Phylogenetic and functional growth form diversification in the Cape grass genus *Ehrharta* Thunb.

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Presented for the degree of Doctor of Philosophy
in the Department of Botany, University of Cape Town

June 2000

Abstract

This thesis uses phylogenetic and comparative data to test an hypothesis of adaptive radiation in the Cape grass genus *Ehrharta* Thunb. sensu stricto.

Morphological data and sequence data from two noncoding regions of DNA (ITS1 and trnL-F) are used to produce a phylogenetic hypothesis for the tribe Ehrharteae. Combined analysis of these data sets resolves four principal clades that approximate the genera *Ehrharta* s. s., *Microlaena*, *Tetrarrhena* and *Zotovia* and this result thus supports a four-genus classification. Poor resolution and a reduction in branch length at the base of a clade nested within *Ehrharta* s. s. suggests past radiation.

Parsimony-based reconstruction of ancestral habitats and growth form attributes indicates that such radiation is associated with a historical transition to seasonally-drier but more fertile habitats, and the coincident or subsequent evolution of several growth form novelties (e.g. buried and swollen culm bases and annualness). These traits are interpreted to reflect divergent strategies for surviving seasonal drought (i.e. via seed or storage). Much higher transpiration rates in summer-deciduous leaves than in perennating culms of two species suggest that the evolution of summer-deciduous foliage was important in the occupation of seasonally-arid habitats.

Controlled growth experiments are used to test the hypothesis that divergence in persistence traits is associated with differences in seedling biomass allocation and relative growth rate (RGR). *Ehrharta* s. s. shows wide variation in seedling RGR and regressions based on phylogenetically independent contrasts suggest that differences are better explained by early biomass allocation than leaf area indices. Species with a high allocation to leaves grow faster and flower sooner, so these traits are typical of seeding species.

Experimental data plus a comparison of species' soil-preferences suggest that high RGR's are sustainable only in comparatively fertile habitats. Thus resource-limited systems favour low RGR-species that invest more heavily in vegetative persistence (e.g. storage). In addition, experimental and field data show that species from resource-limited habitats capitalize on increased resource availability after fire. Data

from a defoliation/ fertilization experiment indicate that apparent fire-stimulated flowering in *E. capensis* is a response to ameliorated growth conditions rather than a strict fire cue.

In conclusion, this study supports the hypothesis that *Ehrharta* s. s. has undergone adaptive radiation in summer-arid habitats at the Cape, presumably following the inception of a summer-arid climate in the late Tertiary. Parallel studies on other taxa are needed to determine whether such radiation is a general feature of the Cape flora.

Contents

Acknowledgements

The financial assistance of the **National Research Foundation** towards this research is hereby acknowledged. Opinions expressed in this work, or conclusions arrived at, are those of the author and are not necessarily to be attributed to the National Research Foundation.

In addition, I wish to thank the following:

- **Peter Linder** and **Willy Stock** for supervision and advice, and for comment on various draft of the manuscript.
- **Nicola Bergh** and my parents, **Annelies Davies** and **Nico Verboom**, for support and encouragement throughout the course of the study. Nicola Bergh also commented on and proof-read the final draft of this thesis.
- The **Department of Botany** at the University of Cape Town (UCT) for logistical support, and for providing employment at the end of the study when funds ran dry.
- The staff at the **Bolus Herbarium (BOL)** at UCT for their encouragement and willingness to accommodate me at all times
- **Melissa Stander** for instruction in DNA isolation, PCR-amplification and DNA purification. **Julie Hawkins** and **Pia Eldenäs** also provided useful advice in this area.
- Various technical staff, especially **Gonzalo Aguilar**, **Des Barnes**, **Henry Botha** and **Joey Jacobs** for their assistance.
- Numerous colleagues, some paid and some unpaid, for assistance and accompaniment in the field: **Ronwyn Brereton-Stiles**, **Peter Carrick**, **Titus Dlamini**, **Allan Ellis**, **Graham Ellis**, **Julie Hawkins**, **Sioban Munro**, **Terry Wilson**.
- **Chris and Carol Martens** and **Pierre and Lisa van den Berg** for hospitality and for facilitating collection work in the Langeberg Conservancy (Grootvadersbosch).
- **Mike Crisp** and **Don Edward** for hospitality and for facilitating collection work in Victoria and Western Australia, respectively.
- **Willie Mostert** for the use of his farm Weltevreden (Paardenberg) as an experimental site.
- **Anthony Hitchcock** of the propagation unit at the National Botanic Institute (Kirstenbosch) for advice on seed treatment and germination, and for the provision of fungicide.

- **Guy Midgley** of the Stress Ecology Unit at the National Botanic Institute (Kirstenbosch) for use of their leaf area meter.

- **Eric Harley** and **Colleen O' Ryan** of the Departments of Chemical Pathology and Biochemistry at UCT for the use of their labs.

- **Wal Whalley** for information describing the habitat and biology of *Microlaena stipoides*.

- **Warwick Harris** and **Henry Connor** for pickled material and silica dried leaf material of *M. stipoides* and *Petriella colensoi*. Henry Connor also shared his knowledge of the Ehrharteae.

- Various herbaria for the loan of material and/ or provision of data: **L** (specimens); **MELB** (data), **NSW** (data, specimens), **P** (specimens); **PRE** (data, specimens, slides) and **WELT** (specimens).

- **Royal Botanic Gardens Kew** for total DNA of *M. stipoides*.

- **Roger Ellis** for permission to use his anatomical preparations.

- Various government agencies for permission to collect plant material required for study. These include the **Cape Department of Nature Conservation**, the **Western Australia Department of Conservation and Land Management**, **State Forests of New South Wales** and the **Victoria Department of Natural Resources and Environment**.

Chapter 1. General introduction

With 24 species, *Ehrharta* Thunb. sensu stricto (hereafter *Ehrharta* s. s., which excludes *Microlaena* R. Br., *Tetrarrhena* R. Br. and *Zotovia* Edgar and Connor) is the largest genus in the Ehrharteae Nevski (Table 1.1), a tribe with a presumed Gondwanan distribution (Fig. 1.1: Linder 1989; Gibbs Russell and Ellis 1987; Linder et al. 1992; Crisp et al. 1999). The bulk (22) of *Ehrharta* species are endemic to southern Africa (Table. 1.1) and, in particular, the winter- and all year-rainfall region of the western Cape (Fig. 1.2), and this area thus emerges as the centre of diversity of the tribe (Fig. 1.1). In order to account for the concentration of ehrharteoid species diversity in the Cape, Clayton and Renvoize (1986) postulated that the group has undergone 'a bout of speciation following adaptation to the winter rainfall regime' of the region, thereby invoking an evolutionary radiation scenario. That *Ehrharta* s. s. also shows remarkable growth form and habitat diversity (Gibbs Russell and Ellis 1987; Linder 1989; Linder and Ellis 1990) suggests that putative radiation may be

TABLE 1.1. Traditional generic delimitation of Ehrharteae, indicating the natural distributions of the species

Genus	Species and distributions
Ehrharta Thunb. (24 species)	Southern Africa: *E. barbinodis* Nees, *E. brevifolia* Schrad., *E. bulbosa* J. E. Sm., *E. calycina* J. E. Sm., *E. capensis* Thunb., *E. delicatula* Stapf, *E. dura* Nees, *E. eburnea* Gibbs Russell, *E. longiflora* J. E. Sm., *E. longifolia* Schrad., *E. longigluma* C. E. Hubb., *E. melicoides* Thunb., *E. microlaena* Nees, *E. ottonis* Kunth, *E. pusilla* Nees, *E. ramosa* Thunb., *E. rehmannii* Stapf, *E. rupestris* Nees, *E. setacea* Nees, *E. thunbergii* Gibbs Russell, *E. triandra* Nees, *E. villosa* Schult. f. Southern and East Africa, and Madagascar: *E. erecta* Lam. Reunion: *E. avenacea* Willd. ex Schult. & Schult.
Microlaena R. Br. (4 species)	New Guinea, New Zealand, Fiji and Tahiti: *M. avenacea* (Raoul) Hook. f. [incl. *M. carsei* Cheeseman] New Zealand: *M. polynoda* (Hook. f) Hook. f. Australia, Malesia, New Guinea, New Zealand and Philippines: *M. stipoides* (Labill.) R. Br. Australia: *M. tasmanica* Hook. f.
Tetrarrhena R. Br. (6 species)	Australia: *T. acuminata* R. Br., *T. distichophylla* (Labill.) R. Br., *T. juncea* R. Br., *T. laevis* R. Br., *T. turfosa* N. G. Walsh, *T. oreophila* D. I. Morris
Zotovia Edgar and Connor (3 species)	New Zealand: *Z. acicularis* Edgar and Connor, *Z. colensoi* (Hook. f.) Edgar and Connor, *Z. thomsonii* (Petrie) Edgar and Connor

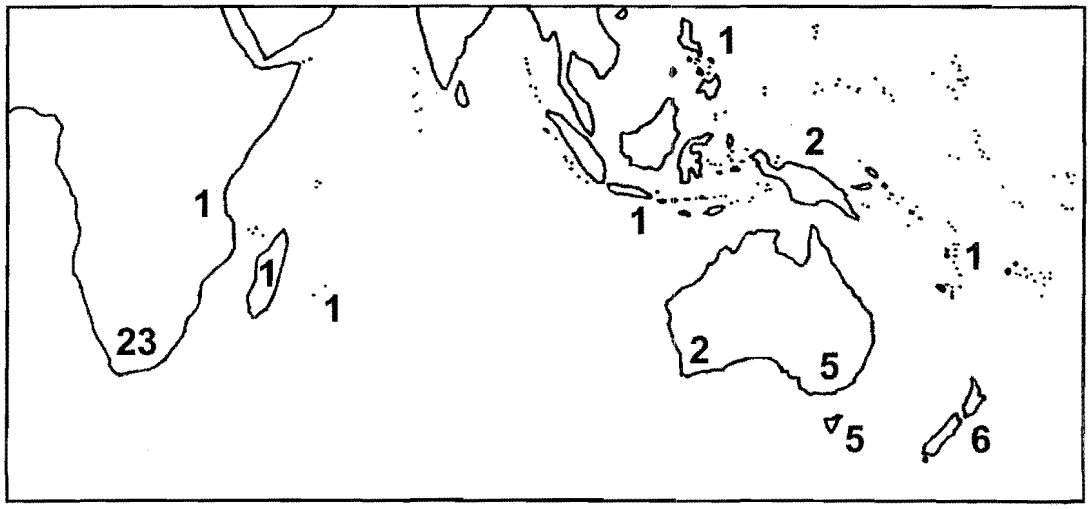


FIGURE 1.1. The natural distribution of species in Ehrharteae, as inferred from herbarium material from BOL, P, PRE, NSW and WELT, specimen data from MELB and literature accounts. Numbers indicate the species totals present on different land masses or in different parts of a single land mass. Note that widespread species may be represented by more than one number.

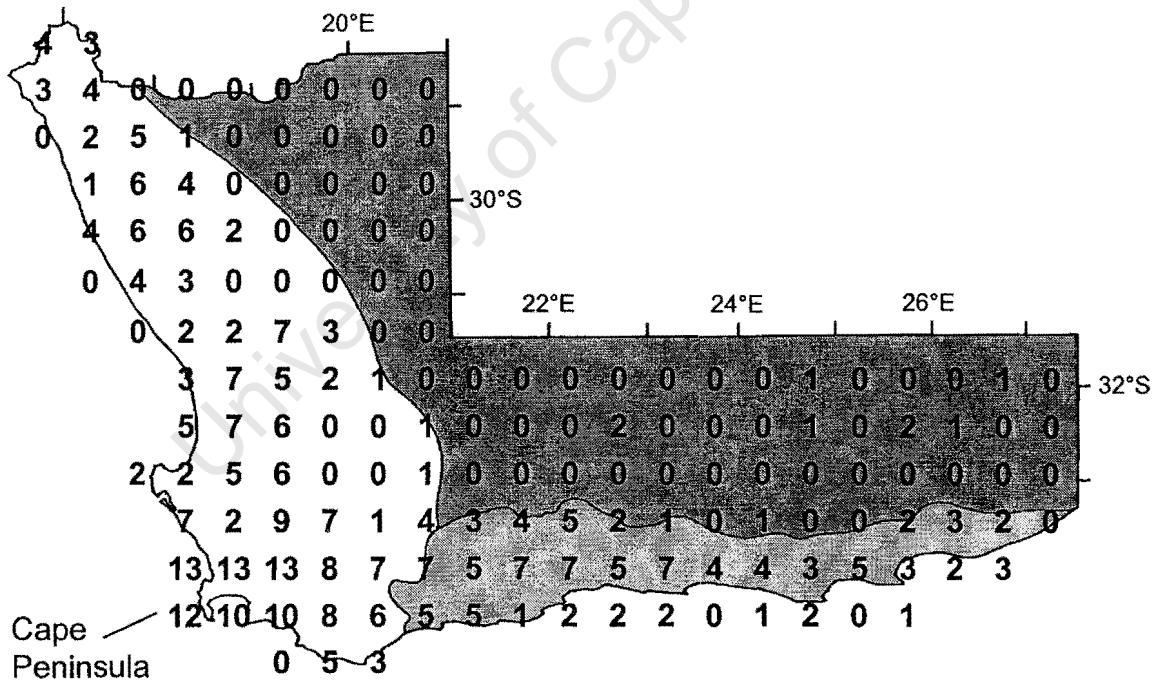


FIGURE 1.2. The natural distribution of species in *Ehrharta* s. s. in the southwestern portion of South Africa, as inferred from material at BOL and that contained in a large, representative sample of material from PRE. Numbers indicate the species totals for each half degree grid square. Areas receiving rainfall predominantly in winter (unshaded), summer (dark grey) and all-year (pale grey) are indicated.

linked to functional divergence. However, the evolution of key traits that characterize these forms remains undescribed and their adaptive significance thus unknown.

The Cape environment and habitat diversity in *Ehrharta* s. s.

A complex geological history (Deacon 1983; Visser 1986; Deacon et al. 1992) involving sedimentation, orogenic folding, erosion and volcanic intrusion underlies the topographic and edaphic diversity of the Cape region (Lambrechts 1979; Ellis and Lambrechts 1986; Schloms et al. 1983; Deacon et al. 1992). This is most marked in the southern portion of the region in which extensive orogenic folding has produced the Cape Fold mountain belt (Fig. 1.3). The sandstones (Cape Supergroup) associated with these mountains are readily leached and weather to produce a sandy, low-pH soil that is critically deficient in plant nutrients, especially phosphorus (Lambrechts 1979; Campbell 1983; Specht and Moll 1983; Deacon et al. 1992; Stock and Allsopp 1992). In contrast, the soils associated with the coastal platform and intermontane valleys as well as the Namaqualand region (Fig. 1.3) are more clayey, being derived from granites (Cape Granite Suite, Namaqualand Complex), shales and mudstones (Malmesbury, Witteberg and Nama Groups). In general, these soils are more fertile and have a higher pH than those derived from sandstone (Campbell 1983; Cowling 1984; Specht and Moll 1983; Deacon et al. 1992; Stock and Allsopp 1992)

The climate of the western Cape region is mediterranean, being characterised by cool, humid winters (July to August) and warm to hot, dry summers (January to March) (Fuggle and Ashton 1979; also see Schulze 1997). Annual rainfall volumes vary considerably, being lowest (less than 200mm) in the extreme north-west and highest in the south (more than 800mm), particularly in the south-east and at higher altitudes (Fig. 1.4). Rainfall seasonality is also variable. While much of the south-east and the high-altitude zone of the Cape Fold mountains experience some rain throughout the year, the area to the north of the Cape Peninsula is for the most part characterized by marked summer aridity (Fig. 1.5; Fuggle and Ashton 1979; also see Schulze 1997).

Rainfall and soil variation are thought to have a strong influence on vegetation type distributions in the Cape region (Kruger 1979; Campbell 1983, 1985; Specht and Moll 1983; Cowling 1984; Cowling et al. 1992). The major vegetation types in the region are fynbos, renosterveld shrubland and succulent karoo scrub. Fynbos, a

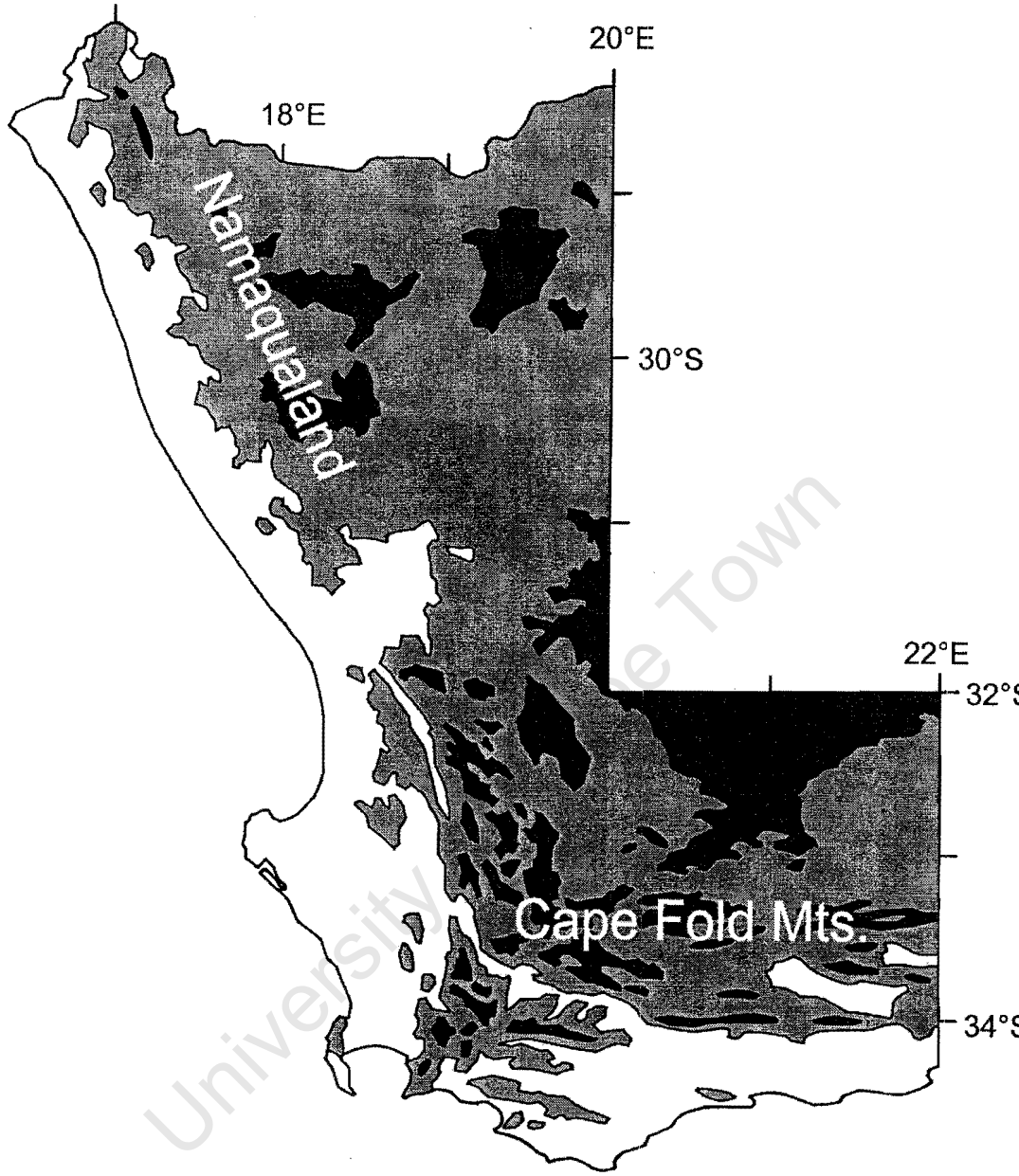


FIGURE 1.3. Altitudinal variation in the western Cape region of South Africa, simplified from Schulze (1997). Areas below 400m (unshaded), 400-1000m (pale grey) and higher than 1000m above sea level are indicated.

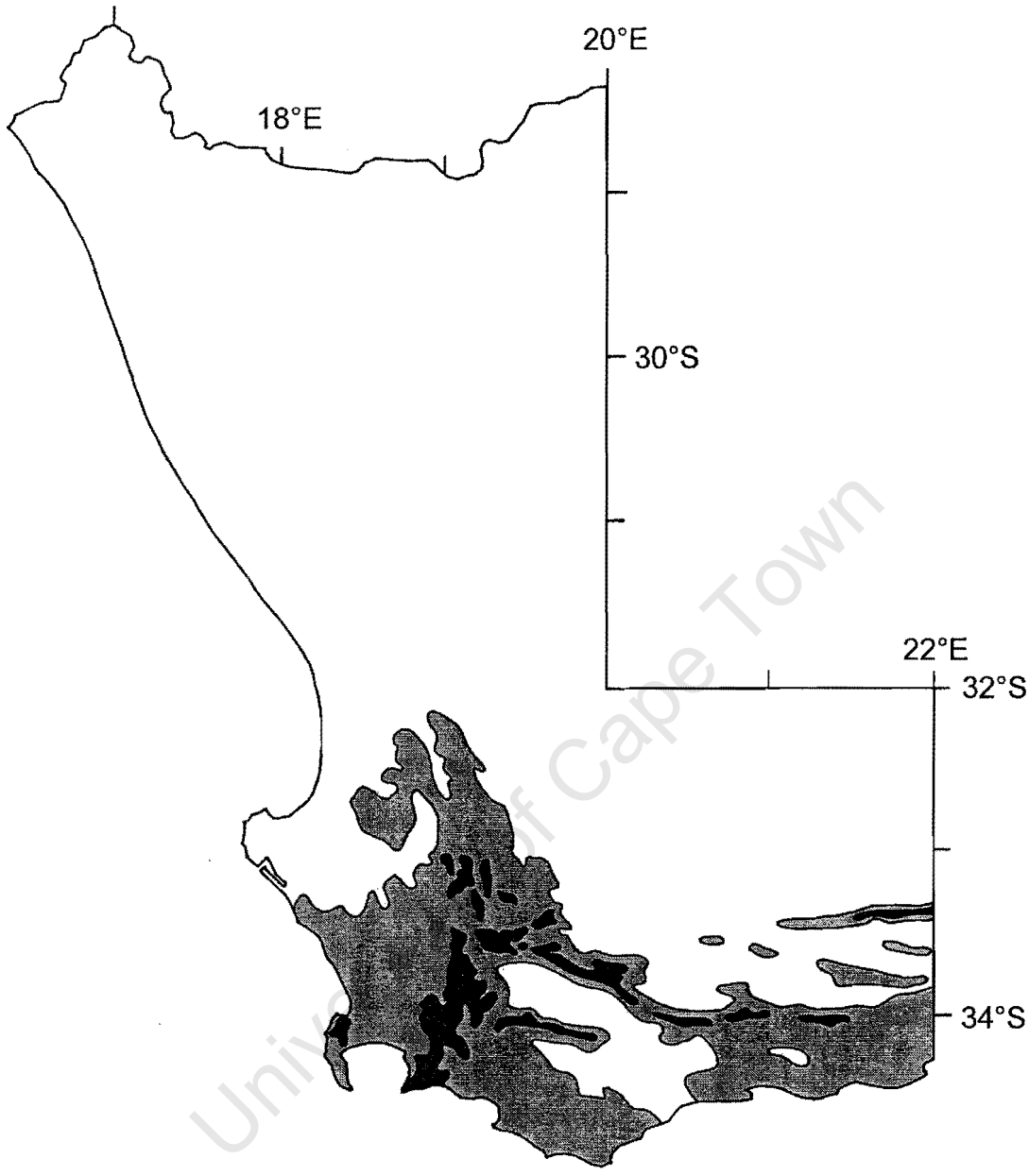


FIGURE 1.4. Distribution of mean annual rainfall in the western Cape region of South Africa, simplified from Schulze (1997). Areas receiving less than 400mm (unshaded), 400-800mm (pale grey) and more than 800mm of rainfall annually are indicated.

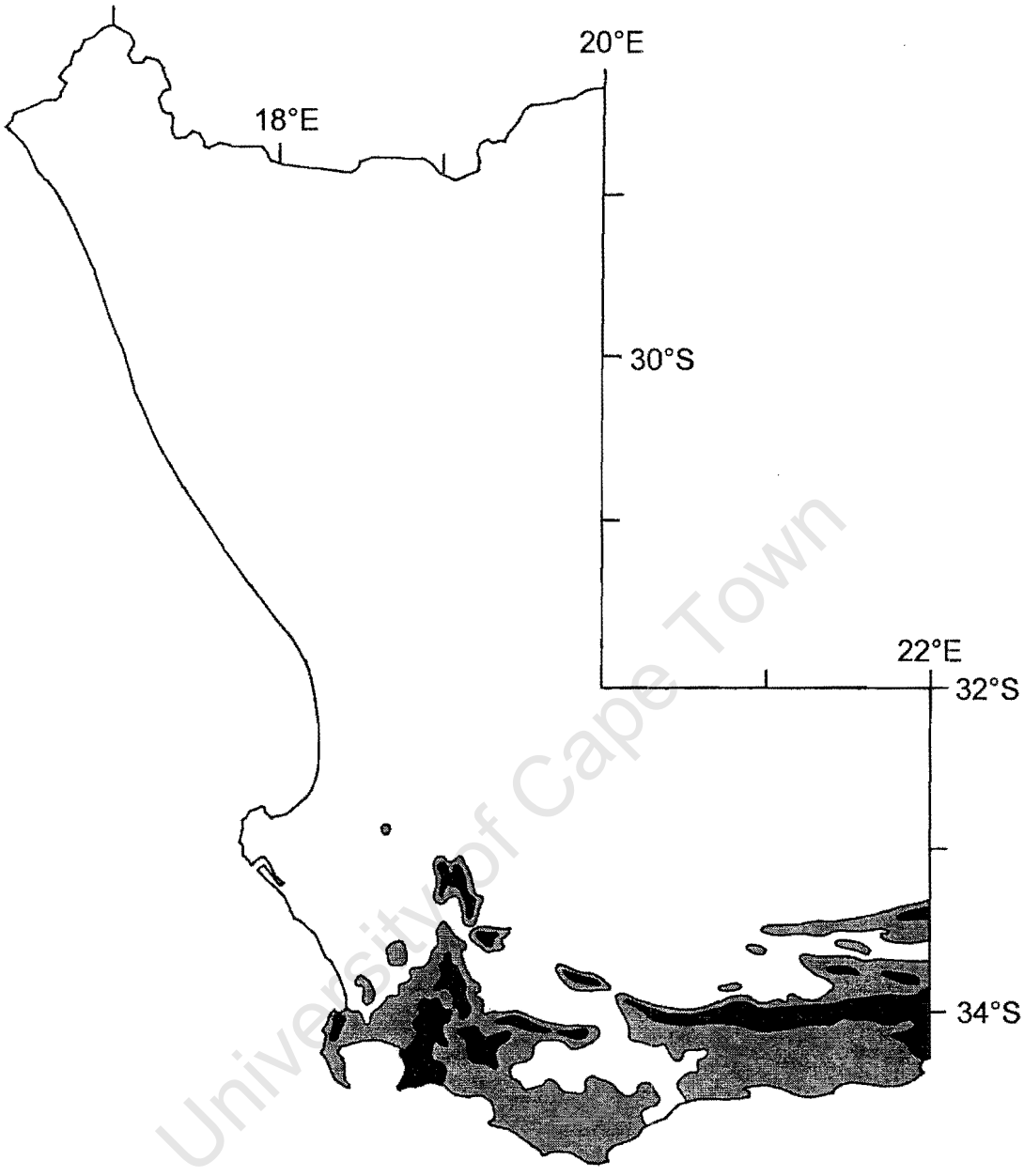


FIGURE 1.5. Distribution of median January rainfall in the western Cape region of South Africa, simplified from Schulze (1997). Areas receiving less than 10mm (unshaded), 10-20mm (pale grey) and more than 20mm of rainfall during January are indicated.

sclerophyllous, heathy shrubland dominated by restioids (wiry, leafless graminoids with photosynthetic culms), is associated with the sandstones of the Cape Fold mountains, while renosterveld shrubland and succulent karoo scrub vegetation occur on more fertile, higher-pH soils. In general, there is a decreasing precipitation gradient from fynbos, through renosterveld, to succulent karoo. Forests and thicket vegetation also occurs in the Cape and is here associated with fertile habitats that are protected from episodic fires which are a feature of fynbos and renosterveld (Kruger 1979; Cowling 1984; Cowling et al. 1992). The grass flora native to all vegetation types in the western Cape is predominantly of the C_3 photosynthetic type, being drawn chiefly from the Danthonieae and Ehrharteae (Linder 1989).

Within the winter- and all-year rainfall region of the western Cape, *Ehrharta* s. s. occupies a diverse array of habitats varying with respect to altitude, substrate, climate and vegetation association. At one extreme, *E. setacea*, *E. rupestris*, *E. dura*, *E. microlaena* and, to a lesser extent, *E. ramosa* and *E. rehmannii* form a distinctive suite of species occupying mid- to high-altitude, mesic, fire-prone fynbos habitats in the Cape Fold mountains (Gibbs Russell 1987a; Gibbs Russell and Ellis 1988; Gibbs Russell 1990). At the other extreme, *E. barbinodis*, *E. brevifolia*, *E. delicatula*, *E. longiflora*, *E. pusilla* and *E. triandra* are centred in the arid but more fertile Namaqualand region (Gibbs Russell 1990) where they are part of the succulent karoo flora. To some extent, the high species diversity in the vicinity of the Cape Peninsula (Fig. 1.2) reflects the overlap of these two suites of species.

Growth form diversity in *Ehrharta* s. s.

Growth form diversity in *Ehrharta* s. s. includes annuals as well as tufted, geophytic and suffrutescent perennials (Fig. 1.6; Table 1.2). Annuals such as *E. delicatula* and *E. pusilla* typically commence growth from seed at the start of the moist, winter period and flower in late winter or spring, set seed and then die at the onset of the dry summer period. In *Ehrharta* s. s., as in other grasses, such species are typically herbaceous with weakly developed bases (Fig. 1.6a). By contrast, all perennial species survive the dry summer period vegetatively although the persistence structures vary. In addition to rhizomes, suffrutescent forms such as as *E. setacea*, *E. ramosa* and *E. barbinodis* possess long-lived culms that persist through the dry season (Fig. 1.6b, c). Because there is subsequent axillary growth from the elevated nodes on these culms, such culms are typically branched and may in some cases assume a restioid appearance (Linder and Ellis 1990). In contrast, the culms of

TABLE 1.2. Categorisation of growth form diversity in *Ehrharta* s. s. (growth form descriptions in text).

Suffrutescent perennial	Geophytic perennial	Tufted perennial	Annual
E. barbinodis	*E. bulbosa*	*E. avenacea*	*E. brevifolia*
E. ramosa	*E. capensis*	*E. calycina*	*E. delicatula*
E. rehmannii	*E. eburnea*	*E. dura*	*E. longiflora*
E. rupestris	*E. longifolia*	*E. erecta*	*E. pusilla*
E. setacea	*E. ottonis*	*E. longigluma*	*E. triandra*
E. thunbergii		*E. melicoides*	
E. villosa		*E. microlaena*	

tufted perennials such as *E. calycina* and *E. melicoides* are short-lived and simple (Fig. 1.6d). In these species only the rhizomes and (sometimes) leaf bases persist through the dry summer period. In geophytic species such as *E. capensis* and *E. eburnea* the lowest internodes of the culms are compressed and laterally swollen to form hard, globose, corm-like structures (Fig. 1.6e) that are buried in the soil and persist through the summer season. Subsequent growth is initiated from axillary buds on these structures, the above-ground culm portion being seasonal.

Thesis objectives and structure

The central goals of this thesis are (i) to test the hypothesis that the high species richness of *Ehrharta* s. s. reflects radiation following adaptation to a rainfall regime characterized by summer aridity (cf. Clayton and Renvoize 1986) and (ii) to test whether the evolution of diverse functional growth forms can be linked to such diversification, thus implying adaptive radiation (Simpson 1953; Futuyma 1986; Grant 1986; Schluter 1996; Givnish 1997). Because both the interpretation of evolutionary diversification (Slowinski and Guyer 1989, 1993; Sanderson and Donoghue 1994, 1996) and the evaluation of trait evolution (e.g. Coddington 1988, 1994; Carpenter 1989; Baum and Larson 1991; Brooks and McLennan 1991; Harvey and Pagel 1991; Pagel 1994; Wenzel and Carpenter 1994; Ackerly and Donoghue 1995; Andersen 1995; Harvey et al. 1995; Rees 1995; Larson and Losos 1996) relies ultimately on phylogenetic pattern, the approach employed by the current study is fundamentally phylogenetic.

