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REGULAR ARTICLE

The roles of climate and soil nutrients in shaping the life histories of grasses native to the Cape Floristic Region

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Abstract
Aims We hypothesized that in seasonally-arid systems the evolution of annuality is controlled by both moisture regime and substrate quality, with infertile soils either precluding annuality or selecting for improved nutrient acquisition in annuals. The grass flora of the Cape Floristic Region to test these ideas.
Methods We compared intrinsic variation in life history and nutrient acquisition traits between populations of *Ehrharta calycina* J. E. Sm. (Poaceae) situated along an aridity gradient and on diverse substrates. We also evaluated the importance of moisture regime and substrate as predictors of life history across 79 Cape grass species.
Results In *E. calycina*, rhizome survivorship, plant growth and reproductive maturation rate were interrelated and closely tied to wet season duration. By contrast, life history variation was poorly correlated with soil nutrients, and there was little evidence of enhanced nutrient acquisition in annual populations. Across multiple species, however, substrate was identified as a significant co-predictor of life history.
Conclusion Our identification of substrate as an important predictor of life history across multiple species but not within *E. calycina* is not paradoxical, since *E. calycina* consistently acts as an annual species in avoiding the ultra-oligotrophic, quartzitic sands that dominate the Cape mountains. Overall, our data support the joint influence of climate and substrate on the evolution of annuality and associated life history traits.

Keywords Annual · Biomass allocation · Cape floristic region · Rainfall · Flowering · Fynbos · Soil nutrients · Perennial · Poaceae · Relative growth rate · Root mass ratio · Succulent karoo

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Introduction

The annual life history, in which survival from one growth period to the next is achieved via seed, is favoured in situations where growth-favourable conditions are episodic and the probability of adult survival from one growing season to the next is low (Bell 1976; Fox 1990; Young and Augspurger 1991). As

such, annuality is common in desert and seasonally-arid ecosystems where it functions in the avoidance of seasonal drought (Schaffer and Gadgil 1975; Gutterman 2002). Because the continued persistence of annual plant species relies fundamentally on seed, annual plants tend to invest more heavily in seed-mediated persistence than do perennials (Hirshfield and Tinkle 1975; Primack 1979; Bonser and Aarssen 2006; Van Kleunen 2007), with reproductive maturation being accelerated to ensure seed set before the end of the growth period (e.g. Hall and Willis 2006; Franks et al. 2007). High specific leaf areas (Garnier 1992; Garnier and Laurent 1994) and shoot:root ratios (Zangerl and Bazzaz 1983; Forseth et al. 1984) may contribute towards this objective by enhancing growth rates (Garnier 1992; Verboom et al. 2004; Van Kleunen 2007). Of course, selection for fast growth and early flowering also extends to perennials whose 'perennation' organs (e.g. rhizomes, corms etc.) are insufficiently well-developed to guarantee survival (i.e. 'weak' perennials). These plants probably constitute the evolutionary springboard from which obligate annual species have arisen, the inability to perennate emerging as a consequence of maximal investment in early reproduction (Gadgil and Solbrig 1972; Law 1979; Roff 1992).

While climatic regime is known to play a central role in selecting for the evolution of annuality in arid systems, the feasibility of a seed-mediated persistence strategy may also be constrained by nutrient availability. This is because nutrient limitation depresses growth rate (Chapin 1980; Fichtner and Schulze 1992; Baraloto et al. 2006), potentially retarding reproductive maturation (Chapin 1980; Ma et al. 1997), and because high nutritional costs (e.g. Hocking 1980; Witkowski and Lamont 1996) may result in the production of fewer, smaller and/or qualitatively inferior seeds (Deng and Woodward 1998; Wagner et al. 2001). Nutrient limitation may also compromise seedling recruitment success (Bisigato and Bertiller 1999; Barger et al. 2003), although this may be offset by the production of larger, more nutritious seeds (Jurado and Westoby 1992).

Nutritional constraints may explain the lack of a significant annual component in some floras. The floras of the South African Cape Floristic Region (CFR, *sensu* Goldblatt and Manning 2000) and the Australian South-West Floristic Province (Lambers et al. 2006; Orians and Milewski 2007), for example, show remarkably low incidences of annuality when compared with other semi-arid and Mediterranean-type floras (Beard et al. 2000; Goldblatt and Manning 2000). Also, within the Greater Cape Floristic Region (GCFR, Born et al. 2006), the proportion of annual species inhabiting the heathy Fynbos biome of the CFR is much lower than that in the adjacent Succulent Karoo biome (*ca*. 4% *versus* 40%; Van Rooyen 1999). While this may be partly attributable to the generally wetter climate of the Fynbos biome, a role for nutrients cannot be ruled out. Within the CFR, most annual species inhabit the richer (Specht and Moll 1983) shale- and granite-derived soils of the coastal platform and intermontane valleys, and the calcareous substrates (limestones and Quaternary sands) of the coastal zone. Where annuals are faced with acute nutrient limitation, however, the high nutritional demands incurred by an annual life history may stimulate the evolution of traits enhancing nutrient-acquisition. Candidate adaptations include increased allocation to roots (Chapin 1980), elongated and/or more finely divided root systems (Fitter et al. 1988; Tjoelker et al. 2005; Roumet et al. 2006), increased transpiration rates (Barber 1995; Cramer et al. 2008, 2009) and the development of cluster roots and/or mycorrhizal associations (Lambers et al. 2006). Increased seed mass (and increased seed reserves) may also serve to improve recruitment success (Lloret et al. 1999; Caddick and Linder 2002).

The grass flora of the GCFR represents an excellent system for testing the influence of nutrients and climate on the evolution of annuality. Besides inhabiting a variety of climatic and edaphic environments, the Cape grasses display diverse adaptations for surviving seasonal drought (Linder and Ellis 1990a; Verboom et al. 2004). Overall, about10% of Cape grass species are annual, most of the remainder being perennial, but with a handful of species (e.g. *E. calycina* J. E. Sm. and *E. erecta* Lam.) showing variability and/or plasticity in life history (Gibbs Russell et al. 1990b). There is some evidence to suggest that substrate has influenced the evolution of growth- and life history-related traits in Cape grasses: within the genus *Ehrharta*, for example, substrate appears to have directed the evolution of alternative drought-survival strategies (Verboom et al. 2004), while in *Pentameris* (*sensu* Linder et al. 2010) it appears to have stimulated the differentiation of orthophyllous and sclerophyllous leaf morphologies (Galley and Linder 2007).

In this paper, we explore further the association of life history with substrate on the one hand, and climate on the other. We used a common garden experiment to

quantify intrinsic variation in life history traits amongst populations of *E. calycina* (Fig. 1a), a species which reportedly varies from perennial to annual across its range (Chippendall 1955; Gibbs Russell et al. 1990a; Zacharias 1990). Then, using the climatic and edaphic attributes of each population source locality, we evaluated the relative importance of climate and soil fertility as evolutionary drivers of life history trait variation (rapid growth and early flowering, high reproductive allocation). We also tested whether, in the context of nutrient-deficiency, a greater dependence on seed-mediated persistence has stimulated the evolution of traits potentially enhancing nutrient acquisition. Finally, looking beyond *E. calycina*, we used GIS data to characterize the climatic (mean annual rainfall, duration of the moisture growing season) and geological niches of 79 Cape species of *Ehrharta* and *Pentameris*, using these once again to evaluate the relative importance of climate and substrate as potential determinants of life history variation. We focused on these genera because they are ubiquitous in the Cape flora, both contain annual and perennial species, and published phylogenies (Verboom et al. 2003; Galley and Linder 2007) and accurately geo-referenced specimen data are available for both.

