

Two haplotypes of *Capsella bursa-pastoris* (Brassicaceae) in Continental Chile support multiple introduction

Dos haplotipos de *Capsella bursa-pastoris* en Chile continental soportan múltiple introducción

SERGIO A. CASTRO^{1,2,*}, CHRISTIAN ESPINOSA¹ & JAVIER A. FIGUEROA^{3,4}

¹Laboratorio de Ecología y Biodiversidad Vegetal, Departamento de Biología, Universidad de Santiago de Chile. Casilla 40, Correo 33, Santiago. Av. Lib. B. O'Higgins 3363, Santiago, Chile.

²Centro para el Desarrollo de la Nanociencia y Nanotecnología, CEDENNA, 917-0124. Av. Lib. B. O'Higgins 3363, Santiago, Chile.

³Centro de Estudios Arquitectónicos, Urbanísticos y del Paisaje, Facultad de Arquitectura, Urbanismo y Paisaje, Universidad Central de Chile. Avenida Santa Isabel 1186, Santiago, Chile.

⁴ONG Entorno, Avenida Vicuña Mackenna 439-A, Santiago, Chile.

*sergio.castro@usach.cl

ABSTRACT

Shepherd's purse (*Capsella bursa-pastoris*; Brassicaceae) is a global weed. In Chile, this species was introduced early during Spanish colonization, however, previous studies based on allozymes and RAPD fragments, show that populations in southernmost Chile could be related to England populations. Here, we analyze the geographical variation of three sequences of cpSSR in 286 individuals of *C. bursa-pastoris* (ATCP46615, ATCP66701 and ATCP31017). We collected plants from 14 populations distributed over a wide latitudinal gradient in continental Chile. Only one of the markers showed variation (ATCP31017), evidencing the presence of two haplotypes along the territory. The geographic distribution of these haplotypes support previous studies based on allozymes and RAPD fragments, suggesting that *C. bursa-pastoris* in continental Chile was introduced at least twice.

KEYWORDS: Biological invasions, colonization history, cpSSR, continental Chile, Shepherd purse.

RESUMEN

La bolsita de pastor (*Capsella bursa-pastoris*, Brassicaceae) es una maleza de distribución global. En Chile, esta especie fue introducida tempranamente durante la colonización española, sin embargo, estudios previos basados en aloenzimas y fragmentos RAPD indican que las poblaciones del extremo sur de Chile estarían emparentadas con poblaciones provenientes de Inglaterra. En el presente estudio se analizó la variación geográfica de tres secuencias de cpSSR pertenecientes a 286 individuos de *C. bursa-pastoris* (ATCP46615, ATCP66701 y ATCP31017). Se recolectaron plantas de 14 poblaciones distribuidas en un amplio gradiente latitudinal en Chile continental. Sólo uno de los marcadores mostró variación (ATCP31017), lo que muestra la presencia de dos haplotipos de *C. bursa-pastoris* en el territorio. La distribución geográfica de estos haplotipos apoya la hipótesis que plantea que *C. bursa-pastoris* en Chile continental fue introducida al menos en dos oportunidades.

PALABRAS CLAVE: Bolsita de pastor, Chile continental, cpSSR, historia de colonización, invasiones biológicas.

INTRODUCTION

Shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.; Brassicaceae) is one of the most widely distributed plants in the world (Randall 2002, Hintz *et al.* 2006). This species, whose original distribution includes Europe, Asia and Northern Africa (Hurka & Neuffer 1997), is currently present in all continents, occupying a wide latitudinal range

(Randall 2002). *C. bursa-pastoris* is considered a weed in most countries where it has been introduced (Randall 2002). In Chile, this species was first noted by Gay (1845), but its introduction probably occurred long before 1800 (Gay 1845, Figueroa *et al.* 2004). Currently, its geographical range covers the whole latitudinal extension between 18 °S and 56 °S (Matthei 1995, Castro *et al.* 2005, Fuentes *et al.* 2012), including Easter Island (Zizka 1991). In Chile,

