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Ecotypic Differentiation in Morphology and Cold Resistance in Populations of *Colobanthus quitensis* (Caryophyllaceae) from the Andes of Central Chile and the Maritime Antarctic

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Abstract

Colobanthus quitensis is one of only two vascular plant species that occur in the Antarctic islands. We evaluated morphological and physiological traits and survival after freezing in individuals of *C. quitensis* collected in two populations: one from the Andes of central Chile and the other from the maritime Antarctic. We addressed whether these populations are ecotypes in terms of shoot morphology and physiology, and cold resistance. We also evaluated genetic differentiation at the internal transcribed spacer (ITS) regions. Under controlled growing conditions, Andean plants had longer and narrower leaves and longer peduncles. However, shoot biomass was similar in both populations. There was a 1.17% sequence divergence of ITS regions between populations. Survival to freezing at temperatures from 0° to −16°C was not different in nonacclimated plants. After cold-acclimation at 4°C, freezing tolerance was greater in Antarctic plants. Both Andean and Antarctic plants increased soluble sugars with increasing acclimation time, but it did not differ between populations. Shoot water content decreased with acclimation time in both populations, but Antarctic plants maintained slightly higher water contents after cold exposure. Shoot growth did not differ during acclimation. The studied populations of *C. quitensis* represent different morphological and cold resistant ecotypes despite their genetic similarity. However, the mechanistic basis of the higher survival to freezing of Antarctic plants was not elucidated.

Introduction

Ecotypic differentiation among plant populations is a widespread phenomenon. Typical approaches to this topic involve common garden comparisons of plant morphology and/or performance in individuals from populations along latitudinal, altitudinal or moisture gradients (Linhart and Grant, 1996). Persistent differences among genotypes regardless of the experimental environment and superior fitness in the original habitat are evidence of the existence of ecotypes (see Joshi et al., 2001).

The occurrence of ecotypic differentiation in plant populations is more likely when environments are consistently stressful (Bradshaw and Hardwick, 1989). Freezing stress is a selective factor that acts over long time-scales and hence it may be assumed that plant populations in cold environments have undergone genetic differentiation that allows them to adapt their functioning to local conditions (Körner, 1999). Several studies have shown ecotypic divergence in arctic and alpine plants, mostly along altitudinal gradients (McNaughton et al., 1974; Chapin and Chapin, 1981; Macdonald and Chinnappa, 1989; Galen et al., 1991; McGraw, 1995; Williams et al., 1995, Cordell et al., 1998), detecting distinct patterns of growth and physiology that are functional to the original habitat. Studies addressing ecotypic differentiation as a response to cold environments that include both fitness consequences and mechanistic approaches are, however, seldom found in the literature.

The cushion-forming plant *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) is one of the two native vascular plant species that have successfully colonized the maritime Antarctic islands. *Colobanthus quitensis* is a selfing species, capable of asexual reproduction, and shows considerable morphological variability along its wide distributional range, which extends from Mexico (17°N) to the southern Antarctic Peninsula (68°S) and from 0 to 4200 m a.s.l. (Moore, 1970; Smith, 2003). In Chile, *C. quitensis* occurs along the Andes, typically in bogs situated at high altitudes in the north, but close to sea level at poleward latitudes (Moore, 1970; Hoffmann et al., 1998). The flora of bogs is rather homogeneous despite its occasional geographical discontinuity along the Andean range (Arroyo et al., 1982; Villagrán et al., 1983). This pattern has been related to the relative constancy of environmental conditions in these habitats despite the differences in latitude and altitude (Arroyo et al., 1982). In view of this, it could be speculated that, at least along the Andean range, ecotypic differentiation of plant species belonging to the community of bogs should not be a common phenomenon. With regard to Antarctic populations of *C. quitensis*, the occurrence of persistent seed banks (McGraw and Day, 1997; Ruhland and Day, 2001) may act as a buffer to the effects of selective pressures and hence prevent local genetic differentiation. On the other hand, it has been argued that the achievement of about 30% of its maximum photosynthetic rate at 0°C is the most important feature for survival of *C. quitensis* in Antarctica (Edwards and Smith, 1988). However, cold resistance mechanisms and biochemical adaptations to cold in *C. quitensis*, which have been recently described (Bravo et al., 2001; Alberdi et al., 2002), are no less important. In cold-acclimated plants, it has been shown that this species is capable of supercooling to –9.4°C (Bravo et al., 2001; Alberdi et al., 2002). This mechanism of freezing avoidance may allow *C. quitensis* to endure low temperatures during the Antarctic growing season.