Materials and methods

Field sampling

Ehrharta calycina populations were sampled at eight localities along a 443 km transect spanning a steep

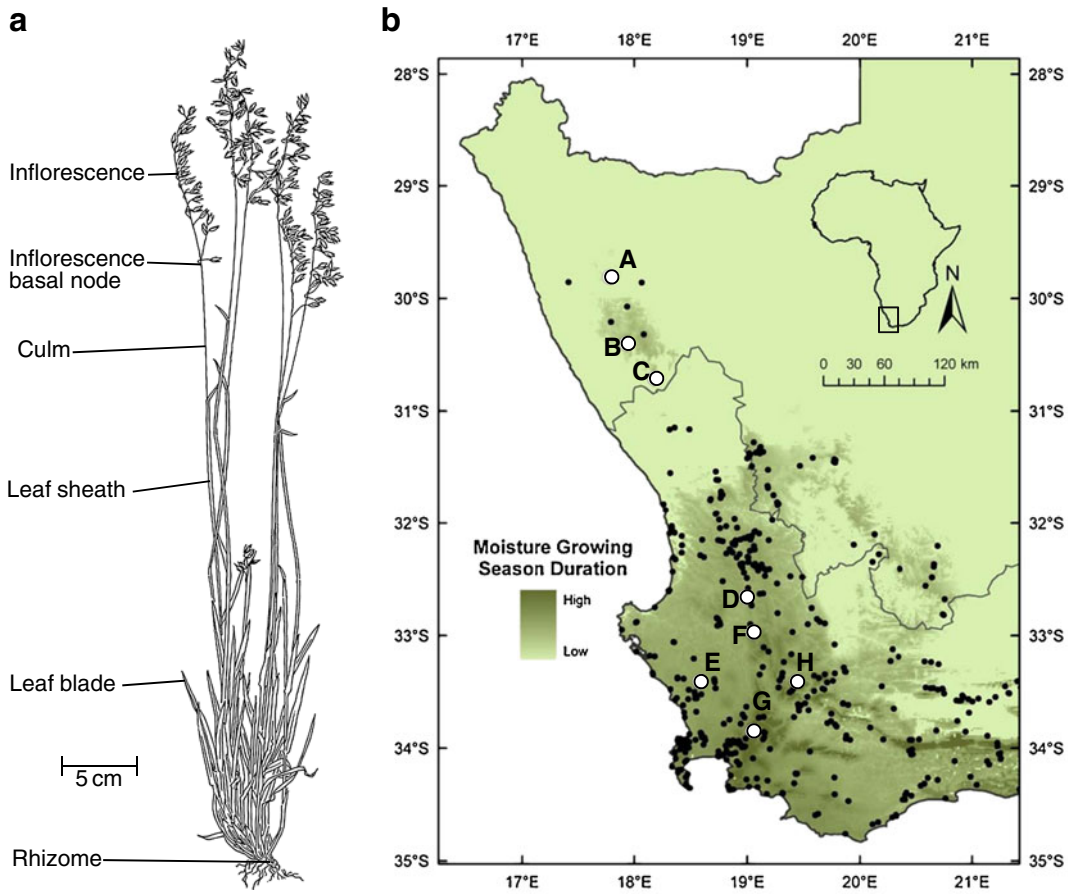

Fig. 1 **a** Growth form of *Ehrharta calycina*, illustrating morphological terms used in the text. Like other grasses, the *E. calycina* plant is composed of tillers, each comprising a single aerial shoot (culm) with its associated leaves, rhizome portion and inflorescence. **b** Distribution of *E. calycina* in the winter-rainfall zone of South Africa, based on collection localities of specimens at BOL and PRE (*black dots*), with study populations marked by white-filled circles. Geographical variation in moisture growing season duration is indicated

aridity gradient within the South African winter-rainfall zone (Fig. 1b; Table 1). Most sampled sites are on granite- or shale-derived soils, with two (F and H) being on a quartzite/shale interface (Table 1). Being surrounded by agricultural lands, site G showed signs of human disturbance, infestation by alien plants, and recent burning. The following material was collected at each locality towards the end of the 2007 flowering season (September 2007): (i) seed, bulk-harvested from multiple plants, (ii) flowering tiller material (for an explanation of grass morphological terminology, see Fig. 1a) from five representative individuals, (iii) leaf samples from five individuals, for isotopic analyses, and (iv) a reference voucher, deposited in the Bolus Herbarium (BOL) at the University of Cape Town. For (ii) and (iii), samples were taken from distinct tussocks separated by >10 m to ensure that each represented a distinct genet. Towards the end of the 2009 dry season (February 2009), five plants at each sampling locality, situated >100 m apart, were sampled for tiller material (four tillers per plant, with basal rhizomes attached), for evaluating summer rhizome survivorship, and soil samples, for chemical analysis.

Climatological characterization of sampling sites

We used two variables to characterize the moisture regime at each population locality: mean annual precipitation (MAP) and the duration of the moisture growing season (MGSD). These variables capture different aspects of the moisture regime, the first measuring the total volume of precipitation during the course of a year and the latter its seasonal availability. As used here, moisture growing season is defined as the annual period when soil moisture is sufficient for crop growth, being approximated as the period when median monthly precipitation equals or exceeds 30% of the median monthly A-pan equivalent potential evaporation (FAO 1978; Schulze 1997). Populations were characterized for each climatic variable using ArcView 3.3 (ESRI Inc. Redlands, California, USA), with Spatial Analyst 1.1 and Grid Analyst 1.1 extensions, to query published GIS layers for these variables (Schulze 1997). The MAP layer used for this purpose was based on interpolation of data from over 6,000 weather stations across southern Africa, while the MGSD layer was based on data obtained from 570 weather stations, for which a minimum of 3 years' worth of monthly temperature, rainfall and A-pan evaporation observations were available (Schulze 1997). Both layers were generated at a resolution of 1′ by 1′ of a degree.

Summer rhizome survivorship

Within 48 h of collection, 0.5 cm-long rhizome fragments were separated from each of the tiller samples collected in February 2009 (five plants per locality, four rhizome sections per plant) and stained with 2,3,5-triphenyltetrazolium chloride (TTC), whose reduction to pink formazan indicates metabolic activity (Roberts 1951). Prior to staining, rhizome fragments were cleaned and sectioned to ensure exposure of potentially-living vascular tissue, after which they were dark-incubated for 1.5 h in a 0.8% w/v solution of TTC in 0.05 M KH_2PO_4, and examined under a dissecting microscope. In addition to the results of TTC staining, rhizome viability was assessed by the presence of moist and/or green bud tissue. Rhizome survivorship at each site was then quantified as the mean proportion of rhizome sections per plant determined to be viable on the basis of the three measures employed, the latter showing a high degree of correspondence. Scoring rhizome viability in this manner allowed for identification of plants whose perennation structures were completely dead (rhizomes viable=0/4=0%) or alive (rhizomes viable=4/4=100%), as well as those whose rhizome systems were partially dead (intermediate values).

Tiller and diaspore analysis

As a measure of reproductive investment, the proportion of above-ground shoot mass invested in inflorescences (inflorescence mass ratio) was quantified using field-sampled tiller material. For each sample (n=5 per population), three flowering tillers were divided into inflorescence (starting at the basal inflorescence node) and non-inflorescence fractions (leaf blades and sheaths, culm and rhizome) fractions. These were oven dried at 80°C for 48 h before being weighed. To assess variation in seed mass, ten fully-developed diaspores from each population were randomly selected and weighed.