No phylogenetic hypothesis exists for *Ehrharta* s. s. and the development of such a hypothesis is, therefore, the principal goal of Chapter 2. Because the validity of generic limits in Ehrharteae is controversial (Willemse 1982; Connor and Edgar 1986; Edgar and Connor 1998) and the monophyly of *Ehrharta* s. s. thus

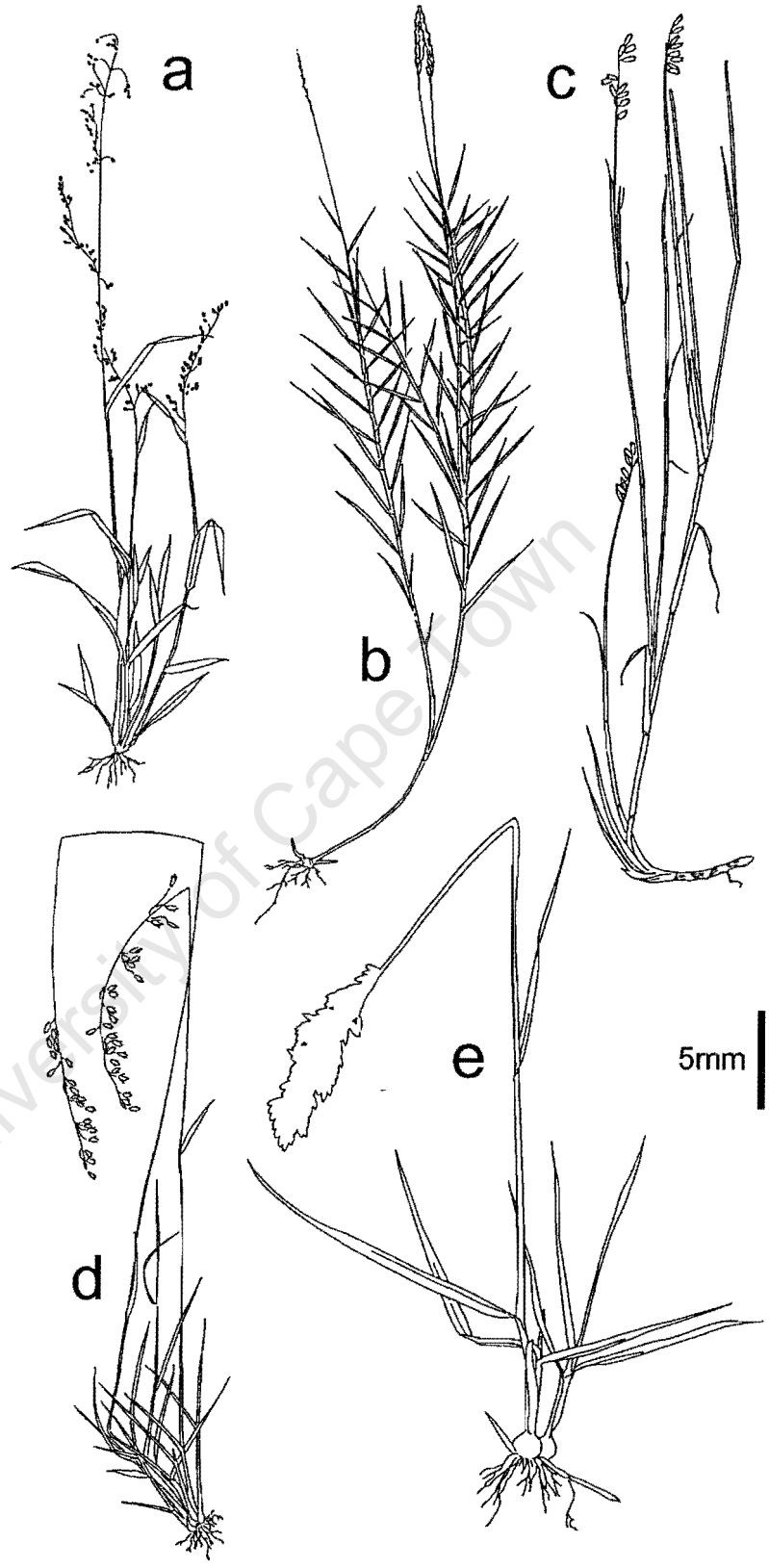


FIGURE 1.6. Growth form variation in *Ehrharta* s. s. *E. delicatula* (a) is annual, *E. setacea* (b) and *E. ramosa* (c) are both suffrutescent, the latter being restioid, *E. calycina* (d) is a tufted perennial and *E. bulbosa* (e) is geophytic.

questionable, this demands consideration of the other genera in the tribe and representatives of these are included in phylogenetic analyses. Using a combination of molecular (ITS1 and trn*L-F* sequences) and morphological data, Chapter 2 sets out (i) to establish a phylogenetic hypothesis for the Ehrharteae, (ii) to document relative character support for individual clades and (iii) to use this information to evaluate generic limits as well as the utility of an infrageneric classification of *Ehrharta* s. s.

In assessing adaptive radiation in *Ehrharta* s. s., Chapter 3 sets out to establish evidence for an increase in speciation rate and to determine the historical events that may have been influential in driving a putative shift in diversification rate. Historical reconstructions of growth form attributes and habitat preferences are used to evaluate the hypothesis that adaptation to a rainfall regime characterized by summer aridity has been influential in triggering rapid diversification in *Ehrharta* s. s. (cf. Clayton and Renvoize 1986). Trait reconstruction is also used to assess whether putative radiation is associated with the evolution of novel growth form attributes and whether these are interpretable as alternative drought survival strategies.

Because functional divergence (particularly with respect to resource utilization) is regarded as an integral aspect of adaptive radiation (Simpson 1953; Futuyma 1986; Grant 1986; Schluter 1996; Givnish 1997), the hypothesis that growth form diversification in *Ehrharta* s. s.reflects such divergence is tested in Chapter 4. In particular, this chapter examines the hypothesis that different allocation strategies associated with alternative growth forms influence early seedling growth and flowering age and thereby determine the regenerative options available to each growth form. The possibility that differences in RGR may be associated with habitats of different fertility (Grime and Hunt 1975; Chapin 1980; Poorter 1989) is also examined.

The penultimate chapter focusses on two species of *Ehrharta* s. s. from fire-prone, fynbos habitats. Because post-fire conditions in resource-limited habitats are known to favour light-loving, fast-growing species (Bond and van Wilgen 1996), many such species show a pulse of flowering, growth, seed release or seed germination immediately after fire. Using data from a field experiment and a field survey, Chapter 5 tests (i) whether *E. ramosa* and *E. capensis* show marked post-fire responses, (ii) whether such responses reflect utilisation of post-fire conditions and (iii) how such responses relate to the particular growth form/ life strategy of either species.

Chapter 2. A phylogenetic analysis of species included in the grass tribe Ehrharteae Nevski

Introduction

Despite substantial attention from Ellis (1987a, b), Gibbs Russell (1984a, b, 1987a, b) and Gibbs Russell and Ellis (1987, 1988), no phylogenetic hypothesis for the endemic African genus *Ehrharta* Thunb. s. s. (excluding *Microlaena* R. Br., *Tetrarrhena* R. Br. and *Zotovia* Edgar and Connor) yet exists. Since a robust phylogeny is fundamental to the interpretation of trait evolution and the evolution of habitat preferences (Lanyon 1993; Losos 1994; Donoghue and Ackerly 1996), the generation of such a phylogeny is central to this thesis and is the focus of this chapter. Since Willemse (1982) could find no justification for distinguishing between *Ehrharta* s. s. and the remaining ehrharteoid genera, *Microlaena* R. Br., *Tetrarrhena* R. Br. and *Zotovia* Edgar and Connor, and Watson and Dallwitz (1992) noted that *Zotovia* (=*Petriella* Zotov) 'seems scarcely distinguishable from *Ehrharta* and *Tetrarrhena*,' any attempt to resolve the phylogeny of *Ehrharta* s. s. must take these other genera into account. Accordingly, this study samples these closely related genera as thoroughly as possible in order to allow the monophyly of *Ehrharta* s. s., as well as that of the remaining ehrharteoid genera to be tested. Because phylogenetic data bear on the delimitation of genera and infrageneric categories in Ehrharteae and *Ehrharta*, respectively, these issues are also addressed here. While the monophyly and relationships of Ehrharteae as a whole are briefly discussed, neither is comprehensively evaluated.

Systematics of Ehrharteae

Discussions of the systematics of Ehrharteae Nevski have focussed on three principal issues: (1) its phylogenetic affinities, (2) its monophyly, and (3) its generic subdivision. These issues are related because, firstly, some knowledge of the tribe's closest relatives is essential for an effective test of its monophyly and, secondly, in the absence of evidence supporting its monophyly, Willemse's (1982) proposal to

TABLE 2.1. Position and generic division of Ehrharteae in the classification of the grass family, as proposed by three recent treatments. Subfamilies are indicated in upper case, supertribes and tribes in lower case, and genera in lower case italics.

Clayton and Renvoize (1986)	Tzvelev (1989)	Watson and Dallwitz (1992, 1994)	GPWG (unpublished)
BAMBUSOIDEAE	BAMBUSOIDEAE	POOIDEAE	ANOMACHLOOIDEAE
Bambuseae	POOIDEAE	BAMBUSOIDEAE	PHAROIDEAE
Anomachloeae	Brachpodieae	Oryzodae	PUELIOIDEAE
Streptochaeteae	Triticeae	Oryzeae	BAMBUSOIDEAE
Olyreae	Bromeae	Olyreae	EHRHARTOIDEAE
Parianeae	Poeae	Centhoceae	Ehrharteae
Phareae	Phleeae	Anomachloeae	Oryzeae
Phaenospermateae	Meliceae	Brachyelytreae	Phyllorachideae
Streptogyneae	Brylkinieae	Diarrheneae	POOIDEAE
Oryzeae	Diarrheneae	Ehrharteae	ARISTIDOIDEAE
Phyllorachideae	Ampelodesmeae	*Ehrharta*	PHRAGMITOIDEAE
Ehrharteae	Stipeae	*Microlaena*	DANTHONIOIDEAE
Ehrharta	Lygeeae	*Petriella*	CENTOTHECOIDEAE
Diarrheneae	Nardeae	*Tetrarrhena*	PANICOIDEAE
Brachyelytreae	Phaenospermateae	Phaenospermateae	CHLORIDOIDEAE
POOIDEAE	Oryzeae	Phyllorachideae	
CENTOTHECOIDEAE	Phyllorachideae	Phareae	
ARUNDINOIDEAE	Ehrharteae	Streptochaeteae	
CHLORIDOIDEAE	*Ehrharta*	Streptogyneae	
PANICOIDEAE	*Microlaena*	Bambusodae	
	Petriella	ARUNDINOIDEAE	
	Tetrarrhena	CHLORIDOIDEAE	
	Centosteceae	PANICOIDEAE	
	Arundineae		
	Thysanolaeneae		
	Micraireae		
	Aristideae		
	Cynodonteae		
	Arundinelleae		
	Isachneae		
	Paniceae		
	Andropogoneae		

include all the Ehrharteae in a single genus seems little better than the traditional route of recognising four genera.

Affinities and monophyly of Ehrharteae

Several early authors treated members of Ehrharteae as belonging to the Phalarideae (e.g. Bentham 1878, Bentham and Hooker 1883, Hackel 1887, Stapf 1900, Bews 1929) presumably due largely to superficial similarities in spikelet structure. Subsequent cytological and embryological studies, however, supported a relationship between Ehrharteae and Oryzeae (Avdulov 1931, Reeder 1957, de Wet 1960) and this relationship has been integrated into most modern treatments of the grass family (Table 2.1: Clayton and Renvoize 1986, Tzvelev 1989; Watson and Dallwitz 1992, 1994; GPWG, unpublished). Tateoka (1963) tabulated a number of anatomical and micromorphological differences between Ehrharteae and Oryzeae (Table 2.2) and on the basis of these suggested that the former 'may be a distinctive

TABLE 2.2. Anatomical and morphological differences between Oryzeae and Ehrharteae as documented by Tateoka (1963).

Feature	Oryzeae	Ehrharteae
Leaf midrib anatomy	Complex, usually with an adaxial-abaxial pair of vascular bundles	Simple, with a single vascular bundle
Leaf chlorenchyma	Usually composed of arm cells	Arms cells completely lacking (but see Ellis 1987a)
Silica bodies	Oryzoid type	Dumbell type to rounded
Glumes	Highly reduced to absent	Always present, usually well developed
Sterile lemmas	Highly reduced	Well developed

group without close relatives.' This perspective is mirrored in treatments of the grass family by Prat (1960) and Watson et al. (1985) which leave Ehrharteae unplaced. Kellogg and Campbell's (1987) morphology-based phylogenetic analysis of the grass family indicated a sister relationship between Ehrharteae and Bambusoideae (including Oryzeae). Both this study and a later study by Kellogg and Watson (1993), however, cast some doubt on evidence for the monophyly of Ehrharteae. Kellogg and Campbell (1987) could suggest no morphological synapomorphies for Ehrharteae, while Kellogg and Watson (1993) found Ehrharteae to form an unresolved residue (neither monophyletic nor positively paraphyletic) at the base of a clade containing, in addition, a woody bambusoid clade and an oryzoid-olyroid clade.

Subsequent phylogenetic analyses using both molecular and morphological data, however, questioned both the topology and rooting of the phylogenies produced by the preceding studies. Separate analyses using variation in morphology plus chloroplast DNA restriction site data (Soreng and Davis 1998) as well as chloroplast DNA sequence data from the gene *ndhF* (Clark et al. 1995) rooted the grass phylogeny robustly on the branch supporting (*Anomochloa* + *Streptochaeta*) and, of greater relevance to the current topic, identified a strongly supported sister relationship between *Ehrharta* and (*Oryza* + *Leersia*) (Fig. 2.1a, b). This arrangement is further supported by a combined analysis of both of these data sets that further includes sequences of five additional loci (Fig. 2.1c: GPWG unpublished). Cummings et al. (1994) similarly identified a sister relationship between *Oryza* and *Microlaena* on the basis of variation in the grass-specific insert of the gene *rpoC2*. However, the omission of additional bambusoid, olyroid and oryzoid grasses from their analyses limits the effectiveness of their data as a test of

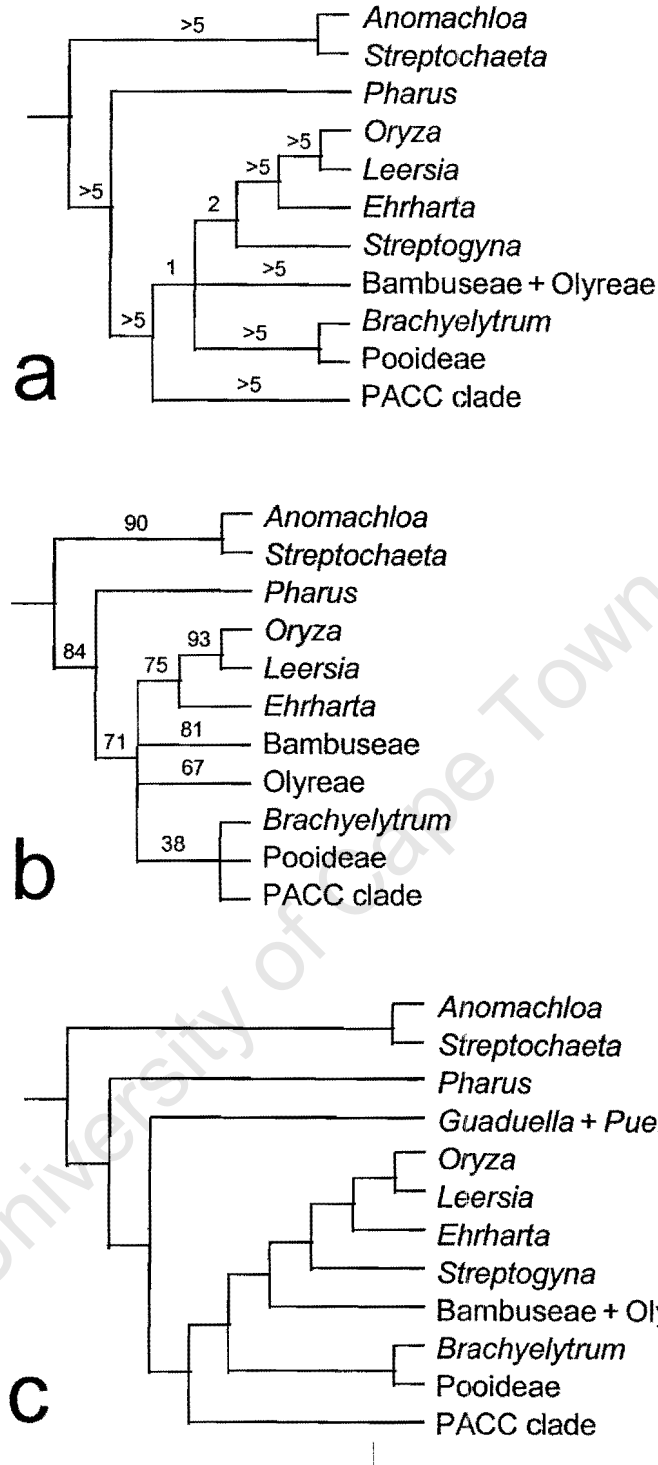


FIGURE 2.1. Recent phylogenetic hypotheses (strict consensus) describing basal relationships in Poaceae, based on (a) ndhF sequence data (Clark et al. 1995), (b) morphology plus chloroplast restriction site data (Soreng and Davis 1998) and (c) these three data sets plus data five additional sequences (GPWG unpublished). Numbers above branches are Bremer supports (a) and bootstrap percentages (b).

the relationship between Ehrharteae and Oryzeae.

Strong phylogenetic evidence for the monophyly of (Ehrharteae + Oryzeae) prompted Soreng and Davis (1998) to propose their combination in a single subfamily, Ehrhartoideae Link (see also GPWG [unpublished]). Relationships within this group, however, remain unclear. While Kellogg and Watson (1993) failed to support the monophyly of Ehrharteae, their most inclusive analysis did support the monophyly of Oryzeae. Clearly, a comprehensive test of the monophyly of these two tribes will be provided only by a phylogenetic analysis that includes a good representation of each. One point seems clear, however: if Ehrharteae is paraphyletic, it is almost certainly so only with respect to the oryzoid grasses. Deciding which of the features in Table 2.2 are synapomorphic for the two tribes must rest on the basal resolution not only of the ehrhartoid clade but of the grass family itself.

Generic taxonomy

Willemse's (1982) próposal to sink all the ehrharteoid grasses into a single genus has been met with some resistance, particularly from workers in Australasia (Watson and Dallwitz 1992; Edgar and Connor 1998) who prefer to distinguish the four genera that have traditionally been included in the tribe. Of these, the largest and oldest is *Ehrharta* s. s. (excluding *Microlaena*, *Tetrarrhena* and *Zotovia*) itself.

Following the publication of a number of Cape ehrharteoids under the name *Ehrharta* Thunb. (Thunberg 1779, 1794; Linnaeus f. 1781; Lamarck 1786; Smith 1790), Brown (1810) erected two new genera, *Microlaena* R. Br. and *Tetrarrhena* R. Br., to accommodate five Australian species, of which two had already been described by Labillardiere (1804) under *Ehrharta*. Subsequent to this, several further Cape species were described by various authors under *Ehrharta*. The first ehrharteoid from New Zealand was described by Raoul (1844) under the generic name *Diplax* Soland. ex Benn., as was a further species from Tasmania by Hooker (1860). Hooker (1864), however, subsequently transferred all three species to *Microlaena*. Hooker (1853) also described a single alpine species from New Zealand under *Ehrharta*, as did Petrie another (1880). Although both of these were later transferred to *Microlaena* (Petrie 1909; Smith 1911), Zotov (1943) removed both alpine species to a new genus, *Petriella* Zotov. This name was later found to be illegitimate (Zotov

1965; Edgar and Connor 1998), prompting Zotov to return the two *Petriella* species to *Microlaena*, from which they have recently been again segregated by Edgar and Connor (1998) under the name *Zotovia*.

In 1982, Willemse, working on the Malesian ehrharteoids, concluded that *Petriella* and *Tetrarrhena* 'could not be delimited satisfactorily against each other, nor against *Ehrharta* and *Microlaena*.' He considered glume size, presence/ absence of a rachilla-process, presence/ absence on corrugation of the sterile lemmas, and presence/ absence of awns on the sterile lemmas too inconsistent to use as generic characters. While he acknowledged the potential value of palea nerve number (two in *Ehrharta* s. s. and *Petriella*; one in *Microlaena* and *Tetrarrhena*) and stamen number (two or four in *Microlaena*, *Petriella* and *Tetrarrhena*, three or six in *Ehrharta* s. s.) as generic delimiters, he argued that lack of correlation with other characters undermined their utility. He therefore recommended fusing all four genera into an expanded *Ehrharta* (sensu lato) and published new combinations, where necessary, for species and infraspecific taxa occurring in Malesia.

While Clayton and Renvoize (1986) accepted Willemse's (1982) changes, Watson and Dallwitz (1992, 1994) did not, although their treatment of the grass family acknowledges a lack of distinctness among the genera (p. 688). Connor and Edgar (1986) refused to accept Willemse's (1982) arguments for an expanded *Ehrharta* due to 'the incompleteness of his study and the simplicity of his approach,' and continued to recognise both *Microlaena* and *Petriella* in their revision of the New Zealand ehrhartoids (Edgar and Connor 1998). However, since no comprehensive cladistic analyses of the Ehrharteae exist, the phylogenetic status of its genera remains unknown.

Relationships within *Ehrharta*

The only recent attempt to establish a series of infrageneric taxa within *Ehrharta* s. s. is that of Gibbs Russell and Ellis (Ellis 1987a, b; Gibbs Russell 1987a, b; Gibbs Russell and Ellis 1987, 1988) who designated seven informal 'species groups' on the basis of spikelet morphological and leaf anatomical characters (Table 2.3). Because no cladistic analysis of *Ehrharta* s. s. has yet been carried out, a rigorous evaluation of the phylogenetic status of these species groups and the apomorphic status of their defining features is lacking.

TABLE 2.3. Membership and distinguishing features of the seven species groups in *Ehrharta* s.s. recognised by Gibbs Russell and Ellis (1987).

Species group	Species	Distinguishing features
Setacea group	*E. rupestris* *E. setacea*	First sterile lemma reduced, glume-like; spikelets fewer than 20; arm cells (sometimes) present in mesophyll
Capensis group	*E. barbinodis* *E. bulbosa* *E. capensis* *E. eburnea* *E. longifolia* *E. ottonis*	Lowest culm node bulbous (except E. barbinodis); second sterile lemma stipitate; spikelets large, hairy at margins
Erecta group	*E. erecta* *E. longiflora* *E. triandra*	Spikelets small; first sterile lemma well-developed; glumes short; sterile lemmas constricted at the base, lacking appendages; lemma sides glabrous; raised abaxial epidermal cells absent; stomata with wax plugs
Calycina group	*E. brevifolia* *E. calycina* *E. delicatula* *E. longigluma* *E. melicoides* *E. pusilla*	Spikelets small; glumes long; first sterile lemma well developed; second sterile lemma base not stipitate, with ear-like appendages; raised mid-intercostal long cells; cubical wax granules associated with stomata
Ramosa group	*E. ramosa* *E. rehmannii*	Spikelets small; sterile lemmas well developed, with tips rounded, sides rough and glabrous, with basal appendages; stomata with distinct rims and no wax deposits
Dura group	*E. dura* *E. microlaena*	Spikelets very large; lemmas glabrous; plants perennial; spikelets awned; tanniferous cells present; stomatal pores obscured by wax platelets
Villosa group	*E. thunbergii* *E. villosa*	Culms over 1m long, suffrutescent; leaf blades reduced, rolled; spikelets very large; lemmas profusely hairy, conspicuously bearded, stipitate, mucronate; stomata with four epidermal flanges

Delimitation of higher taxa

Following the inception of phylogenetic systematics, several workers have argued increasingly against the formal recognition of paraphyletic higher taxa, favouring instead a classification system in which all higher taxa are demonstrably monophyletic (e.g. Funk 1985; Donoghue and Cantino 1988; Humphries and Chappill 1988; de Queiroz and Gauthier 1992, 1994; Schrire and Lewis 1996; Van Welzen 1997; Backlund and Bremer 1998). A phylogenetic system of classification is thought to have three principal benefits (Donoghue and Cantino 1988; Schrire and Lewis 1996). First, because such a classification aims to reflect the one 'true' phylogeny of life, it provides an objective standard against which alternative arrangements can be evaluated and thus accords classification a scientific basis (Humphries and Chappill 1988). Second, a phylogenetic classification, in reflecting

relationships, facilitates the study of evolutionary processes. Finally, the taxonomic distribution of organismal traits may be better anticipated when taxa are defined to be monophyletic, resulting in greater predictivity. In spite of these presumed advantages, several workers have actively resisted monophyly-based classification on the grounds of its inability to convey anagenetic information, the logical inevitability of paraphyletic ancestral species, and the failure of phylogenetics to cope with reticulation (Cronquist 1987; Brummitt 1996, 1997; Sosef 1997). In addition, even if the problem of paraphyletic ancestral species is circumvented, attempts to shoe-horn a monophyly-based classification into the currently-used Linnaean conventions is anticipated to severely disrupt the current taxonomic system, through the introduction of widespread name changes and redundancy. A complete abandonment of the current Linnaean code in favour of a new phylogenetic code (de Queiroz and Gauthier 1992, 1994) has, therefore, received some support (e.g. van Welzen 1997). Pending such a transformation, however, several workers meanwhile advocate maximum adherence to the principle of taxon monophyly (e.g. Schrire and Lewis 1996; Backlund and Bremer 1998), and some examples of phylogenetic classifications now exist (e.g. Linder and Kurzweil 1994; APG 1997). Since the benefits of a phylogenetic system of classification are considerable, the taxonomic recommendations of the present study, with respect to the delimitation of supraspecific taxa in Ehrharteae, adhere to the principle of higher taxon monophyly. In addition, ancillary criteria such as degree of character support, diagnosibility and phylogenetic informativeness may be useful in deciding which monophyletic groups deserve formal recognition (Linder 1991a; Schrire and Lewis 1996; Backlund and Bremer 1998). Linder and Verboom (1996) similarly considered eco-geographical distinctness a useful ancillary criterion for generic delimitation in their treatment of the *Rytidosperma* complex.

Data choice and analytical approach

Although the practice of combining all available data in phylogeny reconstruction is commonly advocated (Kluge 1989; Barrett et al. 1991; Crowe et al. 1992; Eernisse and Kluge 1993; Kluge and Wolf 1993; Chippendale and Wiens 1994; Doyle et al. 1994; Nixon and Carpenter 1996), alternative approaches for coping with multiple, potentially conflicting data sets have been thoroughly debated (de Queiroz et al. 1995; Miyamoto and Fitch 1995; Huelsenbeck et al. 1996). In contrast to the total evidence approach, some authors (Miyamoto 1985; Miyamoto and Fitch 1995) have

argued in favour of analysing data sets separately, on the grounds that these might reflect different phylogenetic histories and/ or evolve differently. After separate phylogeny estimates are produced, consensus methods are used to identify points of agreement. A third approach is that of conditional combinability (Bull et al. 1993; de Queiroz 1993) in which data sets are tested for significant conflicting phylogenetic signal (e.g. Rodrigo et al. 1993; Huelsenbeck et al. 1996; Mason-Gamer and Kellogg 1996) prior to combination. Separate analysis of individual data sets and the evaluation of conflict among them has gained popularity (e.g. Baum et al. 1998; Munro and Linder 1998) because apart from its bearing on the question of data set combinability, such a protocol is heuristically valuable in assessing the relative contribution of each data set to nodal resolution in the combined analysis (Nixon and Carpenter 1996; Gatesy 1999). In assessing the phylogenetic relationships of Ehrharteae, therefore, the present study analyses conflict among the three data sets used, prior to their combination.

In spite of recent objections to the use of morphological data in phylogenetic analysis (Hedges and Maxson 1996; Givnish and Sytsma 1997a, b), this study combines morphological data with two molecular data sets to construct a phylogenetic hypothesis for Ehrharteae. In practice, the combined use of morphological and molecular data in phylogenetic inference often shows remarkable complementarity (e.g. Lafay et al. 1995; Pennington 1996; Eldenäs and Linder, in press) and some authors (e.g. Donoghue and Sanderson 1992; Lee 1997) have emphasised the potential value of morphological characters in phylogeny estimation. Givnish and Sytsma (1997a, b), however, recently argued against the use of morphological data in phylogeny estimation, on the grounds that morphological characters are prone to high levels of homoplasy, and are therefore less reliable for this purpose. In contrast to a total evidence approach, they favoured, instead, studies using molecular data only. In support of their arguments Givnish and Sytsma (1997a) demonstrated significantly lower consistency indices (CI's) for phylogenies based on morphological data than those based on molecular data, something which Sanderson and Donoghue (1989) had earlier failed to do. However, the assumption that the data set having the lowest homoplasy, by dint of its strong phylogenetic signal, best estimates the true organismal phylogeny is flawed. The genealogical histories of individual genes may differ markedly within a single set of organisms (Doyle 1992; Mason-Gamer and Kellogg 1996), indicating that individual gene phylogenies, no matter how

homoplasy-free, may be phylogenetically misleading with respect to the estimation of organismal phylogeny. Because reciprocal comparison among multiple data sets offers the only test of such error, the use of data from as many sources as possible is strongly advocated. Givnish and Sytsma (1997b) acknowledged as much when they noted, albeit fleetingly, that 'some molecular data may be positively misleading' and that, therefore, their conclusions 'should not be read as a blanket endorsement of the use of molecular vs morphological data in phylogenetic reconstruction.'

In plants, molecular phylogenetic inference at or below the generic level presently relies heavily on sequence variation in a small number of noncoding DNA sequences. Most commonly used are the internal transcribed spacer (ITS1 and ITS2) sequences of 18S-26S nrDNA (e.g. Hodges and Arnold 1994; Smith and Klein 1994; Baldwin and Robichaux 1995; Wen and Zimmer 1996; Baum et al. 1998; Xiang et al. 1998), the trnL-F spacer and the rpl16 and trnL intron sequences of cpDNA (e.g. Gielly and Taberlet 1994, 1996; Gielly et al. 1996; Kelchner and Clark 1997; Baum et al. 1998; Small et al. 1998), as well as chloroplast restriction site data (e.g. Sytsma et al. 1990; Donoghue and Sytsma 1993; Givnish et al. 1995, 1997; Pennington 1996; Sakai et al. 1997; Baum et al. 1998). However, because these sources often show limited variation within recently diverged groups, the phylogenetic resolution they offer is poor (Small et al. 1998), and this has prompted a search for additional highly variable sources of sequence data (e.g. Morton et al. 1996; Small et al. 1998). Under these circumstances, the rejection of an independent morphological data set in low level studies remains difficult to justify. Accordingly, this study uses morphological data in conjunction with sequence data from two sources, the nuclear ITS1 region and the chloroplastic trnL-F intergenic spacer, to resolve phylogenetic relationships within Ehrharteae.

Hedges and Maxson (1996) recently argued against the use of morphological characters in phylogeny reconstruction, on the grounds that this invalidates the subsequent use of such phylogenies to study morphological evolution due to the introduction of circular reasoning. This argument is an extension of a principle first articulated by Coddington (1988) that '(cladistic) structure should not be inferred from characters involved in the hypothesis of adaptation (being tested)' as this introduces biases. However, various authors (Deleporte1993; Luckow and Hopkins 1995; Luckow and Bruneau 1997) have argued that character use in phylogeny reconstruction and in evolutionary character interpretation are logically independent.

Thus, the evolution of individual characters may validly be studied using a phylogeny estimated on the basis of a matrix including those characters. Ultimately, the decision to drop certain classes of characters from phylogenetic analysis represents a loss of information and may produce less robust phylogenetic hypotheses (Hopkins and Luckow 1995; Luckow and Bruneau 1997). De Queiroz (1996) has pointed out that analyses of character evolution are biased both by including or excluding characters and suggested that no single approach is uniformly superior. Although specific characters (those under study) may often be justifiably excluded, the exclusion of entire data partitions (e.g. morphological data) simply because they include such characters cannot (de Queiroz 1996). Thus, the inclusion of morphological data in the phylogenetic analysis presented here is fully justified. Nonetheless, because the growth form traits investigated in the following chapter are both few in number and, in most instances, show some intergradation among states, they are excluded from phylogenetic analysis. Thus the final morphological data set relies largely on variation in spikelet morphology and leaf anatomy.

