

Evolutionary changes in correlations among functional traits in *Ceanothus* in response to Mediterranean conditions

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Ceanothus is a widely speciated genus with two sections that differ markedly in size, patterns of biomass allocation and reproductive strategies. On greenhouse-grown seedlings we tested whether divergence occurred in single traits or in suites of traits, and whether taxonomic affinity or the ability to fix nitrogen (N) influenced the relationships among functional traits. Species of the subgenus *Cenastes* differed from species in the subgenus *Ceanothus* in the following characteristics: reproduction by seed rather than primarily resprouting, high rates of photosynthesis and high stomatal conductance, thick leaves, low root allocation, and high leaf allocation. Correlations of traits across the entire genus showed positive correlations among traits that maximize photosynthesis and tradeoffs between root allocation and carbon gain patterns frequently observed in other broad taxonomic comparisons. Trait correlations differed between the two subgenera (divergence in allocation-photosynthesis tradeoffs in *Ceanothus* and divergence of growth-related traits in *Cenastes*). Similarly, N-fixers, which were distributed broadly between the two subgenera, differed in trait correlations (primarily among traits related to photosynthesis) from non-fixers (primarily among traits related to growth and allocation). These results indicate that 1) divergence in genus *Ceanothus* was associated with changes in entire suite of traits, rather than independent changes in individual traits and 2) evolution occurring under different environmental or nutritional circumstances alters the suites of traits exhibited by plants.

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Plant species characteristic of infertile environments reveal consistent correlations among traits such as low growth rate, high root-to-shoot ratio, high concentrations of secondary metabolites, and low nutrient absorption rate which are a response to low availability of resources (Chapin 1980, Aerts and Chapin 2000). This combina-

tion of attributes represents a strategy to cope with high levels of stress and enables plants to successfully colonize infertile environments (Grime 1977, Westoby et al. 1992). The set of trait attributes is sufficiently consistent among different low-resource environments that it constitutes what could be termed a stress resistance syndrome (SRS,

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Chapin et al. 1993). Environmental pressures have presumably selected for this specific profile of attributes in response to stressful environments.

The presence of a similar suite of trait attributes in different species irrespective of evolutionary lineage supports the idea that the SRS evolved in a synchronous manner rather than by parallel selection for each individual trait. Evolutionary changes leading to stress tolerance in plants might occur through only a few mutations in genes with large pleiotropic effects rather than by accumulation of many small mutational events (Chapin et al. 1993).

However, this hypothesis has not been rigorously tested by statistical analysis of the relationships among traits in plant groups whose evolutionary relationships are known.

Different species might have traits in common either because they are linked by a network of shared ancestry or because the traits are adaptations to common environments (Lechowicz 1984, Herrera 1992). Therefore, when analyzing species traits to look for selective pressures or ecological trends, it is important to determine the phylogenetic relationships among species and the extent to which changes in one set of characters is associated with evolutionary changes in another set (Huey and Bennet 1987).

Although there are consistent broad trends in suites of traits, the broad diversity of plant types indicates some flexibility in these associations among traits. What types of changes in trait associations occur through evolution, and how are these influenced by phylogenetic constraints, physical environment, and physiological specializations such as nitrogen (N) fixation? In this paper we report an experiment with ten species of the genus *Ceanothus*, whose phylogeny is relatively well documented (Hardig 1997, Hardig et al. 2000), in which we compared two subgenera, one of which evolved more recently perhaps in response to drier and seasonally cooler environments. We compared correlations among functional traits between these subgenera and between species whose nutritional status was greatly altered by the presence or absence of N fixation capability.

Ceanothus is a highly diversified genus that is widespread in California. It has two well differentiated sections, the subgenera *Ceanothus* and *Cerastes*, both of which have species present in a wide range of environments, from coastal scrub to dry chaparral habitats to sub alpine elevations in Sierra Nevada. Species differ widely in size, allocation patterns and reproductive strategies, which are conditioned by disturbance regime. Adult individuals of *Cerastes* species are killed by fire, but produce a number of large seeds whose germination is triggered by fire. In contrast, most species in the subgenus *Ceanothus* resprout from the root crown when the aerial part is removed by fire or grazing and produce fewer, smaller seeds. Most species of the genus *Ceanothus* now present in California appeared after an Eocene climatic change (Mason 1942), when a drier, more seasonal climate favored the recurrence of fires. Phylogenetic analyses using chloroplast DNA suggest that the two subgenera diverged 18–39 million years ago, whereas

species within each subgenus diverged more recently (Jeong et al. 1997). Subgenus *Ceanothus* species share a large number of traits with other Rhamnaceae species, particularly regarding leaf traits and rooting depth, and can be considered ancestral, while *Cerastes* species with smaller, thicker leaves and shallow root systems differ from other Rhamnaceae, are considered to have evolved later and are better suited to thrive under a Mediterranean climate (Ackerly 2004b).