C. bursa-pastoris occupies ruderal habitats, roadsides, wastelands, prairies, and cultivated lands (Matthei 1995).

In spite of its ubiquity and the extension of its geographic distribution, there is scarce information about introduction of *C. bursa-pastoris* in Chile as well as on the processes that have determined these. Analyzing allozymes and polymorphic DNA obtained by random amplification techniques (RAPD), Neuffer *et al.* (1999) detected two polymorphic variants of *C. bursa-pastoris* in continental Chile. One of them is widely distributed from northern to southern Chile, corresponding to polymorphic variants from Spain (Neuffer *et al.* 1999), while the other is distributed locally in southernmost Chile (Patagonia and Tierra del Fuego), corresponding to polymorphic variants present in England (Neuffer *et al.* 1999). Thus, according to these authors the variation and polymorphic distribution patterns of *C. bursa-pastoris* in Chile would correspond to two routes of arrival.

In the present paper, we study the variation of three microsatellite chloroplast markers (cpSSR) for *C. bursa-pastoris*, seeking to determine haplotypes and respective geographical distributions. With these findings we discuss the colonization model proposed by Neuffer *et al.* (1999) for *C. bursa-pastoris* in Chile.

MATERIALS AND METHODS

SAMPLING

Between 2008 and 2012, specimens of the genus *Capsella* were collected from 14 populations distributed over a wide

latitudinal range (Table I). In the field, fresh leaves were removed from each specimen and were stored in 50-mL Falcon tubes with 20 g of silica gel. The tubes were labeled and stored under laboratory conditions. In the laboratory the *C. bursa-pastoris* and *C. rubella* individuals were distinguished by cloning of the trnD-trnY fragment of the cpDNA carried out by the Amplification Refractory Mutation System (ARMS; Newton *et al.* 1989); thus, 286 specimens of *C. bursa-pastoris* were recognized by ARMS-technique and whereas the *C. rubella*'s specimens were excluded from the following analyses. Samples of *C. bursa-pastoris* were submitted to analyses of three microsatellite loci (cpSSR), amplified from cpDNA. These markers correspond to the ATCP46615, ATCP66701 and ATCP31017 loci, documented by Ceplitis *et al.* 2005 (Table II).

GENOME EXTRACTION AND MARKER AMPLIFICATION

A total extraction of genomic DNA was carried out using CTAB extraction buffer procedure described by Doyle & Doyle (1990) and modified by Allen *et al.* (2006). Each sample was processed under the following conditions: 100 mg of leaf tissue powdered with liquid nitrogen were incubated in 0.7 ml of buffer [120 mM Tris-HCL pH 8.0, 80 mM EDTA pH 8.0, 0.5% triton X-100, 0.5% β-mercaptoethanol] for 20 min at 45 °C. Then, the aqueous phase was removed and the sediment was incubated in 0.7 ml of 2X CTAB buffer [2% w/v hexadecyltrimethylammonium bromide, 5 M NaCl, 2 M Tris-HCl pH 8.0, 0.5 M EDTA, 0.1 M sodium sulfite, 1% w/v PVP40, 0.5 % v/v β-mercaptoethanol] at 60 °C during 20 min, and then 5 μl of proteinase K 10 mg/ml and 1 μl of RNAsa A 10 mg/ml

TABLE I. Localities and geographic location of the plants of *Capsella bursa-pastoris* collected in this study.

TABLA I. Localidades y ubicación geográfica de las plantas de *Capsella bursa-pastoris* colectadas en este estudio.

LOCALITY	COORDINATES		NO. OF PLANTS
Vallenar	28° 35' 00,0" S	70° 46' 00,0" W	7
La Serena	29° 54' 00,0" S	71° 20' 00,0" W	12
Los Vilos	31° 54' 37,3" S	71° 30' 02,7" W	9
San Felipe	32° 45' 00,0" S	70° 43' 00,0" W	14
Los Andes	32° 49' 46,9" S	70° 35' 58,0" W	21
Santiago	33° 26' 45,6" S	70° 41' 18,2" W	35
Curicó	34° 58' 47,6" S	71° 14' 48,3" W	25
Talca	35° 25' 49,9" S	71° 38' 23,5" W	24
Chillán	36° 07' 49,0" S	71° 50' 27,0" W	27
Concepción	36° 49' 17,3" S	73° 02' 30,1" W	35
Los Ángeles	37° 27' 05,8" S	72° 20' 20,6" W	36
Temuco	38° 43' 44,9" S	72° 34' 13,0" W	19
Chile Chico	46° 32' 25,0" S	71° 43' 20,0" W	6
Punta Arenas	53° 09' 45,0" S	70° 55' 21,0" W	16
Total			286