Table 1 The names, vouchers (all at BOL) and locations of populations (Pop.) from which *Ehrharta calycina* seed was sampled, along with the mean annual rainfall (MAP), duration of the moisture growing season (MGSD) and soil properties at each of these localities. Bedrock type, soil pH, electrical conductivity (EC), total P, Bray II extractable P, exchangeable cations (Na, K, Ca, Mg) and total N and C are shown, as are values of the derivative soil nutrient index (SNI). Soil variables are reported as mean±standard error ($n=5$)

Pop.	Co-ordinates	MAP (mm)	MGSD (d)	Soil properties											
				Bedrock	pH	EC (mS/m)	Total P (mg kg^{-1})	P Bray II (mg kg^{-1})	Na (cmol kg^{-1})	K (cmol kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	C (%)	N (%)	Soil nutrient index
A	29.81°S 17.78°E	202	0	Granite	4.68±0.18	6.07±1.30	87.84±6.30	7.8±3.6	0.23±0.01	0.18±0.04	1.05±0.08	0.46±0.10	0.26±0.02	0.04±0.01	2.00±0.55
B	30.40°S 17.93°E	355	96	Granite	4.68±0.34	4.03±0.62	101.61±15.85	8.2±2.0	0.22±0.01	0.14±0.01	1.27±0.38	0.54±0.12	0.35±0.02	0.04±0	1.68±1.05
C	30.71°S 18.18°E	226	0	Granite/ shale	4.85±0.44	3.58±0.61	62.26±8.57	4.5±0.5	0.20±0.01	0.09±0.02	0.54±0.14	0.19±0.03	0.32±0.06	0.04±0.01	−0.44±0.35
D	32.66°S 18.99°E	640	137	Shale	4.02±0.05	2.17±0.23	38.40±4.70	3.6±0.4	0.18±0.01	0.05±0.01	0.29±0.15	0.04±0.01	0.29±0.02	0.04±0	−2.19±0.25
E	33.41°S 18.58°E	551	150	Shale	4.16±0.04	5.20±0.87	32.76±2.13	2.4±0.2	0.20±0.02	0.09±0	0.28±0.04	0.25±0.02	0.37±0.07	0.07±0.03	−1.05±0.30
F	32.97°S 19.05°E	930	184	Quartzite/ shale	3.84±0.07	2.96±0.49	92.83±20.54	3.8±1.0	0.20±0.01	0.07±0.02	0.27±0.07	0.18±0.03	1.28±0.18	0.05±0	−1.08±0.43
G	33.85°S 19.05°E	928	185	Granite	4.36±0.12	5.69±0.98	62.60±9.39	7.4±1.2	0.09±0.01	0.15±0.04	0.92±0.13	0.38±0.05	1.07±0.18	0.10±0.01	3.05±0.60
H	33.41°S 19.44°E	639	142	Quartzite/ shale	3.98±0.12	2.27±0.30	22.68±1.11	2.2±0.5	0.19±0.01	0.06±0.01	0.27±0.08	0.15±0.03	0.44±0.13	0.07±0.01	−2.07±0.33

Soil analyses

In order to quantify soil fertility at each population locality, soil samples were analysed for pH, electrical conductivity and plant nutrient concentrations. Soil samples were oven-dried at 80°C for 48 h and sieved (1 mm mesh). Soil pH was determined by shaking 2 g soil in 20 mL 1 M KCl at 180 rpm for 60 min, centrifuging at 10 000 g for 10 min and measuring the supernatant pH. Soil N and P were determined, respectively, by digestion with an FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, USA), and by extracting 6.6 g soil in Bray II solution (Bray and Kurtz 1945) prior to filtration and analysis using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Varian Vista MPX, Australia). K, Na, Ca and Mg were also analysed using ICP-AES, the exchangeable cations first being displaced from 10 g soil with 25 mL of 0.2 M ammonium acetate, filtered through Whatman No. 2 paper, and made up to 200 mL. No soil textural analyses were done.

Foliar analyses

Field-sampled leaf material was oven-dried at 80°C for 48 h, before being milled using a Wiley mill with a 0.5 mm mesh (Arthur H. Thomas, California, USA), and analysed for tissue N concentration by digestion on an FP-528 Nitrogen Analyser (Leco Corporation, St. Joseph, USA). For isotopic determinations, 2.100–2.200 mg of each leaf sample was weighed into an 8 by 5 mm tin capsule (Elemental Microanalysis Ltd., Devon, UK) on a Sartorius microbalance (Goettingen, Germany) and combusted in a Thermo Flash EA 1112 series elemental analyzer (Thermo Electron Corporation, Milan, Italy). The released gases were fed into a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron Corporation, Milan, Italy) via a Thermo Finnigan Conflo III control unit (Thermo Electron Corporation, Milan, Italy), and their $\delta^{13}C$ and $\delta^{15}N$ values determined. Calibration was done using one IAEA and two in-house standards.

Plant culture

To evaluate genotypic variation in plant growth and flowering behaviour, wild-sampled seed representing all populations was germinated and grown under common garden conditions in a temperature-controlled greenhouse at the University of Cape Town. For this purpose, 60 fully developed diaspores from each population were selected and sown (two seeds per pot) in 0.15 m diameter pots in washed river sand at a depth of 1 cm. Diaspores were sown on 19 July 2006, with seedlings emerging over a four day period from 27 to 30 July, following which excess seedlings were topped. Pots were arrayed randomly (with respect to population and harvest time) on a series of trolleys, which were moved twice weekly in a regular pattern to compensate for heterogeneity of growth conditions within the greenhouse. Temperatures were maintained between 20 and 25°C. An automated sprinkler system irrigated plants for 5 min twice daily, ensuring that the soil was always moist, but not waterlogged. From 1 week post-emergence, plants received 0.1 L of 2 mM Long Ashton nutrient medium (Hewitt 1966) modified to contain 2 mM $NaNO_3$ (pH 6.5) initially once per week, and then twice weekly once the plants became larger.

Growth and flowering of potted plants

For the purposes of monitoring change in specific leaf area and dry mass allocation, and estimating seedling growth curves for each population, five seedlings per population were harvested at 43, 50, 57 and 64 d post-sowing (1, 8, 15 and 22 September, respectively). At each harvest, plants were carefully removed from their pots and sand washed off the roots. Root, stem and leaf (blade only; sheaths included in stem fraction) fractions were oven-dried at 80°C for 48 h and weighed. At the 43 and 64 d harvests, the leaves were arranged between two glass plates and photographed for subsequent analysis of leaf area, prior to drying. Also, at 64 d a sub-sample of the root fraction was removed for estimation of total root length prior to drying and weighing. For measurement of root dimensions, root subsamples were teased apart in a thin layer of water in a transparent tray and the image captured at 300 dpi using an EPSON 4870 PHOTO scanner (EPSON America, Long Beach, California, USA). Leaf areas were determined from leaf images using the histogram tool in Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, California, USA), whilst WinRhizo v2005c (Regent Instruments Inc., Nepean, Ontario, Canada) was used to estimate root dimensions. The remaining ten plants per population were grown for a further 50 d, during which transpiration rates were measured (at 64 d) and flowering monitored

and recorded. Finally, on 12 November (115 d after germination) all remaining plants were harvested, and the mean inflorescence dry mass present on each plant determined.