In the present study, we evaluated morphological and physiological traits and survival to freezing in individuals of *C. quitensis* collected in two populations: one from the Andes of central Chile and the other from the maritime Antarctic. Specifically, we addressed whether the populations of *C. quitensis* from the Andes and Antarctica are ecotypes with respect to (1) leaf and shoot morphology and

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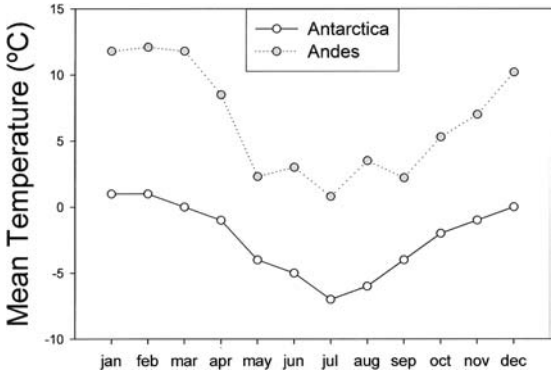

FIGURE 1. Mean temperature in Antarctica and the Andes of Central Chile. ANTARCTICA = Bellingshausen Antarctic Station (62°12′S; 58°55′W; 16 m a.s.l.). Thirty years on record (www.weatherbase.com/weather/weather.php3?s=890500&refer=&units=metric). ANDES = South-facing slopes in Valle Nevado (33°20′S; 70°16′W; 2600 m a.s.l.). Two years on record (Cavieres and Arroyo, 1999).

physiology, and (2) cold resistance. We also examined genetic similarity between the two populations at the level of the nuclear ribosomal internal transcribed spacer (ITS) regions 1 and 2.

Materials and Methods

COLLECTION SITES AND EXPERIMENTAL PLANTS

The Antarctic flora comprises ca. 485 cryptogams (mosses, liverworts, and lichens) and two flowering plants: *Deschampsia antarctica* Desv. (Poaceae) and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae) (Kappen, 1999; Smith, 2003). Plants of *C. quitensis* (about 10 mm height, vegetative stage) were collected in the vicinity of Arktowski Station, King George Island, maritime Antarctic (62°14′S; 58°48′W; sea level). Average summer (January and February) and winter (June to August) temperatures are 1 and –6°C, respectively (Fig. 1). In King George Island, the mean annual temperature is –2°C. Temperature ranges from –2 to 6°C in summer and from –1 to –20°C in winter. Mean annual precipitation on King George Island is 700 mm and falls mainly as snow. Summer fogs are frequent. Antarctic soils have a high content of coarse mineral particles and total organic carbon, low C/N ratio, and acidic pH. Local enrichment of nutrients is due to input by seabirds (Beyer et al., 2000). Vegetation in the area consists mostly of lichens and mosses, although the vascular plants *Colobanthus quitensis*, *Deschampsia antarctica*, and the exotic *Poa annua* are also found. Collected plants were transported in a cooler in plastic bags to the laboratory at Universidad de Concepción in the austral summer of 1997. One week after, they were planted and reproduced in the laboratory at 15°C. For vegetative reproduction, lateral stems without roots were manually detached and planted in plastic pots (20 × 12 × 8 cm; 20 plants per pot). These stems rapidly produced roots. Under these conditions nearly all plants survived. To preserve the genetic constitution, no sexual reproduction was allowed.

