

Differential responses to Na^+/K^+ and $\text{Ca}^{2+}/\text{Mg}^{2+}$ in two edaphic races of the *Lasthenia californica* (Asteraceae) complex: A case for parallel evolution of physiological traits

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Summary

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- Sodium, potassium, calcium, and magnesium ion uptake physiology and tolerance to sodium and magnesium were characterized in two edaphic races (A and C) of two closely related species in the *Lasthenia californica* complex.
- Uptake rates of race A plants were 20-fold higher for Na^+ , and 2-fold higher for Ca^{2+} and Mg^{2+} than those of race C plants. Race A translocated c. 50% of absorbed Na^+ to the shoot compared with < 30% in race C. For Ca^{2+} and Mg^{2+} corresponding values for the two races were > 95% and \leq 50%, respectively.
- Germination, root growth and survivorship estimates indicated greater tolerance by race A to Na^+ and Mg^{2+} . Significant genotype treatment interactions were observed, suggesting that these races are genetically differentiated in their tolerance responses.
- The study suggests parallel evolution of physiological traits in populations belonging to the two species and points to intriguing correlations between the presence of sulfated flavonoids and the capacities for the uptake of and tolerance to specific ions.

Key words: *Lasthenia californica*; $\text{Ca}^{2+} : \text{Mg}^{2+}$ quotient; edaphic races; ion-uptake; parallel evolution; salt tolerance; serpentine; sodium.

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Introduction

The distribution of many plant species is strongly influenced by edaphic conditions. Extreme edaphic conditions such as guano deposits, vernal pools, salt marshes, serpentine soils and mine tailings provide sharp discontinuities in edaphic features leading to intriguing patterns of plant diversity (Antonovics *et al.*, 1971; Holland & Jain, 1981; Vasey, 1985; Flowers *et al.*, 1986; Brooks, 1987). In such extreme cases, a particular factor or a suite of factors can be identified to be vital, either because species are restricted to soils with a particular edaphic status (i.e. edaphic endemics) or because species are excluded from such soils. Although most plant species avoid edaphic extremes, genetic accommodation to stressful edaphic conditions has been shown to take place quite rapidly in some species (Shaw, 1990). In the case of heavy metals, such

accommodation can be achieved even within a few generations (Liu & Godt, 1983; Al-Hiyaly *et al.*, 1990). Thus, edaphic conditions, when manifested in extreme form, can be potent agents of natural selection (Kruckeberg, 1986; Macnair & Gardner, 1998). The study of closely related, edaphically differentiated taxa can shed light on the possible roles of adaptation in plant diversification.

Lasthenia (Heliantheae:Asteraceae) is a predominantly western North American genus of seven sections comprising 21 herbaceous taxa (Chan *et al.*, 2001). Members of the genus have wide edaphic tolerance; species are found in habitats such as coastal bluffs, guano deposits, vernal pools, salt and alkaline flats, serpentine outcrops, deserts, grasslands and open woodlands (Ornduff, 1966, 1993). *Lasthenia californica* DC ex Lindl. *sensu* Ornduff (1993) has the widest edaphic tolerance within the genus, with populations spanning all but guano habitats.

An ecological survey of *L. californica sensu* Ornduff (Rajakaruna & Bohm, 1999) documented that previously described geographical clades of this species (Desrochers & Bohm, 1995) consist of two distinct edaphic races (A and C). A recent molecular phylogeny for *Lasthenia* (Chan *et al.*, 2001) recognizes the two geographical clades within *L. californica sensu* Ornduff as two cryptic species, *L. californica sensu stricto* (*s.s.*) and *L. gracilis* (DC.) Greene. A recent study (N. Rajakaruna *et al.*, unpublished) indicates that previously described edaphic races (Rajakaruna & Bohm, 1999) are found in both these closely related species, suggesting that the biochemical characteristics differentiating the two races (Bohm *et al.*, 1989; Desrochers & Bohm, 1995) may have arisen secondarily in response to edaphic differences. Race A plants, regardless of the species, predominate in habitats subject to ionic stress, specifically, coastal bluffs, alkaline flats, serpentine outcrops and salt flats, suggesting cross-resistance to harsh environments. Race C plants appear to be restricted to relatively 'benign' habitats such as inland pastures, roadsides and open fields. The relatively higher ion concentrations in race A soils have led to significant differences in ionic strength of the soil solutions (ranges for race A and C are 2.23–111.7 mM and 1.4–26.8 mM, respectively) of the sites where the two races grow (Rajakaruna & Bohm, 1999), suggesting that race A plants are adapted to growing in soils of greater ionic strength and/or in soils where concentrations of specific ions may inhibit the growth of race C plants. Race A plants are often found in osmotically extreme soils where electrical conductivities can reach values as high as 7.49 mScm⁻¹ (Rajakaruna & Bohm, 1999); the highest value obtained for a race C soil is 1.79 mScm⁻¹. Electrical conductivity above 4 mScm⁻¹ is highly toxic to most plants (Brady, 1990). In some extreme sites (for example, near Soda Lake in San Luis Obispo Co., CA) where race A plants occur, the soil surface is crystalized with salts.

The study by Rajakaruna & Bohm (1999) suggested that the concentrations of Na⁺ and Mg²⁺ may be important in delimiting the distribution of these races, particularly race C. Exchangeable Na⁺ and Mg²⁺ in race A soils showed a much wider range than for race C soils (Rajakaruna & Bohm, 1999). The highest Na⁺ concentration encountered in race A soils was 13-fold higher than the highest concentration recorded for race C soils. Further, race A plants appeared to predominate in Mg²⁺-rich serpentine habitats where Ca²⁺ : Mg²⁺ quotients were considerably below 1. Low Ca²⁺ : Mg²⁺ quotients in serpentine soils are often thought to restrict species from growing in these soils (Walker *et al.*, 1955; Tibbetts & Smith, 1992).