Measurement of transpiration rate

Transpiration rates were measured on six replicate plants using a LI-6400 Portable Photosynthesis System (Licor, Lincoln, New England, USA) at a saturating photosynthetic photon fluence rate of 1,500 μmol quanta m^{-2} s^{-1} (as determined from preliminary light-response curves) in a Licor LI-6400-02B cuvette. Measurements were made after about 2.5 min equilibration in the cuvette, between 10 h00 and 14 h00, with the cuvette temperature set to 25°C and the CO_2 concentration to 380 μmol mol^{-1}.

Statistical analysis of *E. calycina* data

Relationships between moisture regime, soil nutrient status, rhizome survivorship, and plant traits, were evaluated using standard correlation-regression and multiple regression analyses, with false discovery rates (Benjamini and Hochberg 1995) applied where necessary to compensate for multiple tests. In the absence of direct measures of nutrient supply rate, soil nutrient concentrations were used as proxy measures of soil fertility. Since statistical power was severely restricted by low sample size (n=8 populations), it was necessary to restrict the number of variables examined. Therefore, we used principal components analysis (PCA), as applied to multiple soil chemical variables, to derive a single index of soil nutrient status. In order to evaluate whether plant water use efficiency varied along environmental gradients, we also tested the relationships of the $\delta^{13}C$ ratios of field-sampled leaf material to variation in rainfall and soil nutrient status, with $\delta^{15}N$ ratios of the same leaf material being used as an independent index of aridity. Population-specific relative growth rates (RGR) were determined as the slopes of functions relating the logarithm of plant dry mass to time. All statistical analyses were conducted using R version 2.8.1 (R development core team 2008).

Correlates of annuality in *Ehrharta* and *Pentameris*

For the purpose of quantifying the climatic niches of Cape *Ehrharta* and *Pentameris* species, we assembled a database of coordinate data describing the collection localities of all relevant specimens housed at BOL and PRE whose position could be determined with suitable accuracy. The number of records per species ranged from 3 to 322 (mean=59.7) in *Ehrharta* (n=20 species) and from 1 to 182 (mean=23.4) in *Pentameris* (n=59). The MAP and MGSD at each collection locality was then determined using ArcView 3.3 with Spatial Analyst 1.1 and Grid Analyst 1.1 extensions, to query published GIS layers for these variables (Schulze 1997). The resulting data were then averaged to obtain species means, standard errors, and the 0.1 and 0.9 quantile values (Appendix 1).

Since substrates in the CFR show fine-scale heterogeneity, species' substrate preferences were scored on the basis of personal field observations (GAV) and published information (Gibbs Russell et al. 1990a, b; Goldblatt and Manning 2000; Linder and Ellis 1990a, b; Linder and Davidse 1997; Verboom et al. 2004; Galley and Linder 2007), the latter also being used to score species' life histories. For substrate preference, species were scored (binary) as being either associated predominantly or exclusively with the ultra-oligotrophic, quartzitic sands of the Cape Supergroup or with more eutrophic substrates, whereas for life history they were scored as being either annual or perennial.

To evaluate the importance of MAP and MGSD as determinants of life history, we first used independent sample t-tests to evaluate whether these variables differed significantly between the annual and perennial species pools, using species' means as observations. Since extremes are often more meaningful than means as indicators of a species' range limits, these analyses were repeated using the 0.1 and 0.9 quantile values. We also tested for an association between life history and substrate, using a Fisher contingency test. To account for the possibility that associations were a product of phylogenetic covariance, they were re-evaluated using phylogenetic independent contrasts (PICs; Felsenstein 1985). For the continuous variables (MAP, MGSD), the protocol of Webb et al. (2011) permitted identification of eight annual-perennial PICs (Appendix 2), using the phylogenetic trees of Verboom et al. (2003) and Galley and Linder (2007). For each PIC, the mean value of the annual species set was compared to the mean value of the perennial species set, the existence of a significant association across PICs being evaluated using a paired sample *t*-

test. Finally, to test whether the evolution of annuality was significantly associated with more eutrophic (i.e. non-quartzitic) substrates, we reconstructed the evolution of life history and substrate preference on a pruned *Ehrharta* + *Pentameris* phylogeny using parsimony (DELTRAN), as implemented in Mesquite version 2.5 (Maddison and Maddison 2008). Thereafter, we used the test of Sillén-Tullberg's (1993) to evaluate whether gains of annuality were more frequently associated with non-quartzitic branches than with quartzitic branches, compared with chance expectation. Significance was again evaluated using a Fisher contingency test.

Finally, generalized linear models (GLMs) were used to evaluate the relative importance of MAP, MGSD and substrate as predictors of life history. Since the response variable (life history) was binary, its relationship to the three predictors (two continuous, one binary) was defined using a logit link function. Identification of the optimal model started with a full model containing all three predictors and their interactions. Following Crawley (2007), models were generally simplified by excluding first non-significant interactions and thereafter non-significant main effects. At each step, models were compared using the Akaike Information Criterion (AIC), the optimal model identified as that having the lowest AIC score. Unfortunately, our data set contained too few annual-perennial PICs to offer the the statistical power required to carry out a phylogenetically controlled equivalent of the GLM procedure described above, and attempts to do so failed because the estimation procedure did not converge on a solution.

Since *E. calycina* and *E. erecta* are polymorphic for life history, all statistical tests were run twice, with these species set either as perennial or as annual. All statistical tests were conducted using R version 2.8.1 (R development core team 2008).

Results

E. calycina site characteristics

The sampled *E. calycina* populations span a steep, north–south aridity gradient which varies in both MAP and MGSD (Fig. 1b; Table 1). Both MGSD (Fig. 2a) and MAP (r=0.866, P=0.005) were strongly correlated with latitude, the northernmost populations receiving an average of 200–350 mm of rain annually and having MGSD <100 d, and the southern populations having higher MAP and MGSD. Since MGSD and MAP were tightly correlated (r=0.932, P<0.001), we included only the former in subsequent analyses evaluating the significance of trait variation in *E. calycina*. Evidence for the direct selective influence of growing season duration on maturation rates in annuals (e.g. Franks et al. 2007) identifies MGSD as being a more important determinant of life history traits than MAP.

Field-sampled leaf material of *E. calycina* (Table 2) revealed significant among-population differences in $\delta^{15}N$ ($F_{7,\ 32}$=100.82, P<0.001) and C:N ($F_{7,\ 32}$=

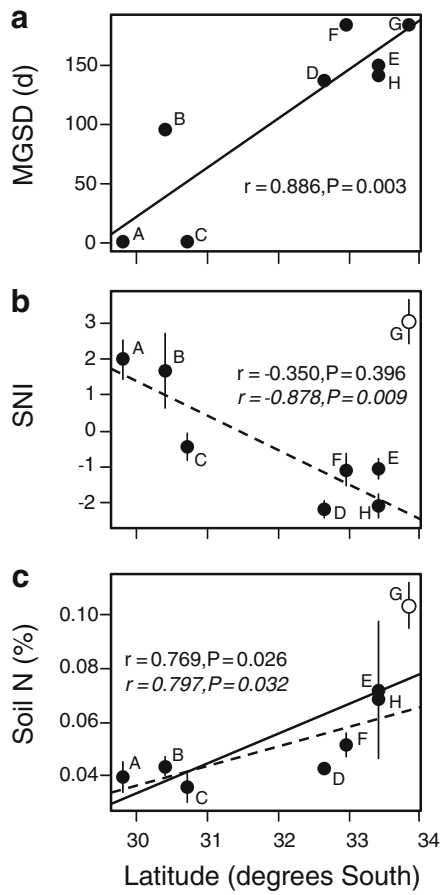

Fig. 2 Variation in (**a**) duration of the moisture growing season duration (MGSD), (**b**) soil nutrient index (SNI), and (**c**) soil N with latitude. Bars are standard errors and point labels are *Ehrharta calycina* population codes (see Table 1). Correlation statistics and fitted regression lines (significant relationships only) are based on the full set of points (stats in normal font, solid lines) or on all sites except G (stats in italic font, dashed lines)

