

EDAPHIC DIFFERENTIATION IN *LASTHENIA*: A MODEL FOR STUDIES IN EVOLUTIONARY ECOLOGY

NISHANTA RAJAKARUNA

Department of Biological Sciences, 371 Serra Mall, Stanford University,
Stanford, CA 94305-5020
nishanta@stanford.edu

ABSTRACT

The current knowledge of the goldfield genus *Lasthenia* is discussed, emphasizing the possible role of edaphic factors in the divergence of this predominantly Californian genus. *Lasthenia* species occupy a wide array of edaphic habitats ranging from serpentine outcrops to salt flats to guano deposits, thriving under specific ion and osmotic conditions that are uninhabitable for the vast majority of species. Studies show that ion accumulation and sequestration are common strategies used to counter osmotic effects, common to most habitats harboring *Lasthenia* species. Inter- and intra-specific variation in tolerance to edaphic conditions has been observed in closely related yet reproductively isolated taxa, suggesting that edaphic factors may have set the stage for the diversification of this genus. *Lasthenia* provides numerous opportunities to examine the link between adaptation, reproductive isolation and speciation.

Key Words: adaptive divergence; California flora; edaphic races; *Lasthenia*; salinity tolerance; serpentine.

According to Lindley and Moore (1876), “they (*Lasthenia*) grow in wet places, and appear to be uninteresting weeds.” They do “grow in wet places,” but studies of *Lasthenia* (Heliantheae: Asteraceae) over the last several decades have shown the genus to consist of anything but “uninteresting weeds.” Commonly known as goldfields, plants of *Lasthenia* occupy large areas of the Californian landscape, casting spectacular carpets of brightly-colored golden yellow flowers in early spring. A recent molecular phylogenetic study recognized 21 species and subspecies belonging to seven sections (Chan et al. 2001). All but one species are endemic to the Californian Floristic Province. *Lasthenia kunthii* (Less.) Hook & Arn., the only member of the genus found outside western North America, is endemic to vernal pools and wetlands in central Chile (Ornduff 1966). Members of the genus have wide edaphic tolerance and 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). Members of *L. californica sensu* Ornduff (Ornduff 1993) have the widest edaphic tolerance within the genus, with populations spanning all but guano habitats. Keck (1959) stated that *L. californica sensu* Ornduff [then *Baeria chrysostoma* (Fischer & C. Meyer) E. Greene] was the most abundant composite in California. Other *Lasthenia* taxa have rather restricted distributions, with seven taxa now listed in California Native Plant Society’s “Inventory of Rare and Endangered Plants of California” (CNPS 2001).

Following Ornduff’s extensive monograph (1966), *Lasthenia* has received considerable attention. Early studies examined inter- and intra-specific variation in flavonoid chemistry (Saleh and Bohm 1971; Ornduff et al. 1973, 1974; Bohm et al.

1989; Desrochers and Bohm 1993) to assess biosystematic relationships and determine trends of biochemical evolution in the genus. Electrophoretic work followed to establish evolutionary relationships among closely related taxa within the genus (Crawford et al. 1985; Crawford and Ornduff 1989) as well as within the highly variable *L. californica sensu* Ornduff (Desrochers and Bohm 1993). More recently, comprehensive phylogenetic studies (Chan et al. 2001, 2002; Desrochers and Dodge 2003) have contributed to our understanding of patterns of divergence in *Lasthenia*. The study by Chan et al. (2001) agrees with observations made earlier (Ornduff 1966, 1976) that divergence patterns in the genus conform to expectations of catastrophic selection and saltational diversification (Lewis 1962).