Andean plants of *C. quitensis* (about 15 mm height, vegetative stage) were collected on slopes below Cerro La Parva (33°22′S; 70°21′W; 2600 m a.s.l.) in March 2001. The climate of this site corresponds to the Mediterranean-type climate with some continental influence of the Chilean Andes (Cavieres and Arroyo, 1999). Mean annual precipitation is about 431 mm, falling mainly as snow between April and October. Occasional hailstorms and light snow may occur at the study site through the summer months (December–March), particularly in El Niño years. Mean annual temperature is about 6.5°C; mean monthly temperature for the summer-autumn months ranges from 7.0°C in November to 8.5°C in April, with a peak of 12.1°C in February (Fig. 1). Soils are loamy and organic matter content is <3%. The dominant vegetation is composed of dwarf shrubs, cushion-forming perennial herbs, caespitose and rosette forming perennial herbs, and some annual species (Cavieres et al., 2000). *Colobanthus quitensis* was collected from bogs where the dominant species are large hard cushion plants such as *Patosia clandestina*, *Oxychloe andina*, and *Werneria* spp.

Under laboratory conditions, collected plants were reproduced vegetatively in plastic pots, using a soil:peat mixture (3:1) and maintained at 13 to 15°C in a growth chamber (Forma Scientific Inc.) with a photon flux density (PFD) of 150 μmol m^{-2} s^{-1} at the top of the canopy and 16/8 h light/dark period. The light source was provided by cool-white fluorescent tubes F40CW (General Electric). Plants were fertilized with Phostrogen® (Solaris, NPK:14-10-27) using 0.2 g L^{-1} once every 2 wk. The cold acclimation treatment consisted of transferring plants above described to a growth chamber set at 4°C with the same light intensity and photoperiod. Independent groups of plants were used for each of the evaluations during the acclimation period.

Antarctic plants were grown in laboratory conditions during 4 yr before the Andean plants were collected. It could be considered that this fact would introduce a bias in the results of the comparative experiments. However, this is unlikely because (1) we did not observe any phenotypic change in the laboratory plants once the rearing started, and (2) we prevented sexual reproduction in the plants, hence keeping the original genotypes, which are the subject of the experimental protocols carried out.

FREEZING RESISTANCE

This was evaluated as survival of plants after freezing at a given temperature. Plants were placed in small pots (30 ml) with the same substrate as described above. For each temperature treatment, 5 pots with 2 plants each were placed in a steel chamber (5.5 × 7.5 × 25 cm) and the whole system submerged in a cryostat. The temperature was decreased at 2°C/min until the desired temperature (–2, –4, –6, –8, –10, –12, –14°C). After 2 h of exposure to the selected temperature, plants were removed and thawed overnight at 4°C, then grown at 15°C for survival evaluation by monitoring the fate of the frozen leaves and progress of new leaf growth during the next 30 d. After that period, plants showing green leaves or regrowth were considered as survivors. The lethal temperature at which 50% of plants died (LT50) was obtained for each population by interpolation at 50% of survival in a survival vs. freezing temperature plot.

SUGAR ANALYSIS

Three samples of shoots of *C. quitensis* from the Andes and Antarctica were harvested at 0, 7, 14, and 21 d of cold acclimation. Each fresh sample was cut in small pieces and extracted with ethanol (86%) for 24 h, and then centrifuged at 12,000 g for 10 min. The supernatant was depigmented in a mixture of 1:3 (v/v) with chloroform, and the aqueous fraction was freeze-dried overnight. The dry residue was resuspended in 500 μl of methanol. Total soluble sugar content was determined spectrophotometrically by the Resorcinol method (Roe, 1934) at 520 nm, using sucrose as a standard.

BIOMASS AND MORPHOLOGY

Samples of shoots of mature plants of *C. quitensis* at flowering stage (flowers with visible seeds) from Andes and Antarctica grown at 15°C were harvested and their fresh weight (FW) determined using an analytical balance (Sartorius-Werke GMBH). Dry weight (DW) was

determined after drying the samples for one week at 60°C until constant mass was attained. Water content (%) was calculated as follows: WC = 100 (FW − DW)/FW. Leaf dimensions (length and width) and flower peduncle length (only flowers with visible seeds) were measured from 50 individual plants from each location. Measurements were done using a calliper (resolution: 0.5 mm).