Questions addressed

Besides providing a baseline for the comparative study of growth form and life history evolution (Chapters 3 and 4), the phylogenetic hypothesis developed in the present chapter is here used to evaluate (i) the phylogenetic status of genera in Ehrharteae, and (ii) the appropriateness of establishing formal taxonomic entities within *Ehrharta* s. s., as proposed by Gibbs Russell and Ellis (1987). In particular, two principal questions are addressed. First, are the genera *Ehrharta* s. s., *Microlaena*, *Tetrarrhena* and *Petriella* monophyletic, such that their continued recognition is justified? If not is an alternative generic division possible, or is the single-genus scenario advocated by Willemse (1982) preferable? Second, is *Ehrharta* s. s. divisible into a series of monophyletic infrageneric taxa, whose recognition is of practical value?

Materials and methods

Data collection

Molecular data

Plant samples and DNA extraction

Total DNAs of 27 species (one collection per species) representing all four ehrharteoid genera (Table 2.4) were extracted from 50-100 mg amounts of silica-dried leaf material following the protocol of Doyle and Doyle (1987). Material of most species was collected in the field, except for leaf material of the two New Zealand species which was kindly provided by the Manaaki Whenua Landcare Research Garden. Total isolated DNA of *M. stipoides* was kindly provided by the Royal Botanic Gardens, Kew. Except for these species all DNA samples are represented by specimens housed at the Bolus Herbarium (BOL), University of Cape Town.

DNA amplification and sequencing

Chloroplast trn DNA

The polymerase chain reaction (PCR), using the primers designed by Taberlet et al. (1991), was used to amplify the trnT-L and trnL-F intergenic spacers as well as the trnL intron from the chloroplast genome. All primers used were synthesised by the oligonucleotide synthesis unit at the Department of Biochemistry at the University of Cape Town. A pilot study using four species of *Ehrharta* thought to represent a phylogenetically diverse ingroup sample indicated that the trnT-L intergenic spacer and the trnL intron were insufficiently variable to resolve relationships in the broader study group, and these were therefore not sampled more widely. The trnL-F intergenic spacer showed some variation, however, and amplification products were prepared for all 28 species listed in Table 2.4 using the c and f primers of Taberlet et al. (1991). All of these yielded successful sequencing results.

PCR was performed on a Hybaid PCR Sprint™ thermal cycler. Each 100µl reaction tube (two reactions per sample) was prepared on ice as follows: 77.5µl of sterile water, 10.0µl of 10X Taq polymerase buffer (Bioline), 2.0µl of 50mM $MgCl_2$, 4µl of 5mM dNTP, 1µl of each primer (50µM), 0.5µl (2.5 units) of Taq (Bioline), and 4µl of template (1/10 stock concentration.) Reaction tubes were sealed with two or three

TABLE 2.4. List of molecular vouchers, collection localities, and corresponding sequences obtained.

Species	Voucher	Collection locality	Sequences
Ehrharta barbinodis Nees	Verboom 103	Spektakel Pass, W of Springbok, S. Africa	ITS1, trnL-F
E. brevifolia Schrad.	Verboom 116	Hondeklip Bay, S. Africa	ITS1, trnL-F
E. calycina J. E. Sm.	Verboom 258	Weltevreden Farm, Paardeberg, S. Africa	ITS1, trnL-F
E. capensis Thunb.	Verboom 147	Weltevreden Farm, Paardeberg, S. Africa	trnL-F
E. delicatula Stapf	Verboom 99	Eselsfontein, W of Springbok, S. Africa	ITS1, trnL-F
E. dura Nees	Verboom 183	Bergfontein Farm, Langeberg, S. Africa	trnL-F
E. eburnea Gibbs Russell	Verboom 97	Nieuwoudtville Reserve, Nieuwoudtville, S. Africa	ITS1, trnL-F
E. erecta Lam.	Verboom 84	Devil's Peak Estate, Cape Peninsula, S. Africa	ITS1, trnL-F
E. longiflora J. E. Sm.	Verboom 91	Clanwilliam, S. Africa	ITS1, trnL-F
E. longigluma C. E. Hubb.	Linder 6698	Katse Pass, Lesotho	ITS1, trnL-F
E. melicoides Thunb.	Verboom 155	Gydo Pass, N of Ceres, S. Africa	ITS1, trnL-F
E. ottonis Kunth	Verboom 176	Camps Bay, Cape Peninsula, S. Africa	ITS1, trnL-F
E. pusilla Nees	Verboom 111	Near Wallekraal, E of Hondeklip Bay, S. Africa	trnL-F
E. ramosa Thunb.	Verboom 256	Table Mt., Cape Peninsula, S. Africa	ITS1, trnL-F
E. rehmannii Stapf	Verboom 257	Wemmershoek Dam, S of Paarl, S. Africa	trnL-F
E. rupestris Nees	Verboom 180	Boesmansbos Wilderness Area, Langeberg, S. Africa	ITS1, trnL-F
E. setacea Nees	Verboom 179	Boesmansbos Wilderness Area, Langeberg, S. Africa	ITS1, trnL-F
E. thunbergii Gibbs Russell	Verboom 92	Near Pakhuis Pass, E of Clanwilliam, S. Africa	ITS1, trnL-F
E. triandra Nees	Verboom 101	Spektakel Pass, W of Springbok, S. Africa	ITS1, trnL-F
E. villosa Schult. f.	Verboom 166	Bloubergstrand, N of Cape Town, S. Africa	trnL-F
Microlaena avenacea (Raoul) Hook. f.	MWLRG 69/92[a]	Unknown	ITS1, trnL-F
M. stipoides (Labill.) R. Br.	RBGK 1973-15875[b]	Unknown	trnL-F
Tetrarrhena acuminata R. Br.	Verboom 245	Grampians National Park, Victoria, Australia	ITS1, trnL-F
T. distichophylla (Labill.) R. Br.	Verboom 243	Grampians National Park, Victoria, Australia	ITS1, trnL-F
T. juncea R. Br.	Verboom 247	Grampians National Park, Victoria, Australia	ITS1, trnL-F
T. laevis R. Br.	Verboom 232	Greenmount Forest Reserve, Darlington, WA, Australia	ITS1, trnL-F
T. turfosa N. G. Walsh	Verboom 248	Grampians National Park, Victoria, Australia	ITS1, trnL-F
Zotovia colensoi (Hook. f.) Edgar et Connor	MWLRG 107/90[a]	Unknown	ITS1, trnL-F

[a]MWLRG=Manaaki Whenua Landcare Research Garden
[b]RBGK=Royal Botanic Gardens, Kew

drops of mineral oil to prevent evaporation during cycling, and preheated for three minutes at 95°C prior to cycling. The thermal cycler program was run for 35 cycles, each consisting of 45 seconds at 94°C, 45 seconds at 52°C, and 2 minutes at 72°C. Cycling was followed by a final extension step of 8 minutes at 72°C. Each batch of reaction tubes was accompanied by two negative control reactions to check for contamination.

Prior to purification, amplification products were checked by electrophoresis on 1% agarose minigels (to which ethidium bromide had been added for visualization purposes) in 1× TAE buffer. About 5-10µl of each reaction product was checked in this way, this being sufficient to allow product visualization over an UVA light source. Gel electrophoresis was also used to purify PCR products. Following cold ethanol precipitation and re-elution in 10-15µl TE, reaction products (the products of multiple reactions based on a single sample being pooled) were resolved electrophoretically on 1% agarose gels at low voltage (typically 50V) for 2-3 hours, after which the band containing the desired product was excised using a clean scalpel blade. Product DNA was extracted from the excised gel slice using a Nucleon GX™ gel purification kit (Amersham), using sterile water to elute the DNA. Final DNA concentration in each sample was determined using a Pharmacia GeneQuant™ RNA/DNA calculator.

Nuclear ITS DNA

As for the trn spacers, PCR was used to amplify the ITS1 spacer of the nuclear ribosomal genome. Primers ITS5 (White et al. 1990) and primer ITS2c (Hsiao et al. 1998) were generally used in amplifications, except in *E. setacea* and *E. rupestris* which were amplified using primers ITSL (Hsiao et al. 1994) and ITS2c. The ITS1 region was successfully amplified and sequenced for 22 species listed in Table 2.4.

PCR was performed on a Techne thermal cycling system (PC-5 pump unit, CH-5 chiller and PHC-2 heating block). Typically, each 50µl reaction tube (three reactions per sample) was prepared on ice as follows: 37.75µl of sterile water, 5.0µl of 10X Taq polymerase buffer (Biotaq), 1.0µl of 50mM $MgCl_2$, 2µl of 5mM dNTP, 1µl of each primer (25µM), 0.25µl (2.5 units) of Taq (Biotaq), and 2µl of template (1/10 stock concentration.) Reaction tubes were sealed with mineral oil and preheated for three minutes at 95°C prior to cycling. The thermal cycler program was run for 35 cycles, each consisting of 35 seconds at 93°C, 35 seconds at 49°C, and 2 minutes at 72°C.

Cycling was followed by a final extension step of 7 minutes at 72°C. In *E. setacea* and *E. rupestris* this amplification protocol yielded a product that separated into multiple bands.Therefore, the annealing temperature for these species was raised to 60°C and the amount of $MgCl_2$ per 50µl reduced to 0.5µl, resulting in a product yielding a single-band.

Amplification products were checked electrophoretically as described above, before being pooled for each sample and purified directly using a Qiaquick™ PCR purification kit (Qiaex), using sterile water (pH 8.0) to elute the DNA. Final DNA concentration in each sample was determined as above.

All sequencing was done by the core sequencing facility at the Department of Chemical Pathology at the University of Cape Town, using an ABI 373 Stretch DNA sequencer (P E Biosystems). Trn primers e and f were used to sequence the trnL-F intergenic spacer, and primers ITS5 and ITS2c the ITS1 region.

Sequence alignment

Sequences of four non-ehrharteoid species, plus an ITS1 sequence for *M. stipoides*, were obtained from Genbank (Table 2.5) and used as a reference for sequence alignment. Sequencing products were visualized using Chromas version 1.43 (C. McCarthy, Griffith University), while sequence alignment and editing was performed by eye using DAPSA version 4.04 (Harley 1997). Following initial alignment and editing, all sites that showed any variation among species were checked to verify that such variation was unambiguously supported. All nucleotide ambiguities were conservatively coded as uncertain (IUPAC code 'N'). Because two regions in the ITS1 sequence appeared hypervariable and could not be meaningfully aligned

TABLE 2.5. List of genbank sequences included in analyses, along with source details. The first four are outgroups, the last an ingroup.

Species	Sequence	Authors	Publication	Genbank accession
Aegilops triuncialis	trnL-F	Gielly and Taberlet	Mol. Biol. Evol. 11: 769-777 (1994)	X75712
Aegilops umbellulata	ITS1	Wang et al.	Unpublished	AF149197
Leersia hexandra	ITS1	Hsiao et al.	Unpublished	AF019793
Oryza sativa	trnL-F	Hiratsuka et al.	Mol. Gen. Genet. 217: 185-194 (1989)	X15901
Microlaena stipoides	ITS1	Hsiao et al.	Unpublished	AF019791

between the ingroup and outgroup taxa (nor among outgroup taxa), these were also coded as uncertain for the outgroups. Aligned trnL-F (contained between the e and f spacers of Taberlet et al. [1991]) and ITS1 (contained between the ITS1 and ITS2 primers of White et al. [1990]). spacer sequences are, respectively, 416bp and 385bp long. The two hypervariable regions of the ITS1 sequence that could not be outgroup-aligned comprise a total of 163bp (positions 51-135 and 189-266, counting from the 5' end of the ITSL primer).

Morphological and anatomical data

Study material

The morphology of all species included in the tribe Ehrharteae (except *T. oreophila* D. I. Morris and *Z. acicularis* Edgar and Connor of which no material was seen) plus that of two oryzoid species (*Leersia hexandra* and *Oryza sativa*) was examined from fresh, pickled and herbarium material, the latter provided by the following herbaria: BOL, L, PRE, NSW, WELT. Almost all of the South African and Australian species were seen growing in their natural habitats. R. P. Ellis kindly granted permission to make use of his extensive collection of leaf anatomical preparations (and photographs thereof) representing most species of *Ehrharta*, and this was supplemented with anatomical preparations obtained from field-collected material that had been fixed in FAA (24 hours) and stored in 70% ethanol, or from herbarium material rehydrated in soapy water.

Preparation and observation

Spikelet morphology was observed both directly, through dissection under low power, or by examining, under higher power, dissections of whole spikelets mounted in a solution of fuchsin in water and glycerin. Where appropriate, measurements were made using either a metal ruler with 0.5mm gradations or an eyepiece graticule precise to the nearest 0.1mm.

Transverse sections and abaxial epidermal scrapes were prepared from the mid-portions of basal leaves. Sections were prepared by hand, using a razor blade, under a dissecting microscope, while epidermal scrapes were made according to the method of Metcalfe (1960). All anatomical preparations were stained in a combined

safranin-Alcian blue stain (Tolivia and Tolivia 1987), dehydrated though an alcohol series and mounted in Canada balsam.

Character treatment and coding

Molecular data

Substitutions at all sites were equally weighted, as were all categories of nucleotide substitution. Where alignment gaps (indels) occurred, these were treated as missing data in the nucleotide matrix, and their absence/ presence then coded in a separate binary matrix. This method of coding indels has been advocated by Baldwin et al. (1995) on the grounds that it avoids the danger of overscoring that results from treating indels as a fifth character, and has been employed in several studies (e.g. Baum et al. 1994; Wojciechowski et al. 1999). Inclusion of indel information in the present analysis was permitted by relatively low alignment ambiguity, homology being inferred only on the basis of identity in respect of both indel length and position. In some species, the absence/ presence of certain indels could not be determined because they were completely overlapped by a larger deletion/ non-insertion. In these cases, an uncertain coding was used.

Morphological and anatomical data

As far as possible characters were coded using the 'conventional' approach sensu Hawkins (2000). Conventional coding treats character states as alternative forms (transformational homologues) of the same thing (i.e. the character) (Platnick 1979) and in so doing maximises the logical independence of characters (Hawkins et al.1997; Hawkins 2000). In addition, because conventional coding assigns character states explicity to reflect hypotheses of primary homology (cf. unspecified homologue coding), the effectiveness of secondary homology testing is maximised. Presence/ absence coding may be problematic because shared absences are assumed to be homologous in spite of minimal evidence. However, the inclusion of a small number of presence/ absence characters was unavoidable. Inapplicable data coding' (Hawkins 2000), in which taxa that lack a specific feature are coded as unknown with respect to variation in that feature, was used only if the multiple 'presence' states were considered homologous relative to the absence state (e.g. characters. 938-941). The presence/ absence was then coded separately. Where this was not the case, simple multistate coding was employed (e.g. character 927).

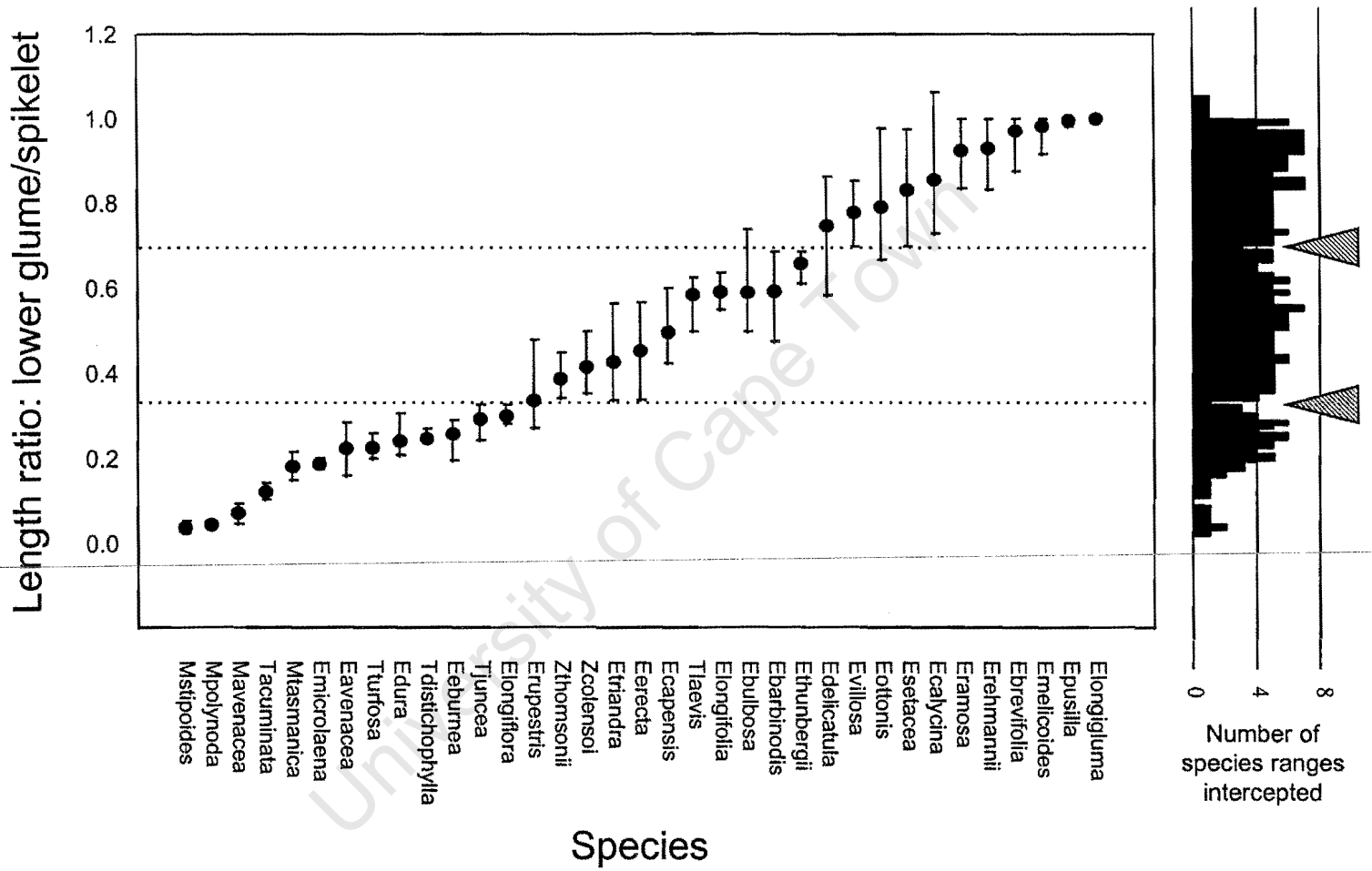


FIGURE 2.2. Value ranges and means (dots) of the ratio of lower glume length to spikelet (excluding glumes) length ratio (character 923) observed in 35 ehrharteoid species. Species are arranged in order of increasing mean value. The histogram on the right reflects the number of species' ranges overlapping at any given value of this ratio. Arrows and horizontal stippled lines indicate two points of minimum overlap or near-gaps used to break this character into discrete states.

Stevens (1991) pointed out the underlying quantitative basis of many morphological characters, and highlighted problems with coding ambiguity in both implicitly and explicitly quantitative characters. While a number of studies have produced schemes for coding continuous data in a discrete manner (e.g. Archie 1985; Baum 1988; Thiele 1993; Strait et al. 1996), some authors argue that complete gaps are the only real justification for state distinction (Pimentel and Riggins 1987; Stevens 1991), while Chappill (1989) has argued for restricted use of continuous data. Unfortunately, the probability of detecting absolute breaks in quantitative character variation decreases as the study group increases so that it may only be realistic to look for near-gaps. Using a similar logic to that of Linder and Mann (1998), I used a graphical technique to identify near-gaps as points of minimum overlap among the value ranges of different species (Fig. 2.2). These were then used to delimit states in three quantitative characters (characters 922, 923, 935: see Appendix 1). Given a choice between recognising three or four states (e.g. character 923) this study favoured the former to minimise the implicit weighting introduced by increased state number (e.g. see Thiele 1993). In addition to the three explicitly quantitative characters used, five additional characters having an implicit quantitative basis (characters 930, 937, 940, 949, 951) were included but, because the states in these were judged sufficiently distinctive, their delimitation was not rigorously examined.

All multistate characters were treated as unordered (non-additive analysis: Fitch 1971) to minimise the influence of potentially false assumptions regarding character evolution. Although such an assumption is implicit even in non-additive coding (Mickevitch and Weller 1990), the latter is 'a less restrictive statement because all possible character state trees are equally probable' (Hauser and Presch 1991). Thus, in the absence of an explicitly defensible hypothesis of character evolution, non-additive coding was consistently preferred. Ordered coding is commonly preferred for discretely-coded multistate characters having an underlying continuous basis (e.g. characters 922, 923,935). However, such ordering was not consistently supported when changes in these characters were traced onto the cladogram produced by treating them as unordered.

Thirty-one morphological and eight leaf anatomical characters were included in the final character list (Appendix 1). The vast majority (30) of morphological characters used describe variation in the reproductive structures, particularly the lemmas, which are structurally diverse in *Ehrharta* s. s. (Gibbs Russell and Ellis 1987). Although all

characters used by Gibbs Russell and Ellis (1987) to construct their scheme of species groups were considered as cladistic characters, many were not included in the data set because they were quantitative and could not be justifiably divided into discrete states. Epidermal wax characters were ignored because their description by Ellis was considered too vague, while the inconsistent presence of arm cells in *E. setacea* (Ellis 1987a; Ellis and Gibbs Russell 1987) renders this character cladistically uninformative.

Phylogenetic analysis

Data set analysis

Three data sets are used in this study: (i) trnL-F, (ii) ITS1, and (iii) morphology (including leaf anatomy). Both molecular data sets include both nucleotide substitution and indel variation components. Data sets were analysed both individually and in two combined (total evidence) analyses, one including only ingroup taxa for which data were available for at least two of the three data sets (combined-most), and one including all ingroup taxa (combined-all). All analyses were performed using PAUP version 3.1.1 (Swofford 1993). The NEXUS file used in the analyses is included in Appendix 2. The morphological data are also presented separately in Appendix 3.

Two outgroups were included in each analysis. For analyses involving molecular data, *Aegilops* (trnL-F and ITS1 data from different species: Table 2.5) and a composite oryzoid (trnL-F data from *Oryza sativa*; ITS1 data from *Leersia hexandra*: Table 2.5) were used. Composite terminals have been used elsewhere with some success (e.g. Mishler et al. 1994; Chase et al. 1995). However, because cladistic analysis assumes terminal monophyly (Bininda-Emonds et al. 1998), the use of a composite terminal requires that its constituent species form a monophyletic entity relative to the remaining species included in a given study. Since the monophyly of the two *Aegilops* species relative to (Oryzeae + Ehrharteae) is beyond question, and the monophyly of Oryzeae has been repeatedly demonstrated (Kellogg and Watson 1993; Clark et al. 1995; Duvall and Morton 1996; Soreng and Davis 1998) the use of both composite *Aegilops* and oryzoid terminals in the present study seems validated. Spikelet specialization in Ehrharteae/ Oryzeae renders the homology assessment of spikelet parts between this group and other grass groups impossible and two oryzoid

outgroups, *O. sativa* and *L. hexandra,* were, therefore, included in the morphological analysis, at the expense of a more distant outgroup.

All searches for optimum length trees were heuristic. In each instance, an initial shallow search, with 10,000 random addition sequences, NNI branch swapping and MULPARS not in effect, was conducted to account for the possibility of multiple islands of parsimony (Olmstead et al. 1993; Olmstead and Palmer 1994). The set of trees identified by this procedure was then subjected to more thorough branch swapping (TBR algorithm, MULPARS in effect) in the absence of a MAXTREES limit.

Branch support

Branch support was estimated using the character bootstrap (Felsenstein 1985a), as implemented in PAUP 3.1.1, and Bremer (1988, 1994) support. The latter was calculated in PAUP 3.1.1 using converse constraints, which were generated using Autodecay version 3.0 (T. Eriksson and N. Wikström, distributed by the authors). Despite several objections to the use of bootstrapping in phylogenetic systematics on statistical, interpretational and philosophical grounds (Carpenter 1992; Hillis and Bull 1993; Kluge and Wolf 1993; Sanderson 1995), the bootstrap continues to be widely used to estimate nodal support. In practice, bootstrap support is often correlated with other support measures (e.g. see Linder 1991a) and its use, alongside the Bremer support index, is thus empirically justified.

Bootstrap analyses were varied according to the properties of different data sets. For both the ITS1 data and the combined-most data, 200 bootstrap replicates were analysed using the following parameters: simple addition sequence, TBR branch swapping, MULPARS in effect, MAXTREES=500. In contrast to the foregoing data sets, the intrinsic resolution contained in both the trnL-F and morphological data sets was too low to permit bootstrap analyses with MULPARS in effect to run to completion within a realistic time-frame. Therefore, for these data sets 100 (morphology) or 200 (trnL-F) bootstrap replicates were analysed using the following parameters: 100 random addition sequences, TBR branch swapping, MULPARS not in effect. Experience with the data sets used in this study indicates that while searches performed with the MULPARS option not in effect were unable to locate multiple trees, they were generally effective at finding trees of optimum length. Since each bootstrap replicate requires at least a representative set of trees, the use of multiple random addition sequences per replicate was intended to circumvent this

limitation. Searches to determine Bremer support values used the following parameters: 10 random addition sequences per converse constraint tree, TBR branch swapping, MULPARS in effect, MAXTREES=500.

Topological conflict

Topological conflict among trees generated from the three individual data sets (i.e. trnL-F, ITS1 and morphology) was tested using Wilcoxon signed rank (WSR) tests in the manner applied by Templeton (1983), Mason-Gamer and Kellogg (1996), Baum (1998) and Munro and Linder (1998). Given potentially significant topological conflict between two data sets, this test determines whether either data set, when reanalysed under a constraint that accommodates the topological conflict presented by the other data set, produces a set of changes in the lengths of individual characters whose directionality is greater than expected by chance alone. Because the constraint trees used in the method may be based on entire conflicting topologies or individual points of conflict, this method has the benefit (as do the test of Rodrigo et al [1993], and the T-PTP test of Faith [1991]) of being able to localise points of conflict. Here the test was applied as follows. First, points of conflict were identified by visual inspection of the strict consensus trees derived for each of the three data sets. Conflict between a pair of branches was considered potentially significant and worthy of further testing only if both competing branches were supported by a bootstrap percentage of 75 or more and/ or a Bremer support of 2 or more. Constraint trees were designed to represent points of conflict individually, rather than in concert, since this provides greater insight into the localisation of inter-data set conflict. Because the set of species coded for each data set was not identical, in designing constraint trees, some species, specifically those not accounted for by the 'constraining' data set, could not be reliably placed. Such species were generally placed in such a way that they would minimise the amount of character change produced (i.e. reducing the probability of detecting conflict). An alternative approach would be to omit taxa of uncertain placement from the constraint trees and then use backbone constraints. This would, however, produce the same results as those generated by the method employed here. Constrained searches were performed heuristically using the following parameters: 1000 random addition sequences, TBR branch swapping, and MULPARS not in effect. Because both unconstrained and constrained searches produced multiple optimum length trees and the Templeton test requires comparison between fully resolved, fundamental trees, ten replicate

TABLE 2.6. Statistics describing the data sets used in cladistic analysis, and the resulting trees. The percentage of data that are missing or ambiguous (coded 'N' or '?') in each data partition is provided, along with the number of informative characters. The number and length of trees produced by analysis of each data set is indicated, as are their consistency (C. I.) and retention indices (R. I.), calculated with autapomorphies excluded.

Data set	Percent data 'N' or '?'	No. inform. chars.	No. trees	Length	C. I.	R. I.
trnL-F	7.1	40	12,537	65	0.80	0.90
ITS1	10.5	76	4	171	0.74	0.88
morphology	8.7	39	15,666	92	0.52	0.85
combined-most	15.3	150	3	364	0.66	0.83
combined-all	30.7	151	84	412	0.69	0.84

comparisons between randomly selected tree pairs were made to test the effect of each constraint. Significance was determined using two-tailed probabilities.

Combined analysis: character support

In order to determine which data sets were most responsible for the retrieval of specific branches (nodes) in the consensus tree based on analysis of the combined-most data, the retrieval of each of these nodes by the separate data set analyses was noted. Further, the effects on node retrieval of sequentially removing each data set from the combined-most analysis were also examined. Finally, characters were optimised onto the combined topology using an ACCTRAN optimisation, in order to identify clade-specific synapomorphies.

Results

Analysis of individual data sets

Strict consensus topologies based on analysis of the individual data sets are provided in Fig. 2.3, along with relevant branch support information. Tree statistics are listed in Table 2.6.

Morphology

Relative to the two oryzoid outgroups included, analysis of the morphology data set provides strong support for the monophyly of Ehrharteae in terms of high bootstrap

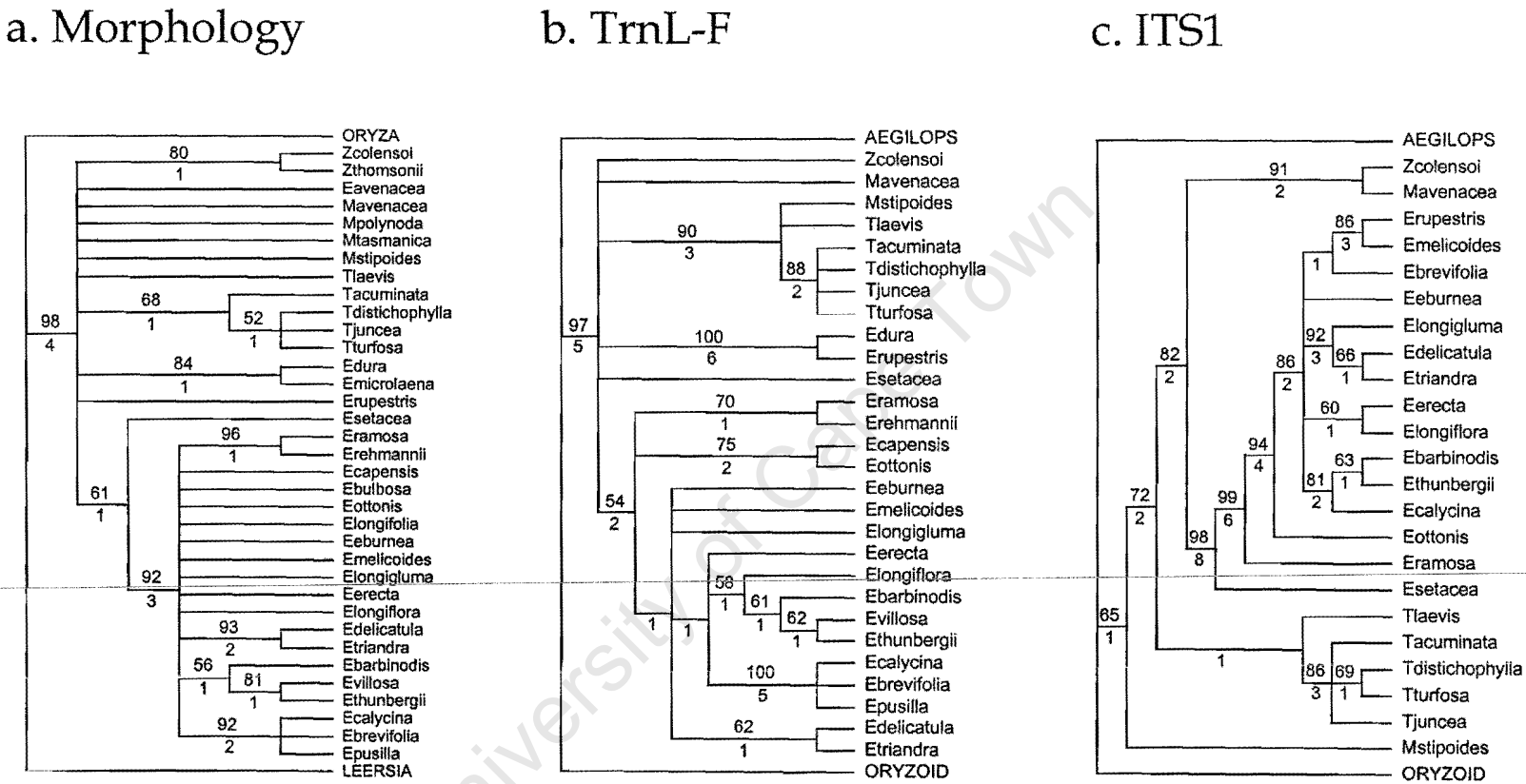


FIGURE 2.3. Strict consensus topologies based on the (a) morphology, (b) trnL-F, and (c) ITS1 data sets. Numbers above and below each branch are, respectively, bootstrap percentages (not indicated if less than 50%) and Bremer support values.

and Bremer support (Fig. 2.3a). Within Ehrharteae, resolution is poor. The largest clade contains the bulk of African species historically included in *Ehrharta* s. s. While branch support for this clade is weak, the exclusion of *E. setacea* leaves a core clade for which support is substantial. Within this latter clade, relationships are largely unresolved. Exceptions are (*E. delicatula* + *E. triandra*), (*E. calycina* + *E. brevifolia* + *E. pusilla*), (*E. ramosa* + *E. rehmannii*), and (*E. barbinodis* + *E. villosa* + *E. thunbergii*), of which the first three are reasonably to well supported. The second largest clade resolved within Ehrharteae has moderate branch support and contains four *Tetrarrhena* species: *T. acuminata*, *T. distichophylla*, *T. juncea* and *T. turfosa*. Except for sister relationships between the two species of *Zotovia* and between *E. dura* and *E. microlaena*, further basal resolution is lacking, so that four species of *Microlaena* (*M. avenacea*, *M. polynoda*, *M. stipoides* and *M. tasmanica*), *T. laevis* and two species of *Ehrharta* (*E. rupestris*, and the poorly known *E. avenacea* from Réunion) are unplaced.

trnL-F

As with the morphological data, relative to the outgroups included (one oryzoid and one pooid), analysis of the trnL-F data strongly supports the monophyly of Ehrharteae (Fig. 2.3b). The core clade of African *Ehrharta* s. s. species retrieved by the morphological data is also resolved but only with moderate branch support. As with morphology, resolution within this clade is poor and weakly supported, except for the branch subtending (*E. calycina* + *E. brevifolia* + *E. pusilla*), which receives 100% bootstrap support. (*E. capensis* + *E. ottonis*) and (*E. ramosa* + *E. rehmannii*) are also moderately supported and occupy basal positions within the core clade. The *Tetrarrhena* clade (containing *T. acuminata*, *T. distichophylla*, *T. juncea* and *T. turfosa*) resolved by morphology is likewise retrieved but receives much greater branch support from the trnL-F data. In addition, this group forms a strongly supported trichotomy with *M. stipoides* and *T. laevis*. The only remaining resolution is a strongly supported sister relationship between *E. dura* and *E. rupestris* which, due to asymmetric sampling, does not represent conflict with the sister relationship between the former species and *E. microlaena* suggested by morphology.