Existing knowledge suggests that *Lasthenia* can provide a model system for studies in evolutionary ecology, specifically, in understanding the role of edaphic factors in differentiation. Species of *Lasthenia* have successfully colonized diverse habitats within California, including those that exclude the vast majority of species (Ornduff 1966, 1993; Kingsbury et al. 1976; Vasey 1985; Rajakaruna and Bohm 1999; Noe and Zedler 2000; Parsons and Whelchel 2000). Further, in some sections (e.g., sect. *Hologymne* [Bartling] A. Gray and sect. *Ornduffia* R. Chan) all species occupy the same edaphic habitat yet generally do not grow intermixed (Ornduff 1966), while in others (e.g., sect. *Ptilomeris* [Nutt.] Ornduff), species occupy contrasting edaphic habitats (Crawford et al. 1985; Vasey 1985). Thus it is plausible to hypothesize that edaphic factors may have played an important role in the diversification of the genus. In this paper, taxa in several sections are discussed with emphasis on the

possible role of edaphic factors in speciation. Since many endemic plants in western North America are edaphic (Kruckeberg 1969), the genus such as *Lasthenia* provides insights into speciation in edaphically diverse genera.

Lasthenia sect. *Amphibia*
L. californica

Lasthenia sect. *Amphibia*
as *L. sect. Baeria* (Fischer) Ornduff recently revised (Chan 2001). Species, *L. californica* DC. ex Ornduff, *L. californica*, subsp. *macrantha* Ornduff, and subsp. *bakeri* (J. T. Howell) Ornduff, *L. ornithoglossa* (DC.) Greene, *L. ornithoglossa* (DC.) Ornduff, *L. gracilis* were previously considered as *L. californica sensu* Ornduff (1993) found within *L. californica sensu* Ornduff (1993). *L. californica* subsp. *californica* has been the subject of intensive taxonomic studies (Rajakaruna et al. 2002; Desrochers and Bohm 1999).

A biosystematic study (Desrochers and Bohm 1995) first suggested the existence of geographical races within *L. californica* based on flavonoid pigmentation. Desrochers and Bohm (1995) described patterns, and cypselae characters. An ecological study (Rajakaruna et al. 2002) suggested that the two races (Desrochers and Bohm 1995) occur in habitats that can be classified on the basis of soil and water availability. Races occur in habitats subject to ionic stress, and in these environments are associated with high clay content is generally low water holding capacity of soils. Races are restricted to moist or even saline environments. In contrast to soils in ionically “benign” environments, soils are often sandy, rocky, and occur early in the growing season at Jasper Ridge Biological Preserve (Stanford University, San Mateo Co., CA). Races in parapatry, mirror the trends of the taxa, with the two races occupying microhabitats: Race A occupies ionically harsh soils at the Jasper Ridge, while race C occupies ionically less stressful upland sites (Desrochers and Bohm 1999). At Jasper Ridge, race C plants belong to *L. californica sensu* Ornduff, while race A plants belong to *L. californica sensu* Ornduff. Races do not always correspond to the taxonomic units recognized by Chan et al. (2001). This study shows that one or both races occur in parallel in both *L. californica sensu* Ornduff and *L. californica sensu* Ornduff.

possible role of edaphic factors in their diversification. Since many endemic species of flowering plants in western North America are edaphic endemics (Kruckeberg 1969, 1986), studies of a model genus such as *Lasthenia* could provide general insights into speciation in a variety of other such edaphically diverse genera.

Lasthenia sect. *Amphiachaenia* (DC.) R. Chan:
L. californica sensu Ornduff

Lasthenia sect. *Amphiachaenia*, formerly known as *L. sect. Baeria* (Fisch. & Mey.) Ornduff, was recently revised (Chan 2001) to include four species, *L. californica* DC. ex Lindl. [consists of subsp. *californica*, subsp. *macrantha* (A. Gray) R. Chan, and subsp. *bakeri* (J. T. Howell) R. Chan], *L. gracilis* (DC.) Greene, *L. ornduffii* R. Chan, and *L. leptalea* (A. Gray) Ornduff. *Lasthenia californica* and *L. gracilis* were previously recognized as *L. californica sensu* Ornduff (1993). The two cryptic taxa found within *L. californica sensu* Ornduff, *L. californica* subsp. *californica* and *L. gracilis*, have been the subject of intense ecological and evolutionary studies (Rajakaruna and Bohm 1999; Chan et al. 2002; Desrochers and Dodge 2003).