We tested whether the two sections of the genus *Ceanothus* diverged in functional traits; whether suites of traits diverged as a group or individually; and finally, whether the ability to fix N affected the assemblage of traits. We expected that in response to new, drier conditions evolution would enhance traits related to stress tolerance, such as small leaves, while the higher frequency of fires in mediterranean-type climate would affect recruitment traits, enhancing the seeder habit.

Study species

Ten species of the genus *Ceanothus* were selected, including five species belonging to the subgenus *Ceanothus* (*C. arboreus*, *C. integerrimus*, *C. sorediatus*, *C. spinosus* and *C. thyrsoiflorus*) and five to subgenus *Cerastes* (*C. cuneatus*, *C. crassifolius*, *C. gloriosus*, *C. megacarpus* and *C. verrucosus*). They included six known N fixers (Becking 1977) and four non-fixers. Nine species were evergreen and one deciduous (*C. integerrimus*). Most species are well represented in coastal ranges and canyon slopes, as well as in inland chaparral (MacMinn 1942, Munz and Keck 1978). *Ceanothus integerrimus*, the deciduous species, is more restricted to the mountain slopes, where it is often found in the understory of conifers, but it also occurs in chaparral (Conard et al 1985). Other species, such as *C. thyrsoiflorus* are also found in the understory of conifers. The selected species differ in size, ranging from the prostrate *C. gloriosus* (0.3 m height) to *C. arboreus* (4–8 m) and in their response to fire. All *Cerastes* species are obligate seeders, while most species in subgenus *Ceanothus* resprout from the root crown following fire (MacMinn 1942). Among the species we studied, *C. sorediatus*, in this subgenus, does not resprout after fire in the central and southern coast ranges but has been reported to stump-sprout in the northern coast ranges (D. Schwilk pers. comm.). Species were considered as N fixers or non-fixers following Bond and Wheeler (1980).

Methods

Seeds, provided by the Rancho Santa Ana Botanical Garden, were placed in boiling water and allowed to cool at room temperature, because most of the *Ceanothus* species need fire to stimulate germination (Quick and Quick 1961). Seeds were then placed in a growth chamber with 25/20°C day/night temperature on wet sand in petri

dishes. Germination occurred between 14 and 21 d. Seedlings of *C. cuneatus* and *C. integerrimus* were taken from the field, as seeds were unavailable. In both cases, young seedlings with cotyledons attached (6–7 cm tall) were collected from silty road slopes 30 to 40 km east of Sacramento, California and cleaned of soil particles. Seedlings of all species were planted in 30 cm plastic containers in a vermiculite-perlite (1:1) medium and received half-strength Hoagland's solution with a mixture of NO_3 and NH_4 as source of N (Hewitt 1966) once a week and deionized water every two days. Seedlings were grown in a greenhouse in Berkeley, California at $26\pm 3^\circ\text{C}$ and natural irradiance and daylength. Two weeks after planting, plants were inoculated with ground radical nodules of *C. cuneatus* taken in the field and diluted in water. At the time of planting in mid May, root and shoot dry weights of 5–10 seedlings of each species were recorded. One month later, another group of 5–10 plants per species was randomly chosen for harvest, and a final harvest was made after 26 weeks. Harvested plants were oven-dried at 65°C for at least 48 h before the dry mass of roots, stems, and leaves was recorded; before drying, total leaf area was measured with a LI-COR 3000 area meter. One week before the final harvest, photosynthetic rate (A) was measured in attached leaves of 3–6 individuals using a LI-COR 6200 portable photosynthesis system with a 0.25 L chamber at ~ 380 ppm CO_2 , and 27°C . Ambient light was supplemented with 1000 W metal-halide lamps and photon flux density averaged $1275 \mu\text{mol m}^{-2} \text{s}^{-1}$. Ground leaf samples were analyzed for N, C and ^{13}C using a Europa Scientific Roboprep-CN elemental analyzer and mass spectrometer. $\delta^{13}\text{C}$ was calculated following Ehleringer and Osmond (1989). Maximum relative growth rate (RGR_{max}) was calculated by linear regression of the natural log of plant mass at each harvest against time over a standard weight change (0.1–0.2 g) because RGR depends on size (Cook and Evans 1983), and its variance was estimated using Tukey's jackknife procedure (Sokal and Rohlf 1981). Net assimilation rate (NAR) was calculated from RGR_{max} and leaf mass ratio (Hunt 1982). Nitrogen use efficiency (NUE) was calculated as the amount of dry matter produced per unit of N, combining root and shoot mass and tissue N concentration. Specific leaf area (SLA) was calculated from total leaf area and mass, and N and C pool sizes in leaves (g m^{-2}) were calculated per unit leaf area. Photosynthetic N use efficiency (PNUE) was obtained by dividing photosynthetic rate by the N pool in the leaf. For comparative purposes only, water use efficiency (WUE) was obtained dividing photosynthetic rate by within-chamber transpiration rate.