Table 2 Population means±standard error of six plant variables measured on field-sampled material. Units are indicated in parentheses and, except for seed mass (n=10), n=5

Population	Trait					
	Rhizome survivorship (%)	Leaf δ^{15}N ratio	Leaf δ^{13}C ratio	Leaf C:N ratio	Inflorescence mass ratio ($g.g^{-1}$)	Seed mass (mg)
A	50±8	9.61±0.41	−28.32±0.32	29.28±1.60	0.20±0.02	2.60±0.14
B	50±8	7.40±0.45	−28.75±0.20	24.27±2.64	0.21±0.01	2.34±0.04
C	0±0	14.17±0.59	−28.05±0.13	16.47±0.81	0.17±0.02	2.11±0.05
D	85±10	3.63±0.47	−28.55±0.59	29.95±1.35	0.14±0.01	2.56±0.10
E	100±0	3.22±0.30	−28.00±0.14	18.97±1.11	0.10±0.02	2.75±0.13
F	100±0	1.49±0.15	−28.11±0.27	33.11±4.74	0.14±0.01	3.21±0.14
G	100±0	−0.30±0.30	−28.49±0.22	39.05±3.63	0.11±0.01	3.12±0.09
H	100±0	2.51±0.73	−28.60±0.15	34.81±1.69	0.11±0.01	3.80±0.19

7.143, P<0.001), but not δ^{13}C values ($F_{7,\ 32}$=0.90, P=0.524). Values of δ^{15}N showed a strong negative relationship with MGSD (r=-0.955, P<0.001) indicating high rates of ecosystem N turnover (Aranibar et al. 2004) at the drier northern sites than at the wetter southern sites. On the other hand, the lack of correlation between leaf δ^{13}C and MGSD (r=−0.165, P=0.696) suggests that plant water use efficiency (Francey and Farquhar 1982; Farquhar et al. 1989) does not vary with aridity. This is probably because *E. calycina* growth is constrained to periods of high moisture availability. Neither tissue N (r=−0.497, P=0.210) nor C:N ratio (r=0.562, P=0.147) were related to MGSD.

The soils at all sites were acidic (Table 1) and relatively oligotrophic, with low cation exchange capacity (CEC), N, and available P and K. Since an initial PCA based on all soil variables identified substantial covariance amongst all variables except N and C (data not shown), the analysis was repeated with N and C excluded. The first PC axis derived from the latter analysis captured 63% of the total variance present, and described a high proportion of the variance in electrical conductivity (EC, r=0.802, P=0.017) and in the availabilities of several important nutrients including P (Bray II P: r=−0.920, P=0.001), K (r=0.931, P<0.001), Ca (r=0.891, P=0.003) and Mg (r=0.875, P=0.004). Consequently, this first axis was deemed a reasonable indicator of soil nutrient status, justifying its use as a soil nutrient index (SNI). Since SNI did not reflect N availability (r=0.285, P=0.494), however, soil N was treated as a separate nutritional variable. Latitude was positively correlated with soil N (Fig. 2c) but uncorrelated with SNI, due to the elevated SNI of soils at site G (Fig. 2b). With site G excluded, both soil N and SNI showed significant relationships with latitude (positive and negative, respectively; Fig. 2b, c), but there was no significant relationship between these soil variables (r=−0.493, P=0.261). Since the elevated nutrient status of soils at site G was probably a consequence of recent anthropogenic influences (see methods), this site was excluded from subsequent analyses evaluating the importance of soil nutrients as an explanation of trait variation in *E. calycina*.

E. calycina summer rhizome survivorship

Summer rhizome survivorship varied substantially among sites (Table 2), ranging from 0% (i.e. all plants functionally annual) in the dry north to 100% in the moist south (all plants functionally perennial). This clear association with moisture gradient yielded strong positive correlations with both MGSD (Fig. 3a) and mean annual rainfall (r=0.821, P=0.012). Consistent with our expectations, rhizome survivorship showed strong, negative relationships with traits describing the rate of reproductive maturation (mean inflorescence dry mass at 115 d: Fig. 3b), growth rate (Fig. 3c) and reproductive allocation (inflorescence mass ratio, IMR: r=−0.750, P=0.032).

Developmental and reproductive traits of *E. calycina*

The common garden plants showed substantial variation in life history traits (Table 3), this variation in many

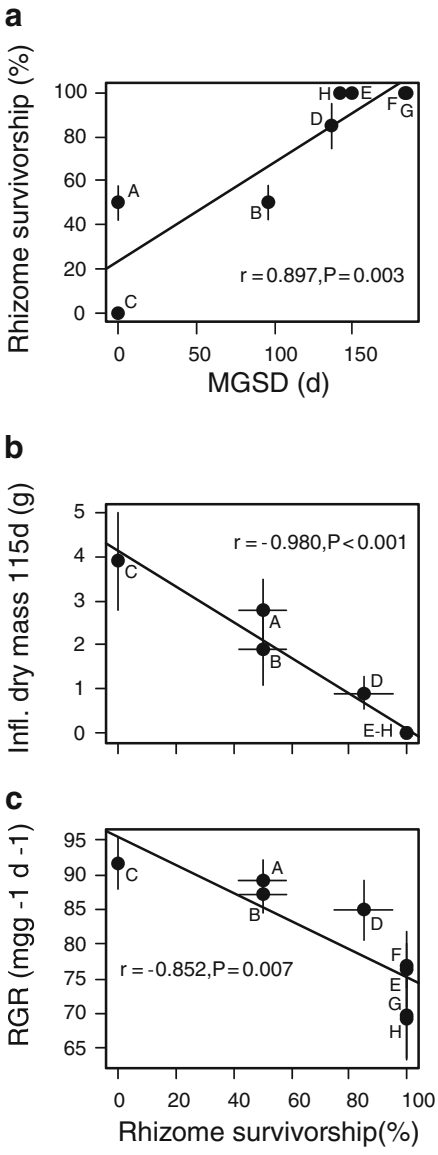

Fig. 3 The relationships of (**a**) *Ehrharta calycina* summer rhizome survivorship (measured on field-based plants) with duration of the moisture growing season (MGSD), (**b**) the mean inflorescence dry mass at 115 d (measured on common garden plants) with rhizome survivorship, and (**c**) relative growth rate (RGR; measured on common garden plants) with rhizome survivorship. Bars are standard errors and point labels are population codes (see Table 1)

cases being closely correlated with environmental conditions at the source population localities. RGR was positively correlated with inflorescence dry mass at 115 d (r=0.892, P=0.002), supporting a role for rapid growth in advancing reproduction. Variation in RGR was also positively related to specific leaf area (SLA), as determined at both 43 (r=0.848, P=0.008) and 64 d (r=0.768, P=0.026). Although RGR was not associated with leaf mass ratio (LMR) at 43 d (r=0.342, P=0.406), a strong negative relationship between RGR and LMR was evident at 64 d (r=-0.942, P<0.001), this being attributable to a late pulse of root growth in high-RGR plants. Owing to the strong relationships of RGR with SLA at 43 d and with LMR at 64 d, RGR was positively and negatively correlated with leaf area ratio at 43 d (r= 0.931, P<0.001) and 64 d (r=−0.794, P=0.019), respectively.