Exclusion of indel information from the analysis of the trnL-F data results in the collapse of five nodes (those subtending the *E. ramosa-E. eburnea*, *E.ramosa-E. rehmannii*, *E. eburnea-E. erecta*, *E. erecta-E. calycina* and *E. barbinodis-*

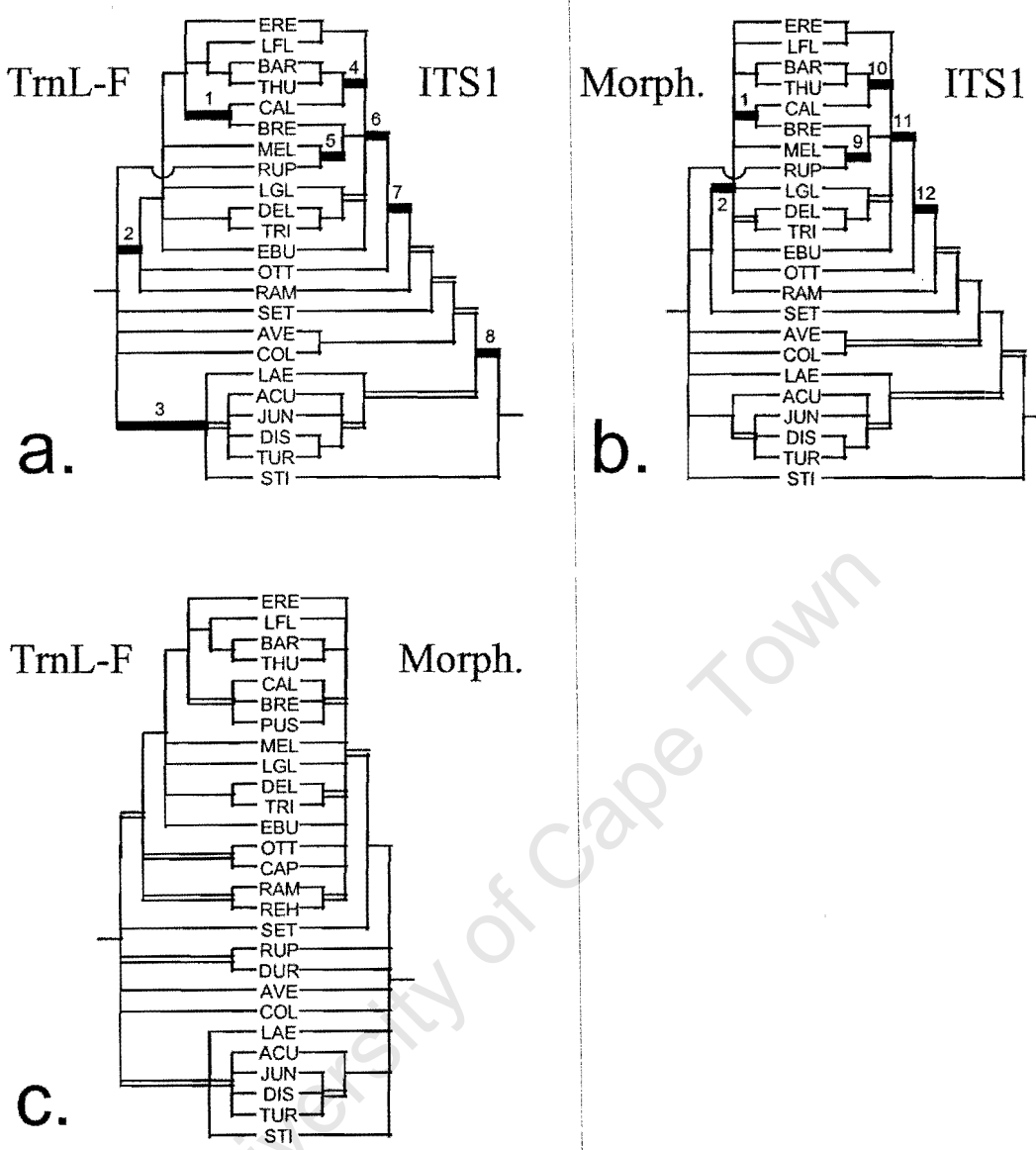


FIGURE 2.4. Schematic comparisons among topologies based on separate data set analyses. Branches supported either by a bootstrap percentage of 75% or more and/ or a Bremer support of 2 or more are indicated by a double line or solid bar. Solid bars indicate cases of reciprocal conflict among such 'supported' nodes, while double lines indicate zero conflict. Numbers above conflicting nodes correspond to constraint trees imposed on conflicting data sets by Templeton tests (see text). Species codes: ACU=*T. acuminata*, AVE=*M. avenacea*, BAR=*E. barbinodis*, BRE=*E. brevifolia*, CAL=*E. calycina*, CAP=*E. capensis*, COL=*Z. colensoi*, DIS=*T. distichophylla*, EBU=*E. eburnea*, ERE=*E. erecta*, DEL=*E. delicatula*, DUR=*E. dura*, JUN=*T. juncea*, LAE=*T. laevis*, LFL=*E. longiflora*, LGL=*E. longigluma*, MEL=*E. melicoides*, OTT=*E. ottonis*, PUS=*E. pusilla*, RAM=*E. ramosa*, REH=*E. rehmannii*, RUP=*E. rupestris*, SET=*E. setacea*, STI=*M. stipoides*, THU=*E. thunbergii*, TRI=*E. triandra*, TUR=*T. turfosa*, VIL=*E. villosa*.

E.thunbergii clades), indicating that indel information is a comparatively important component of this data set. Thus, although the numbers of indels supporting each of these nodes is small (usually one) and associated with low bootstrap support, indel exclusion has a noticeable effect on tree resolution. This seems to be due to the low intrinsic homoplasy of the trnL-F data set, which facilitates the expression of individual characters.

ITS1

The ITS1 data provide greater resolution than both the morphology and trnL-F data sets (Fig. 2.3c). The monophyly of Ehrharteae is again supported, albeit weakly. A monophyletic clade of African *Ehrharta* s. s. species is again retrieved but differs from that retrieved by the morphology and trnL-F data sets by the inclusion of *E. rupestris* in its core, as sister to *E. melicoides*. In contrast, the other data sets leave this species in an unresolved position at the base of Ehrharteae. The relatively basal positions of *E. setacea*, *E. ramosa* and *E. ottonis* at or near the base of the *Ehrharta* s. s. clade concur with results produced from the other data sets. Within the *Ehrharta* s. s. clade, a lack of resolution is apparent (as before) except that the branches subtending (*E. rupestris* + *E. melicoides*), (*E. longigluma* + *E. delicatula* + *E. triandra*) and (*E. calycina* + *E. thunbergii* + *E. barbinodis*) have reasonable to good support. The *Ehrharta* s. s. clade is resolved as sister to a well-supported clade containing *Z. colensoi* and *M. avenacea*, and the whole is sister to a weakly supported clade containing all five *Tetrarrhena* species. An embedded, strongly supported clade of four *Tetrarrhena* species is identical to that retrieved by the other data sets. In contrast to trnL-F, ITS1 identifies *M. stipoides* as sister to the remaining Ehrharteae. The possibility that this arrangement reflects rooting error is, however, increased by the small number of sites that are both informative and alignable between the ingroup and the outgroups.

In contrast to the trnL-F data set, the effect of excluding indel information from analysis of the ITS1 data is minimal, resulting in the loss of just two nodes (those subtending the *T. laevis-Tacuminata* and *T. distichophylla-T. turfosa* clades).

Topological conflict among data sets

Schematic comparisons among the consensus topologies produced by separate analyses of the three data sets are provided in Fig. 2.4. When only resolution with

TABLE 2.7. Results of the Templeton test of topological conflict. Constraints are as indicated in Fig. 2.4. For each constraint, the results of ten constrained-unconstrained comparison replicates are summarised and the two-tailed WSR probabilities listed. Statistical significance at the α=0.05 level is indicated with an asterisk. In six such comparisons, for example, trees produced under constraint 1 show character length increases and decreases of three and one step respectively, resulting in a net tree length increase of two steps. Although this net increase is identical for the remaining four comparisons, in these it is achieved through a gain of four steps and a loss of two. Under each character length change scenario (for constraint 1), however, the observed length increase is non-significant.

Constraint	gains	losses	freq.	prob.	
1	3	1	6/10	P>0.5	
	4	2	4/10	P>0.5	
2	9	2	8/10	P>0.05	
	10	3	2/10	P>0.1	
3	2	0	4/10	N/A	
	3	1	6/10	P>0.5	
4	8	0	10/10	P<0.05	*
5	5	0	10/10	P>0.05	
6	3	1	10/10	P>0.5	
7	3	1	10/10	P>0.5	
8	5	0	10/10	P>0.05	
9	10	3	1/10	P>0.1	
	9	2	7/10	P>0.05	
	8	1	2/10	P<0.05	*
10	12	4	1/10	P>0.05	
	11	3	3/10	P>0.05	
	10	2	2/10	P>0.05	
	9	1	3/10	P<0.05	*
	8	0	1/10	P<0.05	*
11	10	6	1/10	P>0.2	
	9	5	3/10	P>0.2	
	8	4	2/10	P>0.2	
	7	3	2/10	P>0.2	
	6	2	1/10	P>0.2	
	5	1	1/10	P>0.2	
12	10	6	2/10	P>0.2	
	8	4	1/10	P>0.2	
	6	2	3/10	P>0.2	
	5	1	3/10	P>0.2	
	4	0	1/10	P>0.2	

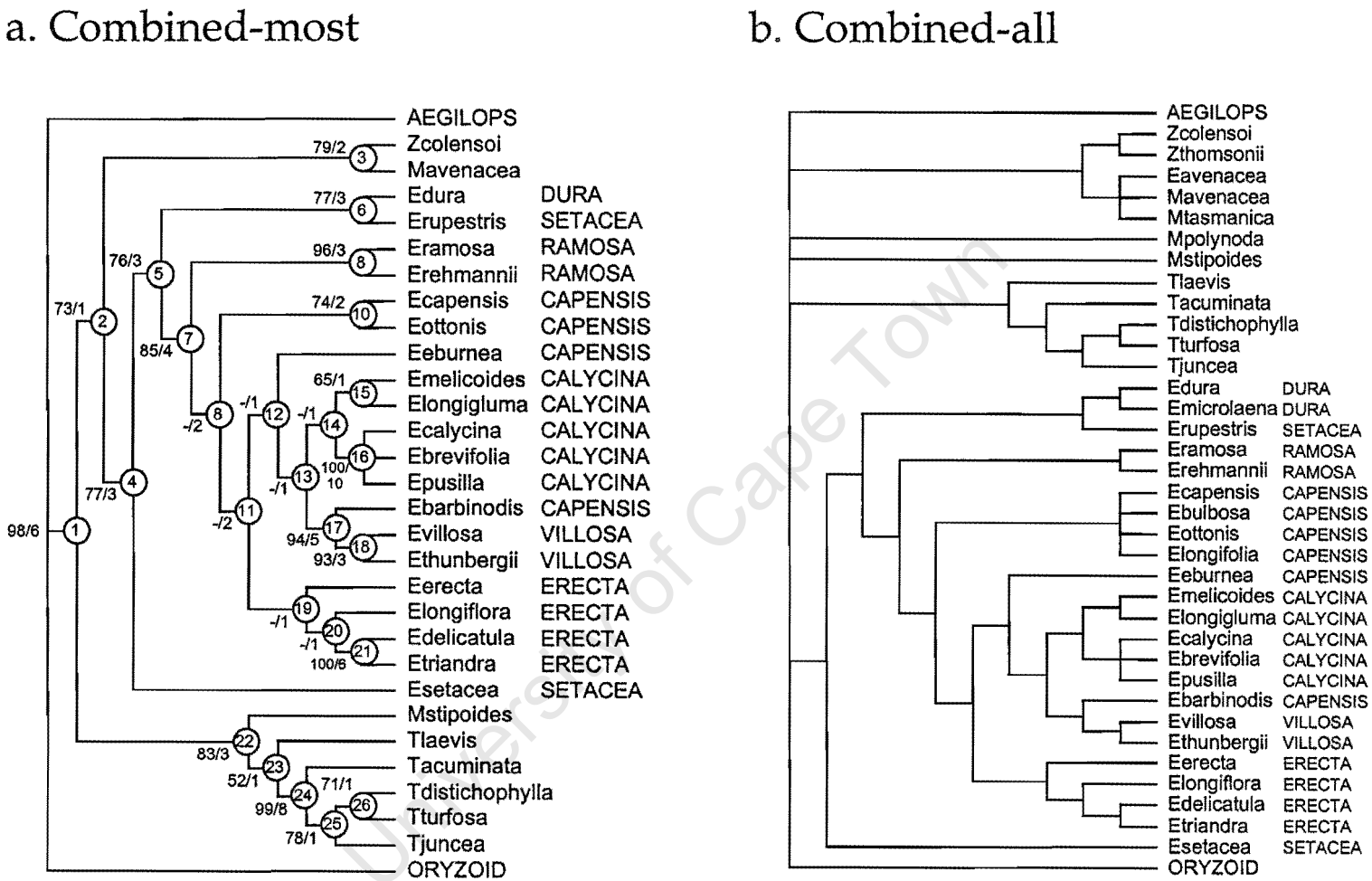


FIGURE 2.5. Strict consensus topologies based on the (a) combined-most and (b) combined-all data sets. In (a) nodes are numbered for reference (in circles), while branch supports are indicated by bootstrap percentages ('-' if less than 50%) followed by Bremer support values. *Ehrharta* s. s. species names are followed by the name of the species group (sensu Gibbs Russell and Ellis 1987: in capitals) to which they belong.

TABLE 2.8. Individual data set support for nodes (numbers as per Fig. 2.5a) retrieved by the combined-most analysis. Columns 2-4 indicate whether each node is supported (bootstrap and Bremer support provided), unsupported ('-') or contradicted ('X') by individual data set analyses. A question-mark indicates a lack of pertinent information. The susceptibility of each node to the removal of individual data sets is indicated in column 5.

Node no.	TrnL-F Support	ITS1 support	Morph. support	Data set removal collapses node
1	97/5	65/1	?	
2	-	82/2	-	mor, ITS
3	-	91/2	80/1	mor
4	-	98/8	-	ITS, trn
5	-	99/6	X	ITS, trn
6	100/6	?	-	trn
7	54/2	X[a](99/6)	92/3	mor, trn
8	70/1	?	96/1	
9	-	X[a](94/4)	-	mor, trn
10	75/2	?	-	trn
11	<50/1	X[a](86/2)	-	mor, trn, ITS
12	X	X	-	mor, trn, ITS
13	X	X	-	mor, trn, ITS
14	X	X	-	mor, trn, ITS
15	-	X	-	mor, trn
16	100/5	X	92/3	
17	61/1	<50/1	56/1	
18	62/1	?	81/1	
19	X	X	-	mor, ITS
20	X	X	-	mor, ITS
21	62/1	66/1	93/2	
22	90/3	X	-	trn
23	-	<50/1	-	ITS
24	88/2	86/3	68/1	
25	-	-	52/1	mor
26	-	69/1	-	ITS

[a]Nodes 7 and 9 are contradicted by the ITS1 data exclusively due to the inclusion of *E. rupestris* in the core of the *Ehrharta* s. s. clade. Otherwise, these nodes are supported by the ITS1 data (branch support values in parentheses).

reasonable branch support is considered (i.e. bootstrap percentage 75 or more and/ or Bremer support 2 or more), three nodes in the trnL-F tree conflict with five nodes in the ITS1 tree (Fig. 2.4a) and two nodes in the morphology tree with four nodes in the ITS1 tree (Fig. 2.4b). No supported conflict occurs between the trnL-F and morphology trees (Fig. 2.4c). These conflicts represent three areas of disagreement between the ITS1 topology and the others: (1) whereas (*E. calycina* + *E. thunbergii* + *E. barbinodis*) is favoured by ITS1, the other data sets prefer (*E. calycina* + *E. brevifolia* + *E. pusilla*); (2) the basal placement of *M. stipoides* in Ehrharteae by ITS1 conflicts with its placement at the base of the *Tetrarrhena* clade by trnL-F; and (3) where ITS1 includes *E. rupestris* in the core of the *Ehrharta* s. s. clade, this species is at best placed basally in this group by the other two data sets. The last disagreement produces three nodal conflicts per comparison. Results of the Templeton test are listed in Table 2.7, with constraints numbered as in Fig. 2.4. Generally, a given constraint produced slightly different character length changes depending on the particular constrained-unconstrained tree pair included in a comparison replicate: therefore, each possible gain/ loss combination is listed along with its frequency (out of ten replicates) and WSR probability. Only three constraints were found to produce significant increases in tree length (Table 2.7). An ITS1-based constraint (constraint 4) enforcing the monophyly of (*E. calycina* + *E. thunbergii* + *E. barbinodis* + *E. villosa*) produced a consistently significant increase of eight steps when applied to the trnL-F data. The reciprocal constraint, however, failed to produce significant change. Constraints 9 and 10, both ITS1-based, produced significant increases in morphological tree length in some constrained-unconstrained comparison replicates (2/10 and 4/10 respectively), but not in others. In each instance, the reciprocal constraint failed to produce significant change.

Combined data analyses

Analysis of the combined-most data (excluding all taxa coded for just a single data set) yielded three optimum length trees (Table 2.6) differing topologically at a single node (subtending *E. calycina*, *E. brevifolia* and *E. pusilla*). The strict consensus of these, with branch support indicated, is provided in Fig. 2.5a, and shows strong bootstrap and Bremer support for the monophyly of Ehrharteae relative to the outgroups included in the analysis. Although a monophyletic Ehrharteae is resolved by all three data sets when analysed individually, the trnL-F data appears to provide

the strongest support for the ingroup node (Table 2.8), which, unsurprisingly, is robust to data set removal. Unexpectedly, the addition of seven taxa coded only for morphology results in loss of ingroup (Ehrharteae) monophyly (combined-all data: Fig. 2.5b: tree statistics in Table 2.6).

Analysis of both the combined-most and combined-all data sets indicate the existence of three principal clades in Ehrharteae: (1) a *Tetrarrhena* clade, (2) a *Microlaena-Zotovia* clade (including *E. avenacea* from Réunion), and (3) an *Ehrharta* s. s. clade. Whereas the combined-most analysis provides a rather weakly-supported resolution of the relationships amongst these clades (Fig. 2.5a: clades (2) and (3) are sisters, with clade (1) the sister of this pair), this pattern is lost in the analysis of the combined-all data (Fig. 2.5b). While support for this resolution seems to be provided principally by ITS1 (Table 2.8: node 2), susceptibility of the node to removal of the morphological data indicates a contribution from the latter.

The *Tetrarrhena* clade

Strong support for the inclusion of *M. stipoides* at the base of a clade dominated by *Tetrarrhena* species (Fig. 2.5a: node 22) appears to be provided principally by the trnL-F data set, whose removal collapses this arrangement (Table 2.8). ITS1 support for a contradictory basal position for *M. stipoides* in Ehrharteae is comparatively weak (Fig. 2.3c) and this arrangement is, therefore, not favoured by the combined-most analysis. Analysis of the combined-all data, however, drops *M. stipoides* into an unresolved basal position within Ehrharteae (Fig. 2.5b). While the sister relationship of *T. laevis* to a clade containing the remaining species of *Tetrarrhena* (node 23) receives only weak support, primarily from the ITS1 data and to some extent the trnL-F data, the inclusion of *T. laevis* in the *Tetrarrhena* clade is robust to data set removal (removal of the trnL-F data collapses node 22 but not node 23, while removal of the ITS1 data collapses node 23 but not node 22). The branch subtending the four remaining *Tetrarrhena* species is highly robust, having high branch support and being unaffected by data set removal (Fig. 2.5a, Table 2.8: node 24). All three data sets resolve this node individually. Relationships within this clade are, however, weakly supported (Fig. 2.5a) - node 25 apparently by morphological data and node 26 by ITS1 data - and are susceptible to data set removal (Table 2.8).

The *Microlaena-Zotovia* clade

The monophyly of (*Z. colensoi* + *M. avenacea*: node 3) is strongly supported by separate analyses of both the ITS1 and morphology data sets (Fig. 2.3a, c) as well as analysis of the combined-most data set (Fig. 2.5a). Because node 3 appears to be uncontradicted by the trnL-F data, its susceptibility to removal of the morphology data set (Table 2.8) is surprising. The strict consensus tree resulting from analysis of the combined-all data includes three additional species in this clade: *Z. thomsonii* is placed as sister to *Z. colensoi*, while *M. tasmanica* and *E. avenacea* form a trichotomy with *M. avenacea* (Fig. 2.5b).

The *Ehrharta* s. s. clade

Both the combined-most and combined-all analyses (Fig. 2.5a, b) support the monophyly of a clade consisting of all the African species historically included in *Ehrharta* s. s., but excluding *E. avenacea* from Réunion (Fig. 2.5b). Branch support for this clade is relatively strong, but appears to be provided largely by the ITS1 data, to whose removal it is susceptible (Table 2.8: node 4). Within this group, node 7 (Fig. 2.5a) defines a strongly supported, embedded clade containing all species except *E. dura* (and *E. microlaena* - its sister species in the combined-all analysis), *E. rupestris* and *E. setacea*. This core clade is also resolved by separate analyses of both the trnL-F and morphology data sets and, except for the position of *E. rupestris*, by the ITS1 analysis as well (Fig. 2.5a, Table 2.8). Both combined analyses identify *E. setacea* as basal within the *Ehrharta* s. s. clade (Figs. 2.5a, b). Support for this arrangement is reasonable but depends heavily on the ITS1 data (Table 2.8: node 5). Strong ITS1 support for the (anomalous) inclusion of *E. rupestris* within the *Ehrharta* s. s. core clade (Fig. 2.3c) plus massive trnL-F support for a relationship between this species and *E. dura* (Fig. 2.3b, Table 2.8: node 6) explain why a more basal position for *E. dura* and *E. rupestris*, favoured by morphology, is not supported by combined analysis. Analysis of the combined-most data with *E. rupestris* excluded (tree not shown) results in the removal of *E. dura* from the *Ehrharta* s. s. clade and its placement as sister to the *Microlaena-Zotovia* clade (with moderate bootstrap support of 75%). Exclusion of the trnL-F data has an identical effect on the position of *E. dura*. Except for the position of *E.dura*, the exclusion of *E. rupestris* leaves the topology produced by analysis of the combined-most data set unchanged, although branch support is increased on nodes 7, 8 and 11 (bootstraps 100%, 91% and

74%,respectively). In addition, the monophyly of *Ehrharta* s. s. (excluding *E. dura* and *E. rupestris*) is more strongly supported (bootstrap 92%). Analysis of the combined-all data set with either *E. rupestris* or the trnL-F data excluded, results in a trichotomy between (*E. dura* + *E. microlaena*) and the *Microlaena-Zotovia* and *Ehrharta* s. s. clades.

Within the core *Ehrharta* s. s. clade, the combined-most data provide good support for the monophyly of (*E. ramosa* + *E. rehmannii*) and that of (*E. capensis* + *E. ottonis*) (Fig. 2.5a). Because ITS1 data were not obtained for *E. rehmannii* and *E. capensis*, resolution of these relationships necessarily relies on the trnL-F and morphological data (Table 2.8: nodes 8 and 10). Analysis of the combined-all data includes *E. longifolia* and *E. bulbosa* in the latter clade. Though receiving just moderate support in the combined-most analysis, the basal position of the *E. ramosa* and *E. capensis* clades within the *Ehrharta* s. s. core is independently resolved by separate analyses of both the trnL-F and ITS1 data sets, as long as the anomalous position of *E. rupestris* implied by the latter is ignored (Fig. 2.3b, c). Both nodes are susceptible to data set removal (Table 2.8: nodes 9, 11). Except for four nodes (nodes 16, 17, 18, 21) that have high branch support and are robust to data set removal, all remaining branches within the *Ehrharta* s. s. core clade are weakly supported (bootstrap percentages below 50, Bremer support below 2) and generally strongly affected by data set removal (Fig. 2.5a). The monophyly of both (*E. barbinodis* + *E. thunbergii* + *E. villosa*) and (*E. delicatula* + *E. triandra*) is supported by all three data sets (Table 2.8: nodes 17 and 21, respectively). Because ITS1 data were not obtained for *E. villosa*, support for the sister relationship of this species and *E. thunbergii* relies on the trnL-F and morphology data. High branch support for the monophyly of (*E. calycina* + *E. brevifolia* + *E. pusilla*) is due exclusively to the trnL-F and morphology data sets, as this relationship is contradicted by the ITS1 data (Table 2.8: node 16). That this node is robust to data set removal indicates the strength of support received from each of these data sets.

Discussion

Data utility and complementarity

In line with the results of Gielly et al. (1996), this study found noncoding ITS sequences to be more variable and to provide greater phylogenetic resolution than noncoding trn intron or spacer sequence variation. Where analysis of the ITS1 data set yielded just four optimum length trees whose strict consensus resolved 17 internal ingroup nodes across a set of 22 ingroup species, the trnL-F data set yielded 12,537 trees having a strict consensus that resolved just 13 ingroup nodes across a set of 28 ingroup species. While the ITS2 region was not investigated in this study, a pilot study revealed that both the trnT-L intergenic spacer and the trnL intron are substantially less variable than the trnL-F intergenic spacer and unsuitable for inferring interspecific relationships in Ehrharteae. These patterns match the expectation of Taberlet et al. (1991) that the trnL intron should evolve more slowly than the trnL-F intergenic spacer due to the catalytic properties of the former. However, they contradict the study of Gielly and Taberlet (1994) which found the two regions to evolve at about the same rate across a range of pooid grasses. The slower evolutionary rate of these sequences suggests that they may be more useful for investigating relationships between the ehrharteoid and oryzoid grasses. Attempts to reliably align entire ITS1 sequences of Ehrharteae against those of two oryzoids, *Leersia hexandra* and *Potamophila parviflora* (Hsiao et al., unpublished data: Genbank accession nos. AF019792 and AF019793), proved futile due to the presence of two hypervariable regions. However, these regions were extremely useful for resolving pattern within Ehrharteae. Given the importance of meaningful alignment to phylogenetic inference (e.g. Morrison and Ellis 1997; Cerchio and Tucker 1998), this suggests that ITS1, at least, is unsuitable for the study of oryzoid-ehrharteoid relationships.

Compared with the molecular data, analysis of the morphological data provided even poorer resolution in Ehrharteae, yielding 15,666 equally parsimonious trees with a strict consensus that resolved just 11 internal ingroup nodes across a set of 35 ingroup species. Possible reasons for the lower resolution associated with the morphological data include the small number of morphological characters relative to the number of terminal taxa and higher levels of character homoplasy. The latter may be due either to the greater number of taxa included in the morphological data

set (Sanderson and Donoghue 1989) or a higher level of intrinsic homoplasy (Givnish and Sytsma 1997a).

Contrary to the situation in *Viburnum* (Donoghue 1983; Donoghue and Baldwin 1993; Donoghue and Sytsma 1993), the distribution of supported topological conflict among analyses of separate data sets does not reflect greater discordance among morphological and molecular data than among different classes of molecular data. Rather, the absence of supported conflict among trees based on morphological and trnL-F data, plus identical areas of conflict between the sets of trees produced by each of these data sets and that produced by the ITS1 data, appears to indicate the association of maximum discordance with the latter. In all instances of conflict, combined analysis favoured the arrangement supported by the trnL-F and morphological data. While these results cannot be taken as evidence that the ITS1 data set is positively misleading in producing these conflicts, they do not support the contention that morphological data are more inclined to mislead than molecular data (cf. Hedges and Maxson 1986; Givnish and Sytsma 1997a, b) and vindicate the inclusion of morphological data in the present study.

One possible explanation for discordance between topologies based on ITS sequences and those based on other data is the possible existence of paralogous ITS copies that differ due to a lack of concerted evolution (Buckler et al. 1997). In the present study, the production of a multiple-banded product during amplification of *E. rupestris* may indicate the existence of paralogous ITS copies in this species. Hence, the anomalous position of this species in the ITS1-based tree may reflect a lack of sequence orthology. In total, three areas of 'supported' topological conflict among separate data set analyses were identified by this study. Since application of the Templeton test failed to attribute reciprocal statistical significance to any of these cases of conflict, I analysed the three data sets in combination without excluding any taxa. However, it is worth noting that broad discordance in the position of *E.rupestris* plus the possibility of ITS paralogy in this species may support its exclusion from combined data analysis.

The observation that data combination yields improved resolution suggests broad complementarity among the data sets and vindicates data combination. Most spectacularly, despite the low resolution yielded by separate analysis of the morphological data, their combination with molecular data greatly improves both

resolution and nodal support. Similar complementarity among morphological and molecular data has been observed elsewhere (e.g. Lafay et al. 1995; Pennington 1996; Eldenäs and Linder, in press), although contrary patterns have been reported (e.g. Barker et al., unpublished).

Monophyly and generic limits in Ehrharteae

Disagreement surrounds the generic taxonomy of Ehrharteae, with some treatments recognising four genera (Watson and Dallwitz 1992; Edgar and Connor 1998) and others preferring to lump these into a single expanded *Ehrharta* (Willemse 1982; Clayton and Renvoize 1986). Under a monophyly criterion (Funk 1985; Donoghue and Cantino 1988; Humphries and Chappill 1988; Schrire and Lewis 1996; Van Welzen 1997; Backlund and Bremer 1998), two questions are central: (i) Are the four component genera demonstrably monophyletic, and (ii) is Ehrharteae as a whole monophyletic? An affirmative answer to (i) and a negative answer to (ii) probably favours multiple genera, while the converse may identify a single genus as the best option. If the answers to both (i) and (ii) are positive, both options are viable and other, ancillary criteria come into play. The most important among these are the maximisation of taxonomic stability, ease of taxon diagnosis and phylogenetic informativeness (Linder 1991a, Schrire and Lewis 1996; Backlund and Bremer 1998). Finally, it is possible that internal structure in Ehrharteae favours a generic alignment different from either of the options presented above.

Monophyly of Ehrharteae

Due to limited outgroup sampling (one oryzoid and one pooid), the present study is not a rigorous test of the monophyly of Ehrharteae. However, relative to the outgroups included, the topologies produced both by separate analyses of all three data sets, as well as by the combined-most analysis, are consistent with ehrharteoid monophyly. The loss of monophyly that results when taxa not coded for the molecular data sets are added is probably artifactual, resulting from missing data, and should not, therefore, be interpreted as questioning monophyly. A comprehensive test of ehrharteoid monophyly is, therefore, still required. Given evidence for a monophyletic (Oryzeae + Ehrharteae) (Clark et al. 1995; Soreng and Davis 1998; GPWG, unpublished), such a test should involve a broad and representative sampling of both the ehrharteoid and oryzoid grasses as well as, possibly, the Phyllorachideae (GPWG, unpublished). In addition, further

representatives from the pooid, bambusoid and PACC clades are required to verify the root position.

