1295, 1301, Table 4).

The populations 1228, 1229, 1295-1301 from the Sierra de Gredos were early to intermediate flowering and were generally tall, but developed only a small number of rosette leaves with different leaf types (Fig. 3).

Pop. 1249 from Sintra (Lisbon) was a "two species site". All plants of both species were late flowering, had small rosettes predominantly with "heteris" leaves, and they remained small with a small number of branches. Their inflorescences had many but small fruits. *C. rubella* of population 1249 from the western Atlantic coast of Portugal had the most common isoenzyme multilocus genotype of this species (HURKA & NEUFFER, 1997).

The populations 1234, 1238 and 1252 from central Spain exhibited various leaf types (Fig. 3). The population 1252 was a "two species site". At this collecting site (Jerez de los Caballeros) we detected one hybrid between *C. bursa-pastoris* and *C. rubella*, which is named as *C. gracilis* Gren. in the literature. This hybrid produced only sterile fruits and therefore was easy to determine. Some *C. rubella* plants showed an unusual isoenzyme multilocus genotype at the *Lap3A* -and *Gdh2A*- loci (*Lap3A-11*, instead of *Lap3A-66*, *Gdh2A-22* instead of *Gdh2A-11*, Table 4) and were later flowering than *C. bursa-pastoris*. These *C. rubella* plants

TABLE 3

## CORRELATION MATRIX OF SPEARMAN'S RANK CORRELATION COEFFICIENTS

[Significant correlations > 0.7 printed bold; (+) positive correlation; (-) negative correlation; \*  $\alpha < 0.01$ , \*\*  $\alpha < 0.001$ ]

	Onset of flowering 4X/2X	Rosette diameter 4X/2X	Leaf number 4X/2X	Plant height 4X/2X	Basal branches 4X/2X	Stem branches 4X/2X	Fruit width 4X/2X	Fruit number 4X/2X
Leaf number	<b>.716**/.865**</b>	.348**/.525**	1.000/1.000	.195**/.669**	.295**/-.370**	.055/-.345	-.007/-.642**	.106**/.458**
Plant height	.116**/-.827**	.412**/.792**	.195**/-.669**	1.000/1.000	.097**/.606**	.408**/.660**	.226**/.768**	-.009/-.676**
Fruit width	-.113**/-.757**	-.008/.694**	-.007/-.642**	.227**/.768**	.038/.382**	.020/.509**	1.000/1.000	-.270/-.569**
Fruit length	-.150**/-.775**	.027/.746**	-.100**/-.666**	.158**/.852**	-.013/.503**	.002/.479*	.457**/.813**	-.121**/-.675**

were tall and bushy with a few but large rosette leaves and few small fruits.

The populations 1272, 1278, and 1279 from the south coast were early flowering with a relatively small number of rosette leaves showing a high amount of "simplex" types and a low number of small to intermediate fruits.

The populations 1288-1291 from the Sierra Cazorla exhibited predominantly the "rhomboidea" leaf type (Fig. 3). Population 1290 was also a "two species site". Both species had the most common Mediterranean isoenzyme multilocus genotype.

The populations 1280-1287 from the Sierra Nevada were early flowering with an intermediate rosette diameter and a very low number of rosette leaves. Plants produced an intermediate number of large fruits. The plants grew tall with the exception of 1282, which had the smallest rosette diameter, and the lowest number of branches but produced the highest number of fruits and a high number of "rhomboidea" leaves. This population originated from the highest point of all collected sites (2000 m).

## DISCUSSION

*Quantitative traits*

*Onset of flowering.*—The onset of flowering in *Capsella* is influenced by geographical, climatic and biotic factors (for a review see HURKA & NEUFFER, 1997). The greater flexibility of *C. bursa-pastoris* compared to *C. rubella* was reflected by a diverse array of flowering ecotypes: Early flowering types in the summerdry and hot Mediterranean climate in the South of Spain (populations 1272-1291), and late flowering types in the alpine climate of the Pyrenees (pop. 830). This stays in contrast to the findings in *Rhinanthus glacialis* PERSONNAT which is earlier flowering when populations are derived from higher elevations (ZOPFL, 1995). In contrast to our findings from other mountainous regions like the Alps in Europe (NEUFFER & BARTELHEIM, 1989) or the Sierra Nevada in North America (NEUFFER &

HURKA, 1999), *C. bursa-pastoris* was early flowering at high elevations in the Sierra Nevada (pop. 1282, Table 2). In alpine climate the higher the altitude of origin, the later the onset of flowering and the more summer annual genotypes were replaced by winter annual genotypes. In the Sierra Nevada, no such clinal variation was observed. We attribute these differences to the different climates in the Alps and in the Sierra Nevada. The latter mountains are summer-dry and have short mild winters. Provenances of the Sierra de Gredos displayed intermediate flower onset (Figure 5). This might be the result of climatic factors which are not as extreme as in the Sierra Nevada (Spain) or in the Pyrenees.