A biosystematic study (Desrochers and Bohm 1995) first suggested the existence of two geographical races within *L. californica sensu* Ornduff based on flavonoid pigments (Bohm et al. 1989; Desrochers and Bohm 1993), allozyme banding patterns, and cypselas characteristics. A detailed ecological study (Rajakaruna and Bohm 1999) suggested that the two races described by Desrochers and Bohm (1995) occur in distinct sets of habitats that can be classified on the basis of ionic stresses and water availability. Race A plants predominate in habitats subject to ionic stress. Although the soils in these environments are ionically harsh, the percent clay content is generally high, increasing the water holding capacity of the soil. Plants are often restricted to moist or even saturated soils in such environments. In contrast, race C plants are found in ionically "benign" inland environments. The soils are often sandy, rocky, and shallow, drying out early in the growing season. The conditions at Jasper Ridge Biological Preserve (Stanford University, San Mateo Co., CA), where the races occur in parapatry, mirror the trends seen across the range of the taxa, with the two races occupying distinct microhabitats: Race A occupying the wet, yet ionically harsh soils at the bottom of the serpentine ridge, while race C occupies the fast-drying, yet ionically less stressful upper reaches (Rajakaruna and Bohm 1999). At Jasper Ridge, race A plants belong to *L. californica* subsp. *californica* while race C plants belong to *L. gracilis*. However, the races do not always correspond to the two taxa recognized by Chan et al. (2001, 2002). A recent study shows that one or both edaphic races have evolved in parallel in both *L. californica* subsp. *californica*

and *L. gracilis*, suggesting that racial features may have evolved secondarily in response to edaphic factors (Rajakaruna et al. in press a).

Recent eco-physiological studies (Rajakaruna et al. 2003) indicate that race A plants from both *L. californica* subsp. *californica* and *L. gracilis* are clearly more tolerant of potentially-toxic Na^+ , suggesting that physiological traits responsible for Na^+ uptake and sequestration may have evolved independently in populations belonging to the two closely related taxa. Schat et al. (1996) provide one of the best examples of parallel genotypic changes in tolerance to an edaphic extreme within a plant species. Their studies have shown that loci conferring copper tolerance have evolved independently in geographically isolated populations of *Silene vulgaris* Garcke (Caryophyllaceae). In *Plantago* (Plantaginaceae), the vacuolar Na^+/H^- antiporter activity, thought to confer tolerance to Na^+ (Apse et al. 1999) is only present in the salt-tolerant *P. maritima* L. but not in the glycophytic *P. media* L. (Staal et al. 1991). This difference is thought to be crucial in the ecological divergence of these two species. Sodium is clearly an important element in many of the habitats where race A is found. Whether the presence/absence or level of expression of the Na^+/H^+ antiporter gene is responsible for the differences in uptake and accumulation in race A plants is an area worthy of investigation. Ion uptake studies conducted on the two races from Jasper Ridge also indicate that race A is more tolerant of high external Mg^{2+} and low $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratios (Rajakaruna et al. 2003). Both of these traits have adaptive significance in magnesium-rich serpentine and coastal habitats where race A is found. The traits that have been studied (Na^+ , Mg^{2+} , $\text{Ca}^{2+}/\text{Mg}^{2+}$) are common features in most habitats where race A is found, thus, it is tempting to hypothesize that differential response to edaphic stresses may have played an important role in the racial divergence of *L. californica sensu* Ornduff. By extending eco-physiological studies to characterize the physiology of hybrids between the two species (and races), it is possible to appreciate better the role of edaphic factors in diversification.