Within Ehrharteae, combined analysis of the three complete data sets (i.e. with *E. rupestris* included) provides reasonable to good support for the monophyly of *Ehrharta* s. s. (excluding *E. avenacea*) and *Tetrarrhena*, while a third clade containing species of *Zotovia* and at least some species of *Microlaena* is also resolved. Relatively low support for a sister relationship between the *Ehrharta* s. s. and *Zotovia-Microlaena* clades leaves the observed ehrharteoid rooting on the branch subtending the *Tetrarrhena* clade open to question, particularly since this appears to be most strongly influenced by the data set that shows the greatest alignment difficulty between the ingroup and the outgroups (ITS1). An alternative rooting cannot, therefore, be entirely excluded.

The *Ehrharta* s. s. clade

The monophyly of *Ehrharta* s. s. (excluding *E. avenacea*) is reasonably well supported by combined analysis of the three complete data sets and is consistent with all separate data set analyses. Although a single synapomorphy, the possession of a large, often inflated rachilla process, defines the clade, this depends on interpretation of the character in *Zotovia*. A monophyletic *Ehrharta* s. s. fits distributional data well and appears to match the conclusions of Gibbs Russell and Ellis (1988) except, perhaps, with respect to the positions of *E. dura* and *E. microlaena*. These authors noted that '*E. dura* and *E. microlaena* are undoubtedly very closely related to each other, yet have very little in common with any other species of *Ehrharta* in southern Africa.' Diffusely-villous sterile lemma calli, the possession of broad-based, tapering lemma awns, and constricted leaf bases suggest an affinity with species of *Microlaena*. In addition, the occurrence of four stamens in both species (four in *E. microlaena*; four or six in *E. dura*) prompted Gibbs Russell and Ellis (1988) to suggest an affinity with *Tetrarrhena*. Surprisingly, these species do not occupy the most basal position within the *Ehrharta* s. s. clade, but are resolved as sister to *E. rupestris*. This arrangement relies on the inclusion of both the trnL-F data and *E. rupestris* in combined data analysis and is lost when either is excluded. Under these circumstances, *E. dura* either occupies a basal position in the *Zotovia-Microlaena* clade (combined-most data) or, with its sister species *E. microlaena*, forms a trichotomy with that clade and the *Ehrharta* s. s.

clade (combined-all data). Because there are too few morphological characters to contest the trnL-F data properly and because ITS1 sequence data were not obtained for *E. dura* and may be problematic for *E. rupestris*, the position of *E. dura* and, therefore, *E. microlaena* remains unsatisfactory.

The *Tetrarrhena* clade

Although *M. stipoides* is placed at the base of the *Tetrarrhena* clade by the combined-most analysis, its position, plus that of *M. polynoda*, is unresolved by the combined-all analysis. The inclusion of *M. stipoides* in a *Tetrarrhena* clade, while resolved chiefly on the basis of trnL-F sequence variation, is consistent with the distribution of four-staminate flowers, which, assuming six stamens to be ancestral (Clifford 1961; Soreng and Davis 1998), then emerges as a synapomorphy for this group. Under this interpretation, the occurrence of four stamens in *E. dura* and *E. microlaena* reflects homoplasy. The possession of four stamens by *M. polynoda*, probably suggests its inclusion in the *Tetrarrhena* clade (with which it also shares one-nerved paleas) but this requires confirmation from molecular data. While two- and even one-, three- or six-staminate flowers have been observed in *M. stipoides*, these are much rarer (Willemse 1982; Edgar and Connor 1998). Nonetheless, the widespread distribution and morphological variability (including polymorphism in stamen number) of *M. stipoides* suggests that this species is potentially paraphyletic (*sensu* Crisp and Chandler [1996]) and basal in Ehrharteae, an hypothesis that could be tested by the inclusion of multiple accessions.

The *Zotovia-Microlaena* clade

Largely because DNA-extractable material of some species of *Microlaena* and *Zotovia* was unobtainable, the membership of a monophyletic *Microlaena-Zotovia* clade remains somewhat tentative. Three species (*Z. thomsonii*, *M. tasmanica* and *E. avenacea*) included in the group by analysis of the combined-all data were not sequenced for either trnL-F or ITS1, and their inclusion is thus entirely morphology-based and requires molecular corroboration. Morphological characters defining the clade include the diffusely villous sterile lemma calli as well as the possession of two-staminate flowers, this representing a reduction from the basic six-staminate condition. The occurrence of tapering awns in basal members of both the *Tetrarrhena* and *Ehrharta* s. s. clades suggests that this 'microlaenoid' attribute is

plesiomorphic. Within the *Microlaena-Zotovia* clade the monophyly of both *Microlaena* and *Zotovia* is resolved.

Generic limits

In identifying, within Ehrharteae, three reasonably well-supported principal clades that correspond broadly to *Ehrharta* s. s., *Tetrarrhena* and (*Microlaena* + *Zotovia*) and are each defined by at least one or two morphological synapomorphies, this study provides tentative support for a three- and, possibly, four-genus classification of Ehrharteae. In terms of generic delimitation, two characters discussed by Willemse (1992), stamen number and degree of development of a rachilla process within the spikelet, seem most important. While not questioned by the results of the present study, it is clear that until the monophyly of Ehrharteae is more thoroughly tested by a larger set of oryzoid grasses, the recognition of a single, expanded *Ehrharta* s. l. is unsatisfactory and the recognition of multiple genera arguably preferable.

Since *Zotovia* and *Microlaena* occupy different niches, the segregation of these genera is favoured by ancillary eco-geographical criteria (cf. Linder and Verboom 1996). Whereas *Zotovia* is a genus of compact, cushion-forming plants restricted to moist high-altitude bogs in New Zealand (Connor and Edgar 1986), *Microlaena* (including *E. avenacea*, *M. avenacea* and *M. tasmanica*) is a genus of tufted, understorey grasses from forest or woodland habitats distributed across a broad geographic range (Australia, Malesia, New Zealand, Polynesia and Réunion). The results of the present study also indicate that, if three or four monophyletic genera are to be recognised, the inclusion of *M. stipoides* in *Tetrarrhena* and *E. avenacea* in *Microlaena* is necessary, while the status of *M. polynoda* remains uncertain. Since *M. stipoides* is the type species of *Microlaena* and this name antedates *Tetrarrhena*, this argues for the transfer of all *Tetrarrhena* species to *Microlaena*, and the formation of a new genus to accommodate *E. avenacea*, *M. avenacea* and *M. tasmanica*. Molecular sampling of species not listed in Table 2.4 is, however, recommended prior to the formation of new combinations. In addition, greater clarity is required regarding the phylogenetic positions of *E. dura*, *E.microlaena* and *E.rupestris*. Finally, it is necessary to exclude the possibility of an alternative ehrharteoid rooting as well as the paraphyly of *M. stipoides*, as both could support an alternative generic delimitation.

Phylogeny and infrageneric classification of *Ehrharta* s. s.

Three of the seven species groups (Table 2.3) described by Gibbs Russell and Ellis (1987) are monophyletic, while relatively minor membership changes would make a further three monophyletic (Fig. 2.5a, b). This indicates broad concordance between the phylogeny presented here and a traditional perspective of relationships in *Ehrharta* s. s. Two unexpected results are, however, the non-basal position of *E. dura* and *E. microlaena* (see above) and the paraphyly of the Setacea group. Although Gibbs Russell (1987a) and Ellis (1987a) both inferred a close relationship between *E. setacea* and *E. rupestris* on the basis of a suite of morphological and anatomical characteristics (Table 2.3), the combined analysis presented in this study strongly supports the paraphyly of the Setacea group. However, inter-data set conflict regarding the phylogenetic position of *E. rupestris*, possibly due to a non-orthologous ITS1 sequence for this species, as well as the lack of ITS1 sequence data for *E. dura* renders the inferred relationships among *E. rupestris*, *E. setacea*, *E. dura* and *E. microlaena* somewhat uncomfortable. Nonetheless, the consistent placement of these species near the base of the *Ehrharta* s. s. clade by both separate and combined data set analyses (except for *E. rupestris* in the ITS1 analysis) supports their generally basal position. This suggests that some of the features defining the Setacea group may be plesiomorphic. Arm cells (see Ellis 1987a), for example, are interpreted to be plesiomorphic in Ehrharteae by at least some optimisations done using the phylogeny of grass family as a whole (GPWG,unpublished). A more detailed analysis of Ehrharteae including representative accessions of all intraspecific taxa in the Setacea group plus all other relevant species would go some way to further evaluate these statements.

Strong branch support for the monophyly of the remaining species of *Ehrharta* s. s. (excluding *E. avenacea*) is corroborated by a suite of unequivocal morphological synapomorphies: corrugated sterile lemmas, collection of the hairs on the sterile lemma calli into discrete tufts, constriction of the upper sterile lemma base, massive, lateral, knob-like swellings near the base of the fertile lemma, and a glabrous rachilla process. While the placement of the *E. ramosa* and *E. capensis* clades near the base of this core group receives just moderate to weak support from combined analysis, separate analyses based on the trnL-F and ITS1 data sets (ignoring the anomalous position of *E. rupestris* by the latter) provide additional support. The basal position of the *E. ramosa* clade is further supported by six morphological

synapomorphies (Fig. 2.5a: node 9), of which three are unequivocal: the presence of microhairs at the base of the upper sterile and fertile lemmas, and the possession of brush-like stigmas. Although several internal branches in the core *Ehrharta* s. s. clade are weakly supported, the clades they define are largely consistent with the scheme of species groups described by Gibbs Russell and Ellis (1987). The monophyly of the Ramosa and Villosa groups is, however, well supported. While neither the Calycina nor Erecta groups are monophyletic, the transfer of just a single species (*E. delicatula*) from the former to the latter is required to rectify this. Similarly, the removal of *E. eburnea* and *E. barbinodis* (the latter to the Villosa group) renders the Capensis group monophyletic with good support. After adjustment to monophyly, five of the species groups in *Ehrharta* s. s., (but not the Erecta and Setacea groups), can be characterised by at least one unequivocal morphological synapomorphy drawn from the morphological data set used in this study (Table 2.9). In addition, both the Villosa and Capensis groups are further characterised by growth form synapomorphies. The former possesses a restioid habit, in which the leaves are short-lived and the culms long-lived, with well-developed subepidermal chlorenchyma (Chapter 3). In contrast, members of the Capensis group are

TABLE 2.9. Unequivocal morphological synapomorphies defining species groups in *Ehrharta* s. s., once membership has been modified to make them monophyletic.

Species group	Species included	Unequivocal synapomorphies
Dura group	*E. dura* *E. microlaena*	Lower sterile lemma base diffusely villous; Upper sterile lemma tapering into an awn
Ramosa group	*E. ramosa* *E. rehmannii*	Basal ear-like appendages on second sterile lemma
Capensis group	*E. bulbosa* *E. capensis* *E. longifolia* *E. ottonis*	Second sterile lemma base stipitate
Calycina group	*E. brevifolia* *E. calycina* *E. melicoides* *E. longigluma* *E. pusilla*	Second sterile lemma callus glabrous; basal ear-like appendages on second sterile lemma; fertile lemma microhairs tightly clustered
Villosa group	*E. barbinodis* *E. thunbergii* *E. villosa*	Lodicules two-parted
Erecta group	*E. delicatula* *E. erecta* *E. longiflora* *E. triandra*	none

geophytic, their culm bases being markedly swollen (Chapter 3).The biological integrity of all six monophyletic species groups is further supported by evidence for inter-group growth rate differences (Chapter 4).

Despite limited character support for their monophyly, evidence of intrinsic biological differences among the six monophyletic species groups in *Ehrharta* argues for their formal recognition at the infrageneric level. However, three species, *E. eburnea*, *E. rupestris* and *E. setacea*, cannot be accommodated in any one of these groups, the last two making up the paraphyletic Setacea group of Gibbs Russell and Ellis (1987). The decision to accord formal status to the six monophyletic groups therefore necessitates the recognition of either one paraphyletic and one monotypic taxon, or three monotypic taxa. Since neither paraphyletic taxa nor monotypic higher taxa are desirable (Funk 1985; Donoghue and Cantino 1988; Schrire and Lewis 1996; Backlund and Bremer 1998), the latter because they increase taxonomic redundancy (Backlund and Bremer 1998), both of these treatments are unsatisfactory. Therefore, because the relatively small size (23 species) of *Ehrharta* s. s. does not render the genus unwieldy, the value of formal subdivision is questionable. I recommend, instead, that the six monophyletic species groups (Table 2.9) be informally recognised as such and that users wishing more detailed phylogenetic information consult this work directly.

Chapter 3. Adaptive radiation in *Ehrharta* s. s.: pattern and explanation

Introduction

Almost two thirds of species included in the grass tribe Ehrharteae are endemic to the western Cape region of southern Africa. In order to explain the high number of species in this area, Clayton and Renvoize (1986) invoked an explicit adaptive radiation scenario, writing:

> '(Ehrharteae) has diverged from the typical oryzoid lifestyle by its occupation of open hillside habitats. It may be a bambusoid relic which has survived extinction by retreating to the southern hemisphere, and is now undergoing a bout of speciation following adaptation to the winter rainfall regime of the South African Cape.'

The central goal of this chapter and, indeed, this thesis in general is to investigate this suggestion more thoroughly, paying particular attention to the pattern of diversification of *Ehrharta* s. s. and its possible causes. The definition of adaptive radiation is contentious (e.g. Erwin 1992; Givnish 1997) and I start, therefore, by clarifying and justifying the use of this term, as well as others that describe related concepts, in this particular study.

Definition of adaptive radiation

Criteria previously used to define adaptive radiations are: (i) increased rate and degree of diversification (speciation) within a single lineage (Simpson 1953; Stanley 1979; Guyer and Slowinski 1993; Schluter 1996), (ii) coincident or subsequent differentiation into diverse ecological roles or lifestyles (Osborne 1910; Simpson 1953; Futuyma 1986; Schluter 1996; Givnish 1997) and (iii) a critical influence of evolutionary novelties or key innovations in facilitating diversification (Guyer and Slowinski 1993). Alternative definitions appear to differ mainly in which of these criteria they choose to emphasise, with increased speciation rate and differentiation into diverse ecological roles being most consistently cited. Several authors (e.g.

Futuyma 1986; Grant 1986; Givnish 1997) have opted, either implicitly or explicitly, to ignore increased speciation rate as an integral feature of adaptive radiation. Most explicit among these is Givnish (1997) who criticised the use of the rate criterion on the grounds that, in ignoring evidence for adaptation, it conflates "'adaptive radiation' with mere speciation." In describing a particular pattern of speciation (accelerated), however, 'radiation' is clearly more restricted in meaning than 'speciation'. The introduction of an 'adaptive' criterion restricts the term further still.

Consistent with a standard definition of the verb 'radiate,' (Concise Oxford Dictionary, seventh edition: Sykes 1986) this study distinguishes evolutionary radiation as a pattern in which lineage divergence events occur rapidly in succession, so that they appear to 'diverge or spread from a central point' or 'disseminate as from a centre.' The definition of radiation in terms of rapid lineage divergence is consistent with Simpson's (1953) criterion of 'more or less simultaneous divergence of numerous lineages from much the same ancestral adaptive type,' and sudden divergence may even be evident in Osborne's (1910) words on the subject: 'From primitive central types branches will *spring off in all directions.*' Reduction in the interval between successive divergence events is expected to result in low phylogenetic signal, so that radiations may be reflected as polytomies (or alternatively a concatenation of very short branches) on a cladogram. However, this does not imply that all polytomies should be taken to reflect radiations, since they may also arise due to conflicting character evidence (soft polytomies) as well as speciational stochasticity. Nonetheless, a number of modern studies have relied on polytomies or localised reductions in branch length to postulate past radiation events (Hodges and Arnold 1995; Baldwin 1997; Hodges 1997; Baldwin and Sanderson 1998; Jackman et al. 1999).

The demonstration of adaptive radiation, in contrast to general radiation, requires evidence that the radiating lineages be shown to occupy 'different, also diverging adaptive zones' (Simpson 1953), 'different ecological niches' (Futuyma 1986), 'different adaptive properties' (Grant 1986) or 'significant interspecific divergence in the kinds of resources exploited and in the morphological and physiological traits used to exploit these resources' (Schluter 1996). The mechanisms by which these differences arise have been studied by Schluter (1996) and are thought to include 'resource environment'-induced phenotypic differentiation, followed by competition-induced character displacement and, ultimately, ecological speciation. In

acknowledging the importance of adaptive divergence in adaptive radiation, I support Givnish (1997) in his criticism of Guyer and Slowinski's (1993) definition based almost exclusively on accelerated speciation. However, unlike Givnish (1993), I interpret rapid divergence as a hallmark of radiation in general and, therefore, follow the classical definition of Simpson (1953) and the more precise definition of Schluter (1996). The latter defines an adaptive radiation as 'a proliferation of species within a single clade accompanied by significant interspecific divergence in the kinds of resources exploited and in the morphological and physiological traits used to exploit these resources.' 'A proliferation of species' is here taken to mean a burst of rapid diversification.

Adaptive radiation, key innovation and the invasion of new 'adaptive zones'

Simpson (1953) considered the occupation of new 'adaptive zones' essential in stimulating radiation. Thus, he considered the evolution of new 'adaptive types,' - forms having characteristics that facilitate successful invasion of new adaptive zones - an important step in many (but not all) evolutionary radiations. Following the lead of Miller (1949), such characteristics have come to be called 'key innovations'

Hunter (1998) reviewed the utility of the key innovation concept and listed a series of alternative definitions of the phrase that differ in subtle, yet critical, ways. Principally, where traditional definitions view key innovations simply as acquired traits that facilitate the occupation of new adaptive zones (e.g. Simpson 1953; Baum and Larson 1991), a number of recent authors have opted, instead, to define key innovations explicitly in terms of a presumed role in accelerating speciation (e.g. Liem 1973, 1990; Guyer and Slowinski 1993; Heard and Hauser 1995). Occupation of a new adaptive zone does not, however, guarantee subsequent diversification (Mayr 1960) since this depends on 'the extent and diversity of the new territory (available)' (Simpson 1953), and it follows, therefore, that these alternative definitions represent distinct conceptual entities. In addition, key innovations may not always be necessary to trigger diversification (Mayr 1960) as changes in the physical environment alone are sometimes probably sufficient to stimulate diversification through the creation of new ecological openings (Vrba 1985). Indeed, several major radiations may be attributable to sudden climatic and/ or geological change (e.g. origin of the Hawaiian archipelago: Funk and Wagner 1995). Importantly, the

implication that key innovation, in the traditional sense, is not prerequisite for radiation suggests that efforts to explain radiations in terms of key innovations may often go unrewarded.

Cracraft's (1990) argument that a causal relationship between key innovation and increased diversification may be impossible to test convincingly is supported by a severe paucity of convincing tests (Heard and Hauser 1995). For this reason and because the relationship between radiation and key innovation is not a strict one, the definition of the latter in terms of increased diversification seems counterproductive. Accordingly, this study opts to define key innovation in the traditional sense, stressing its critical role in 'modifying the selective regime of the lineage in which it evolves' (Baum and Larson 1991). Under this definition testability is improved, as conventional tests of adaptation (Coddington 1988, 1994; Baum and Larson 1991; Andersen 1995; Larson and Losos 1996) apply. Unlike adaptation, however, the evolution of key innovation is expected to precede or be coincident with the functional shift that it effects.

Points addressed in this chapter

The central goals of this chapter are (i) to establish evidence for a radiation in Ehrharteae, specifically in the *Ehrharta* s. s. clade, (ii) to consider historical events (environmental changes, habitat shifts and key innovations) that may have been influential in driving radiation, and (ii) to determine whether such a radiation can be construed as adaptive in the sense described earlier.

Detecting radiation

Recently, several methods have been developed to test for diversification shifts using phylogenetic information (Sanderson and Donoghue 1996). While some of these utilise information on the relative timing of branching events (e.g. Nee et al. 1992; Paradis 1997 1998), others use only topological information (Slowinski and Guyer 1989, 1993; Sanderson and Donoghue 1994). Simplest amongst the latter is the method of Slowinski and Guyer (1989, 1993), which infers accelerated speciation from greater-than-expected imbalances in species number between even-aged sister clades. Because this method utilises a minimal amount of phylogenetic information (a pair of clades), however, it suffers low statistical power, and, in general, a diversity difference of about 40:1 is required to return a significant result (Sanderson and

Donoghue 1996). Since the magnitude of the difference depends on the period of time over which increased diversification has taken place, smaller radiations which have been produced by accelerated speciation over a short period are unlikely to be detectable using a sister group comparison. Even in cases where a diversity imbalance is determined to be significant, however, the results of sister group comparison may be difficult to interpret. Firstly, the test does not permit distinction between cases in which diversity difference reflects exceptionally high diversification (or low extinction) in one of the lineages under comparison, compared with exceptionally low diversification (or high extinction) in its sister group. Secondly, simple sister group comparison may incorrectly associate diversity nested within one of the sister clades under comparison with the sister clade as a whole (Sanderson and Donoghue 1996).

In an attempt to address the low statistical power of the sister-group method, Sanderson and Donoghue (1994) devised a likelihood-based method that, in considering diversity in three related clades (two sister clades, plus their sister clade) instead of just two, increases statistical power slightly. However, the statistical power of this test remains low (Sanderson and Donoghue 1996) so that, while it has been applied with success to at least one recent radiation (Hodges and Arnold 1995), it may overlook smaller and/ or younger radiations and is likely to be most useful in the study of major, ancient radiations (e.g. Sanderson and Donoghue 1994). Neither this method nor the sister group method of Slowinski and Guyer are, therefore, applied in this study.

Both fossil data and the molecular clock assumption (Kimura 1983) may be used to estimate the relative timing of speciation events on phylogenetic trees (Sanderson and Donoghue 1996) and branch length changes can then be used directly to infer shifts in speciation rate. This is particularly true for recent groups, in which observed patterns of diversification are most consistent with models that ignore extinction (Hey 1992). Problems with these techniques are, however, that fossil data are often fragmentary, while assumption of rate homogeneity by a molecular clock is not always tenable (Sanderson and Donoghue 1996; Omland 1997a). In plants, however, the ITS region typically shows low rate heterogeneity, and is commonly used to date divergence events (Baldwin and Sanderson 1998; Baum et al. 1998; Xiang et al. 1998; Vargas et al. 1999).

In *Ehrharta* s. s., conflict between the ITS1-based topology and that based on combined data (Chapter 2), plus a lack of critical species from the ITS1 data set, compromises its utility for investigating diversification shifts. Therefore, this study utilises a modified protocol, as follows, to check for the existence of accelerated diversification within *Ehrharta* s. s. First, consensus cladograms and phylograms based on analyses of different data sets are examined to check for points of consistently low resolution and/ or reduced branch length, these being commonly interpreted as being indicative of rapid or simultaneous diversification (e.g. Baldwin 1997; Springer et al. 1997; Jackman et al. 1999). A simple graphical technique is then used to check for systematic change in internal branch length (based on a combined cladistic analysis of ITS1, trnL-F and morphological data) with depth in the tree, and breakpoint regression used to localise the point of change. The application of a breakpoint regression model is justified by the expectation that radiation will alter the function relating cladogenesis to time. In order to check whether the resulting pattern can be reasonably inferred to reflect actual changes in the tempo of speciation, branch length variation in an ITS1-based maximum likelihood tree, calculated with a molecular clock and containing representatives of the major subclades in *Ehrharta* s. s., is similarly evaluated.

Causes of radiation

The bulk of non-African ehrharteoids favour forested or perennially mesic habitats (e.g. see Connor and Edgar 1986), while many of the Cape species occupy environments experiencing extreme summer aridity suggests that radiation, if it has occurred in *Ehrharta* s. s., may well have taken place in response to successful invasion of the latter habitat (cf. Clayton and Renvoize 1986). Within the western Cape, intense summer aridity characterises the climate principally of the Namaqualand region, as well as the coastal forelands further south. Two key predictions that are testable using parsimony optimisation are (i) that a preference for perennially mesic habitats is plesiomorphic in *Ehrharta* s. s. and (ii) that accelerated diversification coincides with or closely follows a switch to habitats experiencing low rainfall and, possibly, increased evaporation during summer. Because the retention of leaves through the dry summer season represents a transpirational water cost (Orians and Solbrig 1977; Chabot and Hicks 1982), the evolution of seasonal foliage (summer-deciduousness) is here proposed as a key innovation in *Ehrharta* s. s. To test this hypothesis it is necessary to show (i) that the

evolution of leaf deciduousness occurred before or at the same time as a transition to summer-arid habitats and (ii) that the evolution of leaf deciduousness confers a selective benefit with respect to the survival of seasonal aridity (see method described by Baum and Larson [1991]). The first of these predictions is relatively easily investigated through the reconstruction of ancestral leaf characteristics and habitat preferences, while the second, being a functional argument, requires experimental evaluation. Although moisture stress experiments potentially provide the most direct means of assessing relative drought resilience, these are problematic due to the difficulties involved in maintaining constant moisture stress levels (Kramer 1983; Raynal et al. 1985). In addition, such experiments may confound plant responses to water stress with those resulting from concomitant nutrient stress (Raynal et al. 1985). Therefore, the second prediction is here tested indirectly, using two *Ehrharta* s. s. species having the unusual but independently-derived combination of summer-deciduous leaves and perennial culms. Strongly developed subepidermal chlorenchyma plus the presence of epidermal stomata in the culms of these species results in an anatomy similar to that observed in Restionaceae (Cutler 1969; Linder 1984; Linder 1991b) and indicates culm photosynthetic capacity (Linder and Ellis 1990). Since both the leaves and culms of *E. ramosa* and *E. thunbergii* are photosynthetic, both represent a potential transpirational water cost, especially during summer. However, the demonstration of higher tranpiration rates in the leaves of these species than in the perennating culms would support the suggestion that the summer-deciduousness of the latter is linked to water conservation, and this is tested here. Nevertheless, it is worth noting that in Cape plants, generally, the relative significance of variation in leaf phenology as adaptation to moisture- or nutrient-stress is often difficult to distinguish (Stock 1988; Stock et al. 1992).

Adaptive divergence

Growth form diversity in *Ehrharta* s. s. reflects variation in a number of biologically important attributes (Gibbs Russell and Ellis 1987; Linder 1989; Linder and Ellis 1990) including plant lifespan, plant base morphology, position of innovation buds (i.e. whether culms are branched), culm photosynthetic capacity, burial and swelling of the culm bases to form geophytic structures and leaf lifespan and morphology. While hypotheses have been advanced to explain the evolution of these traits, both in *Ehrharta* s. s. and other Cape grasses (e.g. Linder and Ellis 1990), these are based largely on anecdotal evidence and remain for the most part untested. Here, I

attempt to reconstruct the evolution of some important growth form attributes in *Ehrharta* s. s. and to relate these to historical shifts in the selective regime experienced by the group. Such a procedure is an important first step in the assessment of adaptive hypotheses (Coddington 1988, 1994; Baum and Larson 1991; Andersen 1995; Larson and Losos 1996). Using the reconstructions thus produced I test the hypothesis that the generation of growth form diversity in *Ehrharta* s. s. is historically coincident with, or follows, the occupation of summer-arid habitats and thus reflects adaptive and/ or functional growth form diversification. Chapters 4 and 5 then investigate further the suggestion that this growth form diversification reflects 'divergence in the kinds of resources exploited and in the morphological and physiological traits used to exploit these resources' (Schluter 1996).

Materials and methods

Cladistic resolution, branch lengths and breakpoint regression

Single exemplar trees, representing the sets of fundamental trees produced by analyses of the individual data sets as well as the combined-most analysis (Chapter 2), were drawn as phylograms using PAUP 3.1.1 (Swofford 1993).

The topology of the combined-most phylogram was used to calculate the nodal distance (ND) separating each internal node in the *Ehrharta* s. s. clade from the

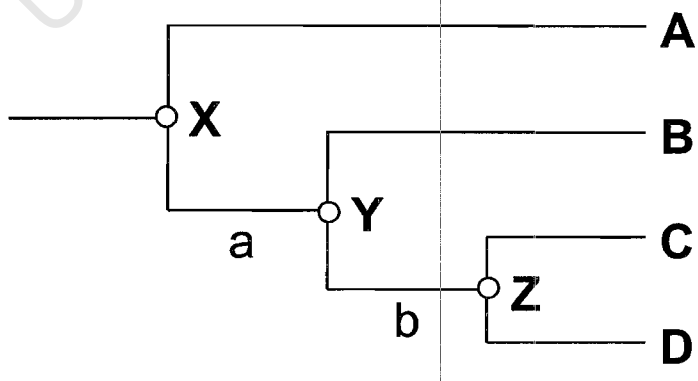


FIGURE 3.1. Hypothetical cladogram describing relationships among four terminal entities, A-D. Internal nodes are labelled X, Y and Z. The letters a and b represent hypothetical branch lengths.

basal node of the entire *Ehrharta* s. s. clade. The ND between a pair of nodes is here defined as the number of internodes separating them. Thus, in a hypothetical clade containing species A, B, C and D (Fig. 3.1) the ND's separating internal nodes X (basal node), Y and Z from the basal node X are, respectively, 0, 1 and 2. Similarly, the total branch length (TBL) separating each internal node in *Ehrharta* s. s. from the basal node of the entire clade was calculated, using branch length data from the combined-most phylogram. The TBL between a pair of nodes is here defined as the summed lengths of the branches connecting them. Hence, in Fig. 3.1 the TBL's separating internal nodes X, Y and Z from the basal node X are, respectively, 0, a and a+b. ND's and TBL's separating all internal nodes in the *Ehrharta* s. s. clade from the basal node were then plotted onto a set of axes (X axis=TBL, Y axis=ND). Points (representing nodes) were connected in such a way as to reflect the topology of the cladogram. Because TBL reflects the amount of character change and ND indicates the amount of cladogenesis, a steep slope on such a plot indicates high branching rate relative to character change, while a shallow slope reflects the converse. Slope changes were pinpointed using breakpoint regression, as implemented in Statistica version 5 (Statsoft 1995). This regression model invokes multiple linear functions to explain apparent non-linearity of the data, and estimates the disjunction(s) (breakpoints) between these functions. Only internal nodes of the combined-most phylogram were included in the breakpoint regression analysis for two reasons. First, since autapomorphic variation was not coded in the morphological data matrix, the branches subtending the terminal nodes are morphologically under-sampled relative to the internal branches and, hence, non-comparable. Second, because only some of the terminal nodes (taxa) included were sampled for ITS1, several of the terminal branches will, in addition, be under-sampled for ITS1 variation. Although exclusion of terminal nodes could potentially result in slope over-estimation by breakpoint regression analysis, this is unavoidable given the non-comparability of the terminal branch lengths with the lengths of internal branches.

Maximum likelihood analysis

In order to estimate the relative timing of major branching events within *Ehrharta* s. s., ITS1 sequences of seven species were analysed in PAUP version 4.0b2 (beta test version: Swofford 1999) using maximum likelihood with a molecular clock. Species included were *E. setacea*, *E. eburnea* and a single representative (the

basal-most one for which ITS1 data were available) of each monophyletic species group in *Ehrharta* s. s., except that containing *E. rupestris*, *E. dura* and *E. microlaena*. While *E. dura* and *E. microlaena* could not be included because ITS1 sequences for these species are unavailable, *E. rupestris* was excluded because its phylogenetic position posited by ITS1 is in disagreement with the topology favoured by combined analysis of all three data sets. Since the seven species sampled contained representatives of all but one of the major clades in *Ehrharta* s. s., it is sufficient to estimate the relative timing of major branching events in the group.

Model parameters are known to have a critical influence on likelihood estimates (Yang et al. 1995) and the data-fit of three models was, therefore, comparatively evaluated using likelihood ratio tests (Felsenstein 1981; Yang et al. 1995; Swofford et al. 1996). The simplest of these, the F81 model (Felsenstein 1981), treats all substitutions as equally likely , while the HKY85 (Hasegawa et al. 1985) model allows for unequal transition and transversion rates, which were here estimated using maximum likelihood. Rate homogeneity among sites is assumed by both of these models but was considered highly unrealistic for the ITS1 data. Therefore, a third model based on the HKY85 model but using a discrete, four-rate approximation to the gamma distribution (shape parameter α estimated by maximum likelihood) to model rate variation among sites (Yang 1993, 1996) was employed (HKY+Γ). Analyses using all three models were performed both with and without the constraint of a molecular clock, and likelihood ratios used to evaluate the appropriateness of a clock assumption. Topological identity among all likelihood trees suggests that use of a chi-square distribution to evaluate likelihood ratios is appropriate (Goldman 1993). For all analyses, proportions of the four bases were set as the observed frequencies in the data set.