Statistical analysis

Differences between the two subgenera of *Ceanothus* were determined by ANOVA, using the GLM procedure and

Tukey's multiple comparison test of SYSTAT 5.2.1. The mean value of each variable for each species was used in statistical analysis, after log transformation when needed to meet normality criteria. Principal component analysis (PCA) based on correlation matrices was first performed including mean values for all ten species, and axes were rotated using the EQUAMAX procedure of SYSTAT; PCA was later performed with *Cerastes* and subgenus *Ceanothus* species separately.

Results

Subgenera differentiation

The major functional differences between the two sections of *Ceanothus* were related to photosynthesis (SLA, A, and g) and allocation (R:S, leaf:stem) (Table 1). The high SLA (high area per unit mass) of subgenus *Ceanothus* species resulted in a low area-based photosynthetic rate (A) and therefore low leaf conductance to water vapor (g) compared to *Cerastes*. These correlations among traits are commonly seen in other species comparisons (Lambers and Poorter 1992, Reich et al. 1992, Poorter and Evans 1998, Niinemets 1999). Seeds of *Cerastes* species (all seeders) tended to be larger than those of crown-resprouting subgenus *Ceanothus* species. Seed mass correlated with adult plant height, so that species reported as trees (>4 m tall) had larger seeds than shrubs (14.4 vs 8.3 mg). At harvest, *Cerastes* and subgenus *Ceanothus* species had similar plant mass (Table 1) because, as plants grow older, interspecific differences in RGR override and compensate for differences in seed mass (Fig. 1; Westoby et al. 1992). Therefore, the correlation was stronger between seed mass and 12-d-old seedlings ($p < 0.00012$) than between seed mass and final plant size ($p = 0.0007$). Maximum relative growth rate (RGR_{max}) did not differ between the two sections of *Ceanothus*. RGR_{max} increased with increasing seed mass in *Cerastes* (the postfire seeders) but showed no relationship to seed mass in subgenus *Ceanothus* (Fig. 1). A high allocation to roots and low allocation to leaves in subgenus *Ceanothus* species contributed to the low NAR (whole plant carbon gain per leaf area) in this section.

The correlation among functional traits differed between the genus *Ceanothus* as a whole and the individual sections (Fig. 2), indicating that the linkages among traits depended on the group of species being compared. The twelve significant correlations across the genus *Ceanothus* showed relationships among traits related to photosynthesis (SLA, A, g, PNUE) and trade-offs in allocation patterns and carbon gain (R:S, leaf:stem, NAR). The five significant correlations in the subgenus *Ceanothus* section related allocation (R:S, leaf:stem) to nitrogen (N, NUE) and photosynthesis (A), whereas the seven significant correlations in the *Cerastes* section related RGR to seed mass, seedling mass, and WUE, and related A to g. Divergence among

Table 1. Seed and seedling mass, root-to-shoot ratio (R:S), leaf-to-stem ratio, maximum relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA), photosynthetic rate (A), leaf conductance to water vapor (g), water use efficiency (WUE), leaf nitrogen (N), photosynthetic N use efficiency (PNUE) and N use efficiency (NUE) of ten species belonging to the *Ceanothus* and *Cerastes* subgenera of *Ceanothus*. Ecological groups are indicated as sprouters (Sp), seeders (Se), N fixers (NF) and non fixers (nNF). Significant differences between subgenera means given by * $p < 0.05$ and ** $p < 0.01$; ns not significant.

	<i>C. arboreus</i>	<i>C. integriramus</i>	<i>C. sordidatus</i>	<i>C. spinosus</i>	<i>C. thyrsiflorus</i>	Average subgenus <i>Ceanothus</i>
Ecological group	Sp, nNF	Sp, NF	Sp/Se, NF	Sp, nNF	Sp, NF	
Sample size (N)	3	9	4	4	14	5
Seed mass (mg)	12.3	4.8	6.3	8.6	3.0	7.0±1.6
Final plant mass (g)	0.97±0.20	1.2±0.1	0.7±0.2	1.1±0.1	1.3±0.1	1.1±0.1
R:S	1.4±0.1	1.6±0.2	1.3±0.6	0.7±0.2	1.1±0.2	1.2±0.2
Leaf:stem	3.2±0.2	1.4±1.1	1.1±0.1	3.1±0.2	2.3±0.2	2.2±0.4
RGR (mg g ⁻¹ d ⁻¹)	12.1±0.4	18.8±0.1	9.3±0.7	15.1±0.2	19.0±0.1	14.9±1.9
NAR (mg g ⁻¹ d ⁻¹)	38.4±1.3	85.2±6.3	40.8±9.1	34.0±3.9	58.5±5.9	51.4±9.4
SLA (cm ² g ⁻¹)	12.8±1.3	6.2±0.62	9.0±0.9	10.0±1.0	10.7±0.9	9.8±1.1
A (µmol m ² s ⁻¹)	11.6±2.2	18.1±1.5	18.9±5.7	9.8±1.2	10.8±0.9	13.8±1.9
g (mol m ² s ⁻¹)	0.11±0.02	0.09±0.03	0.24±0.11	0.09±0.02	0.11±0.01	0.13±0.03
WUE (mmol mol ⁻¹)	11.3±9	244±75	115±28	124±40	75±40	134±29
N (mg g ⁻¹)	22.2±1.1	29.1±0.6	24.4±1.7	18.0±1.4	21.1±0.8	23.0±1.9
PNUE (mmol g ⁻¹ s ⁻¹)	0.66±0.09	0.40±0.04	0.69±0.21	0.56±0.07	0.60±0.17	0.58±0.05
NUE (g g ⁻¹)	45.3±1.7	34.4±1.6	41.5±2.5	56.7±2.5	48.3±1.3	45.3±3.7