Our studies (Rajakaruna & Bohm, 1999) have also shown that the two edaphic races are physiologically distinct with respect to ion accumulation: race A plants accumulate 3–4 times the concentration of Na⁺ found in race C plants. For example, at Jasper Ridge Biological Preserve, Stanford University (San Mateo Co., CA, USA), where the races occur in parapatry on a serpentine ridge, race A accumulates

approximately four times more Na⁺ than race C, although the exchangeable soil Na⁺ for race A is only slightly higher than that for race C at this site (Rajakaruna & Bohm, 1999). This differential accumulation is also evident when examining populations of the two races from throughout the range of *L. californica sensu* Ornduff (Rajakaruna & Bohm, 1999). At certain extreme habitats, Na⁺ accounts for 5.2% of the dry weight of race A plants (N. Rajakaruna, unpublished), approaching levels found in halophytic plants (Flowers *et al.*, 1986; Welch & Rieseberg, 2002). By contrast, the highest Na⁺ concentration ever recorded for race C plants was 0.2%. Thus, under extreme conditions, race A plants had accumulated 26 times as much Na⁺ as race C plants. Interestingly, a detailed field study involving 22 populations of *L. californica sensu* Ornduff (Rajakaruna & Bohm, 1999), revealed a strong correlation between soil Na⁺ and tissue Na⁺ in the case of race A plants ($r = 0.87$; $P < 0.001$) but not race C plants ($r = 0.23$; NS). In comparison with Na⁺, tissue concentrations of K⁺ and the relationship between soil and tissue K⁺ did not differ significantly between race A and C plants. Thus, while tissue Na⁺ appears to vary widely according to soil conditions in race A plants, K⁺ is maintained within relatively narrow limits in both races. Calcium and Mg²⁺ concentrations in plant tissue were also rather similar between the two races. However, the field survey by Rajakaruna & Bohm (1999) indicates differential response to these ions by the two races. For both ions, race A had a strong correlation between soil and tissue concentrations (Ca²⁺ $r = 0.78$; Mg²⁺ $r = 0.81$; $P < 0.05$) while there was no such correlation for race C (Ca²⁺ $r = 0.36$; Mg²⁺ $r = 0.31$; NS). The observations made in the field clearly suggest that the two races are physiologically distinct entities, especially with respect to Na⁺, Mg²⁺ and Ca²⁺ uptake and tolerance.

In the present study we grew plants from two populations of each of the two edaphic races under controlled hydroponic conditions, in order to determine if the physiological differences observed in the field are under genetic control. We addressed the following specific questions:

Are the races distinct in their Na⁺/K⁺ and Ca²⁺/Mg²⁺ ion physiology, that is do the observations made in the field accurately reflect genetic differences in Na⁺/K⁺ and Ca²⁺/Mg²⁺ physiology between the two races? We determined ion uptake rates and accumulation of the four cations and examined germination, survivorship and tolerance responses to potentially toxic Na⁺ and Mg²⁺ levels in the medium.

Has differentiation in the ion accumulating behavior for Na⁺ arisen in parallel in plants belonging to the two recently described phylogenetic species, that is, is there evidence to suggest parallel evolution of this physiological trait?

Materials and Methods

Cypselaes of *L. californica s.s.* and *L. gracilis* were obtained from field collections made during 1996–2002. The populations

Table 1 Race, species, and field soil solution features of the populations used in the hydroponic study. C_G: Upper reaches of the serpentine ridge, Jasper Ridge Biological Preserve, Stanford University, San Mateo Co., CA; A_C: Bottom reaches of the serpentine ridge, Jasper Ridge Biological Preserve; C_C: Andesite deposit on summit of Lower Table Rock, Jackson Co., OR; A_C: Roadside across from Holiday Inn, Avenue Q, Palmdale, Los Angeles Co., CA. Soil features include pH, ionic strength (*I*) in mM, Na⁺, K⁺, Mg²⁺, and Ca²⁺ under field capacity (μM)

Population	Race	Species	pH	<i>I</i>	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺
C _G	C	<i>Lasthenia gracilis</i>	6.4	5.7	245	101	2480	192
A _C	A	<i>Lasthenia californica</i>	6.6	9.5	263	29	2701	89
C _C	C	<i>Lasthenia californica</i>	5.5	2.7	87	203	222	306
A _G	A	<i>Lasthenia gracilis</i>	6.1	17.5	291	136	378	409

used represented both edaphic races from the two phylogenetic species (Table 1). Race A plants from *L. californica* s.s. and *L. gracilis* are hereafter referred to as A_C and A_G while race C plants from the two species are referred to as C_C and C_G. Note first letter represents race while the subscript refers to the specific epithet. The two Jasper Ridge populations are A_C and C_G.

Experiment 1: differential responses to Na⁺ and K⁺: ion uptake and accumulation

Germination and plant growth Approximately 200 cypselae from each population were dipped in 1% bleach, washed three times with deionized distilled water (DDW), placed on moist filter paper in a Petri dish, and moved to a dark, cold room (5°C) for 3 d. Five germinating cypselae each were then placed in washed sand contained in germination tubes. Each germination tube was an open plastic cylinder (1 cm diameter × 1 cm depth) fitted with nylon mesh (1 mm) at the bottom. The germination tubes were fitted onto a styrofoam raft (seven tubes per raft) and rafts were placed randomly in a plastic tub (45 × 45 × 20 cm) containing 8 l of aerated nutrient solution. Each of the four populations was replicated six times, each replicate consisting of *c.* 35 plants. For each population, plants were grown in three extra styrofoam rafts in order to estimate relative growth rates during the experimental period. The edges of the tubs were covered with aluminum foil to limit the entry of light and restrict the growth of algae.

The nutrient solution was prepared based on soil solution concentrations. Soils from the four populations were analyzed for chemical features at field capacity using a method outlined by Proctor *et al.* (1981). Table 1 lists the soil Na⁺, K⁺, Ca²⁺ and Mg²⁺ concentrations, total ionic strength and pH from sites supporting these populations. Since the populations came from fairly distinct habitats, an average value for each element was obtained to make the final solution. A few plants from each population were initially grown in this solution to confirm suitability for growth for plants originating from the various populations.

The composition of the nutrient solution used in the current study is listed below.