Characterisation of vegetative growth form variation

Species were coded for six attributes which describe observed variation in functional vegetative morphology (Table 3.1) using data obtained from three sources: (i) field observation, (ii) examination of herbarium and field-collected material, and (iii) available literature. The complete attribute matrix thus produced is included in Appendix 4.

TABLE 3.1. Delimitation of states used to reconstruct the evolution of vegetative growth form attributes in *Ehrharta* s. s.

Attribute	Coding
1. Plant lifespan	**0**=annual, **1**=perennial
2. Culm branching	**0**=branching, **1**=not branching
3. Culm chlorenchyma	**0**=weakly developed, **1**=strongly developed
4. Culm base swelling	**0**=bulbous, **1**=not bulbous
5. Culm base burial depth	**0**=less than 5cm below ground, **1**=more than 5cm below ground
6. Foliage phenology	**0**=summer-green, **1**=summer-deciduous

Plant lifespan

This trait is typically described in taxonomic accounts. Though essentially developmental, plant lifespan can be reliably inferred from morphology. Typically annual plants have weak, herbaceous bases, while perennials have woody, rhizomatous bases. Although a few specimens of *E. calycina* are annual-like, the vast majority are clearly perennial and the species is accordingly coded as such. *E. erecta* is also coded as perennial, this being the most common state for the species. However, *E. erecta* is facultatively annual (Gibbs Russell and Ellis 1987: Table 2).

Culm branching

Whether a species' culms are branched or unbranched above ground level is easily observed using herbarium material and is also often described in taxononomic accounts. In Ehrharteae, branching culms are typically long-lived and indicate a suffrutescent habit. In *Ehrharta* s. s. culm orientation varies from erect to decumbent. Three species of *Ehrharta* s. s. (*E. erecta* and *E. rehmannii*) as well as two *Tetrarrhena* species have both branched and unbranched culms and are thus coded as polymorphic.

Culm chlorenchyma

The distinction between culms with a weakly and strongly developed photosynthetic capacity is qualitative. Principally, 'strongly developed culm chlorenchyma' is a condition in which the culms are green, typically have long, exposed internodes (sheaths relatively short) and contain a well-developed, palisade-like subepidermal chlorenchyma (Fig. 3.2a). Although culms of most ehrharteoids contain some

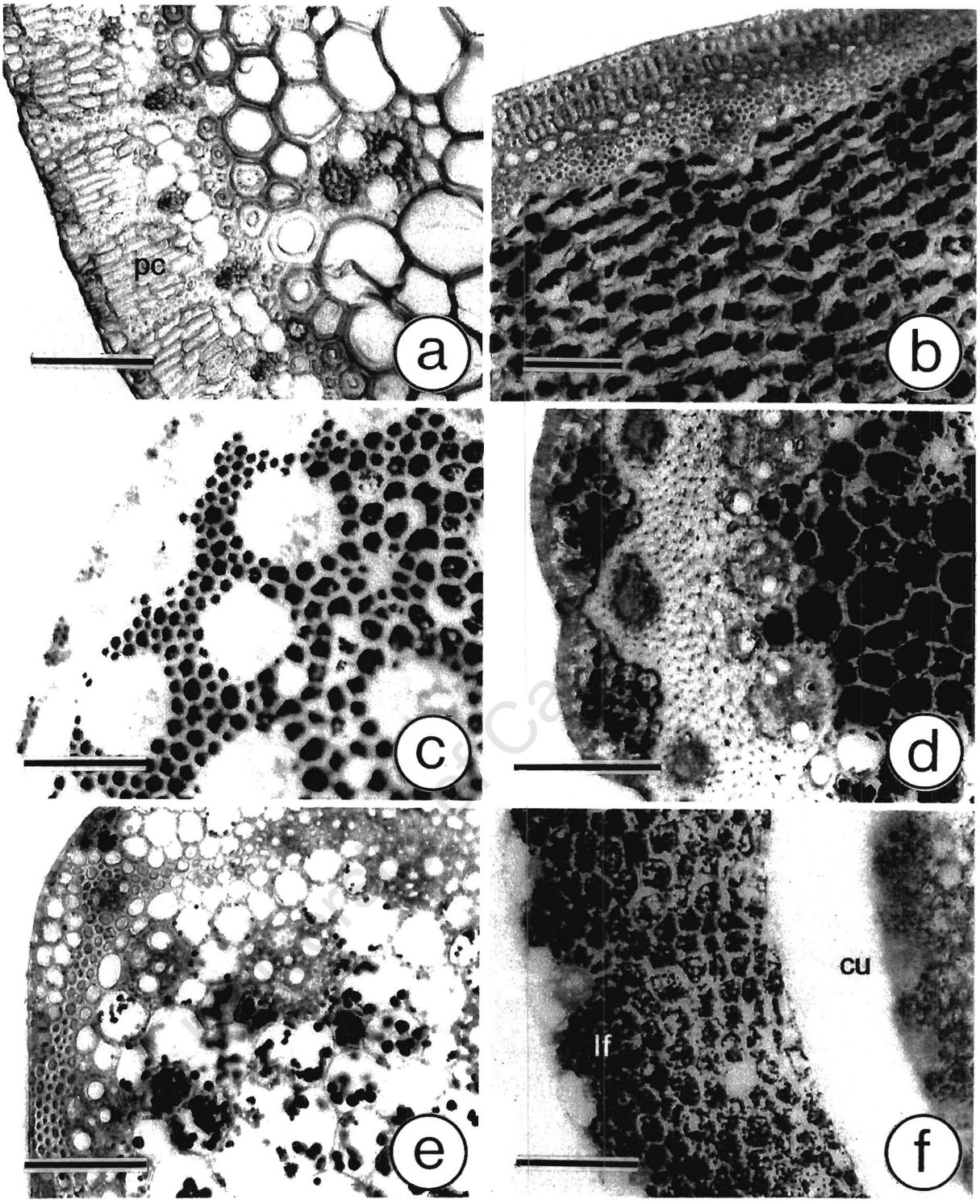


FIGURE 3.2. **(a)** Transverse section through the perennating, photosynthetic culm of *E. ramosa* [Verboom 81] showing the well-developed, palisade-like subepidermal chlorenchyma (pc). **(b)-(e)** Transverse sections through the basal portion of the culms of (b) *E. ottonis* [Verboom 150], (c) *E. barbinodis* [Verboom 158], (d) *E. calycina* [Verboom 156], (e) *E. delicatula* [Verboom 104]. **(f)** Transverse section through the culm (cu) and leaf bases (lf) of *E. melicoides* [Verboom 153]. Sections (b)-(f) are stained with an I_2-KI solution (Johansen 1940) to indicate starch (black). All scale bars are 100μm.

subepidermal chlorenchyma, in most it cannot be considered palisade-like and is then termed 'weakly developed'.

Culm base burial

In some species of *Ehrharta* s. s., the basal portion of the culms are buried below the soil surface and contain large quantities of starch (Fig. 3.2b-e ; Watson and Dallwitz 1992). Accordingly, culm base burial may be associated with a geophytic strategy.

A metal ruler with 0.5mm gradations was used to measure culm burial depth on several herbarium specimens of each species, this being the distance between the culm base (i.e. where it meets the rhizome) and the inferred soil surface level. Soil residues as well as culm chlorophyll distribution were used to estimate the latter. The number of specimens per species varied, mostly between 10 and 30, depending on the degree of intraspecific variation. Where limited material was available sample sizes were smaller. The same delimitation procedure used to define character states in Chapter 2 was used to divide this variable into two discrete states (Table 3.1).

Culm base swelling

Swelling of the culm base into a distinctive, hard, globose, bulb-like structure in *Ehrharta* s. s. is readily observed in herbarium material. Transverse sections through these structures indicate that they are major repositories of starch (e.g. Fig. 3.2b), thus reflecting a well-developed geophytic growth form.

Foliage phenology

Although leaf anatomy may provide clues to leaf lifespan, field observations (Ellis and Linder 1992) made at different times of the year are ultimately required to confirm patterns of foliage phenology. Nonetheless, in grasses mesic leaves are soft and typically persist for just a single growing season, while sclerophyllous leaves are hard and may persist for more than one growing season (Ellis and Linder 1992). Thus where the latter are typically associated with evergreen species, the former usually characterize species whose leaves are seasonally deciduous and shrivel during the dry, non-growing season. Because data describing foliage phenology are rarely included in taxonomic accounts, this attribute was not coded for the non-southern African species. For *Ehrharta* s. s., field observations, anatomical data

(Ellis 1987a, b; Gibbs Russell and Ellis 1987, 1988) and anecdotal reports (e.g. Kruger 1987) were used in combination to infer foliage phenology. Due to incomplete information, *E. dura*, *E. microlaena*, *E. ottonis* and *E. longigluma* were coded as 'uncertain'. The ability of *E. erecta* to retain green foliage throughout the year in moist, shady habitats but not in drier environments underlies the polymorphic coding for this species.

Characterisation of habitat variation

Six environmental variables describing differences in species' habitat preferences were coded as indicated in Table 3.2, the resulting matrix being included in Appendix 5. Habitat variables were selected to reflect putatively important environmental gradients in the western Cape region, this region being the focus of interest with respect to the radiation of *Ehrharta* s. s. Since an infinite number of habitat variables might have been considered, for practical reasons it was decided to include only the set of variables that, on the basis of field observation, appeared to best explain *Ehrharta* s. s. species distribution.

Climatic preferences

The climatic preferences of species of Ehrharteae were determined by overlaying species distributions on maps describing the appropriate climatic variables and reading off the ranges of corresponding values. For this purpose the distributions of South African species were inferred from material at BOL as well as a large and representative sample of the material at PRE, while those of non-South African species were inferred from taxonomic treatments (e.g. Willemse 1982; Edgar and Connor 1998), selected material from NSW, P and WELT and collection locality data of material at MELB.

This map-based technique for inferring species' climatic preferences is comparable to the use of GIS's (geographical information systems) but it is simpler, cheaper and more time effective. Although the method has a limited capacity to account for fine-scale environmental variation (due to restricted data resolution), this limitation is shared with GIS-based methods. Thus, the additional investment associated with the use of GIS-based methods were not considered worthwhile in this study.

Nonetheless, the lack of information on fine-scale climatic heterogeneity remains

TABLE 3.2. Delimitation of states used to reconstruct the evolution of habitat preferences in *Ehrharta* s. s. Variables 1-4 are treated additively (ordered), while variables 5 and 6 are non-additive (unordered).

Variable	Coding
1. Mean annual rainfall	**0**=0-200mm, **1**=200-400mm, **2**=400-600mm, **3**=600-800mm, **4**=800-1000mm, **5**=1000-1200mm, **6**=more than 1200mm
2. Median January rainfall	**0**=0-5mm, **1**=5-10mm, **2**=10-20mm, **3**=20-40mm, **4**=40-60mm, **5**=more than 60mm
3. January potential evaporation	**0**=less than 180mm, **1**=180-200mm, **2**=200-220mm, **3**=220-240mm, **4**=240-260mm, **5**=260-280mm, **6**=280-300mm, **7**=300-320mm, **8**=320-340mm, **9**=more than 340mm
4. Duration of moisture growing season	**0**=0-25d.yr^{-1}, **1**=25-50d.yr^{-1}, **2**=50-100d.yr^{-1}, **3**=100-125d.yr^{-1}, **4**=125-150d.yr^{-1}, **5**=150-175d.yr^{-1}, **6**=175-200d.yr^{-1}, **7**=200-225d.yr^{-1}, **8**=more than 225d.yr^{-1}
5. Substrate parent material	**0**=sandstone, **1**=granite, **2**=shale or dolerite, **3**=basic, quarternary sands
6. Vegetation type	**0**=forest, **1**=fynbos shrubland (sclerophyll heathland), **2**=karroid and renoster shrubland, **3**=succulent shrubland, **4**=grassland, **5**=dune thicket

potentially problematic and may be especially severe in topographically complex areas such as the western Cape region.

The present study takes account of four climatic variables: (i) mean annual rainfall, (ii) median January rainfall, (iii) January potential evaporation and (iv) duration of the moisture growing season. Mean annual rainfall preferences were estimated for both South African and non-South African ehrharteoids but preferences with respect to variables (ii)-(iv) were estimated for the Cape species only. This is because these latter variables were selected explicitly as measures of the intensity ([ii], [iii]) and duration ([iv]) of seasonal (summer) drought in the winter-rainfall western Cape region, so that their coding is meaningful only for species that occur in this area. For the same reason, two species native to the Cape but widespread in southern Africa (*E. calycina* and *E. erecta*), were coded (with respect to variables [ii]-[iv]) exclusively in terms of their preferences in the western Cape region.

Source climatic data for South African species were obtained from Schulze (1997), while mean annual rainfall data for non-South African species were estimated from the Times Atlas (1993). January evaporation data for the western Cape are provided as A pan equivalents (Schulze 1997). The moisture growing season is defined as the period when there is sufficient soil water to sustain crop growth (Schulze 1997). In

South Africa this is estimated to hold when precipitation exceeds 0.3 times the potential evaporation (adapted from the FAO [1978] approach: see Schulze 1997). The duration of the moisture growing season exhibits considerable variation in the western Cape and several species of *Ehrharta* s. s. occur in areas calculated to have no moisture growing season at all. This indicates that these species are able to survive in areas too arid for cultivation. Although the four climatic variables used in this study are fundamentally continuous, they are coded categorically here (Table 3.2) because this is the way the data are presented in Schulze (1997). The delimitation of states thus exactly reflects the original format of the data.

In order to examine the influence of moisture growing season duration on the timing of flowering in different species, the months of last flowering in each species (data obtained from Gibbs Russell [1990]) were plotted against the months representing the end of the moisture growing season experienced by each species. In order to avoid the problems introduced by differences in rainfall seasonality within southern Africa, only species restricted to the western Cape were included in this comparison.

Substrate and vegetation type preferences

Because substrates (soil types) show fine-scale spatial variation, a coarse, map-based approach similar to that used to estimate climatic preferences is unsuitable for inferring species' substrate preferences. Species' substrate preferences were, therefore, scored on the basis of direct field observation as well as herbarium specimen label data and descriptive accounts. These also formed the principal sources of information for inferring the preferred vegetational association of each species. In this regard, accounts by Willemse (1982), Connor and Edgar (1986), Gibbs Russell (1987a, b, 1990), Gibbs Russell and Ellis (1988), Walsh (1989), Wardle (1991) and Edgar and Connor (1998) were particularly helpful. The categorisation of western Cape vegetation types used here broadly follows the classification of Campbell (1985), except that succulent shrubland is distinguished from karroid and renoster shrubland and dune thicket (strandveld) from forest and other classes of thicket. Further, forest is here extended to include, in addition to Afromontane forest, a range of other types (e.g. western thicket [Cowling and Holmes 1992a], open *Eucalyptus* forest [Specht 1970] and *Nothofagus* forest) in which Ehrharteae are known to occur.

Reconstruction of ancestral growth form attributes and habitat preferences

The use of unweighted parsimony to reconstruct ancestral states of any character assumes: (i) an equal probability of change on all branches, (ii) a relatively slow rate of evolution, and (iii) that gains and losses are equally probable (Omland 1999). While several authors have recently started to question the general validity of these assumptions (e.g. Kohn et al. 1996; Omland 1997b, 1999; Schluter 1997; Cunningham et al. 1998), the development of alternative, more explicitly justifiable techniques is in its infancy. Like the majority of studies, this study, therefore, employs parsimony to reconstruct the ancestral states of both growth form attributes and habitat preferences in Ehrharteae.

Parsimony-based character optimisation procedures cope poorly with polytomies (Maddison 1989). Therefore, I principally make use of one of the 84 optimum length trees produced by analysis of the combined-all data (Table 2.6, Fig. 2.5b) to infer historical shifts in species' growth form attributes and habitat preferences. The chosen tree was selected to be topologically compatible with the strict consensus of the combined-most analysis (Fig. 2.5a). Although the latter is taxonomically less complete, it is highly resolved and, judged on the greater completeness of data, more reliable. The set of trees remaining after application of this filter contained conflict in only four areas: (i) the position of *M. polynoda*, (ii) resolution of (*M. avenacea* + *M. diplax* + *M. tasmanica*), (iii) resolution of (*E. capensis* + *E. bulbosa* + *E. ottonis* + *E. longifolia*) and (iv) resolution of (*E. calycina* + *E. brevifolia* + *E. pusilla*). Because topological uncertainties can have a profound influence on character optimisation and attendant evolutionary interpretation (Losos 1994; Donoghue and Ackerly 1996) the effects of all alternative resolutions of these four areas of uncertainty were explored.

Climatic preference variables are fundamentally continuous and so were treated additively (states ordered), being traced onto the selected tree using Wagner optimisation (Farris 1970) as implemented in MacClade version 3 (Maddison and Maddison 1992). In contrast, substrate and vegetation type preferences were treated non-additively (states unordered) and optimised using Fitch optimisation (Fitch 1971), also using MacClade. Where appropriate the association or dependence of evolutionary change in one variable with/ on that in another was tested using the method of Sillén-Tullberg (1993) which is an alternative to the concentrated changes

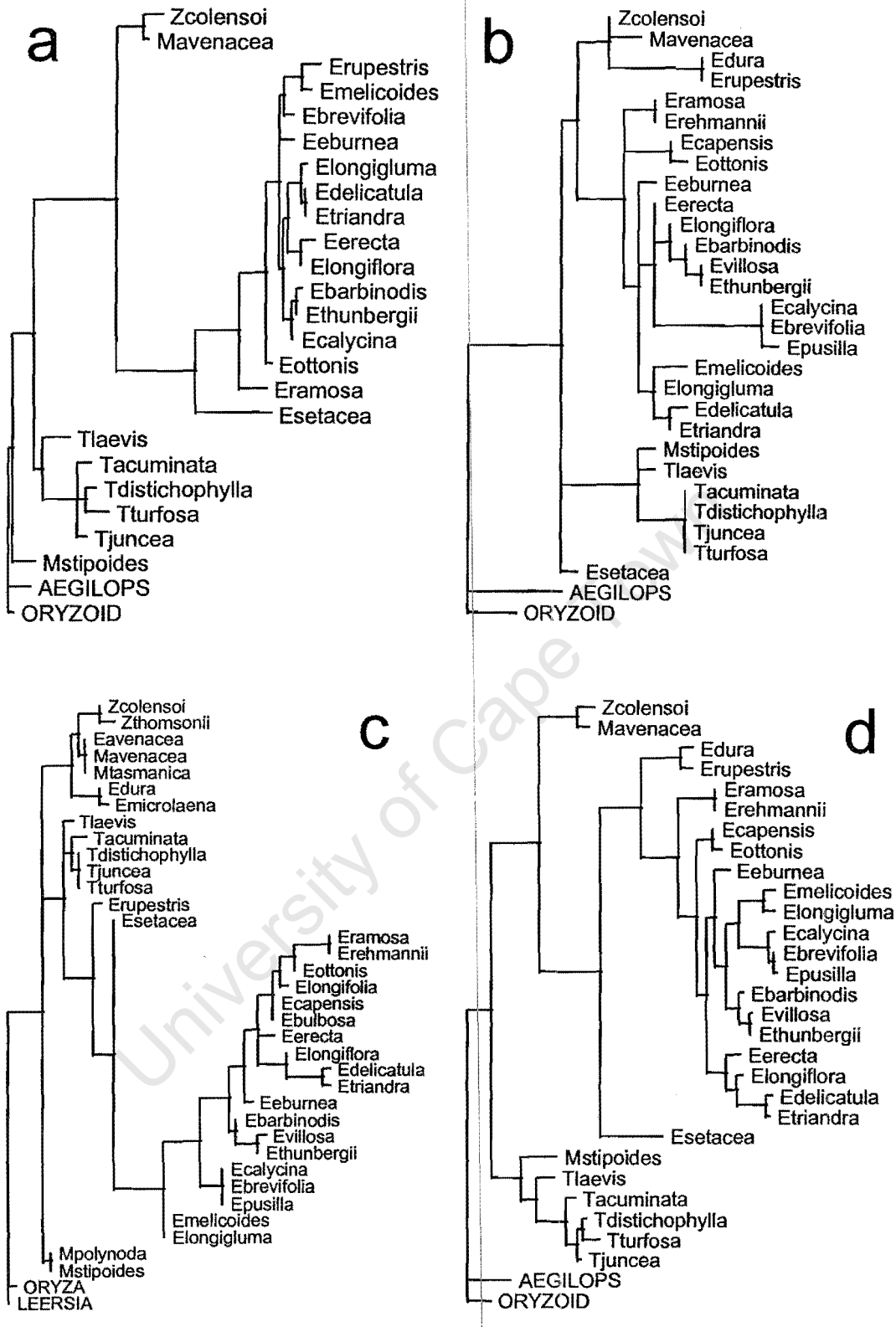


FIGURE 3.3. Phylograms based on the (a) ITS1, (b) trnL-F, (c) morphology and (d) combined-most data sets. Branch lengths indicate amounts of character change. Note that the very short ingroup-outgroup branch in the ITS1 tree is artifactual, reflecting a lack of alignable sequence.

test of Maddison (1990). For this purpose, two-tailed Fisher exact tests as implemented in Statistica version 5 (Statsoft 1995) were employed to check for the significance of evolutionary associations.

Culm and leaf transpiration and photosynthesis rates

Stomatal water conductance ($g_s[H_2O]$) and photosynthetic carbon assimilation rates of leaves and culms of *E. ramosa* and *E. thunbergii* were measured in the field using a CIRAS-1 differential CO_2/H_2O infra-red gas analyser (PP systems, Hitchin, U. K.). Three plants from single populations of each species were sampled in mid-September 1998 during peak growth. Both sample populations are located on the Dasklip Pass near Porterville in the western Cape (32°54'S 19°02'E). Cuvette settings were as follows: CO_2 concentration 360ppm; air humidity 50%; leaf temperature 25°C; photosynthetic photon flux density 1000μmol.m^{-2}.s^{-1}. Earlier laboratory trials on plants of the same species had been performed to confirm that light saturation occurs below a level of 1000μmol.m^{-2}.s^{-1}. Leaf and culm portions included in the cuvette were removed, their surface area calculated and their dry mass determined, these figures being used to calculate dry mass- and area-specific rates.

Results

Phylogram resolution and branch length variation

Strict consensus trees derived from separate analyses of the morphological and molecular data sets consistently show a lack of resolution at or near the base of a clade containing the majority of species of *Ehrharta* s. s. (Fig. 2.3). Species excluded from this clade by all three data sets are *E. setacea*, *E. dura* and *E. microlaena*, while the trnL-F and morphological data sets also exclude *E. rupestris*. In addition, both molecular data sets resolve *E. ramosa* and *E. ottonis* (and their closest relatives) as basal to the remainder of this clade (Fig. 2.3b, c). Phylograms of trees produced by these analyses also typically exhibit branch length reduction within this clade relative the length of the branch subtending it (Fig.3.3a-c). This pattern is particularly marked for the ITS1 trees (Fig. 3.3a). Although having a near-fully resolved strict consensus, phylograms show that the fundamental trees produced by the combined-most analysis are similar to those produced by individual data set

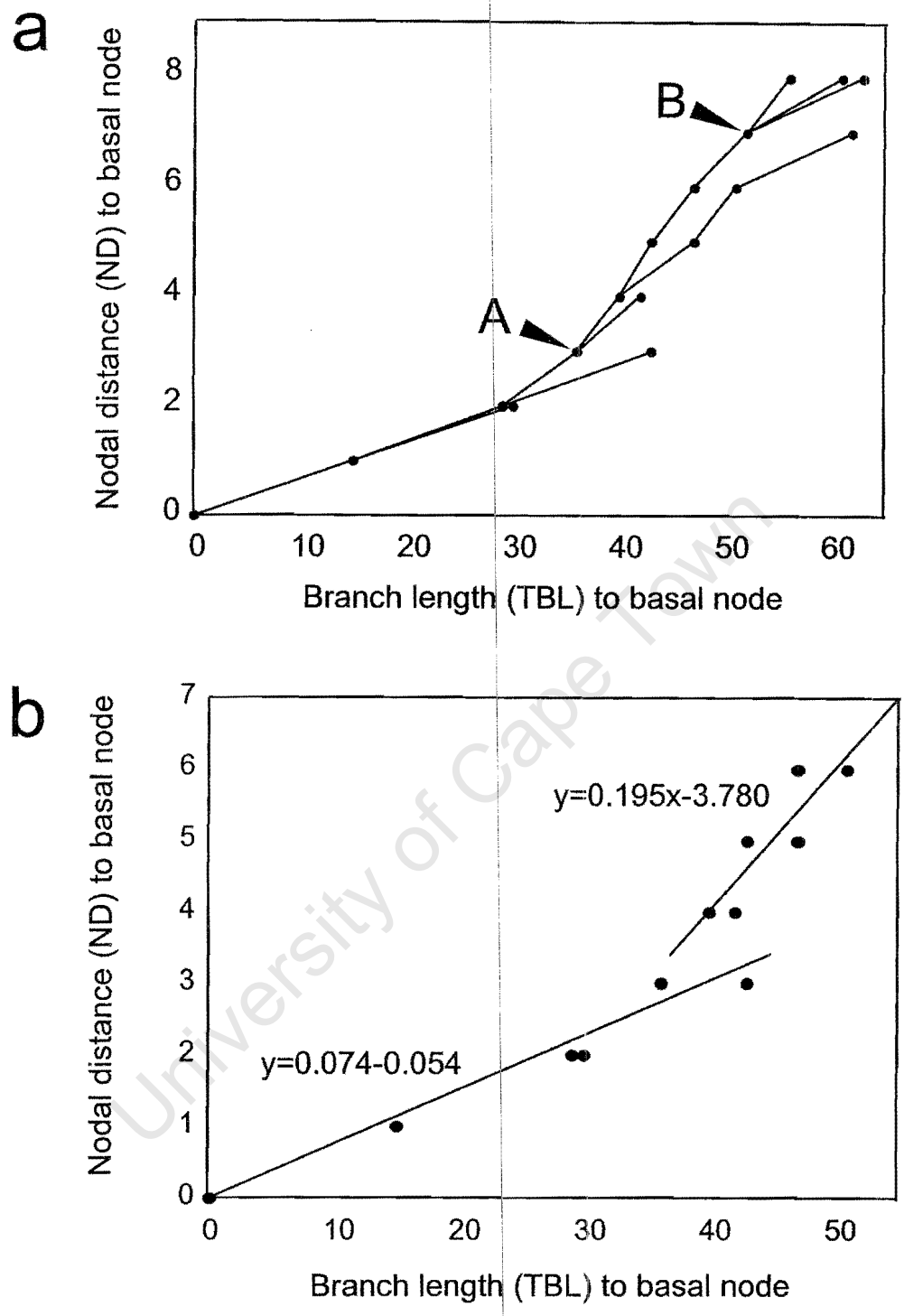


FIGURE 3.4. The relationship between total branch lengths (TBL) and nodal distances (ND) connecting internal nodes within the *Ehrharta* s. s. clade to its basal node, as reflected by the combined-most phylogram. In (a) nodes are connected by lines to reflect topology, while in (b) the linear relationships suggested by breakpoint regression are indicated. Arrows A and B indicate, respectively, points of increase and decrease in the slope of the overall relationship, as inferred on the basis of visual inspection (see text).

analyses in reflecting marked branch length reduction near the base of the *E. ottonis-E.erecta* clade (Fig. 3.3d). A similar pattern, though much smaller in scale, is also observed at the base of the *T. acuminata-T. juncea* clade.

Substantially improved correlation following natural log transformation of the data (r=0.98 transformed, compared with r=0.92 untransformed) confirms that the relationship between the TBL and ND (Fig. 3.4a) separating all internal nodes in the *Ehrharta* s. s. clade (combined-most consensus tree) from its basal node is non-linear. Instead, three separate and more or less linear phases can be subjectively identified: (i) near the base of the tree a low rate of cladogenesis relative to character change is indicated by a shallow slope; (ii) inception of a steeper slope near 'A' indicates increased cladogenesis relative to character change; and (iii) a reduction in slope near 'B' suggests a return to initial conditions. Fig. 3.4b presents the results of a breakpoint regression analysis used to identify the disjunction (breakpoint) between the functions representing phases (i) and (ii) (i.e. 'A'), here assumed to be linear. Because points beyond 'B' (ND=7 and 8) appear to describe a third function, these were omitted from this analysis. The analysis identifies a breakpoint at ND=3.417, the slope of the line above this point (m=0.195) being 2.7 times that below (m=0.074). When points having ND≤4 and those having ND>5 are subjected to separate correlation and linear regression analyses the linear functions associated with these sets of points are identical to those described by breakpoint regression.

These results, therefore, suggest a 2.7-fold increase in the rate of cladogenesis relative to character change in *Ehrharta* s. s., the observed breakpoint value locating this shift on the branch subtending the *E. erecta-E.eburnea* clade.

Although it is possible that slope estimation by breakpoint regression could be biased through the exclusion of terminal nodes, inspection of the data used suggests that the effect of including terminal nodes on the estimation of the position and magnitude of slope change at 'A' would be minimal. In contrast, inferences concerning slope change at 'B' would be substantially affected. Since this study is concerned with the nature of change at 'A', however, these latter effects are of limited relevance.

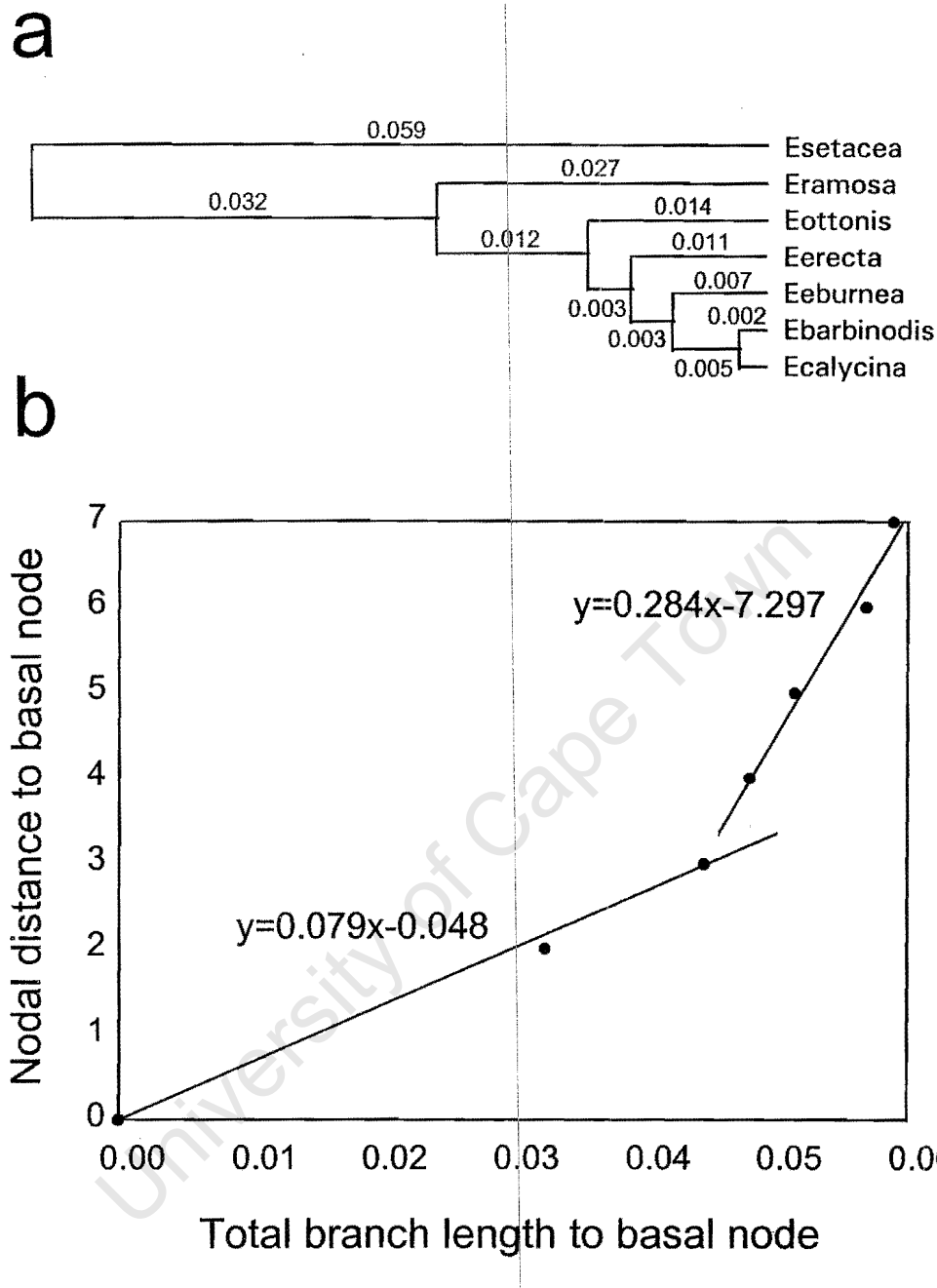


FIGURE 3.5. (a) Maximum likelihood tree based on ITS1 sequences of basal representatives of each of the major subclades (species groups) within *Ehrharta* s. s. The evolutionary model used is the HKY85 model, with a molecular clock assumed. Values on branches indicate the mean number of nucleotide changes per site. (b) The relationship between total branch lengths and nodal distances connecting internal nodes within the *Ehrharta* s. s. clade to its basal node. Linear relationships suggested by breakpoint regression are indicated.