ITS REGION SEQUENCING

Genomic DNA was isolated from 100 mg of young leaves from several plants from each *C. quitensis* population using Plant DNAzol (Gibco-BRL) according to the manufacturer instructions. Good quality DNA was verified by the presence of a clean high molecular band in an agarose gel. The ITS region was amplified from genomic DNA by PCR according to Brauner et al. (1992). Two specific primers against 18S and 28S conserved regions were used (5′–3′ Primer: ACGAATTCATGGTCCGGTGAAGTGTTCG; 3′–5′ Primer: TAGA ATTCCCCGGTTCGCTCGCCGTTAC). PCR products were visualized in 1% agarose gels and ethidium bromide staining and the band was purified form the gel using PCR prep. Purification System Kit (Wizard, Promega). The purified product was sequenced using the primers mentioned above and also two internal primers (5′3′ CTGCGGAAGGATCATTG and 3′5′ TAGTCCCGCTGACCTGG). Sequencing was performed with ABI PRISM-310 Genetic analyzer (Applied Biosystems) using fluorescent dye terminators (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems). Sequences were visually aligned using Clustal W from EMBL-EBI.

STATISTICAL ANALYSIS

Comparisons of plant morphology before the acclimation treatment were carried out applying a one-way ANOVA, with origin (Andes, Antarctica) as the main factor. The effects of plant origin and length of exposure to the acclimation treatment at 4°C on plant survival and physiology were evaluated via a two-way ANOVA.

Results

Andean and Antarctic populations of *C. quitensis* differed in leaf shape. Plants from the Andes population had longer and narrower leaves. Flower peduncles were 3.3 fold longer in the Andean ecotype. Peduncles were longer than those observed in the field for both ecotypes. Shoot biomass was similar in the Andean and Antarctic populations (Table 1). On the other hand, the sequence alignment of ITS regions 1 (212 bp) and 2 (223 bp) revealed that there was a 2.99% sequence divergence among the studied populations when considering gaps or undetermined nucleotides in the calculation (Fig. 2). Following customary practice (Baldwin, 1993; Kropf et al., 2002), if only the actually identified nucleotides (ITS-1 = 207 and ITS-2 = 220, Total 427) are included in this calculation, variation between populations is reduced to 5 nucleotides (Fig. 2, marked *). Therefore, the sequence divergence is only 1.17%.

Survival of plants to freezing temperatures was greater in the Antarctic population and it increased following the acclimation treatment at 4°C at a higher rate. However, survival to freezing was not different in Andean and Antarctic plants before the start of acclimation (Fig. 3, Table 2). The content of soluble sugars in the shoot was slightly higher, albeit not significantly, in the Andes population. Both Andean and Antarctic plants showed an increase of soluble sugars with increasing time of acclimation at 4°C (Fig. 4, Table 2). Shoot water content decreased with acclimation time in both populations but plants from the Antarctic consistently exhibited higher water content as compared to those from the Andes (Fig. 4, Table 2). Shoot growth did not differ among populations during the acclimation treatment (Table 2). Thus, shoot biomass was very similar at the start (Andes 15.3 ± 2.6 mg; Antarctic 13.6 ± 2.3 mg; Mean ± SE) and at the end (Andes 22.3 ± 1.8 mg; Antarctic 22.9 ± 3.3 mg) of the acclimation at 4°C.