Macronutrients were added as (mM): 0.1 KH₂PO₄; 1 MgSO₄·7H₂O; 0.5 CaCl₂·2H₂O; 0.5 NH₄NO₃. Micronutrients

were added as (μM): 20 FeEDTA; 50 H₃BO₃; 12 MnSO₄·H₂O; 1 ZnSO₄·7H₂O, CuSO₄·5H₂O, NiSO₄; 0.2 Na₂MoO₄·2H₂O; 62.5 Na₂SO₄. The pH of the nutrient solution was adjusted to *c.* 6.0 using 0.5 mM Ca(OH)₂. Thus, the final concentrations of the ions of interest, that is Na⁺ and K⁺, in nutrient solution were 125 and 100 μM, respectively.

The nutrient solution was changed once in 10 d initially and every week once the plants were *c.* 1 cm tall. Plants were maintained in a walk-in environment room at 26°C (day) and 18°C (night) cycles; photo-period 16 h (light) and 8 h (dark) cycles. Light was provided by eight Duro-Test Vita Lite (40 W) fluorescent tubes with a spectral composition similar to sunlight (380 μmol s⁻¹ cm⁻² at plant level). Plants were grown under these conditions for 40 d from germination before the ion depletion study was initiated.

Net uptake rates Each styrofoam raft was removed from the tub and placed in fresh solution for *c.* 15 min to equilibrate roots to the fresh uptake solution. Subsequently, each flat was placed in 200 ml of solution, from which 1 ml was withdrawn immediately after transferring the plants (T₀) to measure the initial concentration of Na⁺ and K⁺. One ml of DDW was then added back to restore the initial volume. Each container was then weighed in order to compensate for any loss of volume due to transpiration by corresponding additions of DDW. Subsequently, 1 ml of the medium was taken 1 h after the transfer, and then every 3 h for 24 h. At every measurement, 1 ml of DDW was added back to the container; also, the container was weighed and DDW was added to replace water lost due to transpiration. Net uptake rates expressed are means (± SE) of six replicates (*n* = 6). The experiment was repeated on three consecutive days. At the beginning and end of the depletion study, several plants were harvested from each of the extra rafts to determine relative growth rates.

Concentrations of Na⁺ and K⁺ were measured using IL 443 and IL 943 Flame Photometers (Instrumentation Laboratory Inc., Lexington, MA, USA). After the depletion study, flats containing the plants were placed in 0.5 mM CaSO₄ solution for 5 min to remove Na⁺ and K⁺ from the free space. Plants were then harvested, separating the roots and shoots and then centrifuged to remove surface water prior to determining

f. wt. The plant material was then dried in a forced draft oven at 70°C to a constant weight and dry mass determined. Roots and shoots were then ashed in a muffle furnace at 450°C overnight, and the ash suspended in 10 ml of DDW to estimate total Na⁺ and K⁺ in shoots and roots.

Experiment 2: differential response to Ca²⁺ and Mg²⁺: uptake and accumulation

A_C and C_G plants (Jasper Ridge) were grown using the conditions described previously for experiment 1. Due to limitation in available seed, differential responses to Mg²⁺ and Ca²⁺ were determined only for plants from Jasper Ridge. The concentrations of the ions of interest, that is Ca²⁺ and Mg²⁺, in nutrient solution were 0.5 and 1 mM, respectively. Net uptake rates of Ca²⁺ and Mg²⁺ were determined after 66 d from germination as described above. Uptake rates expressed are means ± SE for three replicates ($n = 3$), each replicate consisting of *c.* 10 plants. The experiment was repeated on three consecutive days. At the end of the third day of measurements, plants were harvested after washing the roots in DDW for 1 min. Roots and shoots were separated and ashed to evaluate total concentrations of the two ions in the roots and shoots. Concentrations of Ca²⁺ and Mg²⁺ were determined by inductively coupled plasma spectroscopy (ICP) using a Varian Vista-Pro CCD Simultaneous ICP-OES instrument (Victoria, Australia).

Experiment 3: differential response in germination, survivorship and tolerance to Na⁺ and Mg²⁺

Sodium Cypselae from race A and C populations at Jasper Ridge (A_C and C_G) were dipped in 1% bleach for 1 min, washed three times with DDW, and then placed on Petri dishes containing filter paper soaked with 0.1, 10, 50, 100, and 200 mM NaCl solutions, in a dark, cold room (5°C) for 3 d. Ten cypselae each were placed on five Petri dishes per

treatment per population. On the third day, dishes were moved to a walk-in environment room (conditions as in experiment 1) and germinants counted on day 8 from sowing. Cypselae showing emergence of radicle were considered successfully germinated. The NaCl concentration at which germination was reduced by 50% (I₅₀ value) was calculated for both populations using either a linear or exponential regression analysis.

Seedlings were then placed on a mesh (0.5 mm) attached to a floating device and placed in plastic containers (6 × 6 × 7 cm) containing nutrient solutions (identical in composition to experiment 1) supplemented with the appropriate NaCl concentration. The initial number of seedlings per treatment per population ranged from 25 to 40. On day 14 from transplant, the seedlings were counted to estimate survivorship.

For each surviving seedling, the length of root was measured. Using either linear or exponential regression analyses, I₅₀ values (NaCl concentration at which root length was reduced by 50%) were estimated for the two populations (Macnair, 1983; Ashraf *et al.*, 1989).

Magnesium Using a similar protocol as for Na⁺, germination, survivorship, and tolerance were estimated for plants from Jasper Ridge (A_C and C_G) exposed to 1, 5, 10, 20, and 50 mM MgSO₄.