Maximum likelihood analysis

All maximum likelihood analyses produced single optimal trees that were topologically identical and compatible with the topology of the cladogram produced by the combined-most analysis. The log-likelihoods associated with each analysis are listed in Table 3.3. Likelihood ratio tests indicate that the HKY85 model provides a significantly better explanation of ITS1 sequence variation in *Ehrharta* s. s. than the F81 model, with or without assumption of a molecular clock (Table 3.3). However, modification of the HKY85 model to accommodate rate variation among sites (HKY-Γ) does not yield significant improvement and the HKY85 model is, therefore, considered sufficient and optimal. Since all likelihood comparisons testing the assumption of a molecular clock under different models of evolution indicate no significant differences (Table 3.3), a molecular clock cannot be rejected. Fig. 3.5a shows the maximum likelihood tree produced under the HKY85 model, with the assumption that a molecular clock is operative.

Breakpoint regression analysis of the relationship between the amount of sequence change and the ND separating all internal nodes in this tree from its basal node, identifies a 3.6-fold slope increase at ND=3.333 (Fig. 3.5b). Under a clock assumption sequence change is proportional to time and so this shift can be interpreted as an increase in time-relative branching rate on the branch subtending the *E. erecta-E. eburnea* clade. Although this shift is positionally identical to the observed slope change in the relationship between TBL and ND calculated for the

TABLE 3.3. Comparison of log-likelihood scores of maximum likelihood trees obtained under different models of molecular evolution, with (c) and without (nc) assumption of a molecular clock. Probabilities associated with likelihood ratios are based on a chi-square distribution, asterisks indicating significance at the α=0.05 level.

Model 1	Model 2	-ln L_1	-ln L_2	-2ln(L_1 /L_2)	d. f.	Prob.
F81(nc)	HKY85(nc)	672.33147	669.29184	6.07926	1	P<0.025 *
HKY85(nc)	HKY85-Γ(nc)	669.29184	667.77754	3.02860	1	P>0.05
F81(c)	HKY85(c)	676.65046	673.61315	6.07462	1	P<0.025 *
HKY85(c)	HKY85-Γ(c)	673.61315	671.97503	3.27624	1	P>0.05
F81(c)	F81(nc)	676.65046	672.33147	8.63798	5	P>0.1
HKY85(c)	HKY85(nc)	673.61315	669.29184	8.64262	5	P>0.1
HKY85-Γ(c)	HKY85-Γ(nc)	671.97503	667.77754	8.39498	5	P>0.1

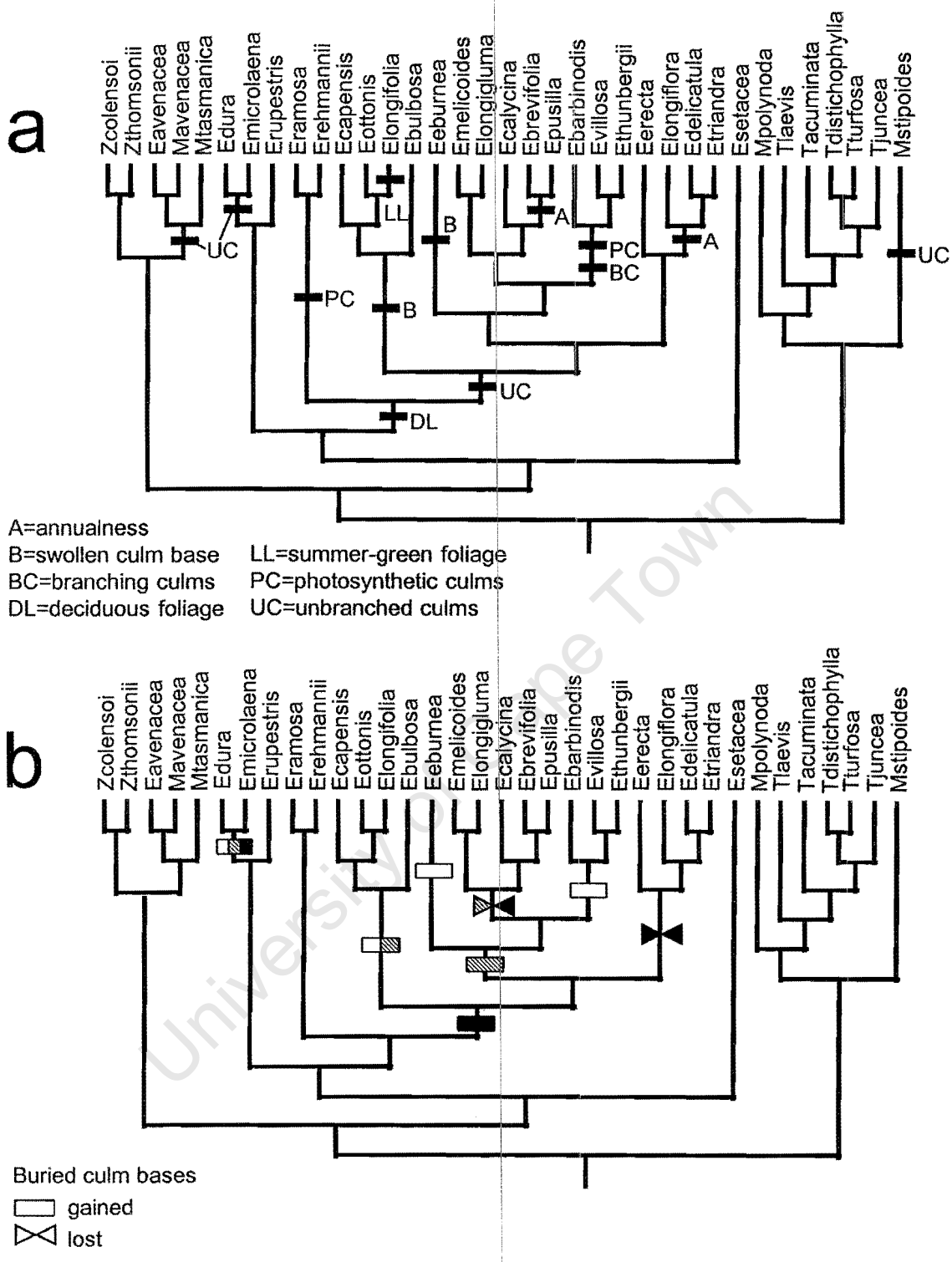


FIGURE 3.6. Optimisation of growth form attributes (Table 3.1, Appendix 4) on a phylogenetic hypothesis for Ehrharteae. The evolution of an annual habit, summer-deciduousness or summer-greenness of foliage, bulbous swelling of culm bases, branching or non-branching of culms and strongly developed culm chlorenchyma is depicted in (a). Three alternative scenarios (empty shapes=DELTRAN, solid shapes=ACCTRAN, hatched shapes=mixed optimisation) describing the evolution of culm base burial are shown in (b). Note that under both alternative resolutions of the *E. calycina-E. brevifolia* clade the evolution of an annual habit is equivocal reflecting either two independent gains (DELTRAN) within this group, or one gain and one loss (ACCTRAN).

combined-most data, the magnitude of this increase is slightly greater. Nonetheless, the amounts of ITS1 sequence change between the basal node of the *Ehrharta* s. s. phylogeny and the internal nodes of the tree shown in Fig. 3.5a (i.e. along the spine of the *Ehrharta* s. s. tree) correlate strongly with the corresponding amounts of change in all characters (TBL, combined-most data) ($r=0.998$, $n=6$, $P<0.0001$).

Reconstructed evolution of growth form attributes

Fitch optimisation of growth form attributes (Appendix 4) onto the phylogeny of the tribe is indicated in Fig. 3.6. The reconstructions suggested by these optimisations are robust to alternative resolution of both the *M. tasmanica* and *E. bulbosa* clades as well as change in the position of *M. polynoda* at the base of the tree. The optimisation of plant lifespan is, however, altered slightly by alternative resolution of the *E. calycina* clade.

The plesiomorphic nature of a perennial habit in both Ehrharteae and *Ehrharta* s. s. is robustly supported by the reconstruction in Fig. 3.6a which indicates two origins of annualness in *Ehrharta* s. s. However, while the reconstruction shown indicates a single acquisition of annualness within the *E. calycina* clade, both alternative resolutions of this clade render optimisation of plant lifespan equivocal, allowing either for separate gains of annualness for *E. brevifolia* and *E. pusilla* under DELTRAN optimisation, or a secondary loss of annualness under ACCTRAN for *E. calycina*.

Like perennial habit, the possession of branching culms is optimised as ancestral in Ehrharteae and *Ehrharta* s. s. (Fig. 3.6a) and is lost on four occasions: in *M. stipoides*, and at the base of the *M. tasmanica-M. avenacea*, *E. dura-E. microlaena* and *E. bulbosa-E. erecta* clades, with a single reversal at the base of the *E. barbinodis* clade. The basal optimisation of this attribute is not, however, robust, since the observed reconstruction could easily change with inclusion of an outgroup having unbranched culms. In addition, the evaluation of culm branching in four polymorphic species (*T. laevis*, *T. acuminata*, *E. rehmannii* and *E. erecta*) is equivocal. If one or all of the first three is/ are coded as having unbranched culms the basal optimisation of this character becomes equivocal.

Summer-deciduousness of foliage is interpreted as derived in *Ehrharta* s. s., evolving once at the base of the *E. ramosa-E. bulbosa* clade, with a single reversal in the *E.*

FIGURE 3.7 (following pages). Optimisation of habitat variables (Table 3.2, Appendix 5) on a phylogenetic hypothesis for Ehrharteae (a, f) or *Ehrharta* s. s. (b-e). For climatic variables (a-d) the discrete state combinations optimised onto the internal nodes are converted back to the continuous value ranges that they represent, and branch shading is used to indicate a transition from less to more seasonally-arid habitats. In each case, the nodal value ranges represent the total ranges suggested by all possible resolutions (ACCTRAN or DELTRAN). Variables traced are as follows: **(a)** Mean annual rainfall. Circled numbers are node numbers referred to in the text and in Fig. 3.8. Optimised nodal ranges are not circled and are in units of 100mm.yr^{-1}. **(b)** January median rainfall (optimised nodal ranges in mm). **(c)** January potential evaporation (optimised nodal ranges in mm). **(d)** Duration of the moisture growing season (optimised nodal ranges in days). **(e)** Substrate parent material. **(f)** Vegetation association. Note that both alternative resolutions of the *E. calycina-E. brevifolia* clade shift the evolution of a strict association with low annual rainfall (0-400mm) as well as a preference for granitic substrates to the basal branch of this clade, while topological rearrangement in the *E. bulbosa-E. capensis* clade shifts the evolution of a strict association with low January median rainfall (10-20mm) to the branch subtending the *E. bulbosa-E. erecta* clade.

Fig. 3.7(a)

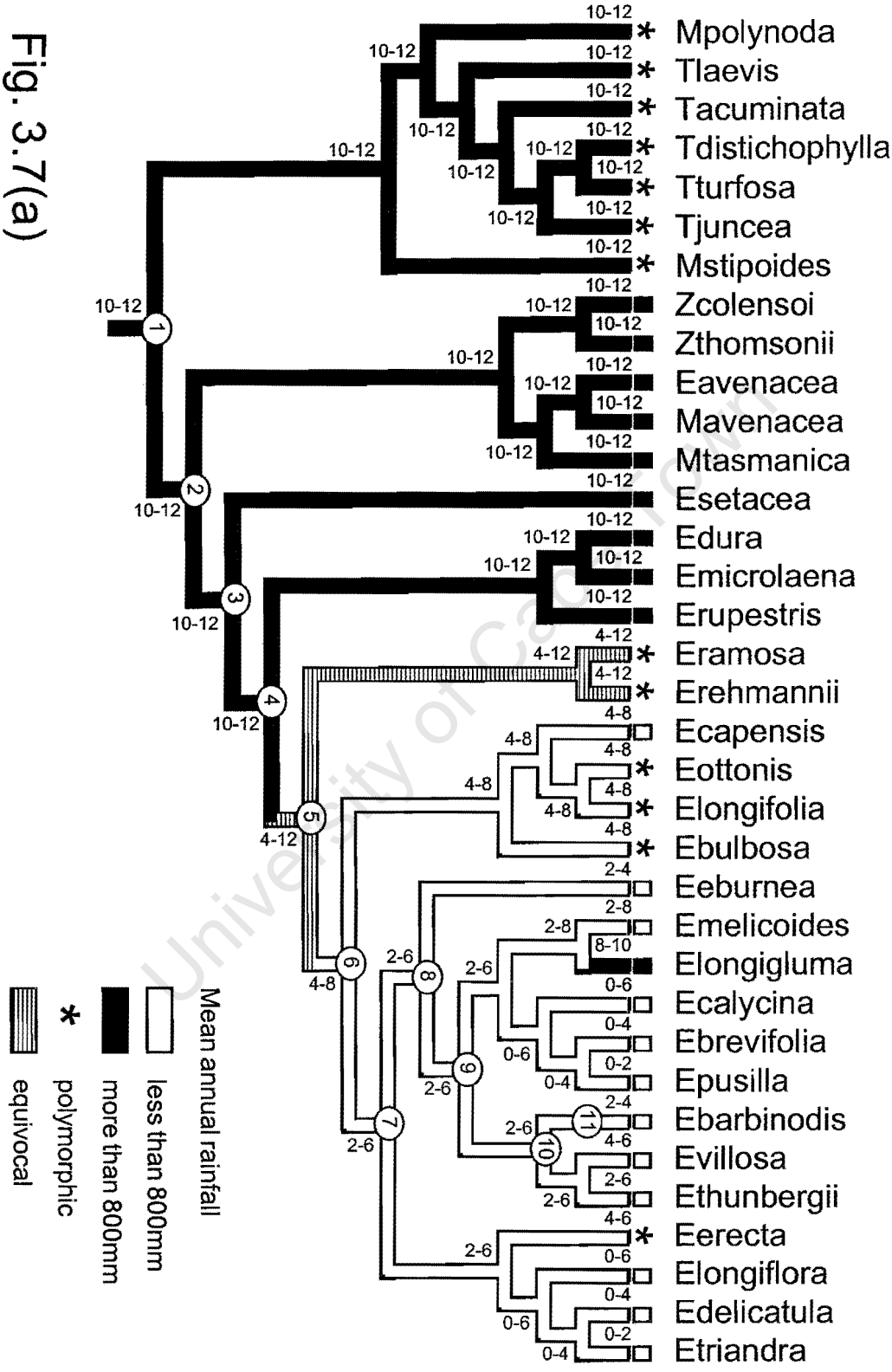


Fig. 3.7(b)

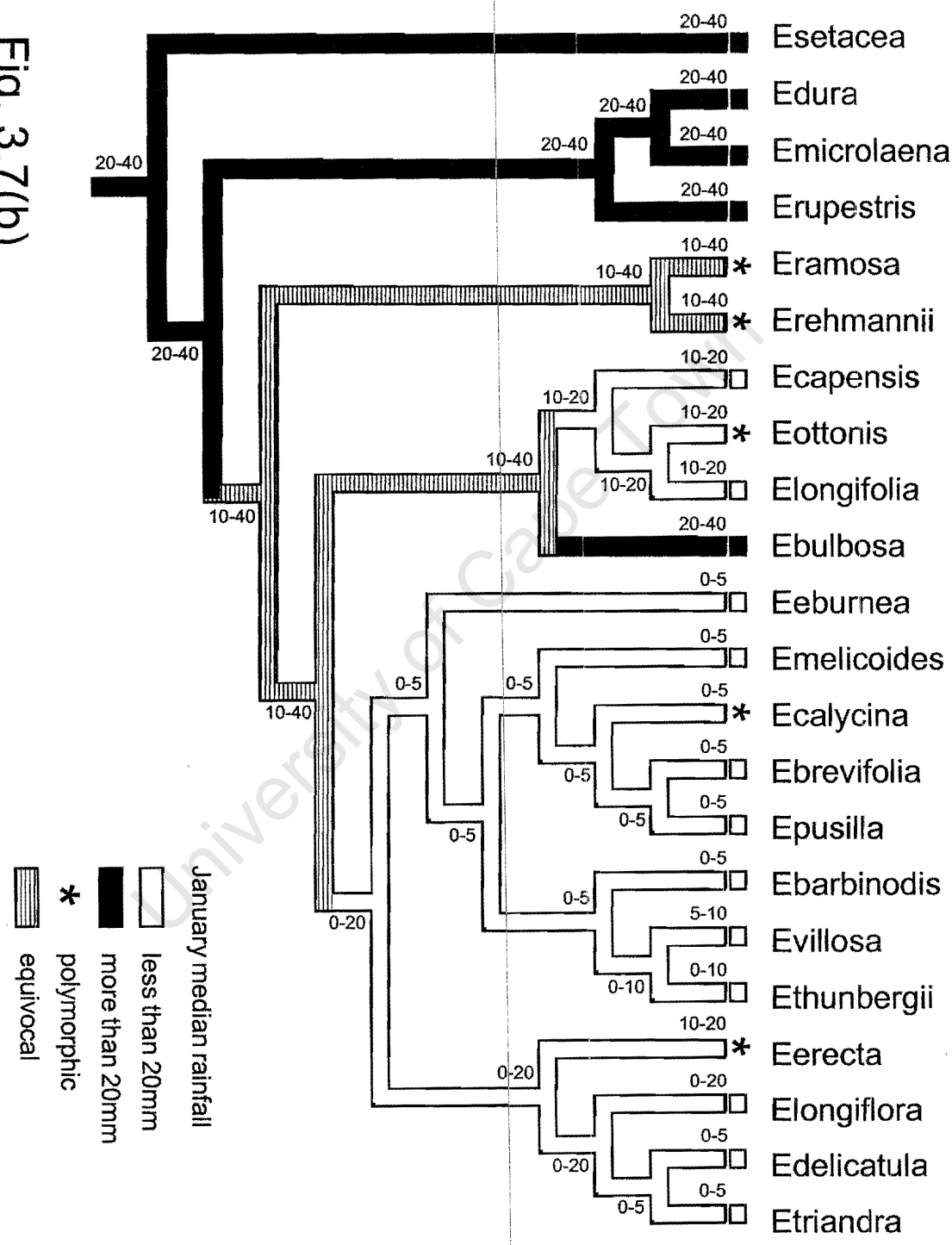


Fig. 3.7(c)

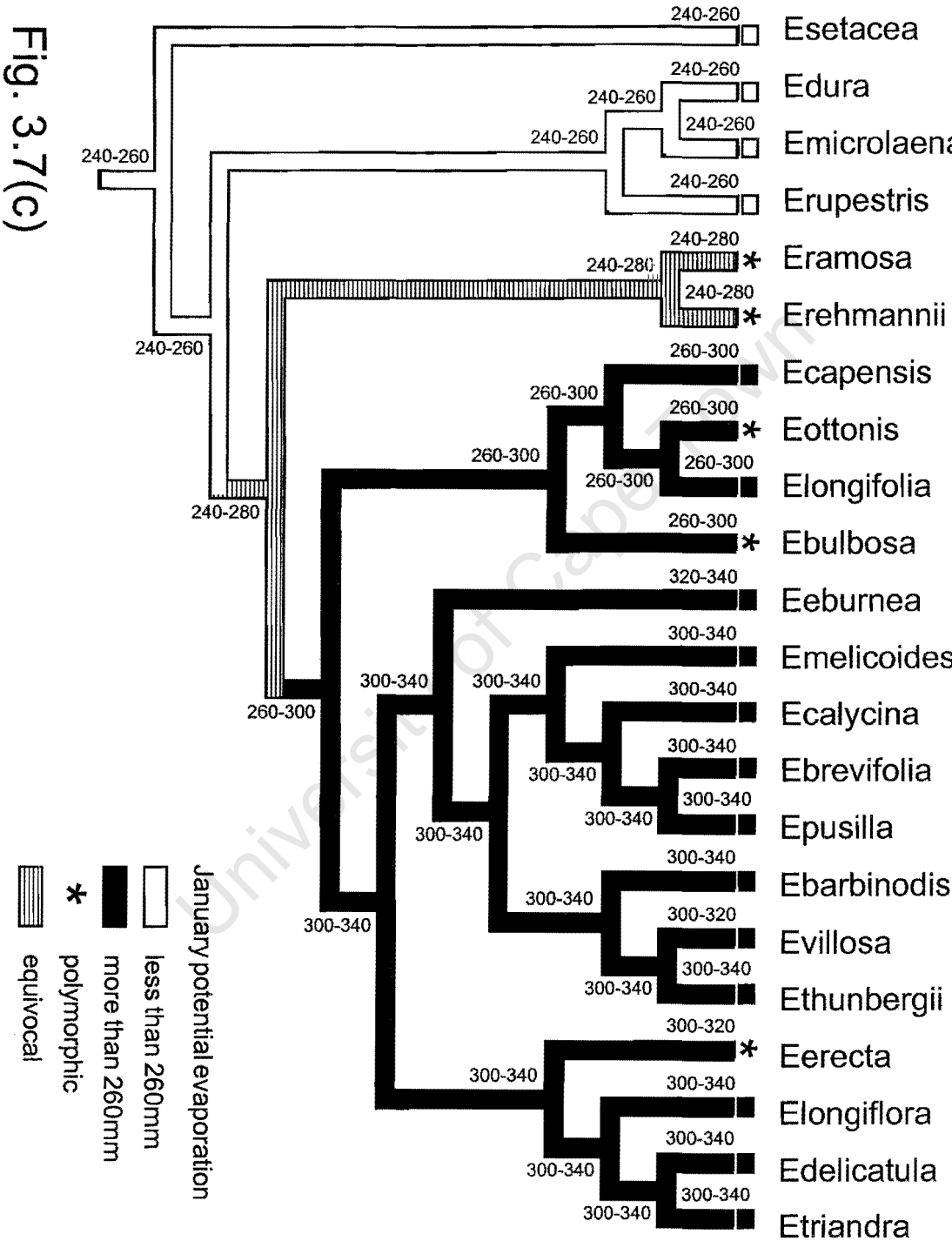
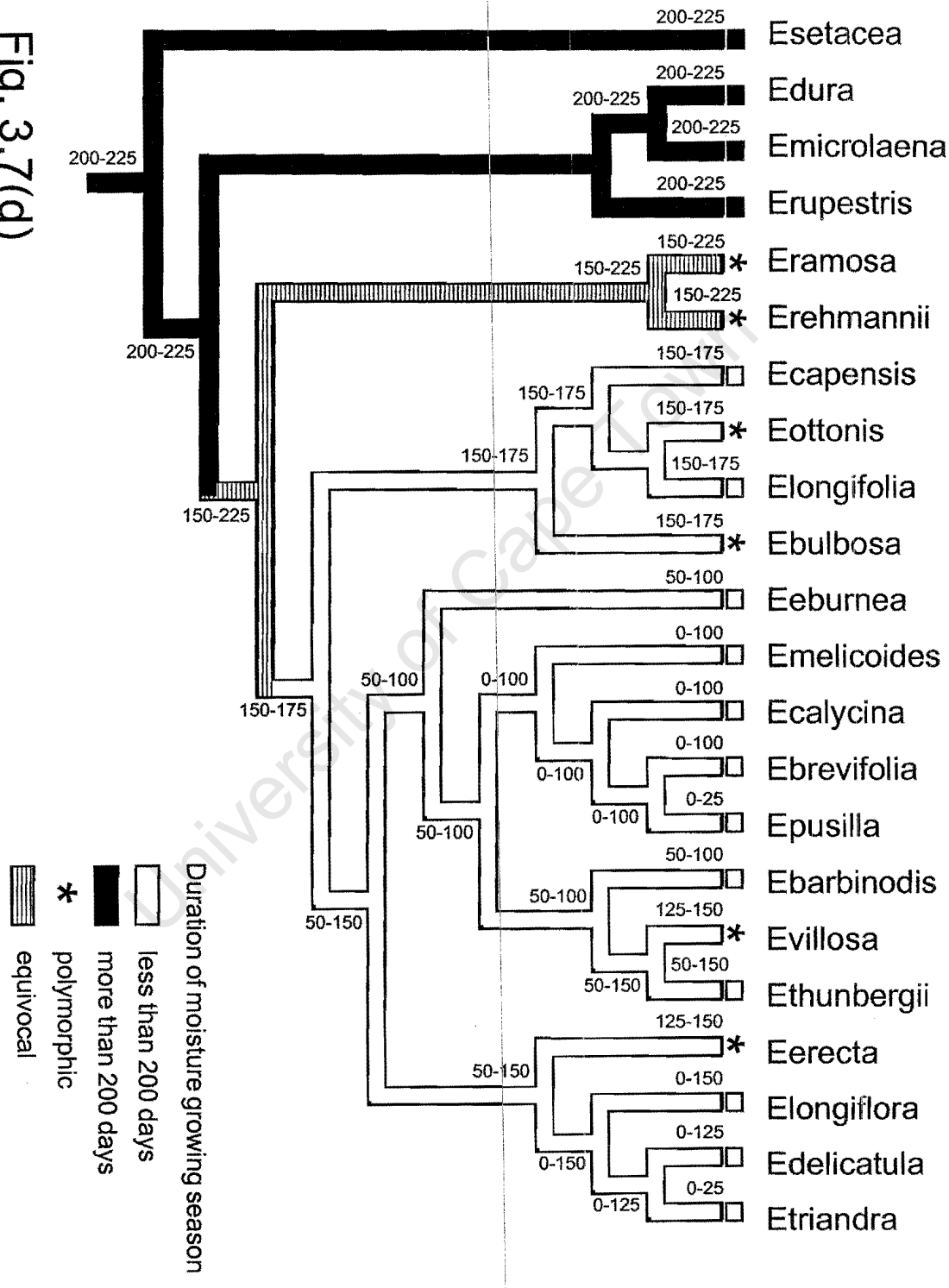


Fig. 3.7(d)



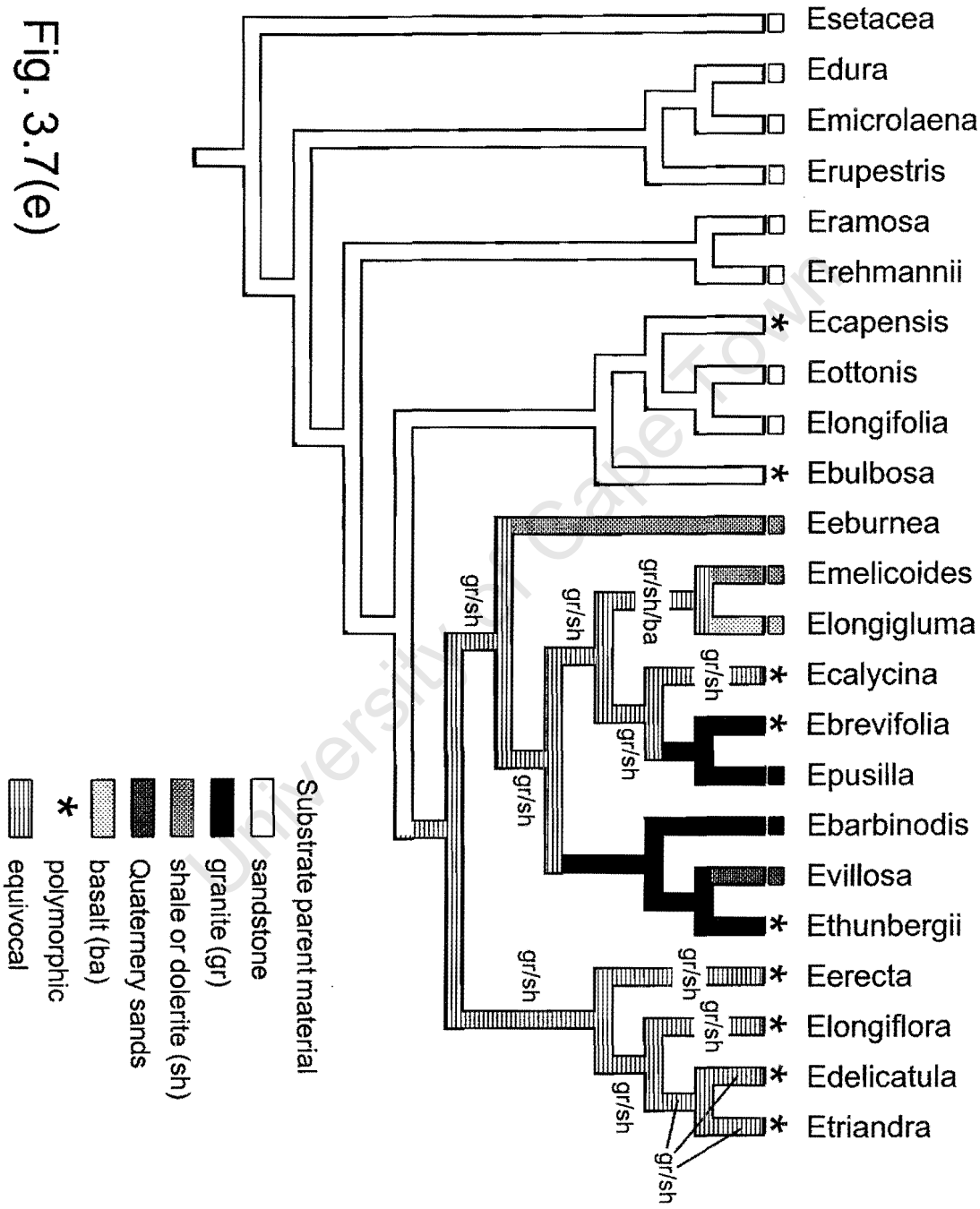


Fig. 3.7(e)

Fig. 3.7(f)

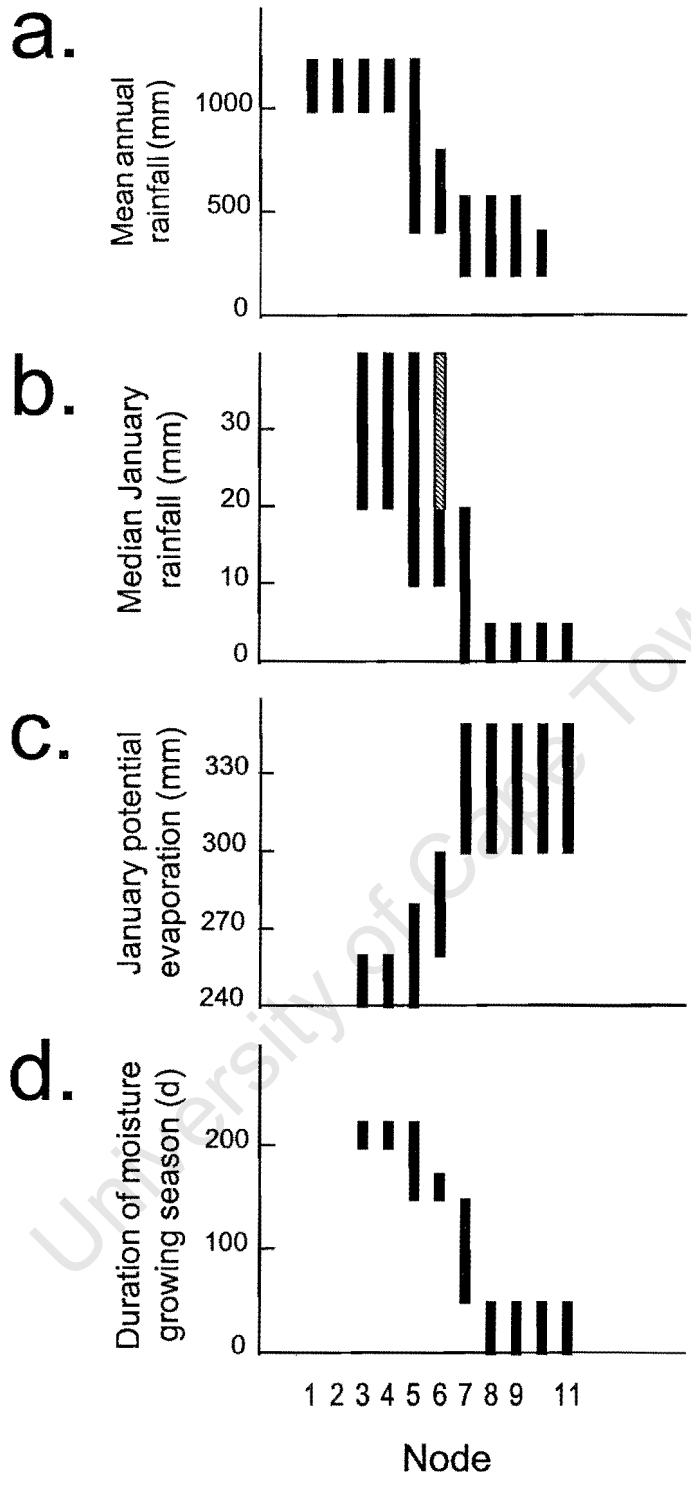


FIGURE 3. 8. Reconstructed changes in climatic preferences (ranges) from the basal node of the *Ehrharta* s. s. clade to one of its terminal nodes (*E. barbinodis*). Nodes are numbered as in Fig. 3.7(a). Variables depicted are (a) mean annual rainfall, (b) January median rainfall, (c) January potential evaporation, (d) duration of the moisture growing season. For January median rainfall, two alternative optimisations exist for node 6 depending on the internal resolution of the *E. bulbosa-E. capensis* clade. These alternatives may be read as black bar only or black bar plus hatched bar.

bulbosa-E. capensis clade (Fig. 3.6a). Because *E. ottonis* is coded 'uncertain' for this feature, the exact position of this reversal is uncertain under some resolutions of this clade. However, available evidence places it on the branch subtending *E. longifolia* (Fig. 3.6a). Interpreting summer-green foliage in *Ehrharta* s. s. as plesiomorphic is not robust as this conclusion relies on the occurrence of non-deciduous foliage in *E. rupestris* and *E. setacea*. Additional data describing the status of this attribute in *E. dura* and *E. microlaena* as well as the non-African Ehrharteae are needed to produce a more reliable interpretation of its evolution below and at the base of the *Ehrharta* s. s. clade.