were added, incubating at 37 °C during 40 min. An organic extraction was carried out with chloroform:isoamyl alcohol (24:1) with centrifugation by 10 min. The aqueous phase was recovered; 3 M sodium acetate, pH 5.2, in an amount equivalent to 1/10 of the recovered volume, and 2 volumes of cold absolute ethanol were added, and the DNA was allowed to precipitate at -20 °C for 6 h. The pellet was then recovered by centrifugation and it was resuspended in 400 µl of 1X TE high salinity buffer [0.01 M Tris-HCl, 0.001 M EDTA, 1 M NaCl, pH 8.0]. Then 40 µl of 3 M sodium acetate and 880 µl of cold absolute ethanol were added, the mixture was allowed to precipitate, and the DNA obtained was re-suspended in 50 µl of 1X TE buffer pH 8.0 [0.01 M Tris-HCl; 0.001 M EDTA, pH 8.0].

The amplifications were made according to the procedure described by Provan (2000) and Ceplitis *et al.* (2005): on 10-µl volume that contained Taq DNA polymerase 1X buffer (Taq DNA recombinant polymerase, Fermentas), 1.5 mM MgCl₂, 0.2 mM dNTP (Fermentas), 0.3 U Taq DNA polymerase (Taq DNA recombinant polymerase, Fermentas), 10 pmol of each primer, and 20 - 50 ng of genomic DNA. The amplification profile consisted of an initial denaturation

by 3-min at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at TAnn for each primer, 30 s at 72 °C; and a final extension of 5 min at 72 °C. The alignment temperatures were adjusted for each pair of primer separately in order to improve the specificity of the PCR. The reaction was carried out using a Techne-Tc-312 thermocycler. The amplification products were resolved electrophoretically in an acrylamide denaturing gel 18% T 5% CBIS, in 1X TBE buffer solution [90 mM Tris, 90 mM boric acid, 2 mM EDTA] (Cregan & Quigley 1997), mounted on the vertical electrophoresis sequencing system (Life Technologies S2001), coupled with a power supply (Biorad Powerpac 3000). The electrophoresis was performed at 1.500 V - 50.000 Vh. The gel was then stained with silver nitrate to visualize the amplification products.

Finally, the size of the alleles observed by denaturing electrophoresis was estimated per calibration curves with respect to the band migration pattern of the molecular size standard (O'RangeRuler 10 bp, Fermentas). It is important to note that the size of the three loci amplified in this study were consistent with the size reported by the sequences deposited in GenBank under accession numbers DQ144475-DQ144500.

TABLE II. Locus and primers for markers of cpDNA of *C. bursa-pastoris*, used in this study.

TABLA II. Locus y partidores para los marcadores de cpDNA of *C. bursa-pastoris*, usados en este estudio.

Locus/ primers	Sequence (5'-3')
ATCP31017	
III	GCC TAC CGC ATC GAA ATA GA
IV	CAA GAA AGT CGG CCA GAA TC
ATCP46615	
trnT(UGU)	AAT TTT TTT CCA TTG CAC ATT G
trnL(UAA)	TCA GAA ATA GTC GAA CGG TCG
ATCP66701	
trnP(UGG)	TCC ACA TCC TCC TTC TTT TTT
psaJ	CAT TTG AAA ACG TAA AGG CC

TABLE III. Molecular sizes (pb) estimated for the studied loci.