Results

Experiment 1: differential responses to Na⁺ and K⁺: uptake and accumulation

Table 2 lists the mean ion uptake rates (μmol h⁻¹g⁻¹ fwt) for the two races from the two species. Rates of Na⁺ uptake by edaphic race C of both species (C_C and C_G) were barely detectable even during the light period, while they absorbed K⁺ at relatively high rates (Table 2). By contrast, edaphic race

Table 2 Mean ion uptake rates (μmol h⁻¹ g⁻¹ f. wt) ± SE during day (8 : 30–20 : 30) and night (20 : 30–8 : 30) for race A and C populations of *Lasthenia californica* and *Lasthenia gracilis*

Population		Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
A _C	Day	0.76 ^a (± 0.11)	1.17 ^a (± 0.08)	0.61 ^a (± 0.37)	1.18 ^a (± 0.29)
	Night	0.37 (± 0.11)	0.21 (± 0.06)	0.32 (± 0.22)	0.914 (± 0.11)
A _G	Day	1.84 ^c (± 0.5)	2.67 ^b (± 0.27)	ND	ND
	Night	0.57 (± 0.66)	1.75 (± 0.24)		
C _G	Day	-0.01 ^b (± 0.06)	1.27 ^a (± 0.1)	0.32 ^b (± 0.34)	0.55 ^b (± 0.7)
	Night	0.02 (± 0.09)	0.09 (± 0.04)	-0.06 (± 0.02)	-0.22 (± 0.13)
C _C	Day	0.06 ^b (± 0.03)	1.05 ^a (± 0.14)	ND	ND
	Night	0.04 (± 0.02)	0.01 (± 0.01)		
F/t		8.07	15.53	10.06	4.65
P		0.001	0.001	0.005	0.02

Means denoted by different superscripts within a column are significantly different (*t*-test or Tukey multiple comparison test: $P < 0.05$). F (ANOVA) and *t*-values and probabilities are also given. $n = 18$ for Na⁺, K⁺ and $n = 9$ for Ca²⁺, Mg²⁺. ND = not determined.

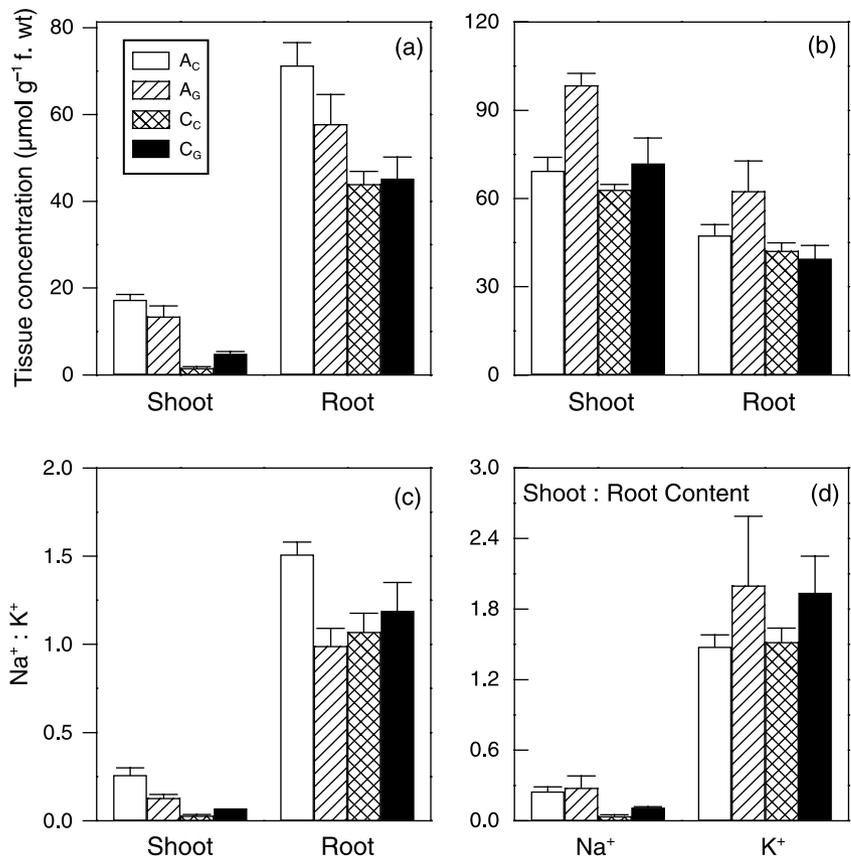


Fig. 1 Total tissue Na⁺ (a), K⁺ (b) and Na⁺/K⁺ (c) and shoot : root content for Na⁺ and K⁺ (d). Bars represent means (± SE). Multiple comparison of means (Tukey Test) suggest significant mean differences for shoot Na⁺: A_C, A_G > C_C, C_G (*P* < 0.001); root Na⁺: A_C > C_G, C_C (*P* < 0.001); shoot K⁺: A_G > A_C, C_G, C_C; root Na⁺ : K⁺: A_C > A_G (*P* < 0.05); shoot Na⁺ : K⁺: A_C > C_G, A_G, C_C (*P* < 0.001) and A_G > C_C (*P* < 0.01); and shoot : root Na⁺: A_C, A_G > C_C (*P* < 0.05).

A of both species (A_C and A_G) absorbed Na⁺ at rates comparable with their K⁺ uptake rates. Race A differed from race C in two other respects: firstly, in A_C and A_G uptake of Na⁺ continued during the dark period albeit at lower rates (18% in A_C and 65% in A_G, of the rates during the light period), while in C_C and C_G net uptake was almost nonexistent during the dark period. During the light period, unlike Na⁺, rates of K⁺ were similar between the two edaphic races of the same species (A_C and C_C) but interspecific differences were significant, e.g. K⁺ uptake rate of A_G was > 2-fold that of A_C (Table 2).

Accumulation and root : shoot partitioning patterns of Na⁺ differed markedly between the edaphic races, irrespective of the species. A_C and A_G accumulated 2–3 times more Na⁺ in the shoot than C_C and C_G (Fig. 1a). In both races, root Na⁺ concentrations were much higher than the shoot concentrations (Fig. 1a,d). Interestingly, the differences observed between the two races in rates of uptake and accumulation of Na⁺ persisted in older (70 d) plants (data not shown). Potassium concentrations of roots and shoots were not significantly different between the edaphic races A and C; however, A_G accumulated significantly more K⁺ than the other three populations (Fig. 1b). Unlike for Na⁺, K⁺ concentration in the shoot was higher than that in the root in all populations; but the differences between root and shoot K⁺ were relatively

small (Fig. 1b,d) compared to the differences observed for Na⁺ (Fig. 1a,d). Shoot weight/root weight ratios (*c.* 4) were not significantly different between the species or between the races. When shoot weight/root weight ratios of these species is taken into account, it is clear that > 85% of the total K⁺ absorbed is translocated to the shoot, compared to < 50% in the case of Na⁺. It is noteworthy that in both race A populations (A_C and A_G), a significantly greater proportion of Na⁺ (48 and 49%, respectively) was translocated to the shoot than in the race C populations (30% in C_G and 12% in C_C). The racial differences between Na⁺ and K⁺ accumulation patterns are well illustrated by Fig. 1(a)–(d).