TABLE 1

Morphological traits (Mean ± SE) of plants from two populations of Colobanthus quitensis*: Antarctica and Andes. Plants grown at 15°C until they attained the flowering stage (flowers with visible seeds). P-values correspond to a one-way ANOVA*

	Antarctica	Andes	*P*-value
Leaf length (mm)	9.94 ± 0.29	26.1 ± 1.01	<0.0001
Leaf width (mm)	1.32 ± 0.05	1.01 ± 0.01	<0.0001
Leaf shape (length/width ratio)	7.93 ± 0.31	25.8 ± 1.05	<0.0001
Peduncle length (mm)	13.8 ± 0.41	43.7 ± 1.89	<0.0001
Shoot biomass (mg)	13.6 ± 2.34	15.3 ± 2.61	0.6416

Discussion

Populations of *C. quitensis* along the Andean range experience rather homogeneous conditions (Villagrán et al., 1983) and hence are not expected to undergo ecotypic differentiation. The maritime Antarctic populations of *C. quitensis* are most probably of South Andean origin, given that Antarctica was the last area where the retreat of the Quaternary glaciation took place (Clapperton, 1994). Consequently, assuming a close genetic relatedness between the Antarctic and Andes populations of *C. quitensis* herein studied, the outcome of the evaluation for ecotypes in this species would rest on the strength of the selective pressure exerted by the significantly colder environment in Antarctica. Ecotypic differentiation is more likely to occur when environments are consistently stressful (Bradshaw and Hardwick, 1989), and freezing stress in Antarctica meets such condition. However, the establishment of genotypes harbored in seed banks (McGraw and Day, 1997) may prevent the formation of ecotypes. Our results suggest that forces favoring the ecotypic differentiation of *C. quitensis* prevailed over those restraining it.

Plants from the Andes and Antarctic populations grown under the same environmental conditions at 15°C differed in their morphological characteristics (leaf size and shape and peduncle length), hence constituting morphological ecotypes. However, shoot biomass was not different, which suggests a differential response in cell elongation rather than in dry matter accumulation. Differences in morphology and growth have been suggested as evidence of ecotypic differentiation in other plant species (e.g., Galen et al., 1991; Williams et al., 1995). In Antarctic individuals of *C. quitensis* leaf anatomy is very similar to that of plants from xeric habitats (Mantovani and Vieira, 2000). Moreover, there are early reports indicating that both peduncle length and leaf length/width ratio in this species are influenced by moisture availability and degree of exposure (Moore, 1970). Likewise, in the other angiosperm inhabiting the maritime Antarctic, *Deschampsia antarctica*, leaf anatomy showed more xerophytic characteristics (smaller epidermal cells, higher leaf thickness and stomatal density) than the leaves of plants grown at mild temperatures in laboratory (Romero et al., 1999). Reduced shoot height and changes in leaf shape are associated with prevention of desiccation by convection due to wind in environments of high altitude or latitude (Körner, 1999). However, it is important to point out that, in the case of *C. quitensis*, individual morphology might not be as important as in other species growing under cold environments because of the cushion-like growth of this plant, which moderates the hazardous effects of such harsh environments (Armesto et al., 1980).

The ITS region of nuclear ribosomal DNA is a valuable source of characters to assess relationships among plant taxa (Baldwin et al.,

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1 Cq-Antarc  CTGCGGAAGGATCAT TGTCGAAACCTGCTT AGCAGAACGACCAGC GAACATGTTTACAAC
2 Cq-Andean  CTGCGGAAGGATCAT TGTCGAAACCTGCTT AGCAGAACGACCAGC GAACATGTT-ACAAC

1 Cq-Antarc  CCACGGGCGTAGGGT GCGGGTAGGTGCCTT GTGTGCTTGCTTGCC CCTTGCCTGCGCCTT
2 Cq-Andean  CCACGGGCGTAGGGT GCGGGTAGGTGCCTT GTGTGCTTGCTTGCC CCTTGCCTGCGCCTT
                                                                    ITS 1
1 Cq-Antarc  ACGGCGTTCCCTTAC AGGGCGTTGGTTTG GCAAAAAAACGAACC CCGGCGCGAAAAGCG
2 Cq-Andean  ACGGCGTTCCCTTAC AGGGCGTT--GTTTG GCAAAAAAACGAACC CCGGCGCGAAAAGCG
                             *

1 Cq-Antarc  TCAAGGAACATAAAC TAATGTGCGCTCTTG TCCTTTGGCCGGTTA GCCGGCTCGAAGGGT
2 Cq-Andean  TCAAGGAACATAAAC TAATGTGCGCTCT-G TCCTTTGGCCGGTTA GCCGGCTCGAAGGGT