**Growth forms.**—Growth performance of plants from the Pyrenees (pop. 830) was similar to what is observed in other mountains appearing low and bushy with large rosettes and many small fruits (NEUFFER & HURKA, 1986b; NEUFFER & BARTELHEIM, 1989). Plants from high elevations in the Sierra Nevada, however, grew taller, had smaller rosettes, and were less branched. They resembled those from the Sierra Nevada valley populations.

### Leaf morphology

Leaf form has implications for thermoregulation, water-use efficiency, photosynthesis, productivity, branching and rooting strategies, and competitive ability (GATES, 1968; PARKHURST & LOUCKS, 1972; TAYLOR, 1975; GIVNISH, 1979; DUDLEY, 1996). A subdivided leaf is believed to decrease the "effective leaf size", thereby reducing overheating and transpiration in dry and exposed conditions, while a continuous leaf surface increases the photosynthetic area and allows the leaf to warm up above air temperature in cool and shady conditions (GIVNISH, 1987).

Most of the *C. rubella* plants showed the "heteris" leaf type, which is in full accordance with the findings of ALMQUIST (1907, 1929), SHULL (1909, 1929), NEUFFER & ESCHNER (1995), and NEUFFER & ALBERS (1996). In

*C. bursa-pastoris*, the "rhomboidea" leaf type occurred at a very high frequency (Fig. 3). Frequency of this leaf type is high in general and the distribution is world-wide, in contrast to "tenuis" which is of low frequency in general (HURKA & NEUFFER, 1997). The present study is in accordance with the general picture (Fig. 3). This unequal distribution of the leaf types may be due to the higher adaptive value of the "rhomboidea" leaf type in regard to water use efficiency or may be just a stochastic phenomenon. In favour of a non-stochastic distribution of the "rhomboidea" leaf type within the Sierra Nevada region is the observation that in lower elevation the leaf types "heteris" and "simplex" occurred. These types disappeared in the mountains (Sierra Nevada and Pyrenees, Figure 3, see also NEUFFER & BARTELHEIM, 1989) and were replaced by the "rhomboidea" leaf type.

### Allozyme analyses

Most *Capsella bursa-pastoris* plants studied are characterized by the multilocus genotype *Aat1A-11 Aat1B-11, Aat2A-11 Aat2B-44, Aat3A-11 Aat3B-55, Lap3A-22 Lap3B-77, Gdh2A-22 Gdh2B-22* (Table 4). This genotype was named the Mediterranean Multilocus Genotype (MMG) because of its prevalence in the Mediterranean area (NEUFFER & HURKA, 1999). It coincides with an early flowering genotype (Table 2 and 4). It stands for discussion to what extent selection will influence the geographic pattern of allozyme variation. In a study of colonial Californian and ancestral Spanish populations of *Avena barbata* L. it was argued that the main force responsible for geographic patterns of isozyme multilocus structures was selection for particular combinations in response to different environments (PÉREZ DE LA VEGA & *al.*, 1991; ALLARD & *al.*, 1993). AINOUCHE & *al.* (1996) detected correlations of isozyme multilocus composition with climatic variables in *Bromus lanceolatus* Roth, and EMERY & *al.* (1994) observed distinct clusters of allozymic variation separating high elevation

TABLE 4. ALLELE FREQUENCIES OF AAT, LAP, GDH OF *CAPSELLA*  
(in brackets frequencies of *C. rubella*)