The findings from ecophysiological studies (Rajakaruna et al. 2003) suggest a correlation between flavonoid differences, edaphic features, and ion accumulation, suggesting a previously unknown adaptive role for flavonoid differences. The primary feature that distinguishes the edaphic races in *L. californica* subsp. *californica* and *L. gracilis* 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 and Bohm 1993) not found in race C plants. Ecological roles for flavonoid pigments have often been postulated (Bohm 1987), and the case for a correlation with habitat and sulfated flavonoids has been brought forward (Harborne 1975). A large number

of taxa found in habitats with water logged and saline conditions contain sulfated flavonoids (Harborne 1975; Barron et al. 1988). In *L. californica* subsp. *californica* and *L. gracilis*, sulfated flavonoids occur only in Na⁺-accumulating plants that predominate in ionically-stressed environments. It is possible that sulfation of flavonoids may be beneficial in sulfate-rich environments where race A occurs. Thus, Rajakaruna et al. (2003) hypothesized an edaphically-linked ecological role for the flavonoid differences that first suggested the existence of these two races (Bohm et al. 1974, 1989).

A study on the response to water stress suggests that the two races from Jasper Ridge show different life history strategies in response to water stress (Rajakaruna et al. in press b). Race C plants (*L. gracilis*) adopt a drought avoidance strategy often referred to as phenological escape (Fox 1990; Aronson et al. 1992) and have significantly greater relative fitness than race A plants under increasing water stress. In contrast, race A plants (*L. californica* subsp. *californica*) adopt a slow-growing strategy and allocate more biomass to root than shoot. This strategy is commonly seen in herbaceous annuals growing under edaphic stress, specifically under water and nutrient stresses (Kramer 1980; Grime 1994). Since the study was conducted in potting soils, to seek effects of water stress in isolation, it is unclear how the findings relate to conditions experienced in the field. A large-scale experiment using natural soils is required to clarify further the relationship between ionic strength, water availability and fitness within environments, and to further explore factors that affect the distribution of these races at Jasper Ridge and across the species' range. Given our understanding of the environmental variables associated with differences in fitness, it is now possible to conduct detailed analysis of selection gradients (Wade and Kalisz 1990; Dudley 1996) to infer the role of natural selection in achieving fitness differences under field conditions.

Previous studies (Desrochers 1992) suggested low crossability as well as reduced gene flow between races (Desrochers and Bohm 1995) of *L. californica sensu* Ornduff. A recent study using seven populations of *L. californica* subsp. *californica* and *L. gracilis* show reduced crossability between the two closely related cryptic taxa (Rajakaruna and Whitton unpublished). Examination of intra- and inter-racial seed set as an effect nested within species suggests that the edaphic races are also reproductively isolated. By comparing patterns of seed set with observations of pollen tube growth, it is clear that most of the reduction in seed set is due to post-pollination, prezygotic effects. Interestingly, there is evidence for enhanced prezygotic isolation in the parapatric location at Jasper Ridge. This pattern suggests a possible role for reinforcement in the observed isolation. Flowering times have diverged in the parapatric location with *L. gracilis* plants always flowering 7–10 days prior to *L. cal-*

ifornica subsp. *californica* plants; the pattern is maintained in the greenhouse. Germination tests also revealed evidence for reduced hybrid fitness. Strong ecological selection at the site (Rajakaruna and Bohm 1999) may severely limit introgression. The fate of any hybrids produced is unknown. A transplant study of hybrids in parental habitats would shed light on possible post-zygotic mechanisms of isolation. Further studies are needed to clarify the roles of ecological selection versus reinforcement on the patterns of reproductive isolation observed between races and the two cryptic plant taxa.

An understanding of the relationship between traits for adaptation and reproductive isolation is critical to further the hypothesis of edaphic differentiation in *L. californica* subsp. *californica* and *L. gracilis*. Studies suggest that reproductive isolation can be achieved as a by-product of a physiological adaptation to unusual soil conditions (Macnair and Gardner 1998). Macnair and colleagues have shown that the linkage block associated with copper tolerance in *Mimulus guttatus* Fischer ex DC. (Scrophulariaceae) also produces hybrid inviability; however, it is unclear if inviability is achieved via pleiotropy or hitchhiking. Nevertheless, their work has clearly documented that natural selection for an adaptive trait (copper tolerance) has caused a gene for post-zygotic isolation to spread through the population (Macnair and Christie 1983; Christie and Macnair 1987). Whether such relationships exist between edaphic tolerance and observed reproductive isolation in *L. californica sensu* Ornduff is worthy of investigation.