Experiment 2: differential responses to Ca²⁺ and Mg²⁺: ion uptake and accumulation

Table 2 shows ion uptake rates for Ca²⁺ and Mg²⁺ in race A and C plants from Jasper Ridge (A_C and C_C). Uptake rates for both ions are two-fold greater in A_C than C_C. During the dark period of the diurnal cycle, rates of Ca²⁺ and Mg²⁺ uptake declined in A_C by 48% and 23%, respectively; compared with C_G where uptake had ceased completely.

Figure 2(a)–(b) show total tissue concentrations for Ca²⁺ and Mg²⁺, respectively, for 70-d-old A_C and C_G plants. A_C had *c.* 127-fold higher Ca²⁺ and *c.* 28-fold higher Mg²⁺ in its

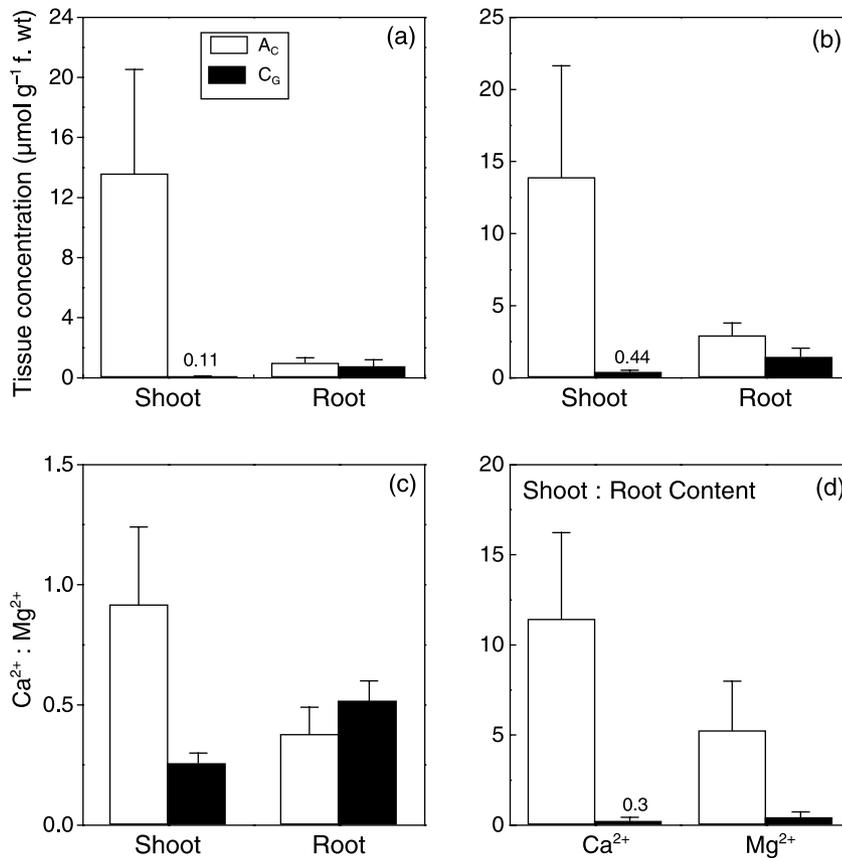


Fig. 2 Total tissue Ca^{2+} (a), Mg^{2+} (b) and $Ca^{2+} : Mg^{2+}$ (c) and shoot : root content for Ca^{2+} and Mg^{2+} (d) for Jasper Ridge plants. Bars represent means (\pm SE). Significant mean differences (*t*-test) observed for shoot Ca^{2+} : $A_C > C_G$ ($P < 0.03$); shoot Mg^{2+} : $A_C > C_G$ ($P < 0.05$); shoot $Ca^{2+} : Mg^{2+}$: $A_C > C_G$ ($P < 0.05$); and shoot : root Ca^{2+} , Mg^{2+} : $A_C > C_G$ ($P < 0.05$).

shoot than C_G , however, in the roots concentrations of both ions were similar in the two populations (Fig. 2a,b). The populations also showed marked differences in their ion allocation patterns: A_C freely translocated the two cations to the shoots, while C_G localized the two ions in its root (Fig. 2d). Further, the shoot $Ca^{2+} : Mg^{2+}$ quotient (Fig. 2c) was significantly higher in A_C (*c.* 1) compared with C_G plants (*c.* 0.2). It is noteworthy that A_C translocated > 95% of the two cations to its shoot, while C_G translocated only 36% of Ca^{2+} and 54% of Mg^{2+} to its shoot.

Experiment 3: differential responses in germination, survivorship and root elongation to Na^+ and Mg^{2+}

Germination, survivorship and root elongation responses of 14-d-old-race A (A_C) and C (C_G) plants (seed source: Jasper Ridge) to varying external Na^+ or Mg^{2+} concentrations are shown in Fig. 3(a)–(f). For each parameter, the results were subjected to a Two-Way ANOVA separately for Na^+ and Mg^{2+} (Table 3). Note that in the 200 mM Na^+ and 50 mM Mg^{2+} treatments both A_C and C_G showed virtually no germination in most replicates, and root elongation measurements in these treatments represent the few surviving seedlings; thus these treatments were excluded from statistical analyses. In all cases, genotype (G) \times treatment (T) interactions for both Na^+ and

Table 3 Results of Two-Way ANOVA for germination and root length (tolerance) for Jasper Ridge A (A_C) and C (C_G) plants grown under $NaCl$ and $MgSO_4$ treatments

Treatment Variable	Genotype (G) (df = 1)	Treatment (T) (df = 3)	G \times T (df = 3)
$NaCl$ Germination	3.7 ns	5.95*	4.23*
$MgSO_4$ Germination	0.19 ns	51.9**	3.5*
$NaCl$ Tolerance	1.34 ns	65.8**	12.77**
$MgSO_4$ Tolerance	9.3**	73.3**	5.9**

Values given are *F* statistics and significance levels; ns = $P > 0.05$; * = $P < 0.05$; ** $P < 0.001$.