	Average subgenus <i>Cerastes</i>	<i>C. crassifolius</i>	<i>C. cuneatus</i>	<i>C. gloriosus</i>	<i>C. megacarpus</i>	<i>C. verrucosus</i>
Ecological group	Se, NF	Se, NF	Se, NF	Se, NF	Se, nNF	Se, nNF
Sample size (N)	9	5	5	5	10	5
Seed mass (mg)	14.5±5.2	17.9	9.2	6.4	33.3	5.5
Final plant mass (g)	1.0±0.2	1.1±0.1	0.9±0.2	0.4±0.0	1.8±0.1	0.7±0.1
R:S	0.7±0.1	0.6±0.1	0.8±0.1	0.6±0.1	0.7±0.1	1.0±0.2
Leaf:stem	3.1±0.3	3.2±0.2	2.5±0.3	4.0±0.5	2.8±0.6	2.9±0.6
RGR (mg g ⁻¹ d ⁻¹)	14.2±3.5	15.8±0.6	12.6±0.1	5.4±0.1	26.5±0.1	10.8±0.2
NAR (mg g ⁻¹ d ⁻¹)	34.1±8.6	33.1±0.9	32.9±2.2	10.7±0.8	64.0±2.2	29.7±2.6
SLA (cm ² g ⁻¹)	4.9±1.0	4.6±0.4	4.5±0.4	8.8±0.8	3.9±0.4	2.8±0.2
A (µmol m ² s ⁻¹)	23.7±2.1	23.1±1.3	24.3±5.0	21.5±1.1	18.4±1.3	31.2±1.0
g (mol m ² s ⁻¹)	0.31±0.06	0.23±0.01	0.33±0.09	0.31±0.03	0.17±0.04	0.52±0.16
WUE (mmol mol ⁻¹)	100±12	103±15	76±8	70±5	139±60	109±43
N (mg g ⁻¹)	22.9±1.6	19.0±0.6	26.0±0.8	27.0±1.4	19.5±0.3	23.0±0.3
PNUE (mmol g ⁻¹ s ⁻¹)	0.51±0.06	0.57±0.04	0.44±0.07	0.71±0.09	0.37±0.02	0.47±0.04
NUE (g g ⁻¹)	44.8±3.2	53.1±1.7	38.6±2.2	38.5±2.2	51.4±1.6	43.4±2.5

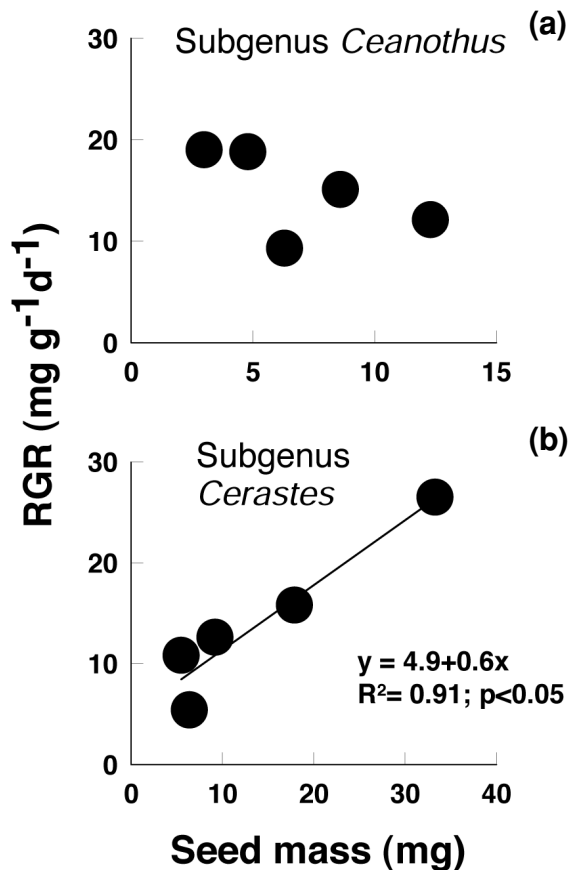


Fig. 1. Relationship between RGR_{max} and seed mass of subgenera *Ceanothus* (a) and *Cerastes* (b) species of *Ceanothus*.

subgenus *Ceanothus* species thus involved allocation-photosynthesis tradeoffs, whereas divergence within *Cerastes* involved growth-related traits.