Lasthenia sect. *Ptilomeris* (Nutt.) Ornduff:
L. minor and *L. maritima*

Lasthenia minor (DC.) Ornduff occurs in a variety of habitats such as alkali flats, coastal bluffs, sand dunes, pond margins, and disturbed sites while *L. maritima* (A. Gray) M. Vasey is restricted almost exclusively to islands and offshore rocks harboring seabird nesting and roosting sites (Ornduff 1965, 1966; Vasey 1985). The soils on these sites are high in nitrogen, low in pH, and highly disturbed from the activities of the birds (Vasey 1985). In addition, constant wind and salt spray produce an outright hostile environment.

Ornduff (1966) considered the self-compatible *L. maritima* to be a recent descendent from the self-incompatible *L. minor*. An electrophoretic study (Crawford et al. 1985) further supported this hypothesis and suggested that speciation probably involved a switch to self-compatibility, development of autogamy, and subsequent divergence driven by edaphic factors. Given that variation for tolerance is first required to colonize the extreme guano habitats, it is likely that self-compatibility arose post-colonization. Whether the switch to self-compatibility arose as a by-product of an adaptation to gua-

no or is directly linked to tation is not known.

A recent phylogenetic study showed a close relationship between the two species, but it did not conclusively support ancestor-descendent relationship between the two taxa. The high fertility of artichokes (Ornduff 1966). However, Vasey (1985) found only truly sympatric site km. Only a few plants appear to be the result of a recombination of parapatric populations at three localities failed to show evidence of natural interspecific hybridization. It is possible that strong ecological selection is responsible for limiting introgression.

The physiological basis for reproductive isolation in *L. maritima* is not known. Studies by Vasey (1985) show that the tolerant of guano-modified soils is likely that extensive screening of individuals among populations is critical to our understanding of the origin of *L. maritima*. Limited studies of *L. maritima* accumulate nitrate (Ornduff 1965), and Vasey (1985) found nitrate content in the cells of *L. maritima* to grow in these sites. Nitrate uptake has been studied (Tischner 2000), and it is unclear in the laboratory whether physiological differences exist between the two species. Physiological differences between the two species such as tolerance to excess salt, high osmotic stress, also have not been examined. Further work to be done to assess the role of guano soils and the L. maritima complex provides an ideal opportunity to study trait differences are established between the two species and their adaptive evolution. It will be possible to examine whether traits contributing to reproductive isolation in soils also contribute to isolation between the two species or reduced hybrid fitness.

Lasthenia sect. *Hologon*
L. chrysantha, *L. glabrata*

All three species in this section are found in habitats and form vigorous populations. The origin of the sterile artificial hybrids (Ornduff 1966) was suspected in the laboratory. Ornduff, putatively from *L. glabrata* Lindl. subsp. *concolor* (Gray) Ornduff and *L. chrysantha* (Green) Ornduff (Ornduff 1966). The study of these hybrids show minimal molecular variation in this section and is uninformative for the hybrid origin of *L. ferrisia*.

no or is directly linked to a gene conferring adaptation is not known.

A recent phylogenetic study (Chan et al. 2001) showed a close relationship between the two species, but it did not conclusively support the proposed ancestor-descendent relationship. The close relationship between the two species is reflected by the high fertility of artificial crosses (Ornduff 1966). However, Vasey (1985) reports that at the only truly sympatric site known for the two species, only a few plants appear to be intermediates. Examination of parapatric populations of the two species at three localities failed to reveal any indication of natural interspecific hybridization (Vasey 1985). It is possible that strong ecological selection is responsible for limiting introgression.