1 Cq-Antarc  TTGATGCCATATCAT AACAATAAACGACTC TCGGCAACGGATATC TTGGCTCTCGCATCG
2 Cq-Andean  TNGATGCCATGTCAT AACAATAAACGACTC TCGGCAACGGATATC TTGGCTCTCGCATCG
                  *

1 Cq-Antarc  ATGAAGAACGTAGCG AAATGCGATACTTGG GGTGAATTGCAGAAT CCCGTGAACCATCGA
2 Cq-Andean  ATGAAGAACGTAGCG AAATGCGATACTTGG TGTGAATTGCAGAAT CCCGTGAACCATCGA

1 Cq-Antarc  GTTTTTGAACGCAAG TTGCCCCCGAAGCTC TCGG-CTTAGGGCAC GTTTTGCTTGGGCGT
2 Cq-Andean  GTTTTTGAACGCAAG TTGCGCCCGAAGCTC TCGGGCTTAGGGCAC GTCT-GCCTGGGCGT

1 Cq-Antarc  CACGCATCGCGTCTC CCCCAAACCACCTAA AGTGGAAGGGGGAGG AAGATGGTTTCCCCG
2 Cq-Andean  CACGCATCGGGTGTC CCC-AAACCACCTAC AGTGGAAGGGGGAGG AAGATGGTTTCCC-G
                  * *                 *

1 Cq-Antarc  TCCCTCATCGGGGGC GGTTGGCCTAAATTT GGAGCCAGA-GGCAC TGAGCTTTTGTGGCG
2 Cq-Andean  TCCCTCATCGGGGGC GGTTGGCCTAAATTT GGAGCCAGAAGGCAC TGAGCTTTTGTGGCG   ITS 2

1 Cq-Antarc  ATAGGTGGTGAACAA GGCTTAGGCCGTGCA AGCAACCCGTCACGT AGCACTTTGCAAACT
2 Cq-Andean  ATAGGTGGTGAACAA GGCTTAGGCCGTGCA AGCAACCCGTCACGT AGCACTTTGCAAACT

1 Cq-Antarc  TGAGCTCGAAGGACC CTGTGATGTCGCCTC GTTGCGATTGAACGC TGCGACCCCAGG
2 Cq-Andean  TGAGCTCGAAGGACC CTGTGATGTCGCCTC GTTGCGATTGAACGC TGCGACCCCAGG
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FIGURE 2. ITS region of ribosomal DNA from Andean (Cq-Andean) and Antarctic (Cq-Antarc) C quitensis. *Bold letters indicate 18S and 28S partial sequences at each end and the whole sequence of 5.8 S segment at the center. ITS-1 and ITS-2 are delimited by those sequences in bold. Underlined letters indicate all potential nucleotide differences in the whole sequence of ITS regions, including gaps and undetermined bases (N). Asterisks (*) indicate the actual differences identified within ITS-1 and 2.*

1995). Intraspecific comparisons of ITS sequences among populations have shown a broad range of variation. Minimum values of sequence divergence (0–1%; Baldwin, 1993; Soltis and Kuzoff, 1993), low intraspecific variation (1–3.7%; Baldwin, 1993; Jobst et al., 1998; Kropf et al., 2002), and high values of intraspecific divergence (7–11%; Jobst et al., 1998) have been reported. In the present study, ITS analysis of *C. quitensis* evidenced a relatively high genetic similarity among the studied populations (sequence divergence = 1.17%) despite the considerable geographical distance (>3300 km). This merits an examination of hypotheses for likely vectors of gene flow between Andean populations of *C. quitensis* and throughout its Antarctic and Subantarctic range. Migratory birds (*Muscisaxicola* spp., Tyrannidae), which are common on the bogs where *C. quitensis* thrives in the Andes (Cavieres, pers. obs.), are candidates for such a role. Assuming dispersal via these agents, an Andean origin of the Antarctic populations seems probable. The fact that *C. quitensis* is a selfing plant species, and that it is capable of asexual reproduction, could explain why the putative migrant population of the Antarctic has not yet diverged genetically. A high level of overall genetic similarity among distant populations of widespread, selfing plant species has been reported (Werth et al., 1984; Novak et al., 1991).