We then used principal component analysis (PCA) to identify the suites of traits that accounted for functional variation in the genus *Ceanothus*. The first two PCA axes with all examined *Ceanothus* species accounted for 61% of the variance (Table 2). The first axis, which explained 32% of the variance, was related to allocation patterns and showed the same allocation trade-off that emerged from the correlation analysis. Species with large seeds allocated more biomass to aboveground parts and leaves, had low NAR because of low N concentration but were more efficient at using N. The second axis explained 30% of the variance and was related to final plant size and its positive correlation with RGR. The two sections of *Ceanothus* separated clearly in the basis of the PCA axes (Fig. 3), mainly because of differences allocation patterns (axis 1).

The first axis of a separate PCA performed on subgenus *Ceanothus* species only (Table 3) loaded on allocation patterns, N, and photosynthetic traits just as in the correlation analysis, explaining 52% of variance. Plants with high leaf

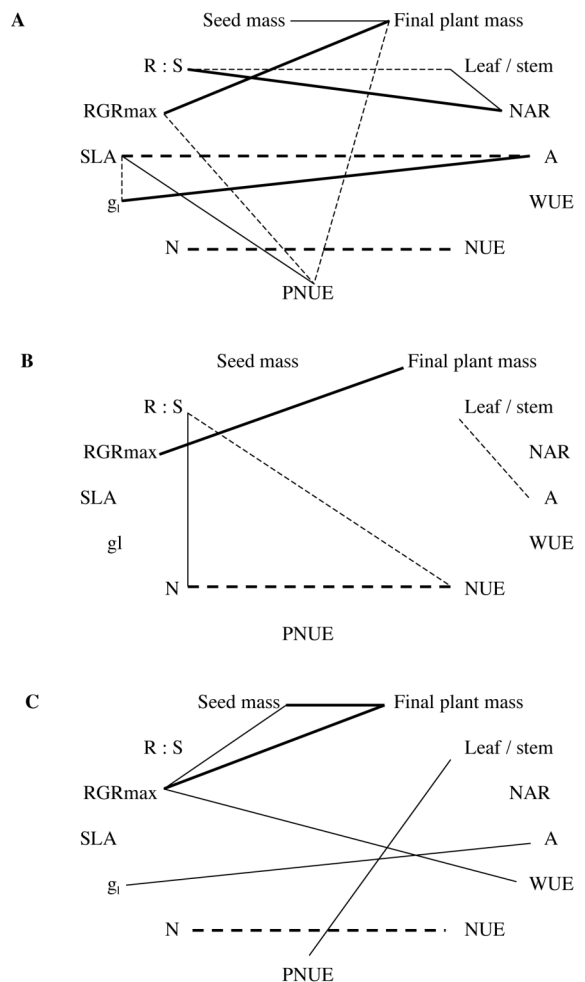


Fig. 2. Correlograms among morphological and physiological traits including all *Ceanothus* species (A), only subgenus *Ceanothus* (B) and only *Cerastes* species (C). Solid lines indicate significantly positive correlations and dashed lines negative correlations. Thick lines indicate $p < 0.01$ and thin lines $p < 0.05$.

N were less efficient at using it in photosynthesis ($r = -0.98$; $p = 0.02$), though they had a high assimilation rate and a high NAR. A high allocation to roots meant a higher leaf N ($r = 0.91$; $p = 0.03$), thicker leaves, and lower leaf-to-stem ratio. In the PCA including *Cerastes* species only, the first axis explained 52% of variance and loaded on size parameters, RGR, and use of N and water (Table 3) just as in the correlation analysis. Plants with large seeds produced large seedlings ($r = 0.97$; $p = 0.02$) that had high RGR ($r = 0.79$; $p = 0.04$), low leaf N and PNUE, and low WUE.

Thus the PCA analyses confirmed the general conclusions of the correlation analyses and identified the major suites of traits that differ between sections.

Table 2. Correlation of functional plant characters of *Ceanothus* species with PCA axes.

	Axis 1	Axis 2
Seed mass	0.717	0.478
Final plant mass	0.276	0.940
Root/shoot	-0.858	0.301
Leaves/stem	0.659	-0.451
RGR	0.281	0.925
NAR	-0.811	0.333
SLA	-0.378	-0.059
A	0.190	-0.456
G ₁	0.467	-0.539
WUE	-0.442	0.634
N	-0.712	-0.302
PNUE	-0.181	-0.604
NUE	0.688	0.343
% variance explained	31.6	29.6

Nitrogen-fixer species vs non-fixers

Seeds of non-N-fixing species tended to be larger than seeds of N-fixers, across all *Ceanothus* species (Table 4) and within sections, the differences being larger in *Cerastes* (19.4 mg in non-fixers vs 11.2 mg in N-fixers) than in subgenus *Ceanothus* (10.4 vs 4.7 mg). Most of the functional traits were similar in both groups, however, because of high inter-specific variability.

