Molecular Ecology (2003) **12**, 1675–1679 doi:10.1046/j.1365-294X.2003.01843x

SHORT COMMUNICATION

# Edaphic races and phylogenetic taxa in the *Lasthenia californica* complex (Asteraceae: Heliantheae): an hypothesis of parallel evolution

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**Abstract**

***Lasthenia californica sensu* Ornduff consists of two races that differ in their flavonoid pigments and edaphic tolerances. Recent phylogenetic studies of *Lasthenia* have revealed that members of *L. californica sensu* Ornduff belong to two phylogenetic species. The relationship of the edaphic races to these new species and to each other is the focus of this study. Characterization of flavonoid profiles and phylogenetic placement of 33 populations demonstrates that races and phylogenetic taxa are not concordant, suggesting that one or both edaphic races evolved in parallel in the two clades. We hypothesize an edaphically linked ecological role for flavonoid differences that first revealed the existence of two races.**

*Keywords*: Asteraceae, California flora, cryptic species, edaphic races, *Lasthenia californica*, parallel evolution, serpentine

*Received 29 November 2002; revision received 12 Fenruary 2003; accepted 12 February 2003*

## Introduction

The independent and recurrent evolution of adaptive traits in closely related lineages is best explained by the action of natural selection (Levin 2001; Schluter & Nagel 1995). Thus, case studies of parallel evolution have the power to uncover the role of ecological selection in speciation. The literature on heavy metal tolerance provides several examples of recent, rapid and recurrent evolution of traits in plants from both mine-tailings (Wu *et al*. 1975; Al-Hiyaly *et al*. 1993; Schat *et al*. 1996) and serpentine habitats (Westerbergh 1996), suggesting that plants growing under edaphic extremes are good candidates for the study of parallel evolution.

*Lasthenia californica* DC. ex Lindl. *sensu* Ornduff (1966, 1993), the common goldfields of California, provides an ideal system for the study of parallel evolution driven by edaphic forces. Common goldfields, as previously delimited, displays the widest range of edaphic tolerances within the genus, occupying diverse habitats within the Californian Floristic Province (Ornduff 1966, 1993). An ecological survey (Rajakaruna & Bohm 1999) concluded that flavonoid races previously described for the complex (Bohm *et al*. 1989; Desrochers & Bohm 1993) correspond to edaphic races. Plants designated as members of race A predominate in ionically extreme habitats such as coastal bluffs, alkaline flats, vernal pools and serpentine outcrops; these plants are characterized by the presence of sulphated flavonoids (Bohm *et al*. 1989). Plants treated as race C are found in ionically benign but drier sites such as road-side pastures and oak woodlands; these plants lack the sulphated flavonoids diagnostic of race A. The two races grow in parapatry on a serpentine outcrop at Jasper Ridge Biological Preserve (Stanford University, San Mateo County, CA), with each race occupying microhabitats that correspond to the distinct edaphic conditions under which plants with the same flavonoid profiles are found across the range of the complex. Recent studies have shown that the two edaphic races differ in their ion uptake physiologies

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and that race A is better equipped to deal with potentially toxic ions such as sodium (Rajakaruna *et al*. 2003a). Greenhouse (Rajakaruna & Bohm 1999; Rajakaruna *et al*. 2003b) and hydroponic (Rajakaruna *et al*. 2003a) studies also demonstrate that the races achieve greater fitness under soil conditions that best match their natural environment, suggesting that edaphic factors may have played an important role in the origin of these races.

Recent molecular phylogenetic studies (Chan *et al*. 2001, 2002; Desrochers & Dodge 2003) indicate that *L. californica sensu* Ornduff is not monophyletic. Based on a comprehensive internal transcribed spacer (ITS)/external transcribed spacer (ETS)/chloroplast DNA (cpDNA) phylogenetic study, Chan *et al*. (2001, 2002) found that *L. californica sensu* Ornduff represents two geographically based, nonsister clades. Chan *et al*. (2001, 2002) recognized the clades as two cryptic taxa, *L. californica* ssp. *californica* representing the northern clade [populations previously treated as *L. macrantha* (A. Gray) Greene constitute the other subspecies of *L. californica*] and *L. gracilis* DC. (Greene) representing the southern clade. The molecular phylogeny has led us to ask about correspondence of the edaphic races to the phylogenetically distinct taxa. If ecological selection has played a role in the origin of edaphic races, as seems plausible, then similar edaphic tolerances may have evolved in parallel within one or both species. Indeed, a previous study of allozyme variation (Desrochers & Bohm 1995) indicated that single populations of race A and race C cluster with sets of populations belonging to the opposing race. To address this intriguing issue, several specimens from the two molecular phylogenetic clades of Chan *et al*. (2001, 2002) were tested for their flavonoid-pigment profiles, and nuclear ribosomal DNAs (rDNA) of several representatives from the two edaphic races were characterized to determine their placement in the context of Chan *et al*.'s molecular phylogeny.