Population	830	1228	1229	1234	1238	1249	1249	1252	1252	1272	1278	1279	1280	1281	1282
Ploidy-level	4X	4X	4X	4X	4X	4X	2X	4X	2X	4X	4X	4X	4X	4X	4X
Alleles															
<i>Aat1A-1</i>	1.0	0.9	1.0	0.95	1.0	1.0		1.0		1.0	1.0	1.0	1.0	1.0	1.0
2							(1.0)		(1.0)						
4		0.1		0.05											
<i>Aat1B-1</i>	1.0	0.9	0.6	0.9	1.0	1.0		0.67		1.0	0.67	1.0	0.9	0.7	1.0
4		0.1	0.4	0.1				0.33			0.33		0.1	0.3	
<i>Aat2A-1</i>	1.0	1.0	1.0	0.1	1.0	1.0	(1.0)	1.0	(1.0)	1.0	1.0	1.0	1.0	1.0	1.0
4				0.9											
<i>Aat2B-4</i>	1.0	1.0	1.0	1.0	1.0	1.0		1.0		1.0	1.0	1.0	1.0	1.0	1.0
<i>Aat3A-1</i>	1.0	1.0	0.5	1.0	1.0	1.0	(1.0)	1.0	(1.0)	1.0	1.0	1.0	1.0	1.0	1.0
2			0.3												
3			0.2												
<i>Aat3B-2</i>	0.25		0.3												
5	0.75	1.0	0.7	1.0	1.0	1.0		1.0		1.0	1.0	1.0	1.0	1.0	1.0
<i>Lap3A-1</i>									(0.25)						
2	1.0	1.0	1.0	1.0	1.0	1.0		1.0		1.0	1.0	1.0	1.0	1.0	1.0
5															
6							(1.0)		(0.75)						
<i>Lap3B-5</i>	1.0	0.5	1.0	0.9	0.56									0.3	0.5
6			0.1	0.17					0.2						
7		0.5			0.28	1.0		1.0		1.0	1.0	1.0	0.5	0.5	1.0
9															
<i>Gdh2A-1</i>	0.6	0.4	0.3	0.7	0.5		(1.0)				0.67		0.6	0.4	
2	0.4	0.6	0.7	0.3	0.5	1.0		1.0	(1.0)	1.0	0.33	1.0	0.4	0.6	1.0
<i>Gdh2B-2</i>	0.6	1.0	1.0	1.0	1.0	1.0		1.0		1.0	1.0	1.0	1.0	1.0	1.0
3	0.4														
number of individuals tested															
AAT	8	10	10	10	9	1	9	6	4	10	3	8	10	10	10
LAP	8	10	10	10	10	1	9	6	4	10	3	8	10	10	10
GDH	5	10	10	10	10	1	9	6	4	10	3	8	10	10	9

populations of *Stellaria longipes* Goldie from those of lower elevations. However, even in the case of coincidence between ecotypic and allozyme variation pattern as has been shown for *C. bursa-pastoris*, this is by no means proof of selection on isozyme loci. One would rather assume that in the case of *C. bursa-pastoris* the MMG is a molecular marker for

an early flowering ecotype. A cosegregation analysis (quantitative trait loci analysis) is presently underway to substantiate this hypothesis (NEUFFER & LINDE, 1999).

From the results of our study we conclude that variation pattern of certain quantitative traits observed in *Capsella* along altitudinal gradients in temperate climates is different

1283	1284	1285	1286	1287	1288	1289	1290	1290	1291	1295	1296	1301	Total	number of individuals	
4X	4X	4X	4X	4X	4X	4X	4X	2X	4X	4X	4X	4X	4X	2X	4X 2X
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	(1.0)	1.0	1.0	0.55	1.0	0.973	199	
											0.45		0.027	5	(15)
1.0	1.0	0.9	0.3	1.0	1.0	0.5	0.5		0.9	1.0	0.4	1.0	0.849	174	
		0.1	0.7			0.5	0.5		0.1		0.6		0.151	31	
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	(1.0)	1.0	1.0	1.0	1.0	0.954	195	(15)
													0.046	9	
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		1.0	1.0	1.0	1.0	1.0	205	
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	(1.0)	1.0	1.0	1.0	0.25	0.951	195	(15)
												0.015	0.015	3	
												0.75	0.029	6	
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		1.0	1.0	1.0	1.0	0.039	8	
													0.961	197	
1.0	1.0	1.0	1.0	1.0	1.0	0.8	0.5		1.0	1.0	1.0	1.0	0.971	198	
						0.1	0.5						0.025	5	
						0.1		(1.0)					0.005	1	(14)
0.1	0.5	1.0	0.9		0.75	0.7	1.0		1.0	0.7	0.1	0.25	0.402	81	
1.0	0.9	0.5		0.1	0.3					0.3		0.174	35		
					0.25					0.3	0.6		0.409	83	
												0.75	0.015	3	
1.0	0.9	0.8	0.1	1.0	0.75	0.3	0.625	(1.0)	1.0	0.5	0.7		0.488	98	(11)
	0.1	0.2	0.9		0.25	0.7	0.375			0.5	0.3	1.0	0.512	103	
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0		1.0	0.7	1.0		0.955	192	
										0.3		1.0	0.045	9	
3	10	10	10	10	4	10	8	2	10	10	10	4			
3	10	10	10	10	4	10	8	2	10	10	10	4			
3	10	10	10	10	4	10	8	2	10	10	10	4			