TABLA III. Tamaño molecular (pb) estimado para los loci estudiados

Locus	No. of observed alleles	Observed size (pb)
ATCP46615	1	62 ± 1 (n= 168)
ATCP66701	1	102 ± 2 (n= 171)
ATCP31017	2	111 ± 2 (haplotype A) (n= 155) 115 ± 1 (haplotype B) (n= 13)

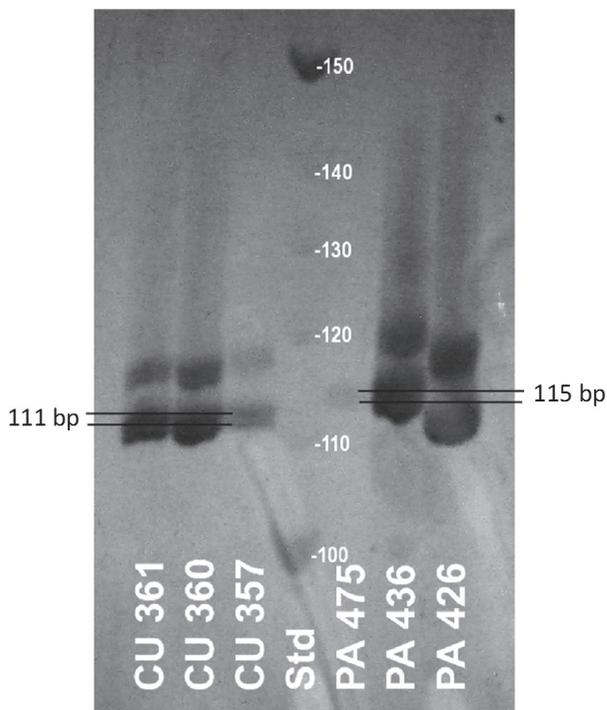


FIGURE 1. Electrophoresis of cpSSR locus for the ATCP31017 marker. CU: Curicó; PA: Punta Arenas; Std: ladder.

FIGURA 1. Electroforesis del locus cpSSR para el marcador ATCP31017. CU: Curicó; PA: Punta Arenas; Std: estándar.

RESULTS

The locus ATCP46615 of *C. bursa-pastoris* was amplified only in 168 of the 286 specimens (58.7%), showed a single haplotype with a size estimated at 62 pb (Table III). Similarly, the locus ATCP66701 was amplified in 171 plants (59.8%) showed no haplotype variation; the estimated size of this locus was 102 pb (Table III). In contrast, locus ATCP31017 was amplified in 168 plants (58.0%; Table III), showing two alleles, one of 111 bp (haplotype A) and other of 115 bp (haplotype B) (Fig. 1; Table III). Haplotype A was found along continental Chile with a frequency equal to 100% in 13 of the 14 sampled localities (Fig. 2); the exception was Punta Arenas where haplotype A was represented in 18.8% of the analyzed plants. Whereas, haplotype B was found only in plants from Punta Arenas (Fig. 2) in 13 of the 16 plants analyzed (i.e., 81.2%; Fig. 2).

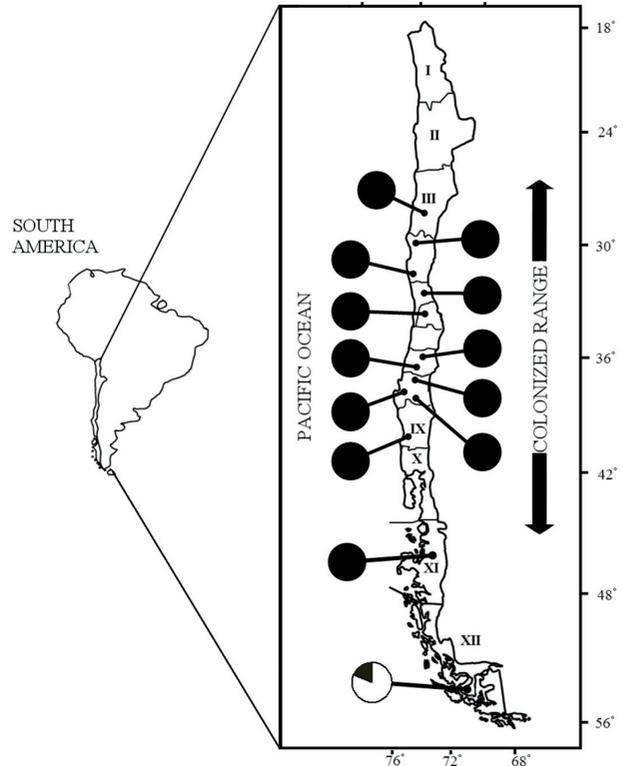


FIGURE 2. Geographic distribution of haplotypes identified for locus ATCP31017 (i.e. haplotypes A and B). The distribution and representation of both haplotypes on the sampled localities are shown as pie charts (filled pie for haplotype A; open pie for haplotype B).