Mg^{2+} were significant. Treatment had significant independent effects in all cases; however, a significant independent G effect was only observed in the case of root elongation under Mg^{2+} . The most notable feature of the data was that at low concentrations of both ions, according to all three parameters examined, C_G performed significantly better than A_C (Fig. 3).

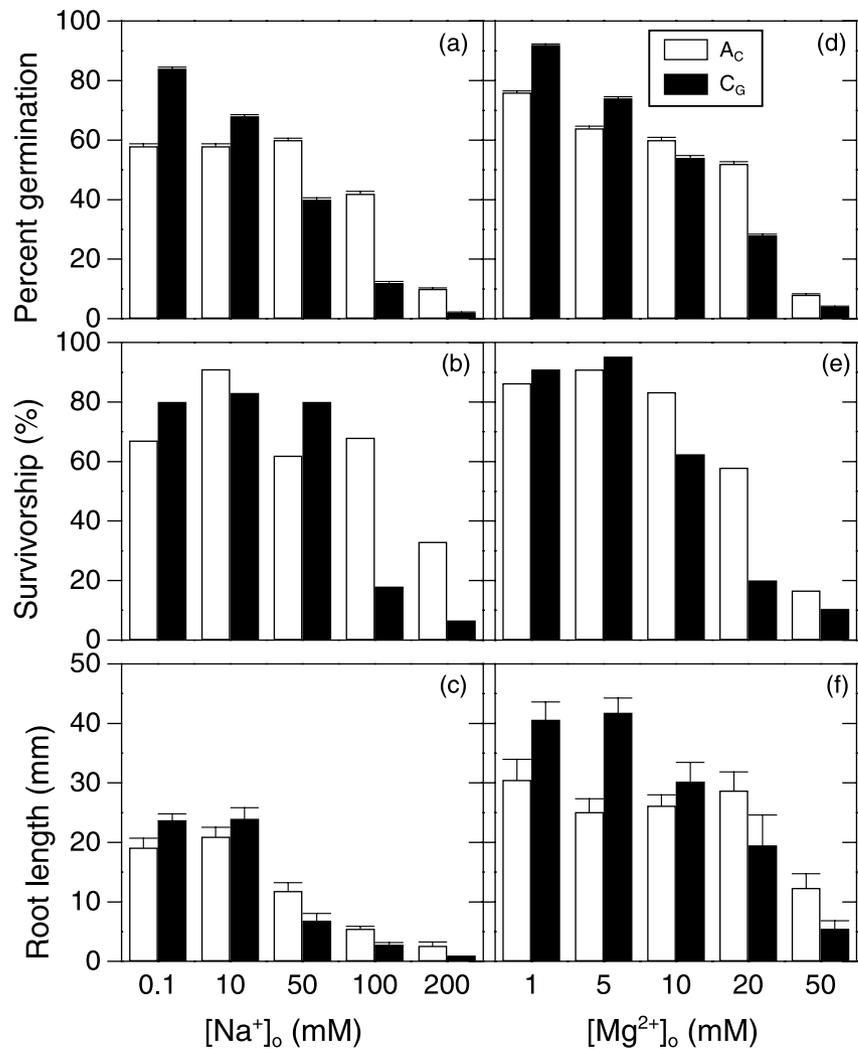


Fig. 3 Measures for tolerance to NaCl (a. germination; b. survivorship; c. root length) and MgSO₄ (d. germination; e. survivorship; f. root length) for race A (A_C) and C (C_C) plants from Jasper Ridge.

Table 4 I₅₀ values (mM) for germination and root length for the two races from Jasper Ridge

Population/Treatment	Germination	Root length
A _C : NaCl	139 mM (R ² = 0.99; P < 0.001) ¹	71.7 mM (R ² = 0.97; P < 0.01) ²
A _C : MgSO ₄	27.8 mM (R ² = 0.99; P < 0.001) ²	46.2 mM*
C _C : NaCl	38.2 mM (R ² = 0.99; P < 0.001) ²	29.4 mM (R ² = 0.94; P < 0.01) ²
C _C : MgSO ₄	12.3 mM (R ² = 0.99; P < 0.001) ²	19.2 mM (R ² = 0.99; P < 0.01) ²

Linear¹ and exponential² models utilized to obtain R² values. *No R² value for root length in A_C/MgSO₄ since a significant change was only observed between 20 and 50 mM treatments.

C_C, however, was more susceptible to increases in Na⁺ and Mg²⁺ concentrations (≥ 50 and 10 mM, respectively) and, at these higher concentrations, performance of A_C was superior to C_C. For both Na⁺ and Mg²⁺, in germination as well as root elongation, I₅₀ for A_C was > twice that for C_C (Table 4).

All three measures described above suggest considerable differences between Jasper Ridge A (A_C) and C (C_C) plants in their response to NaCl and MgSO₄, with A_C clearly appearing to be the more tolerant of the two populations. The significant G × T interaction for both cations confirms that the two

edaphic races from Jasper Ridge are differentiated in their tolerance responses. Interestingly, at both 100 and 200 mM concentrations of NaCl and the 20 and 50 mM treatments of MgSO₄, shoots of A_C plants showed signs of toxicity and the plants resembled the phenotype (stunted, succulent) of those race A populations found on some coastal bluffs (N. Rajakaruna, pers. obs.).