from that observed in Mediterranean climate. Temperature, humidity and radiation during the summer period differ sharply between alpine and Mediterranean mountains. These different ecological regimes apparently favour different ecotypes and thus would explain the different variation patterns in alpine and Mediterranean mountains. One of

the most striking differences concerns the onset of flowering: whereas in alpine climates late flowering replace early flowering ecotypes along an altitudinal gradient, no such replacement occurred in Mediterranean mountains. High mountain populations of *Capsella* comprise early flowering plants only. Frequencies of leaf morphology types

may also be governed by temperature and humidity regimes. In general, it would appear that certain variation patterns of quantitative traits in *Capsella* on a macro- and micro-geographical scale are adaptive despite all the random processes due to its colonizing abilities. It is unlikely, however, that the observed variation pattern in isozyme allelic composition is also the outcome of direct environmental selection pressures. We rather interpret our data by a linkage between isozyme genotypes and phenotypic traits. Analyses are currently under progress to investigate cosegregation of allozymes and quantitative traits.

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#### REFERENCES

- AINOUCHE, M., M.T. MISSET & A. HUON (1996). Patterns of genetic differentiation in two annual bromegrass, *Bromus lanceolatus* and *B. hordeaceus* (Poaceae). *Pl. Syst. Evol.* 199: 65-78.
- ALLARD, R.W., P. GARCÍA, SÁENZ DE MIERA & M. PÉREZ DE LA VEGA (1993). Evolution of multilocus structure in *Avena hirtula* and *Avena barbata*. *Genetics* 135: 1125-1139.
- ALMQUIST, E. (1907). Studien über die *Capsella bursa pastoris* (L.). *Acta Horti Berg.* 4: 1-91.
- ALMQUIST, E. (1929). Zur Artbildung in der freien Natur. *Acta Horti Berg* 9: 37-75.
- BAKER, H.G. (1965). Characteristics and modes of origin of weeds. In: H.G. Baker & G.L. Stebbins (eds.), *The genetic of colonizing species*: 147-172. Academic Press, London, New York.
- BARRETT, S.C.H. & B.J. RICHARDSON (1986). Genetic attributes of invading species. In: R.H. Groves & J.J. Burdon (eds.), *Ecology and biological invasions*: 21-33. Cambridge University Press, Cambridge.
- BROWN, A.H.D. & D.R. MARSHALL (1981). Evolutionary changes accompanying colonization in plants. In: G.G.E. Scudder & J.L. Reveal (eds.), *Evolution today*: 351-363. Proc. 2nd Int. Congr. Syst. Evol. Biol. Pittsburgh.
- CLEGG, M.T. & A.H.D. BROWN (1983). The founding of plant populations. In: C.M. Schonewald-Cox, S.M. Chambers, B. Macbride & L. Thomas (eds.), *Genetics and conservation*: 216-228. Biological Conservation Series 1. Benjamin-Cummings, Menlo Park, California.
- DUDLEY, S.A. (1996). Differing selection on plant physiological traits in response to environmental water availability: A test of adaptive hypothesis. *Evolution* 50: 92-102.
- EMERY, R.J.N., C.C. CHINNAPPA & J.G. CHMIELEWSKI (1994). Specialization, plant strategies, and phenotypic plasticity in populations of *Stellaria longipes* along an elevational gradient. *Int. J. Pl. Sci.* 155: 203-219.
- GATES, D.M. (1968). Energy exchange in the biosphere. In: F.E. Eckhardt (ed.), *Functioning of terrestrial ecosystems at the primary producing level*: 33-43. Proceedings of the Copenhagen Symposium, Unesco Natural Resources Research 5, Unesco, Paris.
- GIVNISH, T. (1979). On the adaptive significance of leaf form. In: O.T. Solbrig, S.K. Jain, G.B. Johnson & P.H. Raven (eds.), *Topics in plant population biology*: 375-407. Columbia Univ. Press, New York.
- GIVNISH, T. (1987). Comparative studies of leaf form: Assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytol.* 106 (Suppl.): 131-160.
- HURKA, H. & S. DÜRING (1994). Genetic control of plastidic L-glutamate dehydrogenase isozymes in the genus *Capsella* (Brassicaceae). *Heredity* 72: 126-131.
- HURKA, H., S. FREUNDNER, A.H.D. BROWN & U. PLANTHOLT (1989). Aspartate aminotransferase isozymes in the genus *Capsella* (Brassicaceae): subcellular location, gene duplication, and polymorphism. *Biochem. Genet.* 27: 72-90.
- HURKA, H. & B. NEUFFER (1991). Colonizing success in plants: Genetic variation and phenotypic plasticity in life history traits in *Capsella bursa-pastoris*. In: G. Esser & D. Overdieck (eds.), *Modern ecology: basic and applied aspects*: 77-96. Elsevier, Amsterdam, New York.
- HURKA, H. & B. NEUFFER (1997). Evolutionary processes in the genus *Capsella* (Brassicaceae). *Pl. Syst. Evol.* 206: 295-316.
- NEUFFER, B. (1990). Ecotype differentiation in *Capsella*. *Vegetatio* 89: 165-171.
- NEUFFER, B. & S. ALBERS (1996). Phenotypic and allozyme variability in *Capsella* populations with different ploidy levels from different continents. *Bot. Jahrb. Syst.* 118: 433-450.
- NEUFFER, B. & S. BARTELHEIM (1989). Genecology in *Capsella bursa-pastoris* from an altitudinal transect in the Alps. *Oecologia* 81: 521-527.
- NEUFFER, B. & S. ESCHNER (1995). Life history traits and ploidy levels in the genus *Capsella* (Brassicaceae). *Can. J. Bot.* 73: 1354-1365.
- NEUFFER, B. & H. HURKA (1986a). Variation of development time until flowering in natural populations of *Capsella bursa-pastoris* (Cruciferae). *Pl. Syst. Evol.* 152: 277-296.