The physiological basis for the substrate tolerance in *L. maritima* is not known. Preliminary studies by Vasey (1985) show that *L. minor* is not tolerant of guano-modified soils. However, it is possible that extensive screening may reveal tolerant individuals among populations of this species. Such studies are critical to our understanding of the origin of *L. maritima*. Limited work suggests that *L. maritima* accumulates nitrates in its foliage (Ornduff 1965), and Vasey (1985) suggests that high nitrate content in the cells may have allowed *L. maritima* to grow in these osmotically-challenged sites. Nitrate uptake has been well characterized (Tischner 2000), and it is feasible to determine in the laboratory whether physiological differences exist between the two species in this regard. Other physiological differences between the two species, such as tolerance to excess cations such as Na⁺ and high osmotic stress, also common to these sites, have not been examined. Clearly, there is much work to be done to assess mechanisms of tolerance to guano soils and the *L. maritima*-*L. minor* complex provides an ideal opportunity to do so. Once trait differences are established between the two species and their adaptive significance determined, it will be possible to examine their genetic basis. Whether traits contributing to adaptation to guano soils also contribute to isolation (i.e., self-compatibility or reduced hybrid fitness) can then be examined.

Lasthenia sect. *Hologymne* (Bartling) A. Gray:
L. chrysantha, *L. glabrata*, and *L. ferrisiae*

All three species in this section occupy saline habitats and form vigorous, moderate to highly fertile artificial hybrids (Ornduff 1966). Hybrid speciation was suspected in the origin of *L. ferrisiae* Ornduff, putatively from hybridization between *L. glabrata* Lindl. subsp. *coulteri* (A. Gray) Ornduff and *L. chrysantha* (Greene ex A. Gray) Greene (Ornduff 1966). The study by Chan et al. (2001) show minimal molecular variation for species within this section and is uninformative on the possible hybrid origin of *L. ferrisiae*. An interesting obser-

vation made by Ornduff (1966) is that these three closely related species, with edaphic requirements that appear to be identical, are never sympatric. A pure population of one of these species may exist within a few meters from a pure population of another. Ornduff (1966) attributes this pattern to occupation of a site on a "first come, first served" basis, yet admits that a detailed ecological study probably would reveal factors that are different among such sites. It is likely that the three taxa differ in their tolerance regime to edaphic features associated with salinity. The species-trio provides yet another setting to explore the relationships among adaptation, reproductive isolation, and diversification.

Lasthenia sect. *Ornduffia* R. Chan:
L. fremontii, *L. conjugens*, and *L. burkei*

All three species in this section are inter-crossable (Ornduff 1966, 1969). The ranges for *L. conjugens* Greene and *L. fremontii* (Torr. ex A. Gray) Greene marginally overlap, however, natural hybrids between the species are very rare (Ornduff 1969). Artificial hybrids between *L. conjugens* and *L. fremontii* show remarkable similarity to *L. burkei* (Greene) Greene, leading Ornduff (1966) to suggest a hybrid origin for this taxon. *Lasthenia burkei* occurs in vernal pool habitats similar to those occupied by its two relatives, but its geographical range is distinct. Many research tools have been utilized (Saleh et al. 1971; Crawford and Ornduff 1989; Chan et al. 2001) to resolve relationships within this section but have not been able to support conclusively Ornduff's hypothesis for the hybrid origin of *L. burkei*.

Although all three species colonize vernal pools, their distributional pattern within a pool seems to be related to water level, soil moisture, and salinity (Ornduff 1966). Ornduff (1966) claimed a similar situation to that described for the three halophytes in sect. *Hologymne*, where the species are rarely sympatric although they may be occupying sites just a few meters apart. Again, specific edaphic tolerances may exist among these vernal pool taxa and the sites they are restricted to may in fact show micro-scale differences in edaphic features. Only a close examination of these sites, such as our detailed study of the serpentine outcrop at Jasper Ridge (Rajakaruna and Bohm 1999), will reveal patterns of soil heterogeneity in these apparently uniform edaphic habitats. In a study conducted in an artificial vernal pool created at Berkeley, Ornduff (1966) demonstrated that *L. conjugens* was always restricted to the soil immediately above and below the water level while *L. fremontii* occurred from water level to the upper limit of soil moisture (Ornduff 1966). *Lasthenia conjugens* appeared to be the more water-dependent of the two species and may be occupying deeper vernal pools that dry out later in the growing season. Though physiological