## Materials and methods

Two heads from each of three populations belonging to the two molecular clades (Chan *et al*. 2001, 2002) were tested for their flavonoid profiles employing one-dimensional thin-layer chromatography, as described earlier in Desrochers & Bohm (1993). The location and clade of the populations tested are described in Table 1 (populations 1–6).

In order to characterize variation in ITS, genomic DNAs were isolated from approximately 1 g of plant tissue following the modified CTAB protocol of Doyle & Doyle (1987). Samples were further purified using the Elu-Quick DNA purification kit (Schleicher and Schuell). The rDNA ITS region was sequenced, as described previously by Chan *et al*. (2001), from DNA isolates of two plants from each of three race C and six race A populations (Table 1, populations 7–15). Although the phylogenetic study of Chan *et al*. (2001, 2002) was based on analysis of ETS, ITS and cpDNA regions, 26 nucleotide differences, and a single 11 base-pair (bp) indel distinguished the ITS sequences of all populations of the two phylogenetic species sequenced thus far. Therefore ITS variation is sufficient for assigning populations to one or the other phylogenetic species.

The 11 bp indel described above is located in the ITS I region, flanked by the 18S and the 5.8S ribosomal genes. This size difference was used as a marker for the assignment of 18 additional populations of known flavonoid type to phylogenetic lineage (Table 1, populations 16–33). Populations 7–15, sequenced as above, were used as size controls. The ITS I region was amplified using primers ITS2 and ITS5 (White *et al*. 1990). Amplifications were carried out in 25-μL volumes including 10 ng of template DNA, 30 mM Tris–HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mM each dNTP, 10 pmol of each primer, 5% acetamide and 1.5 units of DNA polymerase. Amplification was carried out on a 60-well PT-100 thermal cycler (MJ Resreach, Waltham, MA), programmed for an initial denaturation of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C, with a final extension of 7 min at 72 °C. A 2-μL aliquot of each PCR product was separated by electrophoresis on 2% (w/v) SeaPlaque agarose (BMA, Rockland, ME) in 0.5× TBE buffer. After staining in ethidium bromide, the gel was visualized under UV light with an AlphaImager 1200 gel documentation system (AlphaInnotech Corporation).

## Results and discussion

Examination of flavonoid profiles and ITS sequences reveals that edaphic races are not concordant with the newly circumscribed taxa *Lasthenia californica* ssp. *californica* and *L. gracilis*. Flavonoid typing (Table 1, populations 1–6) indicates that two of the populations recognized as *L. gracilis* are race A (RO10079 and RC98011), while the third is race C. The three *L. californica* ssp. *californica* populations are race C. Although the flavonoid profiles do not correspond to the two clades, both populations with flavonoid profile A were found in alkaline flats, supporting the correlation between flavonoid profile and edaphic habitat.

New ITS sequences of the three race C and six race A populations revealed a similar pattern (Table 1, populations 7–15). Here, populations of each race nest within each of the phylogenetic species, further verifying that edaphic races do not correspond to the two molecular clades.