In view of the consistently lower temperature in their original habitat, it was expected that Antarctic plants would show higher resistance to freezing than Andean plants. This was verified in the survival experiment, where the temperature corresponding to a 50% lethal treatment was clearly lower for Antarctic plants. Since this difference was observed at any of the stages of acclimation at 4°C but not before the start of acclimation, it may be concluded that Antarctic plants develop further resistance to freezing as a consequence of an

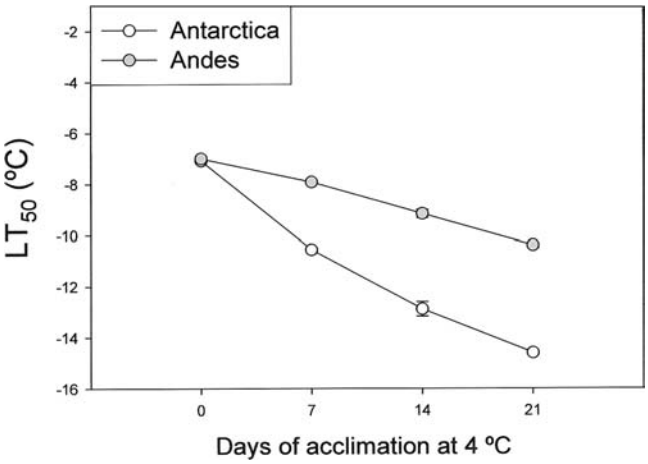

FIGURE 3. Survival to freezing in Antarctica and Andes populations of Colobanthus quitensis. *Lethal temperature of 50% of an experimental group of plants (LT_{50}, Mean ± SE, n = 5) subjected to freezing temperatures after 0, 7, 14, and 21 d growing at 4°C (cold acclimation treatment). See text for details.*

TABLE 2

Analysis of Variance of the effect of acclimation time at 4°C (T) and origin (O: Andes and Antarctica) on survival after freezing temperatures, physiological traits, and growth of plants of Colobanthus quitensis. *See text for methodological details. P-values are shown*

	Time	Origin	T × O
Survival to freezing	<0.0001	<0.0001	<0.0001
Soluble sugars content	<0.0001	0.0579	0.5098
Water content	<0.0001	<0.0001	0.2185
Biomass	0.0166	0.7342	0.7736

environmental cue ("autumn" signal = 4°C) of forthcoming colder conditions ("winter" = subzero temperatures) that triggers a mechanism of freezing tolerance. Although seven days of acclimation were sufficient to produce a significant difference in freezing survival between populations, this difference dramatically increased after 14 and 21 d. In summary, the Andes and Antarctica populations of *C. quitensis* are ecotypes for cold resistance.

The mechanistic basis of the detected ecotypic differentiation for cold resistance could not be clearly determined. The accumulation of soluble sugars is considered a functional response to cold in plants (Alberdi and Corcuera, 1991). Thus, sugar accumulation plays a role in the depression of the freezing point of cell sap and the avoidance of plasmolysis due to protoplast dehydration caused by freezing (Livingston et al., 1989; Santarius, 1992). Consequently, a higher content and/or rate of accumulation of carbohydrates should be expected the more cold resistant a plant population is. In the present study, both populations responded to cold acclimation by accumulating soluble sugars, as expected. However, there was no significant difference between populations for either the amount or the rate of accumulation of total soluble sugars. Further research should address other potential mechanisms of freezing resistance in *C. quitensis*. For instance, the involvement of specific proteins conferring cryoprotection to cold-acclimated plants, as has been described for the Antarctic *C. quitensis* (Doucet et al., 2000).