Covariance among traits differed depending on the N-fixing ability of *Ceanothus* species. N-fixing and non-fixing species shared 8 correlations among traits (Fig. 4). In addition, N-fixer species had 9 pairs of traits that showed significant correlations (many of which related to photosynthesis), whereas 16 were significant in non-fixing species (many related to RGR and allocation). Thus N-fixing ability altered the relationships among traits.

The distribution of N-fixing and non-fixing species in both sections suggests that this ability may have been gained or lost several times during the diversification of *Ceanothus*.

Discussion

Divergence in physiology and allocation patterns in the genus *Ceanothus* was associated with changes in the entire suite of traits rather than with independent changes in individual traits. At this broad taxonomic level, correlations among traits were similar to patterns described previously for entire floras or for functional types such as trees (Lambers and Poorter 1992, Reich et al. 1992, Poorter and Evans 1998, Niinemets 1999). However, at finer taxonomic levels, different relationships among traits emerged in response to different selective pressures (i.e. *Cerastes* in drier habitats). For example, there was a positive relationship among seed mass, final plant size, and RGR in *Cerastes* but not in subgenus *Ceanothus*. Across growth forms and species, seed size and RGR are inversely

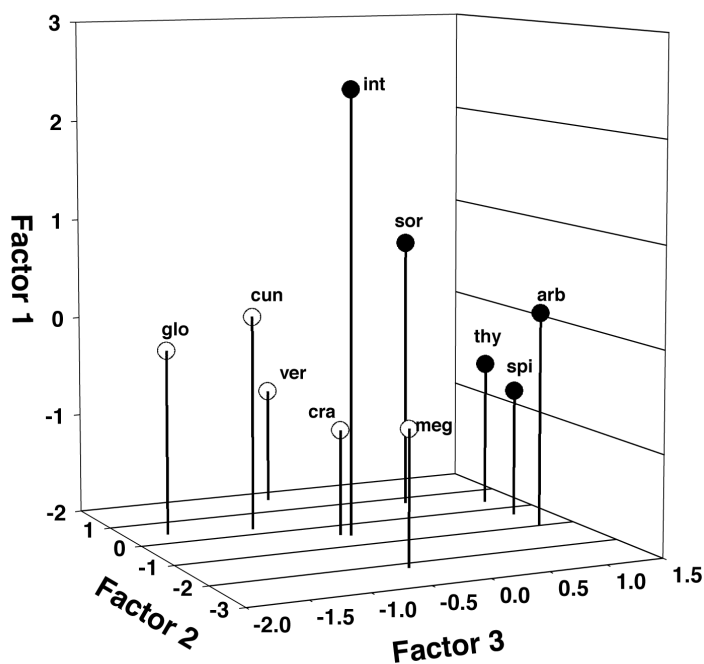


Fig. 3. Graphical representation of species distribution along the 3 first axis obtained by PCA using functional traits of ten species of *Ceanothus*. The first axis was related to allocation patterns; the second to plant size and RGR; and the third axis related to photosynthesis. (subgenus *Ceanothus*, solid dot: arb – *C. arboreus*, int – *C. integrerrimus*, sor – *C. sorediatus*, spi – *C. spinosus* and thy – *C. thyrsiflorus*; subgenus *Cerastes*, clear dot: cun – *C. cuneatus*, cra – *C. crassifolius*, glo – *C. gloriosus*, meg – *C. megacarpus* and ver – *C. verrucosus*).

Table 3. Correlation of functional plant characters of subgenera *Ceanothus* and *Cerastes* of genus *Ceanothus* with PCA axes, and percentage of explained variance.

	Subgenus <i>Ceanothus</i>			Subgenus <i>Cerastes</i>		
	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
Seed mass	-0.563	-0.188	-0.788	-0.937	0.346	0.003
Final plant mass	0.379	0.897	0.068	-0.989	0.091	-0.115
Root/shoot	0.733	-0.339	-0.406	0.019	-0.914	-0.245
Leaves/stem	-0.749	0.524	-0.405	0.542	0.673	0.432
RGR	0.418	0.871	0.197	-0.995	0.059	-0.075
NAR	0.924	0.336	0.021	-0.313	-0.679	0.165
SLA	-0.881	0.014	-0.231	0.559	0.800	-0.029
A	0.757	-0.641	0.024	0.428	-0.874	0.196
G _i	-0.015	-0.937	0.346	-0.471	-0.635	0.542
WUE	0.827	0.133	-0.373	-0.899	-0.151	0.228
N	0.933	-0.262	-0.220	0.828	0.060	-0.545
PNUE	-0.711	-0.624	0.099	0.764	0.485	0.420
NUE	-0.873	0.350	0.239	0.812	0.026	0.559
% variance explained	52.0	30.7	10.9	51.8	30.4	11.2