MGSD was strongly and negatively related to inflorescence dry mass at 115 d (Table 4), suggesting a central role for seasonal aridity in selecting for early flowering. RGR and IMR were also negatively related to MGSD, though these relationships were somewhat weaker (Table 4). Although SNI was positively correlated with IMR (Table 4), it was unrelated to inflorescence dry mass at 115 d and RGR, implying a limited role for SNI-associated nutrients (e.g. P, K, Ca, Mg) in explaining differences in maturation rate. Also, while soil N was significantly related to inflorescence dry mass at 115 d, RGR and IMR (Table 4), the negative sign of these relationships precludes the possibility that development rates in *E. calycina* are limited by low N availability.

Our sample size (n=7 populations, site G excluded) was inadequate to permit a full three-way evaluation of MGSD, SNI and soil N, with interactions, as predictors of reproductive maturation (inflorescence dry mass at 115 d). We therefore ran three separate multiple regression analyses, one comparing MGSD and SNI (interactions included), a second comparing MGSD and soil N (interactions included), and a third comparing MGSD, SNI and soil N (main effects only). While the second analysis identified no significant effects (Table 5b), the first and the third identified only MGSD as a significant predictor of inflorescence dry mass at 115 d (Table 5a, c).

E. calycina diaspore characteristics

Mean diaspore mass varied considerably amongst populations, ranging from 2.11±0.05 to 3.80±0.19 mg, and being smallest at the drier northern sites (Table 2). Diaspore mass was positively associated with rhizome survivorship (r=0.775, P=0.024) and negatively correlated with inflorescence dry mass at 115 d (r=−0.772, P=

Table 3 Population (Pop.) means ± standard error of ten traits measured on plants grown in the common garden. Units are indicated in parentheses and, except for inflorescence dry mass at 115 d (n=10), n=5

Pop.	Trait									
	Specific leaf area at 43 d (m^2 kg^{-1})	Leaf mass ratio at 43 d (g g^{-1})	Root mass ratio at 43 d (g g^{-1})	Specific leaf area at 64 d (m^2 kg^{-1})	Leaf mass ratio at 64 d (g g^{-1})	Root mass ratio at 64 d (g g^{-1})	Transpiration at 64 d (mmol m^{-2} s^{-1})	Specific root length at 64 d (cm kg^{-1})	Relative growth rate to 64 d (mg g^{-1} d^{-1})	Inflorescence dry mass at 115 d (g)
A	45.08±4.19	0.59±0.01	0.24±0.02	28.99±2.05	0.34±0.02	0.46±0.04	4.70±0.20	8.24±1.41	89.1±2.94	2.78±0.70
B	47.23±1.86	0.56±0.03	0.29±0.02	31.12±2.67	0.34±0.02	0.51±0.03	4.58±0.20	12.34±2.21	87.12±2.56	1.90±0.81
C	43.22±2.14	0.61±0.03	0.21±0.02	30.84±0.93	0.28±0.03	0.53±0.04	5.17±0.22	10.19±1.04	91.57±3.68	3.89±1.10
D	45.32±2.62	0.60±0.01	0.23±0.01	26.43±2.44	0.36±0.04	0.46±0.04	4.93±0.24	16.73±0.81	84.88±4.39	0.91±0.37
E	39.22±2.73	0.61±0.04	0.20±0.05	25.63±0.55	0.39±0.01	0.41±0.02	5.64±0.28	13.82±2.01	76.41±5.35	0±0
F	37.39±1.28	0.59±0.03	0.23±0.04	26.03±0.67	0.39±0.03	0.38±0.04	4.58±0.53	15.50±2.32	76.90±3.16	0±0
G	39.32±3.91	0.56±0.03	0.24±0.04	27.13±2.12	0.45±0.06	0.32±0.07	5.53±0.32	15.45±6.96	69.71±6.14	0±0
H	33.98±2.14	0.58±0.01	0.23±0.03	25.60±2.10	0.45±0.07	0.34±0.07	5.06±0.46	13.56±1.85	69.33±5.89	0±0

0.025) and RGR (r=−0.889, P=0.003). Surprisingly, diaspore mass was not significantly correlated with MGSD (Table 4) or with IMR (r=−0.663, P=0.073), nor was it related to SNI or soil N (Table 4).

Nutrient acquisition-related traits in *E. calycina*

Except for a weak, negative relationship between RMR at 64 d and soil N, and a marginal, positive

Table 4 Pearson product–moment correlations describing the associations between plant traits and environmental variables for *Ehrharta calycina*. Bold type indicates correlations that were significant (α=0.05) prior to correction using false discovery rates, while asterisks indicate correlations that remained significant with application of this correction

Plant trait	Environmental variable		
	Moisture growing season duration (MGSD)	Soil nutrient index (SNI)	Soil N
Inflorescence dry mass at 115 d	***r*=−0.953**	r=0.595	***r*=−0.814**
	P*<0.001	P=0.159	***P*=0.026**
Relative growth rate (RGR)	***r*=−0.824**	r=0.618	***r*=−0.906**
	***P*=0.012**	P=0.139	***P*=0.005***
Inflorescence mass ratio (IMR)	***r*=−0.743**	***r*=0.849**	***r*=−0.840**
	***P*=0.035**	***P*=0.016**	***P*=0.018**
Root mass ratio (RMR) at 43 d	r=−0.067	r=0.576	r=−0.418
	P=0.875	P=0.176	P=0.350
RMR at 50 d	r=−0.294	r=−0.013	r=−0.282
	P=0.480	P=0.978	P=0.540
RMR at 57 d	***r*=−0.833**	r=0.484	r=−0.750
	***P*=0.010**	P=0.271	P=0.052
RMR at 64 d	***r*=−0.776**	r=0.563	***r*=−0.818**
	***P*=0.023**	P=0.188	***P*=0.024**
Specific root length (SRL)	***r*=0.908**	***r*=−0.759**	r=0.395
	P*=0.002	***P*=0.048**	P=0.381
Transpiration rate (E)	r=0.251	r=−0.448	r=0.563
	P=0.549	P=0.313	P=0.188
Diaspore mass	r=0.644	r=-0.507	r=0.713
	P=0.085	P=0.246	P=0.072

Table 5 Output of multiple regression analyses fitting for *Ehrharta calycina* inflorescence dry mass at 115 d as a function of (a) moisture growing season duration (MGSD) and soil nutrient index (SNI) (interactions included), (b) MGSD and soil N (interactions included), and (c) MGSD, SNI and soil N (main effects only)

Parameters	Estimate	SE	t	P (>|t|)
(a)				
Intercept	3.621	0.377	9.628	0.002
MGSD	−0.019	0.003	−5.572	0.011
SNI	−0.417	0.269	−1.549	0.219
MGSD * SNI	0.005	0.003	1.827	0.165
		Full model: r^2=0.907, P=0.017		
(b)				
Intercept	7.328	3.048	2.404	0.096
MGSD	−0.035	0.022	−1.580	0.212
Soil N	−104.0	79.3	−1.312	0.281
MGSD * Soil N	0.490	0.552	0.888	0.440
		Full model: r^2=0.937, P=0.009		
(c)				
Intercept	4.742	0.712	6.665	0.007
MGSD	−0.016	0.004	−4.402	0.022
SNI	−0.043	0.136	−0.312	0.775
Soil N	−35.49	16.56	−2.142	0.122
		Full model: r^2=0.923, P=0.013		

relationship between SRL and SNI, none of the potential nutrient acquisition traits examined (measured in the common garden plants) correlated with the nutrient status of the field-sampled soils (Table 4). Also, except for RMR at 57 and 64 d, none of the nutrient acquisition traits was positively associated with any of the life history traits (Table 6), contradicting the notion that their variability is related to meeting the greater nutritional costs of an annual life history. However, since the rapidly-developing northern populations of *E. calycina* exhibited a disproportionate increase in root mass immediately prior to the onset of flowering, RMR at 57 d and at 64 d were strongly and positively related to both RGR and inflorescence dry mass at 115 d (Table 6).