Water content of the shoot decreased slightly with the cold-acclimation treatment in both populations. The same pattern has been observed in *Deschampsia antartica* (Bravo et al., 2001). Plants of cold-acclimated *C. quitensis* from Antarctica showed slightly higher levels of water content than plants from the Andes. Temperature is permanently lower in the Antarctic, while greater daily temperature oscillations occur in the Andes. As expected, Antarctic plants appeared better suited to conserve their water status under permanent cold treatment than the Andes plants. The biological meaning of this small but significant difference in water content under cold treatment is not yet understood. On the other hand, biomass at the end of the acclimation treatment was similar for the two *C. quitensis* populations. This implies that Antarctic plants achieved greater cold resistance without reducing their growth or biomass. Such putative "costs of resistance" have been detected in other systems (Vanoosterom and Ceccarelli, 1993).

Regardless of the particular mechanism underlying the observed pattern of freezing tolerance, this study has shown that populations of *C. quitensis* may be regarded as cold-resistant ecotypes. Several examples of plant species developing ecotypes under extreme conditions such as drought, heavy-metal pollution and herbicide treatment have been reported (Linhart and Grant, 1996). There are also examples of the occurrence of ecotypes along altitudinal gradients (McNaughton et al., 1974; Macdonald and Chinnappa, 1989; Galen et al., 1991; Williams et al., 1995; Cordell et al., 1998). To our knowledge, this is the first example of ecotypic differentiation in a species from the permanently cold Antarctic in comparison with a population already adapted to cold conditions (the high Andes).

The wide distribution of *C. quitensis* along altitudinal and latitudinal gradients might be a consequence of ecotypic differentiation to cope with the limiting environmental factors (e.g., cold, drought, or shade) at each habitat. Whether the ecological breadth of this species rests mainly on such a specialist strategy or is combined with the existence of plastic "multipurpose" genotypes (see Sultan, 1995) is a central question to be addressed by further work.

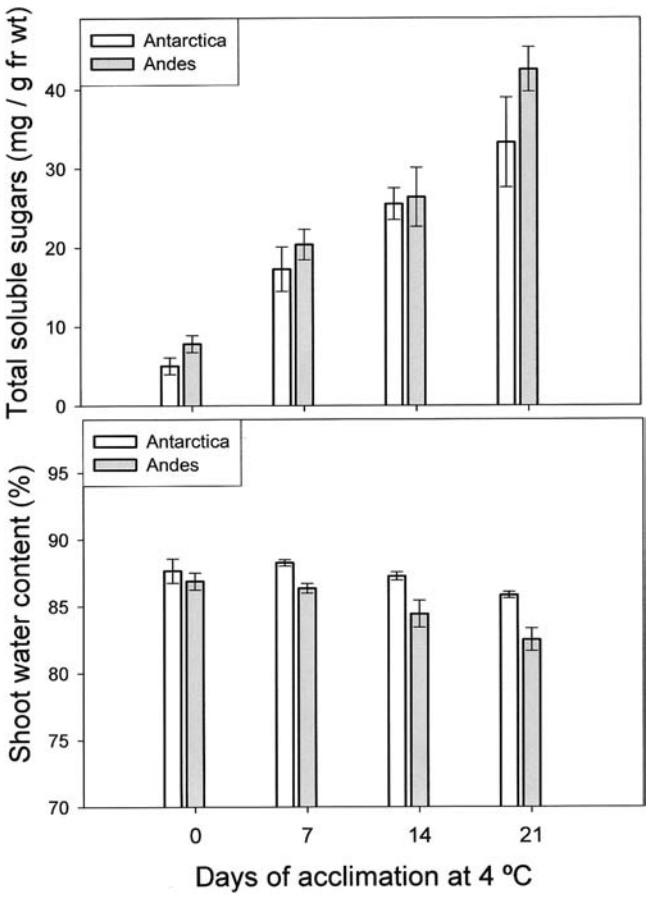

FIGURE 4. Content of total sugars and water (Mean ± SE, n = *3) in the shoot of* Colobanthus quitensis *plants from the Antarctic and the Andes after 0, 7, 14, and 21 d of cold acclimation at 4°C.*

Acknowledgments

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