Finally, PCR-amplified ITS 1 fragments assigned six of seven race C populations to *L. californica*. Of 11 race A populations, four had the longer fragment characteristic of *L. californica*, and seven displayed the shorter fragment characteristic of *L. gracilis* (Table 1, populations 16–33). While the longer ITS 1 product is also characteristic of

**Table 1** Populations of the *Lasthenia californica* complex (*L. californica* ssp. *californica* and *L. gracilis*) sampled in this study

| No | Population | Locality | Race | Species |
|---|---|---|---|---|
| 1 | RO10079 | Alkali flats W and N of Byron Hot Springs Road, E of the airport S of Byron, Contra Costa Co., CA | A | *L. gracilis* |
| 2 | RC 98011 | Alkaline field beyond E corner of Hartford Ave., SW of Livermore, Alameda Co., CA | A | *L. gracilis* |
| 3 | RC98010 | Grassy Hillside ~50 m E of Hwy 33, 0.25 miles N of its junction with Palmer Ave., N of Coalinga, Fresno Co., CA | C | *L. gracilis* |
| 4 | RO10160 | Parking area at Point Reyes Lighthouse, Marin Co., CA | C | *L. californica* |
| 5 | RC98017 | Grassy Field ~100 m W of Hwy 29, 2.4 miles S of State Hospital, Napa Co., CA | C | *L. californica* |
| 6 | RC98020 | Grassy pasture NW of Hwy 120, SW of road leading to Two-Mile Bar Rec. Area, Knights Ferry, Tuolumne Co., CA | C | *L. californica* |
| 7 | RS (AF550682–83) | Serpentine soils of north-facing slope of Rattlesnake Rock, Jasper Ridge Biological Preserve, San Mateo Co., CA | A | *L. californica* |
| 8 | JRA (AF550680–81) | Bottom reaches of the serpentine outcrop, Jasper Ridge Biological Preserve, San Mateo Co., CA | A | *L. californica* |
| 9 | AVEQ (AF550689) | Pasture along Avenue Q, across from Holiday Inn, Palmdale, Los Angeles Co., CA | A | *L. gracilis* |
| 10 | COA (AF550690) | Pasture, 1 km E of Coalinga Springs Road on Route 198, Fresno Co., CA | A | *L. gracilis* |
| 11 | CA3 AF550691 | Serpentine hillside, 4 km E of Paskenta Bridge, Paskenta/Covelo Road, Paskenta, Tehama Co., CA | A | *L. gracilis* |
| 12 | TEHA (AF550688) | Along Tehachapi Willow Springs Road, 3.3 km S of intersection with Highline Road, Kern Co., CA | A | *L. gracilis* |
| 13 | JRC (AF550686–87) | Upper reaches of the serpentine outcrop, Jasper Ridge Biological Preserve, San Mateo Co., CA | C | *L. gracilis* |
| 14 | TR (AF550684) | Andesite deposit, summit of Lower Table Rock, Jackson Co., OR | C | *L. californica* |
| 15 | OR1 (AF550685) | Roadside, Kirtland Road, 0.5 km E from intersection with Table Rock Road, near Water Treatment Plant, Jackson Co., OR | C | *L. californica* |
| 16 | SPS | On coastal bluff, Salt Point State Park, Sonoma Co., CA | A | *L. californica* |
| 17 | PR | Along roadside on way to Light House, Point Reyes, 4 km from Drake Beach, Marin Co., CA | A | *L. gracilis* |
| 18 | MT | On serpentine substrate at Mount Tamalpais State Park, Marin Co., CA | A | *L. californica* |
| 19 | KCN | On serpentine substrate, N-facing slope of Kerby Canyon, W of Coyote Ridge, Santa Clara Co., CA | A | *L. californica* |
| 20 | 25 | Roadside pasture along Route 25, 8.5 km S of Hollister, San Benito Co., CA | A | *L. gracilis* |
| 21 | PV | Pasture along Route 198, 4 km NW of County line of Monterey and Fresno Counties, Priest Valley, Monterey Co., CA | A | *L. gracilis* |
| 22 | AS | Hillside N of 16-M. Road on trail to stream, ~4 km from Arroyo Seco Campsite, Los Padres Nat. Forest, Monterey Co., CA | A | *L. californica* |
| 23 | CASR | Roadside ditch. Intersection California Ave. & Stowe Rd. W of Stetson & Warren Rd, Near Hemet, Riverside Co., CA | A | *L. gracilis* |
| 24 | MR | On soils derived from granite. Motte Rimrock Reserve, Riverside Co., CA | A | L. gracilis |
| 25 | AZ | Sandy soil along dry streambed. N of Apache Junction on Route 88, near M. 203, on left (W) side of road. Maricopa Co., AZ | A | *L. gracilis* |
| 26 | ARZ | Sandy ridge near a sidetrack ~1.5 miles along Dripping Springs Road, N of Mile 153 marker on AZ Route 77. Gila Co., AZ | A | *L. gracilis* |
| 27 | OR2 | 0.5 km from intersection Kirtland Road and Table Rock Road. Roadside pasture, Water Treatment Plant, Jackson Co., OR | C | *L. californica* |
| 28 | OR3 | On gravelly pasture across road from population OR2. Jackson Co., OR | C | *L. californica* |
| 29 | 44 | Near Millville, 2 km from Old 44 Road/Route 44. On oak woodland at Route 44/A17, Shasta Co., CA | C | *L. californica* |
| 30 | 44A | Near Millville, 1 km from population 44 heading towards Redding, Shasta Co., CA | C | *L. californica* |
| 31 | RB | Pasture along Route 36, 1.7 km from intersection with Highway 99. Near Red Bluff, Tehama Co., CA | C | *L. californica* |
| 32 | R20 | Roadside pasture along Route 20 between Colusa and Clear Lake, Colusa Co., CA | C | *L. gracilis* |
| 33 | R29 | Roadside pasture, 4 km N of Calistoga along Route 29. Napa Co., CA | C | *L. californica* |