Discussion

Previous studies have suggested cross-resistance to harsh edaphic conditions such as serpentine and saline habitats (Kruckeberg, 1954; Proctor, 1971; Goodwin-Bailey *et al.*, 1992) and serpentine and mine tailings (Baker *et al.*, 1992; Brooks, 1998; Macnair & Gardner, 1998). Saline and serpentine habitats are characterized by harsh conditions, such as high soil surface temperatures, poor soil structure, low osmotic potentials and high concentrations of specific ions, including Mg²⁺ (Kruckeberg, 1984; Flowers *et al.*, 1986; Fitter & Hay, 1987). Jenny (1980) suggests that successful colonization of harsh environments such as serpentine habitats requires tolerance to the serpentine syndrome characterized by multiple stresses. Once accommodation to one harsh environment has been achieved, however, a plant may be able to successfully venture onto another similarly harsh environment, although adaptations to specific local conditions will determine the ultimate success in the new habitat.

The findings of the present study confirm that racial differences in ion accumulation, previously observed under field conditions, are not exclusively the outcome of differences in soil ionic composition, since they are sustained even under identical growth conditions in the laboratory. This strongly suggests a genetic basis for the observed patterns of ion accumulation. Race A plants, regardless of the species, are clearly more tolerant of potentially toxic Na⁺ concentrations. In the case of Mg²⁺, race A plants from Jasper Ridge (A_C) are more tolerant than the race C plants (C_C) from this site and it is tempting to speculate that Mg²⁺ tolerance may also be a feature common to race A plants from both species. Both Na⁺ and Mg²⁺ are prevalent in some or all of the habitats where race A is found and our measures for germination, survivorship, and root length have shown that race A from Jasper Ridge is better adapted to grow in Na⁺- and Mg²⁺- rich habitats. For both cations, there was a significant G × T interaction, suggesting that the two races from Jasper Ridge are differentiated in their tolerance responses. This tolerance is not based upon ionic exclusion, because root absorption and shoot accumulation of Na⁺ and Mg²⁺ by race A plants was substantially higher than by race C plants.

Previous studies on plant-soil relations provide examples of intraspecific differences in accumulation of and/or tolerance to Na⁺ (Ashraf *et al.*, 1989), K⁺ (Flowers & Lauchli, 1983; Siddiqi & Glass, 1983), Ca²⁺ (Snaydon & Bradshaw, 1961,

1969; Ramakrishnan & Singh, 1966), Mg²⁺ (Main, 1974), and heavy metals (Antonovics *et al.*, 1971). In the case of heavy metals, evolution of intraspecific tolerance can be rapid, taking place within only a few generations (Wu *et al.*, 1975). The case with *Lasthenia* is intriguing, however, because we find parallel physiological changes in two closely related cryptic species [note that both these species were recognized as *L. californica sensu* Ornduff prior to the recent taxonomic revision (Chan, 2001)]. Schat *et al.* (1996) provide one of the best examples to date of parallel genotypic changes in tolerance to an edaphic extreme within a plant species. Studies of copper tolerance in *Silene vulgaris* Garcke (Caryophyllaceae) show population specific alleles for copper tolerance in geographically isolated populations, suggesting independent evolution of the same genetic loci at different localities. Our studies add to this list whereby physiological traits (i.e. tolerance to Na⁺ and Mg²⁺) responsible for adaptation to extreme edaphic conditions such as saline/serpentine habitats appear to have evolved in parallel in two closely related species. However, we should add that it is as yet unclear whether it is more parsimonious to assume that race A, race C or both have evolved in parallel. It is also not yet known whether identical genetic changes are responsible for these differences.

Our findings on the putatively parallel physiological changes in edaphic races of *L. californica sensu lato* raise questions about an intriguing correlation between ion physiology and flavonoid chemistry. The primary feature that distinguishes race A from race C plants is the flavonoid pigment profile; race A contains sulfated compounds, namely sulfated kaempferol and quercetin diglycosides plus prominent eriodictyol glycosides (Bohm *et al.*, 1974, 1989; Desrochers & Bohm, 1993) not found in race C plants. Marine, alkaline and serpentine habitats are high in sulfates and it is tempting to suggest that sulfation of flavonoids may be beneficial in such environments, as a means of detoxifying excess sulfate. For example, the more hydrophilic sulfated flavonoids would be better contained within vacuoles, and furthermore, by reducing the chemical activity of inorganic sulfate, precipitation of compounds such as calcium sulfate that are characterized as having low solubility products may be avoided. Ecological roles for flavonoid pigments have often been postulated (Bohm, 1987; Clegg & Durbin, 2000), and the case for a correlation with habitat and sulfated flavonoids has previously been noted (Harborne, 1975, 1977). For example, a large number of taxa found in habitats with waterlogged and saline conditions contain sulfated flavonoids (Harborne, 1975; Barron *et al.*, 1988). A study by Nissen & Benson (1964) showed that over 50% of radioactive sulfate fed to *Zostera* (sea grass) was later found in the flavonoid fraction. In addition, limited work on salt-marsh plants suggests that Na⁺ can act as the counter cation for these negatively charged sulfated flavonoids (Tomas-Barberan *et al.*, 1987), although K⁺ is usually regarded as the predominant counter cation. These negatively charged sulfated flavonoids may play a role similar to that of

sulfur containing glucosinolates in providing tolerance to excess metal ions (Mathys, 1977) or in providing ionic balance in light of excess cations such as those found in race A habitats. Thus, we recently hypothesized an edaphically linked ecological role for flavonoid differences (N. Rajakaruna *et al.*, unpublished) that first suggested the existence of these two races (Bohm *et al.*, 1989).