Table 6 Pearson product–moment correlations describing the associations between nutrient acquisition traits and traits describing variation in reproductive investment and maturation rate for *Ehrharta calycina*. Bold type indicates correlations that were significant (α=0.05) prior to correction using false discovery rates, while asterisks indicate correlations that remained significant with application of this correction

Nutrient acquisition trait	Reproductive maturation/investment trait		
	Inflorescence dry mass at 115 d	Relative growth rate (RGR)	Inflorescence mass ratio (IMR)
Root mass ratio (RMR) at 43 d	*r*=0.152	*r*=0.231	*r*=0.653
	P=0.720	*P*=0.583	*P*=0.080
RMR at 50 d	*r*=0.195	*r*=0.212	*r*=0.321
	P=0.643	*P*=0.614	*P*=0.439
RMR at 57 d	***r*=0.921**	***r*=0.873**	*r*=0.644
	P*=0.001	***P*=0.005***	*P*=0.085
RMR at 64 d	***r*=0.864**	***r*=0.965**	***r*=0.802**
	P*=0.006	***P*=0.001***	***P*=0.017**
Specific root length (SRL)	***r*=−0.795**	*r*=−0.602	*r*=−0.660
	***P*=0.018**	*P*=0.114	*P*=0.075
Transpiration rate (E)	*r*=−0.288	*r*=−0.453	***r*=−0.713**
	P=0.489	*P*=0.260	***P*=0.047**

Correlates of life history in *Ehrharta* and *Pentameris*

Of the 20 *Ehrharta* species sampled, four were annual, 15 perennial and two, *E. calycina* and *E. erecta*, polymorphic with respect to life history (Appendix 1). The corresponding figures for *Pentameris* were ten annual and 49 perennial. Considered across both genera, and treating *E. calycina* and *E. erecta* as functionally perennial, life history showed clear associations with both climatic variables. Annual species were associated with environments having lower species mean MAP (400.9±32.4 mm, mean±SE) and MGSD (114.5±8.5 d) than those occupied by perennials (MAP=716.4±33.3 mm; MGSD=204.3±6.7 d), both differences being significant whether the comparisons were done using species values (MAP: t_{77}=4.452, P<0.001; MGSD: t_{77}=6.213, P<0.001) or PICs (MAP: t_7=4.292, P=0.002; MGSD: t_7=9.721, P<0.001). Identical patterns of significance were obtained when 0.1 and 0.9 quantile values were used instead of species means (data not shown). Life history variation was also significantly associated with substrate, with annuals being under- and over-represented on quartzitic and non-quartzitic substrates, respectively (Table 7a), and the evolution of annuality being more frequently associated with non-quartzitic branches than expected on the basis of chance (Table 7b). None of these results were affected by scoring *E. calycina* and *E. erecta* as annual (data not shown).

A strong correlation between MAP and MGSD (r=0.835, P<0.001) and strong associations of substrate with both MAP (t_{77}=4.156, P<0.001) and MGSD (t_{77}=7.191, P<0.001) necessitated analyses in which the effects of all three variables and their interactions, as predictors of life history, were jointly assessed. Stepwise simplification of GLMs identified most interaction terms to be non-significant, their exclusion being justified by comparisons (Akaike information criterion, AIC) of model optimality. The sole exception was an interaction between MGSD and MAP which was retained in the optimal model when *E. calycina* and *E. erecta* were scored as perennial (Table 8a). Regardless of how these two species were scored, both GLM analyses identified as optimal models containing substrate plus one or both climatic variables, the coefficients of both substrate and at least one climatic variable being significant or very nearly significant in each case (Table 8a, b). These results indicate the joint importance of substrate and climate as predictors of life history in *Ehrharta* and *Pentameris*.

Table 7 Associations of (a) life history and substrate preference amongst Cape species of *Ehrharta* and *Pentameris*, and (b) phylogenetically-inferred life history shifts (starting from a perennial state) and reconstructed substrate preference on branches in a phylogeny of Cape *Ehrharta* and *Pentameris* species (Sillén-Tullberg 1993). Numbers outside parentheses are the observed numbers of species (a) or life history shifts (b) associated predominantly with quartzitic, Cape Supergroup sands or with other substrates (including soils derived from shale, granite and calcrete parent material, as well as Quaternary sands). Numbers inside parentheses are the numbers of species or life history shifts in each category that would be expected on the basis of chance. The Fisher contingency test identifies both associations as highly significant (P<0.001).

Substrate	Life history/life history shift		Totals
	Perennial/ Perennial to perennial	Annual/ Perennial to annual	
a)			
Quartzitic sands	49 (42.4)	1 (7.6)	50
Other substrates	18 (24.6)	11 (4.4)	29
Totals	67	12	79
b)			
Quartzitic sands	106 (101.0)	1 (6.0)	107
Other substrates	24 (33.0)	7 (2.0)	35
Totals	134	8	142

Discussion

Our common garden experiment revealed substantial genotypic variation amongst populations of *E. calycina* in life history traits, notably those describing differences in biomass allocation (LMR, SLA) and the rates of growth and reproductive maturation (RGR, inflorescence dry mass at 115 d). In the context of a seasonally-arid system, in which the window for growth and flowering is temporally limited, this variation almost certainly reflects population-level differences in the potential of plants to function successfully as annuals. Also, since this variation is closely correlated with climatic gradients across the range of the species, it is probably adaptive, reflecting adaptation to local climatic conditions. Although our data provide little evidence to show that life history trait variation has been directed by substrate properties within *E. calycina*, a comparison of multiple Cape grass species identifies climate and substrate type as significant co-predictors of life history variation. These results are not contradictory, however, since the suites of substrates

Table 8 Optimal (lowest AIC score) generalized linear models fitting life history (annual versus perennial) as a function of moisture growing season duration (MGSD), mean annual precipitation (MAP) and substrate type (quartzitic, Cape Supergroup sands versus other substrates, the latter including soils derived from shale, granite and calcrete parent material, as well as Quaternary sands), across all Cape species of *Ehrharta* and *Pentameris* (n=79), with *E. calyina* and *E. erecta* scored as (a) perennial, and (b) annual. The relationship was defined using the logit link function because the response variable (life history) is binary

Parameters	Estimate	SE	z	P (>|z|)
(a)				
Intercept	−20.13	10.59	−1.901	0.057
MGSD	0.153	0.080	1.900	0.057
MAP	0.067	0.035	1.889	0.059
Substrate	3.232	1.519	2.128	0.033
MGSD * MAP	−0.001	<0.001	−2.068	0.039
(b)				
Intercept	2.346	2.102	1.116	0.264
MAP	−0.035	0.013	−2.807	0.005
Substrate	2.346	1.142	2.055	0.040

involved in the two comparisons are different. Where the significance of substrate in the multi-species comparison reflects the general absence of annuals from the ultra-oligotrophic, quartzitic (Cape supergroup) soils which dominate the Cape mountains, the lack of a substrate effect amongst populations of *E. calycina* reflects this species' general avoidance of these substrates.