Populations 1–6 of known phylogenetic species, were tested for flavonoid profile. Populations 7–15, of known edaphic race, were sequenced to confirm the phylogenetic status. GenBank accession numbers for these new sequences are included in parentheses next to population designations. Populations 16–33 of known edaphic race, were assigned to a phylogenetic lineage by assessing the relative size of the ITS 1 amplicon. Vouchers for populations 1–6 are deposited in the Jepson Herbarium, UC Berkeley; those for populations 7–33 are at the UBC Herbarium.

*L. californica* ssp. *macrantha* (formerly *L. macrantha*), this taxon is distinguished by morphological and flavonoid features, and thus we are confident in assigning all samples here to *L. californica* ssp. *californica*. Note that the size difference was also scored for the nine sequenced samples (populations 7–15) and was concordant in each case with phylogenetic assignment based on the complete ITS sequence.

Thirty-three populations were characterized as part of this study. Of the 16 populations of *L. gracilis*, 13 are race A and three are race C. Of the 17 populations of *L. californica* ssp. *californica*, 11 are race C and six are race A (Table 1). Both edaphic races occur in parallel in both taxa. The pattern is further confirmed by carefully assessing the results of the allozyme analysis of Desrochers & Bohm (1995) and the ITS phylogeny of Desrochers & Dodge (2003), where the edaphic races do not always correspond to the geographical clades.

Although race C populations generally nest within *L. californica* ssp. *californica* and race A populations are most often assigned to *L. gracilis*, the pattern is reversed at Jasper Ridge as well as in several other populations. In many such cases soil features seem to be a better predictor of flavonoid profile (Rajakaruna & Bohm 1999) than either the allozyme data (Desrochers & Bohm 1995) or molecular phylogenies (Chan *et al*. 2001, 2002; Desrochers & Dodge, 2003). For example, in every instance where a plant from an extreme serpentine exposure (JRA, RS, MT, KCN), coastal bluff (SPS, PR) or alkaline flat (RO10079 and RC98011) was tested, the plant had the flavonoid profile of race A, although the plants belonged to different geographical clades, and thus to different taxa. This intriguing finding suggests parallel evolution of edaphic races; racial features may have evolved in response to the contrasting soil conditions under which these plants are found.

Race A plants predominate in habitats of ionic stress while race C plants grow in drier but ionically benign soils. The two races are physiologically differentiated. Race A plants have greater tolerance to sodium and magnesium, ions that predominate in their habitats. Race C plants are better able to tolerate drought, a feature that is common to their habitats (Rajakaruna *et al*. 2003b). A recent ion physiology study shows that race A plants, regardless of the species, have higher sodium ion uptake rates and total tissue sodium concentrations, suggesting that race A from both taxa may have a similar biochemical/genetic basis for this trait (Rajakaruna *et al*. 2003a). Whether similar changes also occur for magnesium tolerance in all race A plants and for drought tolerance in all race C plants is yet unclear.