related (Shipley and Peters 1990, Marañón and Grubb 1993, Fenner 2000) but in *Cerastes* they were positively correlated, perhaps because a large seed size provides reserves to support early seedling growth in this group of species. Earlier studies did not measure RGR at a common plant size and therefore may have confounded developmental changes in RGR with species differences in RGR (Cook and Evans 1983). Differences in covariation among traits between the two sections suggest that 1) there is no single monolithic pattern of trait correlations that characterizes all species and 2) phylogenetic history did not constrain the evolution of new correlations among traits in *Cerastes*.

Consistent differences between sections (Jeong et al. 1997) support paleontological evidence that *Cerastes* evolved during the Eocene in response to the seasonally lower temperatures and aridity of the Mediterranean-type climate that followed the warm, moister climate of the late Tertiary (Mason 1942, Axelrod 1989). In contrast, species in subgenus *Ceanothus* are close to other Rhamnaceae species, in both functional traits and phylogenetic proximity (Hardig 1997, Hardig et al. 2000), suggesting that there was a single evolutionary trend within this subgenus, which could be explained by either natural selection or historical factors such as genetic drift or selection for traits unrelated to physiology.

Table 4. Seed and final plant mass, root-to-shoot ratio (R:S), leaves-to-stem ratio, maximum relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA), photosynthetic rate (A), leaf conductance to water vapor (g_i), water use efficiency (WUE), leaf nitrogen concentration (N), N use efficiency (NUE), and photosynthetic N use efficiency (PNUE) of seedlings of N fixing and non-fixing species of *Ceanothus* grown from seeds under greenhouse conditions for 6 months. Statistical significance of differences (ANOVA) between fixers and non-fixers at p < 0.001, 0.01 and 0.05 given by ***, ** and *, respectively (ns = not significant). Values are mean ± 1 SE; n = 6 for fixers and 4 for non-fixers.

	Non fixers	p	N fixers
Seed mass (mg)	14.9±5.6	ns	7.9±2.4
Final plant mass (g)	13.1±0.9	*	10.0±0.6
R:S	1.0±0.1	ns	1.0±0.2
Leaves/stem	3.0±0.1	ns	2.4±0.5
RGR (mg g ⁻¹ d ⁻¹)	16.1±3.2	ns	13.5±2.4
NAR (mg g ⁻¹ d ⁻¹)	41.5±6.9	ns	43.5±12.2
SLA (cm ² g ⁻¹)	7.4±2.1	ns	7.3±1.0
A (μmol m ⁻² s ⁻¹)	17.8±4.3	ns	19.5±1.2
g _i (mmol m ⁻² s ⁻¹)	0.2±0.1	ns	0.2±0.0
WUE (mmol mol ⁻¹)	121.3±6.0	ns	113.8±31.7
N (mg g ⁻¹)	20.7±1.0	ns	24.4±1.7
PNUE (mmol g ⁻¹ s ⁻¹)	0.5±0.1	ns	0.6±0.1
NUE (g g ⁻¹)	49.2±2.7	ns	42.4±3.2

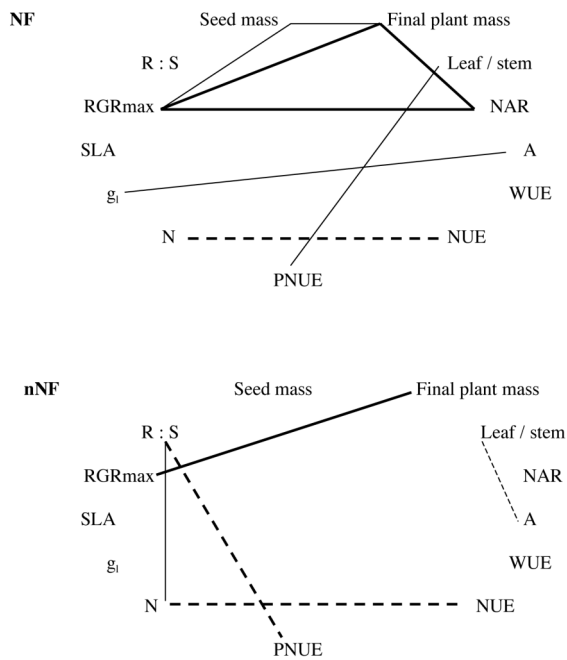


Fig. 4. Correlograms among morphological and physiological traits of fixing (NF) and non-fixing (nNF) species of *Ceanothus*. Solid lines indicate significantly positive correlations and dashed lines negative correlations. Thick lines show $p < 0.01$ and thin lines $p < 0.05$.