- NEUFFER, B. & H. HURKA (1986b). Variation of growth form parameters in *Capsella* (Cruciferae). *Pl. Syst. Evol.* 153: 265-279.
- NEUFFER, B. & H. HURKA (1988). Germination behaviour in populations of *Capsella bursa-pastoris* (Cruciferae). *Pl. Syst. Evol.* 161: 35-47.
- NEUFFER, B. & H. HURKA (1999). Colonization history and introduction dynamics of *Capsella bursa-pastoris* (Brassicaceae) in North America. *Molecular Ecology* (in press).
- NEUFFER, B. & M. LINDE (1999). *Capsella bursa-pastoris* – colonization and adaptation. In: L. Van Raamsdonk, H. den Nijs & R. Van der Meijden (eds.), *Plant evolution in man-made habitats*. Proc. VII IOPB Symp. (in press).
- NEUFFER, B. & M. MEYER-WALF (1996). Ecotypic variation in relation to man made habitats in *Capsella*: field and trampling area. *Flora* 191: 49-57.
- PARKHURST, D.F. & O.L. LOUCKS (1972). Optimal leaf size in relation to environment. *J. Ecol.* 60: 505-537.
- PÉREZ DE LA VEGA, M., P. GARCÍA & R.W. ALLARD (1991). Multilocus genetic structure of ancestral Spanish and colonial Californian populations of *Avena barbata*. *Proc. Natl. Acad. Sci. USA* 88: 1202-1206.
- SCHMITHÜSEN, J. (ed.) (1976). *Atlas zur Biogeographie*. Bibliographisches Institut AG. Zürich.
- SHULL, H. (1909). *Bursa bursa-pastoris* and *Bursa heegeri*: biotypes and hybrids. *Carnegie Inst. Wash. Year Book* 112: 3-56.
- SHULL, H. (1911). Defective inheritance-ratios in *Bursa* hybrids. *Verhdl. Naturhistor. Ver. Brünn* 49: 157-168.
- SHULL, H. (1918). The duplication of the leaf lobe factor in the sheperd's purse. *Brooklyn Bot. Gard. Mem.*: 427-433.
- SHULL, H. (1923). The species concept from a point of view of a geneticist. *Amer. J. Bot.* 10: 221-228.
- SHULL, H. (1929). Species hybridisations among old and new species of sheperd's purse. *Proc. Internat. Congr. Plant Sci.* 1: 837-888.
- STEINMEYER, B., K. WÖHRMANN & H. HURKA (1985). Phänotypenvariabilität und Umwelt bei *Capsella bursa-pastoris* (Cruciferae). *Flora* 177: 323-334.
- TAYLOR, S.E. (1975). Optimal leaf form. In: D.M. Gates & R.B. Schmerl (eds.), *Perspectives of biophysical ecology*: 73-86. Springer, Heidelberg, New York.
- WALTER, H. & H. LIETH (1967). *Klimadiagramm Weltatlas*. VEB Fischer, Jena.
- ZOPFI, H.-J. (1995). Life history variation and infraspecific heterochrony in *Rhinanthus glacialis* (Scrophulariaceae). *Pl. Syst. Evol.* 198: 209-233.

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