The primary feature that distinguishes these edaphic races is the flavonoid pigment profile; race A contains sulphated compounds, namely sulphated kaempferol and quercetin diglycosides plus prominent eriodictyol glycosides (Bohm *et al*. 1974, 1989; Desrochers & Bohm 1993) not found in race C plants. While an ecological role for sulphated flavonoids has never been demonstrated, there is ample evidence from the literature showing an abundance of these compounds in plants found in ionically extreme soils (Harborne 1975, 1977; Barron *et al*. 1988). Marine, alkaline and serpentine habitats are high in sulphates and it is tempting to suggest that sulphation of flavonoids may be beneficial in these environments, as a means of detoxifying the excess sulphate. A study by Nissen & Benson (1964) showed that over 50% of radioactive sulphate fed to *Zostera* (sea grass) was later found in the flavonoid fraction. In addition, limited work suggests that sodium can act as the counter cation for these negatively charged sulphated flavonoids (Tomas-Barberan *et al*. 1987; De Beck *et al*. 1998), although potassium is usually regarded as the predominant counter cation. Interestingly, race A plants are sodium accumulators (Rajakaruna & Bohm 1999; Rajakaruna *et al*. 2003a) and it is intriguing to find that sulphated flavonoids occur only in these sodium-accumulating plants, which in turn are found in sulphate- and sodium-rich environments.

The repeated evolution of traits in similar environments strongly implies that natural selection is the cause of these changes because genetic drift will not commonly produce concerted shifts in the same direction (Schluter & Nagel 1995). Introgression could also produce the patterns that we observe. For example, *L. californica* ssp. *californica* could represent a lineage that was ancestrally race C, with race A populations arising within this lineage as a result of introgression of racial features. The converse could be true for *L. gracilis*. We would predict that this type of process might result in topological incongruence between chloroplast- and nuclear-based phylogenies. Chan *et al*. (2002) did not detect any such incongruence. This could only occur if backcrossing was consistently in the direction of the maternal parent and always resulted in fixation of the maternal ITS and ETS alleles, thus gene flow seems unlikely to account for the parallel occurrence of the races in the two divergent lineages. We favour an hypothesis of parallel evolution at this time, although we stress that further evidence is required to substantiate our hypothesis. For example, phylogenetic trees presented to date lack sufficient resolution to infer whether one or both races has evolved in parallel. In any case, the strong association of edaphic features of localities from which populations were collected with the presence/absence of both sulphated flavonoids and traits responsible for sodium tolerance and drought tolerance strongly suggests that these features are the product of natural selection imposed by edaphic forces. The study reported in this paper documents a novel example of parallel trait evolution in two closely related, phylogenetically distinct taxa, and suggests a previously unknown edaphically linked adaptive role for flavonoid sulphates.

Schluter & Nagel (1995) and Levin (2001) suggest four criteria necessary to demonstrate parallel evolution of

phenotypically similar taxa. First, separate populations that occur in similar environments must be phylogenetically distinct. Second, the shared characteristics must be the products of natural selection. Third, separate descendant populations that are found in similar environments must be reproductively isolated from the ancestral populations. Finally, the separate descendant populations must not be reproductively isolated from one another. Few cases in which all four criteria have been addressed exist (Schluter & Nagel 1995), and Levin (2001) indicates that there are almost no unassailable examples of parallel origins in the plant literature. We believe that our studies (this study, Rajakaruna *et al*. 2003a,b) provide evidence to satisfy the first two criteria. Current studies on the extent of reproductive isolation between these edaphic races (Rajakaruna and Whitton, unpublished results) will perhaps provide additional supportive evidence to further our hypothesis of parallel evolution of edaphic races in the *L. californica* complex.

## Acknowledgements

The authors wish to thank Bridget L. Wessa (Jepson Herbarium) for sequencing the DNA from populations 7–15 listed in Table 1, and Rick Ree and three anonymous reviewers for useful comments on the manuscript.

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This work constitutes part of the doctoral thesis of N.R. on the evolutionary ecology of *Lasthenia californica*; B.G.B. studies the evolution of Californian and Hawaiian floras, with special focus on the origin and relationships of neoendemic lineages; R.C. is a post-doctoral fellow working on the molecular systematics of the Arctoteae and Vernonieae (Compositae); A.M.D.'s research focuses on relationships among perennial species of *Helianthus*; B.A.B.'s primary interest is in plant geography with emphasis on the geographical distribution patterns of micro- and macromolecules; J.W.'s research focuses on the variety of factors and processes that serve as barriers and/or connections between plant populations and species.

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