On the evidence of field-based estimates of rhizome survivorship, *E. calycina* varies from functionally-perennial at the southern end of its range in the CFR (typically 100% rhizome survivorship; Table 3, Fig. 3a) to functionally-annual at the northern end of its range (0–50% rhizome survivorship; Table 3, Fig. 3a). A strong relationship with MGSD suggests that differential rhizome survivorship is dictated by the duration and, possibly, the intensity of seasonal aridity, with *E. calycina* rhizomes apparently being incapable of surviving seasonal drought periods of 250 d or more. Unfortunately, since we did not test whether variation in rhizome mortality was expressed in a common garden, it remains unknown whether it is intrinsically as opposed to environmentally (i.e. phenotypically plastic) determined. Also, because we surveyed rhizome survivorship in only one season, we have little idea how it might vary between years. Regardless, our data indicate that field-based variation in rhizome mortality is associated with genotypically-based (determined in a common garden) differences in flowering behaviour which likely influence the ability of plants to produce seed before the end of the moist season and, thus, to function successfully as annuals.

Echoing the results of earlier studies (Del Pozo et al. 2002; Hall and Willis 2006; Franks et al. 2007; Van Kleunen 2007; Volis 2007), our data identify early flowering as a key target of selection in environments in which the growing season is short and the duration and adversity of the non-growing period sufficient to compromise perennation. Both MGSD and rhizome survivorship showed remarkably strong negative relationships with traits describing the rate of reproductive maturation (e.g. Table 4, Fig. 3b). For example, whereas a high proportion of *E. calycina* plants sampled from the driest, northernmost sites flowered within 4 months of germination in a common garden, none of those from the moist southern sites did. We deduce that the latter either do not flower in their first season of growth or that, as in *Mimulus guttatus* (Hall and Willis 2006), flowering is delayed until late in their first year, this being enabled by longer periods of moisture availability. Moreover, supporting the idea that rapid growth is necessary to ensure early flowering and seed set (Arendt 1997) because plants need to attain a threshold size before they can flower (Weiner 1988; Schmid et al. 1995), reproductive maturation in common garden plants of *E. calycina* was strongly correlated with RGR. Growth rate, in turn, appeared to be strongly influenced by SLA, corroborating the importance of leaf construction in powering growth in herbaceous plants (Poorter and Remkes 1990; Poorter and Van der Werf 1998). The more annual nature of the northernmost populations of *E. calycina* was also reflected in their seed attributes. Like many other annuals (Guo et al. 1999), plants in these populations generally produce large numbers (G. A. Verboom, personal observation) of small diaspores, resulting in diaspore mass being positively associated with rhizome survivorship and negatively associated with RGR and the rate of reproductive maturation (Table 4). The fundamental seed-dependency of annuals is thought to favour the production of numerous, small seeds because it promotes vagility and occupancy of suitable sites, improves seed longevity, reduces rates of seed predation, and offers more scope for risk-

spreading in a spatiotemporally heterogeneous environment (Greene and Johnson 1993; Ehrlen and Eriksson 2000; Guo et al. 2000).

While our data demonstrate the powerful role of climate in directing the evolution of life history traits in *E. calycina*, they provide little indication of a role for soil nutrients. Although reproductive allocation (IMR) was positively associated with SNI, and plant maturation rates were highest in high-SNI sites (especially sites A, B and C), traits describing reproductive maturation (RGR, inflorescence dry mass at 115 d) were uncorrelated with SNI overall and, contrary to expectation, negatively correlated with soil N (Table 4). Moreover, neither SNI nor soil N explained any population-level differences in these traits which were not already accounted for by MGSD (Table 5). Within *E. calycina*, therefore, the incidence of annuality and its associated life-history attributes appears to be dictated predominantly by climate. However, since *E. calycina* largely avoids the nutrient-deficient quartzitic soils (derived from Cape Supergroup quartzites) that underlie much of the CFR, being restricted to richer substrates, these results do not negate the broader potential importance of substrate as a determinant of the distribution and evolution of annuality in the CFR (Verboom et al. 2004). To the contrary, GLMs applied to all Cape species of *Ehrharta* and *Pentameris* consistently identified substrate as a significant co-predictor of life history (Table 8), annuals being associated with low MAP and/or low MGSD, and being significantly under-represented on quartzitic substrates. Although the differential exploitation of fertile and infertile substrates for agriculture might influence species' distributions, thereby biasing the niche characterizations that underpin this analysis, it seems unlikely that this would generate a spurious association between substrate fertility and life history. Certainly, such a bias cannot explain the general absence of annuals from quartzitic substrates, which are largely untransformed. Significantly, the only native annual grass occurring predominantly on these substrates (*P. triseta*) is a strict post-fire ephemeral (Linder and Ellis 1990b) whose growth and reproductive activities coincide with post-fire flushes in N, P and possibly other nutrients (Brown and Mitchell 1986; Stock and Lewis 1986).

The almost-complete avoidance of nutrient-deficient, quartzitic substrates by annual Cape grasses implies that annuality is incompatible with the development of adaptations enabling plants to extract nutrients from such substrates. Consistent with this idea, traits linked to early and/or profuse flowering in *E. calycina* did not, for the most part, associate positively with potential nutrient acquisition traits (Table 6), though this pattern may also reflect this species' general association with richer soils. Of the traits examined, only a late pulse of root formation in faster-developing *E. calycina* plants is interpretable as an adaptation for enhanced nutrient uptake, though it might also be interpreted as an adaptation for improved water uptake. Since the availabilities of water and nutrients are linked (Henkin et al. 1998; Sardans and Peñuelas 2004), however, this may be a false distinction. Coming late in the flowering season and immediately prior to the onset of flowering and fruiting, we interpret the late pulse of root development in fast-developing *E. calycina* plants as a case of allocation being adjusted to match changing resource requirements (Bloom et al. 1985). Specifically, whereas the need to grow rapidly during early development necessitates high investment in leaves, the onset of flowering and seed-filling increases the demand for below-ground resources. Unfortunately, whether shifts of this type are a general feature of annual grasses, particularly in those from semi-arid systems, remains unclear since most studies have focussed on a much earlier developmental window (usually within the first 30 d post-germination: e.g. Poorter and Remkes 1990; Garnier 1992; Atkin et al. 1996; Villar et al. 1998).

Although the analyses presented here focus exclusively on Poaceae, there is some evidence to suggest that the association of annuality with conditions of high fertility is general. Most significant, perhaps, is a striking correspondence between the sets of lineages which contribute most to the annual flora of the CFR (euasterids [57% of CFR annuals], especially Scrophulariaceae [24%] and Asteraceae [18%]; Poales [15%], especially Poaceae [11%] and non-schoenoid Cyperaceae [4%]; Caryophyllales [10%]; Brassicaceae [6%]; papilionoid Fabaceae [3%] and Crassulaceae [3%]; data from Goldblatt and Manning 2000) and which, in the context of Mediterranean-type floras worldwide, reflect exceptionally high foliar P concentrations (Caryophyllales, core papilionoid Fabaceae; euasterids, non-schoenoid Cyperaceae, Malpighiales, Malvales, Poaceae and Rosales: Stock and Verboom 2012). Whether foliar nutrient concentrations are environmentally or genetically determined, the association of annuality with high foliar P implies that this life

history has generally evolved in lineages that associate with fertile substrates.

Conclusion

Our data suggest that the evolution and distribution of life history variation in *E. calycina* is dictated by climate but not soil nutrient status, the latter result probably reflecting this species' consistent association with richer substrates. Importantly, analyses comparing a broader array of grass species, including those inhabiting the full spectrum of substrates in the CFR, identify both climate and substrate as significant determinants of annual evolution and distribution. Whilst the importance of climate as a driver of annual evolution is beyond dispute, the role of substrate fertility has hitherto been under-appreciated.

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