FIGURA 2. Distribución geográfica de los haplotipos identificados para el locus ATCP31017 (i.e. haplotipos A y B). La distribución y representación de ambos haplotipos en las localidades de recolección es ilustrada en gráfico circular, donde el área ennegrecida representa al haplotipo A y blanco al haplotipo B.

DISCUSSION

Our results show that for the populations of *C. bursa-pastoris* in Chile, the ATCP46615 and ATCP66701 loci are monomorphic because exhibit only a single common haplotype in all analyzed samples, whereas locus ATCP31017 showed two haplotypes, A and B. The geographic distribution of these haplotypes showed a well defined geographical pattern, because haplotype B was present only in Punta Arenas (Southernmost Chile), whereas haplotype A was obtained from all continental Chile, including Punta Arenas. The reduced genetic diversity recorded in populations of *C. bursa-pastoris* in Chile could result from effects of genetic drift occurring upon small populations recently introduced in a new range (Avisé 2000, Cox 2004, Dlugosch & Parker 2008).

At least two hypotheses can explain the distributional

pattern shown by locus ATCP31017. On the one hand, it could be argued that one of them (haplotype B) arose in Chile by mutation from the other previously introduced (probably the haplotype A). We believe that this is improbable because it has been stated that the evolutionary rate (μ) for these markers is too low, because it varies between 2.5×10^{-5} and 2.9×10^{-4} mutations per locus per generation (Cregan & Quigley 1997, Provan *et al.* 1999, 2001, Ceplitis *et al.* 2005). This would imply, assuming a single mutation event, an introduction time for *C. bursa-pastoris* longer than 500 years, which is clearly an erroneous estimate.

A second hypothesis states that the presence of these haplotypes in *C. bursa-pastoris* may be the result of the history of the introduction of the species into the territory (Neuffer *et al.* 1999, Neuffer & Hurka 1999). So, the widely distributed haplotype A can be explained at least by one event of introduction, probably associated with the Iberian colonization occurred since the 16th century, as has been suggested by Neuffer *et al.* (1999) and Ceplitis *et al.* (2005). The restricted distribution of the haplotype B suggests that this may have arrived from another introduction event. Evidence from allozymes and RAPD fragments related to Patagonian populations of *C. bursa-pastoris* with England populations (Neuffer *et al.* 1999, Ceplitis *et al.* 2005). According to Neuffer *et al.* (1999), Neuffer & Hoffrogge (2000) and Neuffer *et al.* (2011), this pattern would be consequence of a second point of introduction led by English colonizers on Argentinian and Chilean Patagonia.

In the absence of a comprehensive botanical record, the spatial analysis of variation of molecular markers is a tool to infer possible spread dynamics for invasive species (Cox 2004). Thus, *C. bursa-pastoris* is particularly interesting because its global distribution and ability to colonize contrasting environments. In this line, future investigation can to advance upon the recognition of haplotypes of *C. bursa-pastoris* that exhibit differential invasibility, in order to prevent or control the introduction or/and spread of more “aggressive” or detrimental ones. Although more research is needed to clarify the dynamics invasive of *C. bursa-pastoris* in Chile, our results based on the analysis of haplotypes of cpSSR would support –but do not demonstrate– the proposal of Neuffer *et al.* (1999).

In summary, our results show that the populations of *C. bursa-pastoris* distributed in continental Chile offer genetic variability of one of the cpSSR markers analyzed. This variation indicates the presence of two cpDNA haplotypes. The present geographical distribution of these haplotypes supports the hypothesis that states that the introduction of *C. bursa-pastoris* in continental Chile at least came from two separated introduction.

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