Plants avoid ionic toxicity by either excluding toxic ions or by accumulation and sequestration (Fitter & Hay, 1987). In the case of Na^+ , some tolerant species are able to maintain higher concentrations in the shoot and sequester the ions in the vacuole via Na^+/H^+ antiporters (Apse *et al.*, 1999). This necessitates the generation of compatible solutes within the cytosol to compensate for adverse osmotic effects of high vacuolar Na^+ concentrations (Flowers *et al.*, 1986). While glycophytic (i.e. nonhalophytic) species depend primarily on the exclusion of Na^+ at the plasma membrane, halophytic species accumulate large amounts – up to 700 mM – in the vacuole (Flowers *et al.*, 1977) by the use of the Na^+/H^+ antiporter. In *Plantago*, the vacuolar Na^+/H^+ antiporter activity is only present in the salt-tolerant *P. maritima* L., but not in the glycophytic *P. media* L. (Staal *et al.*, 1991) and this difference is thought to be critical in the ecological divergence of these two species. Although both races in our study restricted a greater proportion of internal Na^+ to their roots, race A plants were clearly able to accumulate greater concentrations of Na^+ in the above-ground tissues than race C plants (*c.* 5-fold) and translocate *c.* 50% of Na^+ to the shoot, showing greater tolerance to this ion. Whether the presence/absence or level of expression of the Na^+/H^+ antiporter gene is responsible for the differences in uptake and accumulation is an area worthy of investigation. Such a study is essential in order to address the underlying biochemical/genetic basis of these traits.

Serpentine substrates are characterized by high concentrations of Mg^{2+} , a factor that appears to have a major influence on the ecology of serpentine vegetation (Proctor & Woodell, 1975). Chelation of Mg^{2+} by soluble carboxylates appears to play an important role in vacuolar accumulation and sequestration of this ion (Woolhouse, 1983) and has been shown to be an important mechanism for tolerance in *Sedum anglicum* Hudson (Crassulaceae) found in serpentine and other Mg^{2+} -rich soils (Tibbetts & Smith, 1992). Race A plants from Jasper Ridge translocated greater concentrations of Mg^{2+} to the shoots than race C plants (> 95% in A_C compared with 54% in C_C) and it is possible that tolerance mechanisms such as those documented for *Sedum* also exist in race A plants, allowing them to accumulate higher concentrations of potentially cytotoxic Mg^{2+} in their shoots.

Of the other two cations tested, K^+ and Ca^{2+} , K^+ is typically the major cation in plant tissues (Glass, 1988). This fact may account for the relatively similar uptake rates and accumulation of K^+ in the two races. However, for Ca^{2+} , the uptake rate and the accumulation level in the shoots were 2-fold and 124-fold higher, respectively, in race A plants from Jasper Ridge. It

is noteworthy that race A (A_C) translocated > 95% of total internal Ca^{2+} to its shoot compared to only 36% in race C (C_C). Higher Ca^{2+} uptake and accumulation levels have been previously reported for other taxa found in Ca^{2+} -poor serpentine soils (Madhok & Walker, 1969), suggesting that race A (A_C) is perhaps better equipped to deal with serpentine soils.

Plants typically have highly selective uptake systems, capable of distinguishing physicochemically similar pairs of ions such as Na^+/K^+ and $\text{Ca}^{2+}/\text{Mg}^{2+}$. At low K^+ concentrations (normally under 0.5 mM), Na^+/K^+ selectivity of barley roots is high, but at high concentrations, this selectivity is only found in halophytes (Epstein, 1969). One of the key elements in salinity tolerance is the capacity to maintain high cytosolic K^+/Na^+ regardless of the external concentrations of these two ions (Yeo, 1998; Maathuis & Amtmann, 1999). Similarly, at low soil $\text{Ca}^{2+} : \text{Mg}^{2+}$ quotients, such as in serpentine habitats, excess Mg^{2+} accumulates in tissues of nontolerant plants with toxic effects (Proctor, 1971). The general paucity of species in serpentine habitats is thought to result from this condition, although various other features, biotic and abiotic, also contribute to the exclusion of species from serpentine habitats (Kruckeberg, 1984). Limited studies show that species that are found in serpentine habitats take up Ca^{2+} and Mg^{2+} to a greater extent in proportion to the external concentration than do susceptible genotypes, suggesting that the resistance mechanism is internal (Walker *et al.*, 1955; Lyon *et al.*, 1971; Main, 1974). This is clearly the pattern seen in race A plants from Jasper Ridge, the race that predominates in serpentine habitats. Race A (A_C) has *c.* 127-fold higher Ca^{2+} and *c.* 28-fold higher Mg^{2+} in its shoot than race C (C_C) and is able to maintain a favorable $\text{Ca}^{2+} : \text{Mg}^{2+}$ quotient (*c.* 1) despite the higher external Mg^{2+} concentrations. Extensive field collections also suggest that race A is clearly more tolerant of adverse $\text{Ca}^{2+} : \text{Mg}^{2+}$ quotients (Rajakaruna & Bohm, 1999). In other species, for example in *Secale* 'rye' (Olsen, 1971), ability to withstand low $\text{Ca}^{2+} : \text{Mg}^{2+}$ quotients lies in the ability to discriminate in favor of Ca^{2+} ; this same phenomenon is applicable to *Helianthus bolanderi* ssp. *exilis* A. Gray, the serpentine endemic sunflower (Madhok & Walker, 1969). These few studies to date show that differences observed between the serpentine and non-serpentine populations may not be due to a single mechanism but, rather, a combination of several possible mechanisms, i.e. differences in root morphology, uptake, translocation, and interactions between cations. It will be important to look at K^+/Na^+ and $\text{Ca}^{2+}/\text{Mg}^{2+}$ selectivity and tolerance under different external concentrations of these ions to determine if the edaphic races from both species differ in their capacity to maintain favorable ion ratios.

An important aspect of our eco-physiological studies that needs further investigation is the ability of the races to withstand low osmotic potentials. Although we have not determined water potential for soils where the races grow, it is clear from other parameters (Rajakaruna & Bohm, 1999) and field observations that race A plants grow in soils with a lower

osmotic potential. Ability to accumulate and tolerate greater concentrations of cations such as Na^+ , Mg^{2+} and Ca^{2+} will undoubtedly allow race A plants to survive under osmotically harsh conditions in which they are often found. Our studies also show that race C plants generally are not found in habitats such as coastal bluffs, serpentine soils and alkaline flats (Rajakaruna & Bohm, 1999). It is possible that the lack of ability for osmotic adjustment, rather than or in addition to tolerance to a specific ion, may be influential in this clear-cut distribution. We are currently designing studies to document tolerance to other specific ions as well as osmotic effects and to determine the relative importance of these factors in the ecological divergence of the edaphic races.

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