Cerastes diverged most from subgenus *Ceanothus* in sclerophylly and in reproductive traits. Differentiation in seed size between the two sections of *Ceanothus* (Table 1) was associated with changes in other traits such as allocation patterns that affect both physiology and adaptive strategies of species (Chapin et al. 1993). *Cerastes* species are seeders that do not resprout after fire, a trait most likely evolved in response to increasing fire frequency (Keeley and Keeley 1977). Because of their shorter lifespan and reproduction mainly by seeds, seeder species track climatic changes, both evolutionarily and spatially, more closely than do resprouter species and show a higher speciation rate (Wells 1969). This is particularly true for *Cerastes* species, because they hybridize easily (Nobs 1963). Finally, a greater reproductive allocation ensures a greater seedling recruitment, a trait that may offset the potential advantage of resprouter species after disturbance by fire (Thomas and Davis 1989).

Usually, the seeder habit is characterized by a low R:S ratio, as in *Cerastes* (Table 1; Keeley and Zedler 1978, Enright and Lamont 1989, Pate et al. 1990, Hansen et al. 1991), causing seeder species to experience more negative water potentials (= greater water stress) than ecologically similar resprouter species (Barnes 1979, Davis and Mooney 1986, Thomas and Davis 1989, Ackerly 2004a). Nevertheless, seedlings of *Cerastes* species survive summer drought better than seedlings of deep-rooted, subgenus

Ceanothus species (Barnes 1979, Keeley and Zedler 1978, Miller and Poole 1979, Bowman and Roberts 1985, Davis 1989), and are more abundant than resprouters on south-facing slopes and dry places in California (Keeley and Keeley 1988, Meentemeyer and Moody 2002), pointing to an increased drought tolerance in *Cerastes*. This tolerance could be based on anatomical modifications of xylem vessels (Carlquist and Hoekman 1985) that tolerate greater water stress by preventing cavitation (Kolb and Davis 1994, Langan et al. 1997). In addition, physiological responses characteristic of warm, dry environments like heat shock protein expression and biochemical photoprotection of photosystems differed between both sections and were associated with leaf physiological parameters and related aspects of life history (Knight and Ackerly 2001).

Cerastes species also have high rates of functional processes (higher leaf allocation, A and g_i , thicker leaves, and lower WUE). These functional differences produce a new adaptive strategy in *Cerastes* that depends strongly in seedling recruitment and rapid growth in the favorable part of the growing season (when shallow roots would be more efficient) and resistance to high levels of stress at other times of the year.

The large initial seedling sizes of *Cerastes* species may have evolved to deal with drier climatic conditions and strong seasonality during establishment (Salisbury 1942, Keeley 1992, Lord et al. 1995). A large seed with large nutrient stores reduces the dependence of seedling growth on external supply of both water and nutrients (Lee and Fenner 1989, Jurado and Westoby 1992) and improves seedling survival under a variety of hazards (Leishman et al. 2000). In addition, *Cerastes* seeds germinated earlier than resprouters seeds (13 vs 18.2 d after sown), a trait of short-lived species (Auld and Myerscough 1986) which could be related to their shorter generation time. Chaparral post-fire seeders may represent a novel group combining early successional traits with traits proper of stress-tolerant species (Ackerly 2004a).

Loss or gain of N fixation ability appeared to be a major evolutionary event in both sections, because it altered the relationships among functional traits. Trait correlations in N-fixers were related more to photosynthesis and those in non-fixers more to allocation (Fig. 4). This suggests that the latter developed new links among traits. That N-fixing species appear in both sections suggests that N-fixing ability has been lost and gained several times through the history of the genus *Ceanothus*. Alternatively, it may be an ancestral trait inherited by early species in both sections of *Ceanothus* that has been kept or lost depending on microhabitat conditions. For example, N fixation may be an old (Tertiary) character associated with wetter conditions, that tended to disappear in drier environments because N fixation is sensitive to water shortage (Irigoyen et al. 1992, Pratt et al. 1997) and causes low water use efficiency (Schulze et al. 1991).

We conclude that, although the linkages among traits in the genus *Ceanothus* as a whole were similar to patterns observed in other broad taxonomic comparisons, speciation within the two subgenera led to distinct physiological, genetic or developmental linkages. The gain or loss of N-fixing ability also influenced linkages among physiological or functional traits. Evolution in response to variation in both physical environment (e.g. drought and fire) and nutritional physiology (N fixation) appears to have shaped the suites of traits currently observed in these species and their ecological function. The evolution of *Cerastes* from earlier species of *Ceanothus* may have led to the acquisition of traits that improved seedling survival in seasonally drier environments. In contrast to subgenus *Ceanothus* species, evolution in *Cerastes* tended to increase seed size, which ensured seedling development before the onset of summer drought. We hypothesize that loss of N fixation capability would allow a faster growth during the seedling stage, which would be favored in arid environments.

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