The possession of culms having strongly developed subepidermal chlorenchyma is inferred to have evolved twice in *Ehrharta* s. s., once on the branch subtending the *E. ramosa-E. rehmannii* clade and again on that subtending the *E. barbinodis-E. thunbergii* clade (Fig. 3.6a). Some uncertainty regarding the status of this feature in *M. polynoda* and some species of *Tetrarrhena* suggests that a third origin is possible.

Three equally parsimonious reconstructions explain the distribution of buried culm bases in Ehrharteae (Fig. 3.6b): (i) ACCTRAN favours two origins of the trait, one of these being followed by two reversals; (ii) DELTRAN favours four independent gains; and (iii) an intermediate option favours three gains with one reversal. While the choice amongst these alternatives is arbitrary, all three reconstructions interpret culm burial as derived within *Ehrharta* s. s. Bulbous culm bases are also interpreted as derived, being inferred to have originated twice independently in *Ehrharta* s. s.. In both instances this has occurred along branches possessing, under all reconstructions, buried culm bases. A two-tailed Fisher exact test of the association between the evolution of these attributes (following Sillén-Tullberg [1993]) yields a significant result under all reconstructions of culm base burial ([i]: P=0.047; [ii]: P=0.034; [iii]: P=0.023)

Reconstructed evolution of habitat preferences

Reconstructions of ancestral habitat preferences using either Fitch or Wagner optimisation on the phylogeny of Ehrharteae or *Ehrharta* s. s. are provided in Fig. 3.7a-f (data in Appendix 5). Where these are altered by topological rearrangement at the points of uncertainty discussed above (i.e. with respect to the position of *M. polynoda* and the resolution of the *M. tasmanica-M. avenacea*, *E. bulbosa-E.*

capensis and *E. calycina-E. brevifolia* clades), the specific changes involved are listed in the relevant figure caption. For all climatic variables the discrete state combinations optimised onto the internal nodes in Fig. 3.7a-d are converted back to the continuous value ranges that they represent. Figure 3.8 traces changes in optimised climatic preferences through a succession of internal nodes from the basal node of either Ehrharteae (Fig. 3.7a: node 1) or *Ehrharta* s. s. (Fig. 3.7a: node 3) to one of the terminal nodes within the *Ehrharta* s. s. clade (Fig. 3.7a: node 11 [*E. barbinodis*]).

A strict association with high annual rainfall (more than 1000-1200mm.yr^{-1}) is inferred to be ancestral in both Ehrharteae and *Ehrharta* s. s. (Fig. 3.7a: black shading). From this ancestral condition there is a transition to a broader annual rainfall range of 400-1200mm on the branch subtending the *E. ramosa-E. bulbosa* clade (Fig. 3.7a: node 5). Comparison of optimised values on successive nodes, from the base of Ehrharteae to *E. barbinodis* indicates that this shift is marked and relatively sudden (Fig. 3.8a: node 5). A subsequent transition to a range of 400-800mm on the branch subtending the *E. bulbosa-E. erecta* clade (Fig. 3.7a: node 6) reflects a shift to a strict association with drier habitats. This is reinforced on the branch subtending the *E. erecta-E. eburnea* clade on which a further transition, to a range of 200-600mm, is inferred (Fig. 3.7a: node 7). A single reversal to a range of 800-1000mm occurs on the branch subtending *E. longigluma*. Within the *E. erecta-E. eburnea* clade there are two transitions to an even lower annual rainfall range (less than 200mm), once on the branch subtending the *E. longiflora-E. delicatula* clade and again on that subtending the *E. calycina-E. brevifolia* clade. A two-tailed Fisher exact test (following Sillen Tullberg [1993]) indicates that the evolution of annualness in these clades is significantly associated (P=0.003) with transitions to such low-rainfall conditions (rainfall less than 200mm.yr^{-1}) even under a conservative reconstruction of the former (i.e. two gains).

In *Ehrharta* s. s., an association with conditions of high January rainfall (more than 20mm; Fig. 3.7b: black shading) and low January evaporation (less than 260mm; Fig. 3.7c: white shading) is ancestral. While these reconstructions lack robustness in that they span just two basal nodes, the fact that all species included in the *M. tasmanica-Z. colensoi* clade occupy forested or montane grassland habitats that experience high annual precipitation (Fig. 3.7a, f) suggests that these experience little, if any, seasonal drought and thus supports the reconstructions shown. From an

ancestral January rainfall range exceeding 20mm there is a transition on the branch subtending the *E. ramosa-E. bulbosa* clade to a range of 10-40mm (Fig. 3.7b). A subsequent transition to low January rainfall (less than 20mm) indicates the evolution of a strict association with conditions of more intense summer-aridity and is suggested by Fig. 3.7b to occur on the branch subtending the *E. erecta-E. eburnea* clade. However, under all alternative resolutions of the *E. bulbosa-E. capensis* clade, this transition occurs sooner, on the branch subtending the *E. bulbosa-E. erecta* clade. Thus, under these alternative reconstructions the transition from a January rainfall preference exceeding 20mm to one less than 20mm is rapid, spanning just two internal nodes (Fig. 3.7b; Fig. 3.8b: nodes 5 and 6).

This rapid transition is mirrored by shifts in ancestral preferences with respect to January potential evaporation. From an initial association with low January evaporation (less than 260mm), there is a transition to a wider range that includes higher evaporation values on the branch subtending the *E. ramosa-E. bulbosa* clade, and this is followed by a shift to a strict association with high January evaporation on the branch subtending the *E. erecta-E. eburnea* clade (Fig. 3.7c; Fig. 3.8c: nodes 5 and 6). Within the *E. erecta-E. eburnea* clade, two transitions to a January rainfall range of 0-5mm and one transition to a January potential evaporation range of 300-340mm (on the branch subtending the entire clade) reinforce the association with more intense summer aridity.

Inferred changes relating to the duration of the moisture growing season in *Ehrharta* s. s. mirror changes in January rainfall and evaporation. On the branch subtending the *E. ramosa-E. bulbosa* clade there is a transition from an ancestral association with a moisture growing season exceeding 200 days to one of 150-225 days (Fig. 3.7d). Subsequent transitions on the branches subtending the *E. bulbosa-E. erecta* (150-225 days to 150-175 days), *E. erecta-E. eburnea* (150-175 days to 50-150 days) and *E. eburnea-E. barbinodis* (50-150 days to 50-100 days) clades (Fig. 3.7d, Fig. 3.8d) indicate successive transitions into habitats with increasingly shorter growing seasons.

Reconstruction of historical substrate preferences in *Ehrharta* s. s. identifies an association with Table Mountain Group sandstones as ancestral (Fig. 3.7e). A principal shift from sandstones to granites, shales and dolerites is inferred on the branch subtending the *E. erecta-E. eburnea* clade. The frequent occurrence of *E.*

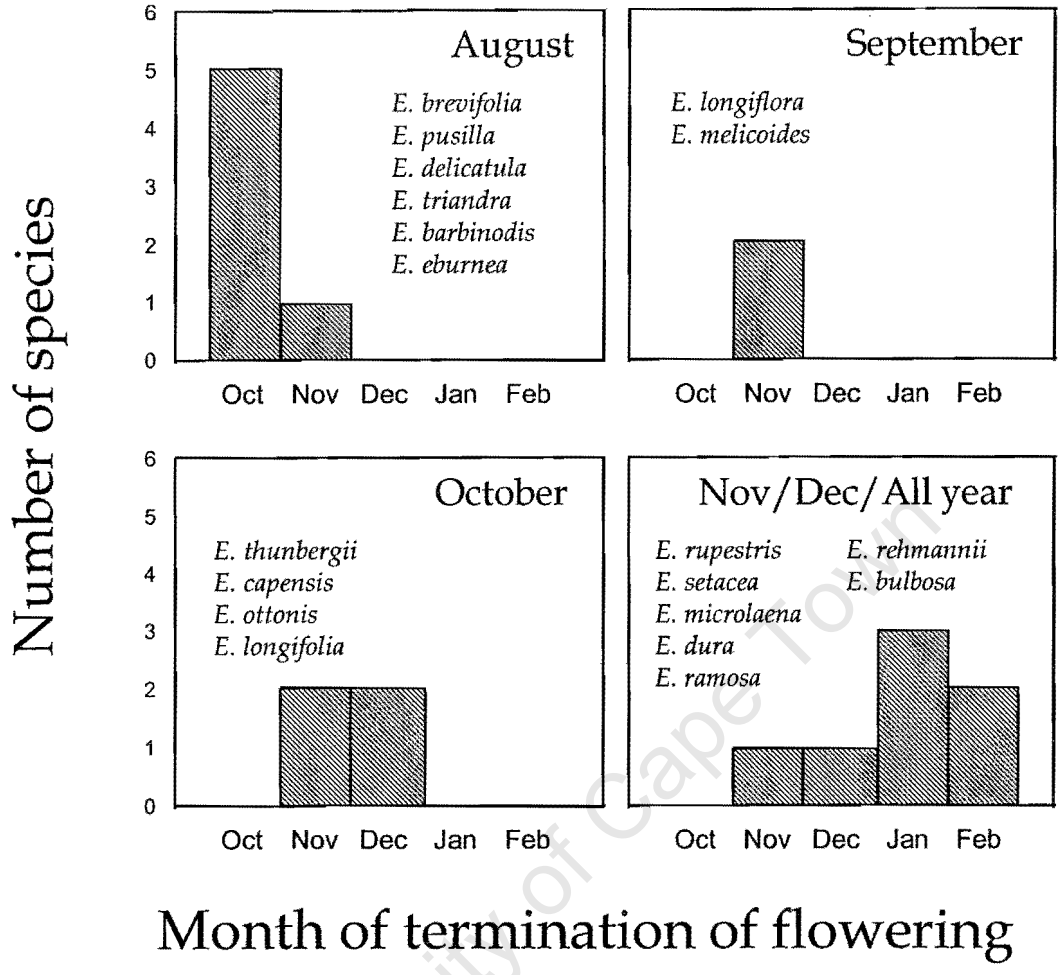


FIGURE 3.9. Histograms describing variation in the month of last flowering among *Ehrharta* s. s. species whose distribution ranges coincide with a moisture growing season ending before the end of August (i.e. moisture growing season is very short to non-existent), before the end of September, before the end of October, or after October (i.e. moisture growing season is longer-lasting to all-year). Only species endemic to the western Cape are included in this comparison.

capensis and *E. bulbosa* on shales and granites in addition to sandstones, however, suggests that, contrary to the pattern shown, initiation of this transition could be associated with the node subtending the *E. bulbosa-E. erecta* clade. A number of species within the *E. erecta-E. eburnea* clade occur on granites, shales and dolerites and thus the optimisation of the basal branches within this clade is equivocal with respect to these substrate types (indicated in Fig. 3.7e). Some subsequent transitions appear to reflect cases of substrate specialisation (e.g. *E. pusilla* on granite, *E. villosa* on Quaternary sands and *E. longigluma* on basalts). Of all species included in the *E. erecta-E. eburnea* clade, only *E. thunbergii* appears to occur commonly on sandstone substrates, representing a partial reversal.

Reconstruction of changes in preferred vegetational association (Fig. 3.7f) indicates an ehrharteoid origin in forests, with subsequent transitions to montane grasslands in the *Zotovia* clade and fynbos heathland on the branch subtending the *Ehrharta* s. s. clade. Within the latter, a further transition to succulent shrubland occurs on the branch subtending the *E. erecta-E. eburnea* clade, while a number of localised transitions within this clade have led to the occupation of other vegetation types (grassland, renoster shrubland and dune thicket).

Flowering in relation to the end of the moisture growing season

Four Cape annuals terminate their flowering in October or earlier (Fig. 3.9), apparently reflecting their preference for habitats in which there is no moisture growing season or in which the moisture growing season ends early (August). In contrast, species occupying habitats with a later-ending to all-year moisture growing season terminate flowering deep into summer (until as late as January and February) (Fig. 3.9). In general, therefore, flowering termination seems to be related to the end of the moisture growing season, lagging behind it by about two months.

Culm and leaf photosynthesis and transpiration rates

Stomatal conductance data (Table 3.4) indicate consistently higher transpiration rates in leaves than in culms of both *E. ramosa* and *E. thunbergii*, whether expressed in terms of transpirational surface area or dry mass. In the latter species, this difference is substantial, whether expressed on a dry mass- (about 12-fold) or surface area-basis (about 4-fold). In *E. ramosa*, however, area-specific leaf

TABLE 3.4. Field-measured carbon assimilation and stomatal conductance ($g_s[H_2O]$) rates of leaves and culms of *E. ramosa* and *E. thunbergii*, expressed on a surface area- and dry mass-specific basis.

Species	Organ	Area-specific C-assimilation rate ($\mu mol.m^{-2}.s^{-1}$)	Mass-specific C-assimilation rate ($\mu mol.kg^{-1}.s^{-1}$)	Area-specific stomatal conductance ($mmol.m^{-2}.s^{-1}$)	Mass-specific stomatal conductance ($mmol.kg^{-1}.s^{-1}$)
E. ramosa	Leaf	7.1±0.7	162±10.8	155.0±13.3	3583.3±401.8
	Culm	0.6±0.1	5.7±1.2	77.9±30.1	722.6±380.9
E. thunbergii	Leaf	4.8±0.8	86.7±23.1	123.9±19.1	2226.2±529.8
	Culm	0.3±0.3	1.9±1.7	30.1±5.3	187.1±41.2

transpiration rate is only about double that of culms, while the dry mass specific-rate is about five times as high. For both species, carbon assimilation rates were found to be at least an order of magnitude higher in leaves than in culms, whether expressed in terms of dry mass (27- to 43-fold difference) or surface area (12- to 16-fold difference).

Discussion

Evidence for radiation in *Ehrharta* s. s.

Evolutionary radiation, the rapid divergence of multiple lineages, is commonly invoked to account for localised lack of resolution in phylogenetic analysis (e.g. Baldwin 1997; Springer et al. 1997), a conclusion that is strengthened by the observation that such a pattern spans multiple data sets (Avise 1994). The failure of three independent data sets used in this study (one morphological and one each from the chloroplast and nuclear genomes) to resolve relationships within the *E. ramosa-E. bulbosa* clade, particularly above the node subtending the *E. erecta-E. eburnea* clade, is therefore consistent with a radiation hypothesis. The observation that the ITS1 data are able to strongly resolve several of the deeper nodes in the tree, while the trn and morphological data provide strong support for at least some relationships above the putative radiation, suggests that the array of data sets used was capable of resolving relationships at all levels within Ehrharteae. While combination of the data sets yields an almost fully-resolved topology for *Ehrharta* s. s., several nodes in the *E. ramosa-E. bulbosa* clade, particularly those immediately

above its basal node, have low character support (Chapter 2). This pattern is matched by a reduction in internal branch lengths relative to those deeper in the tree.

Under the assumption that character evolution in Ehrharteae is more or less clock-like, this pattern implies a shift in absolute speciation rate over time. Many recent studies assume molecular evolution to be clock-like (e.g. Baldwin and Sanderson 1998; Baum et al. 1998; Xiang et al. 1998; Vargas et al. 1999) on the premise of selective neutrality (Kimura 1983; Friday 1994) but the same perspective is not usually applied to morphological change (Friday 1994). Omland (1997a), however, has shown that rates of molecular and morphological evolution are in many instances correlated. This study indicates a sharp increase in the effective rate of cladogenesis relative to character change. This increase is similar in terms of both position (on the branch subtending the *E. erecta-E. eburnea* clade) and magnitude (2.7- or 3.6-fold) whether character change is measured in terms of numbers of changes (branch length) in all characters (i.e. morphological and molecular) or numbers of ITS1 nucleotide substitutions estimated under the assumption of a molecular clock. Indeed, the fact that amounts of total character change along the spine of the *Ehrharta* s. s. phylogeny are highly correlated with corresponding levels of change in ITS1 sequences (estimated under a molecular clock model), suggests that use of the former as a time-surrogate in the present study is reasonable.

Although increased cladogenesis relative to time (character change) may indicate an increase in diversification rate on the branch subtending the *E. erecta-E. eburnea* clade, some points deserve mention. Firstly, the observed increase in cladogenesis may reflect higher levels of extinction on branches deeper inside the *Ehrharta* s. s. clade (cf. Kubo and Iwasa 1995) rather than a recent burst of speciation. Since phylogenetic pattern in recent groups (e.g. genera) is maximally consistent with evolutionary models that ignore extinction (Hey 1992), however, this possibility would be of serious concern only if *Ehrharta* s. s. were found to be an old genus. Two further concerns relate to taxonomic sampling of extant taxa. The inference of increased diversification in *Ehrharta* s. s. relies on a phylogeny (combined-most) that includes most but not all extant species and is based on one sampled individual per species. Thus, the possibility exists that the conclusions reached could be altered by the inclusion of additional species as well as more individuals per species. A more species-inclusive phylogenetic analysis (combined-all) suggests that the subset used adequately samples all major branching events in the *Ehrharta* s. s. clade and that

the inclusion of additional species is, therefore, unlikely to alter the main conclusions. However, the possibility of species paraphyly (Crisp and Chandler 1996) allows that some species may comprise multiple lineages, so that a single sample per species may be inadequate to detect all cladogenetic events. If, for example, *E. setacea* is paraphyletic with respect to the *Ehrharta* s. s. clade, then it is possible that the basal branches of the clade have been undersampled and that actual branch lengths here are shorter. This highlights the need for a more detailed phylogenetic analysis of the basal portion of the *Ehrharta* s. s. clade in which species monophyly is thoroughly evaluated.

Breakpoint regression analysis, as applied in this study, provides a simple (if somewhat rough) method for estimating the position and magnitude of a shift in diversification rate of a lineage on the basis of phylogenetic data. The technique differs from other methods (e.g. Slowinski and Guyer 1989, 1993; Sanderson and Donoghue 1994) in that it does not test the significance of such shifts and so is fundamentally astatistical. Rather, it is designed merely to localise diversification shifts within lineages and in this regard benefits from using a greater amount of phylogenetic information than do these other methods. Due to its astatistical nature, however, it is advisable, wherever possible, to employ breakpoint regression in conjunction with a more rigorous test such as that designed by Sanderson and Donoghue (1994). The first can then be used to detect a putative shift in diversification rate and the second to test its significance. Unfortunately, application of this approach to small or recent radiations is not feasible as the more popular statistical tests lack sufficient power to evaluate these effectively.

The expectation that radiation should alter the mathematical function relating cladogenesis to time, supports the application of a breakpoint model to evaluate changes in diversification rate. In this study, improved correlation following data transformation confirms that the observed relationship between TBL (branch length) and ND (nodal distance) in *Ehrharta* s. s. is non-linear. However, the specific choice of a breakpoint model over an exponential model by this study is based on inspection.

Causes of radiation

The identification of the branch subtending the *E. erecta-E. eburnea* clade as a putative point of radiation in *Ehrharta* s. s. implies that clues to the historical events

influencing radiation should be sought here and on the branches immediately below it. Reconstruction of historical changes in habitat requirements of *Ehrharta* s. s. indicates a number of dramatic shifts closely preceding this node. A transition to habitats receiving much lower annual rainfall (to 400mm.yr^{-1}) than that determined as ancestral (more than 1000mm.yr^{-1}) in Ehrharteae is inferred to have occurred suddenly on the branch subtending the *E. ramosa-E. bulbosa* clade. Primarily, this shift reflects the occupation of habitats experiencing more protracted and intense summer aridity than is prevalent in the ancestral habitat. Although the association of *Ehrharta* s. s. with conditions of summer-aridity is not initially a strict one, the formation of a strict association occurs subsequently, on the branch subtending the *E. bulbosa-E. erecta* clade. The association of *Ehrharta* s. s. with summer-arid habitats is further reinforced on the branch subtending the *E. erecta-E. eburnea* clade, as well as within this clade.

Since the successful entry of *Ehrharta* s. s. into a seasonally arid adaptive zone closely precedes radiation, this particular ecological transition has potential value in explaining radiation in the clade. In contrast, the transition from forest to fynbos heathland that occurs at the base of the *Ehrharta* s. s. clade is probably too distant to qualify as influential. However, causality may be impossible to prove (Cracraft 1990). Two shifts in habitat parameters, one from fynbos heath vegetation to succulent shrubland and another from sandstone substrates to granites and shales, occur on the branch subtending the *E. erecta-E. eburnea* clade. Since the distribution of vegetation types both in the Cape and in mediterranean systems generally is known to be strongly influenced by edaphic properties (Kruger 1979; Cambell 1983, 1985; Specht and Moll 1983; Cowling 1984; Cowling et al. 1992), the correlation of these habitat transitions is expected. Their coincidence with the onset of radiation in *Ehrharta* s. s., however, is suggestive of an influence in facilitating increased speciation. Edaphic specialization is thought to be an important speciational force in the Cape flora (Linder 1985; Cowling 1990; Linder and Vlok 1991; Cowling and Holmes 1992b; Cowling et al. 1992, 1994; Linder and Davidse 1997) and the occupation by *Ehrharta* s. s. of an array of richer soil types such as those derived from shales and granites may, therefore, help to explain a sudden increase in speciation rate. In particular, access to such substrates may have been critical in permitting the evolution of new growth forms, particularly those dependent on high-RGR's (Chapin 1980; Poorter 1989; Chapter 4). Within the Cape region, for

example, native annual grasses are largely restricted to richer soils (Linder and Ellis 1990), which suggests that the evolution of a true annual habit is feasible only on such substrates. Resource availability in oligotrophic fynbos environments is periodically augmented by fire events which thus provide improved opportunities for growth and reproduction (Chapter 5). However, both the episodic and temporary nature of such events may constrain life history possibilities in these habitats. Besides the shift from sandstones to shales and granites, subsequent transitions to other substrate types reflect the evolution of several narrow edaphic specialists. These include *E. pusilla* and *E. barbinodis* which are restricted to granitic gravels, *E. villosa* which occurs on Quaternary coastal sand dunes and *E. longigluma* which is restricted to basalts of the Drakensberg mountains.

The broad association of richer substrates (e.g. shales and granites) in the western Cape with conditions of acute seasonal aridity suggests that adaptation to the latter has been critical in providing access to such substrates. Adaptation to seasonal aridity therefore remains of key importance. Palynological and geological evidence indicates that summer-aridity has not always been a feature of the Cape climate. Analysis of pollen deposits at Arnot (Palaeocene: Scholtz 1985), Noordhoek and Langebaanweg (Miocene: Coetzee 1978; 1983; Coetzee et al. 1983), and possibly even Koingnaas (de Villiers and Cadman 1997) suggest that Tertiary vegetation in the western Cape region was of a woodland or forest type experiencing a higher and more equable rainfall regime than observed at present. Inception of a summer-arid climate is thought to have occurred towards the end of the Tertiary (Deacon 1983; Deacon et al. 1992; Linder et al. 1992; Partridge 1997; Meadows and Watkeys 1999) largely as a result of the establishment of seasonal upwelling of cold water (Benguela Current) along the west coast of southern Africa (Siesser 1980), as well as the development of the South Atlantic high pressure cell around three million years before present (mybp) (Deacon et al. 1992). Since the evolution of a strict association with seasonally arid habitats is inferred on the branch subtending the *E. bulbosa-E. erecta* clade (most reconstructions), an origin for this clade after the inception of such a climate around 3mybp is suggested.

The relatively rapid inception of summer-aridity in the western Cape is likely to have generated 'new, ecologically open territory' (Linder et al. 1992) of the type envisaged by Simpson (1953) as influential in stimulating adaptive radiation. While such diversification may have been further stimulated by subsequent climatic fluctuations

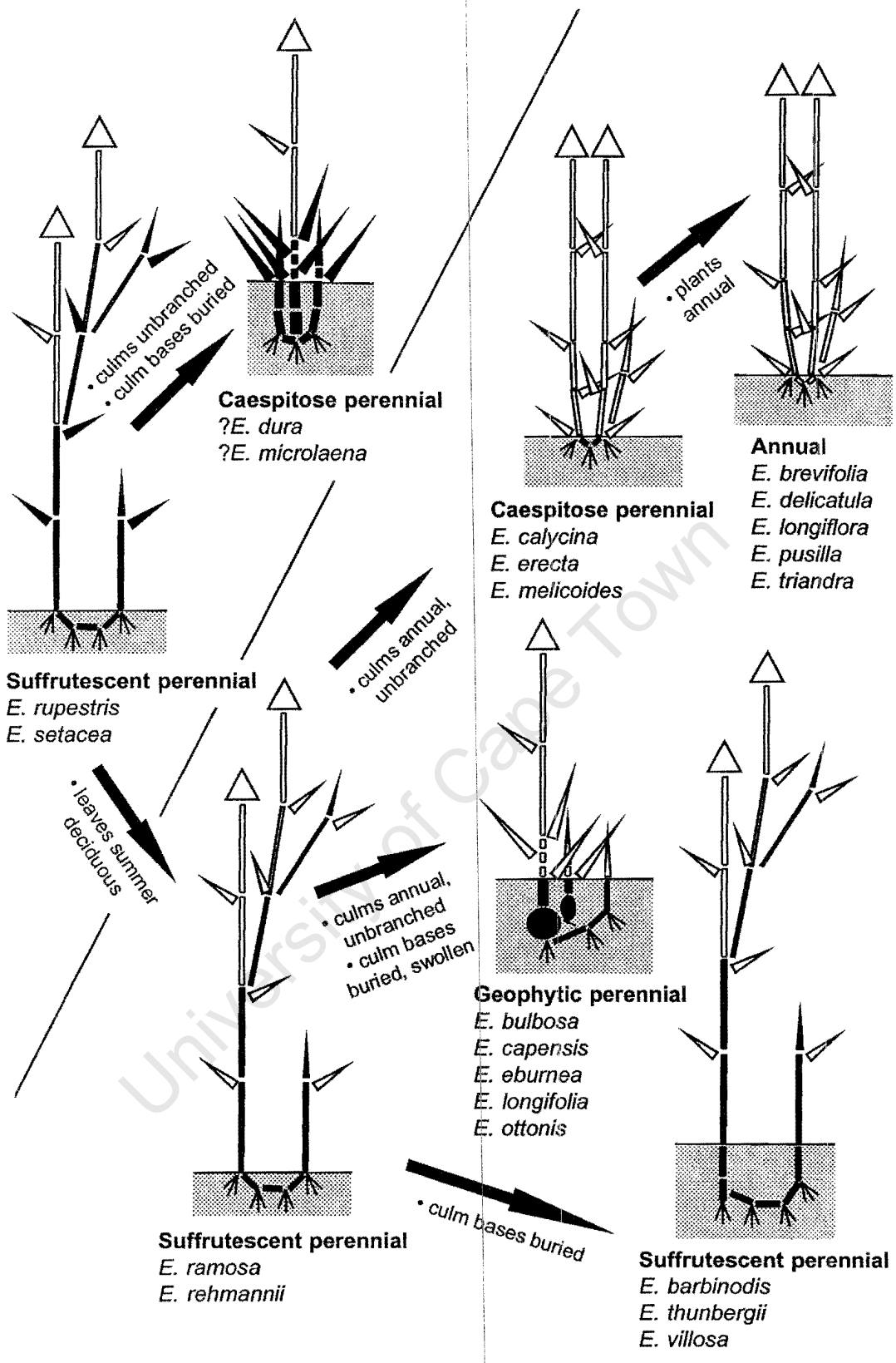


FIGURE 3.10. Schematic diagram depicting the evolution of alternative growth forms in *Ehrharta* s. s. based on the trait optimisations presented in Fig. 3.6. Culm and rhizome internodes are represented by rectangular shapes and leaves and inflorescences by triangles. Perennating structures are filled, while ephemeral structures are not. Western Cape representatives are listed below each form depicted. *E. dura* and *E. microlaena* are marked with '?' to indicate uncertainty regarding their foliage phenology. Note that the summer-greenness of foliage in *E. longifolia* is not shown. A diagonal line separates taxa that experience intense seasonal aridity (to the right) from that do not (to the left).

(Deacon and Lancaster 1988), this is speculative. The remarkable success of *Ehrharta* s. s. in exploiting the graminoid niche in the Cape arid zone deserves further exploration. While the relative lack of success of C_4 grasses is probably adequately accounted for by the winter growing season (Vogel et al. 1978; Ellis et al. 1980), the relatively marginal success of some danthonioid genera (especially *Pentaschistis*, *Pentameris* and *Merxmuellera*), particularly in the Namaqualand region, is unexplained. The general absence of Restionaceae from fertile shale- and granite derived soils that dominate the western Cape lowlands probably reflects their low competitive ability on these soils. It has been suggested that Restionaceae may be more effective than grasses at extracting nutrients from oligotrophic soils (Linder 1991b). However, their lack of leaves may compromise their ability to exploit, through rapid growth, conditions of high nutrient availability (Chapin 1980). In particular, the high degree of lignification and the relatively low surface area: volume ratio of restioid culms may reduce their photosynthetic efficiency (see Lambers and Poorter 1992; Poorter and Bergkotte 1992; van Arendonk and Poorter 1994). In *Ehrharta* s. s., at least, much higher rates of carbon assimilation in leaves than in photosynthetic culms of *E. thunbergii* and *E. ramosa* confirm the importance of leaves in maintaining a capacity for faster growth.

The inferred evolution of leaf deciduousness on the branch subtending the *E. ramosa-E.bulbosa* clade is correlated with the acquisition of increased tolerance of reduced annual rainfall and increased seasonal aridity, and this suggests a functional relationship. Uncertainty regarding the foliage phenology of non-African ehrharteoids leaves the ancestral interpretation of summer-greenness in *Ehrharta* s. s. open to question. However, year-round flowering in *M. avenacea* and *M. stipoides* (Willemse 1982) suggests that year-round growth and related foliage perennation is widespread in Ehrharteae and supports its interpretation as ancestral. This is further supported by examination of herbarium material, which suggests that *M. polynoda*, all species of *Zotovia* and at least some species of *Tetrarrhena* retain green foliage throughout the year. Field-based confirmation is, however, essential.

Transpirational water costs associated with carbon acquisition are typically high (Mooney 1972) and leaf abscission or die-back during the dry, non-growing season may, therefore, prevent unnecessary water loss (Orians and Solbrig 1977; Chabot and Hicks 1982; Sandquist and Ehleringer 1998). The combination of summer-deciduous leaves and persistent, photosynthetic culms in two separate lineages of

Ehrharta s. s. (i.e. the *E. ramosa-E. rehmannii* and *E. barbinodis-E. thunbergii* clades) leads to the prediction that transpiration rates of such culms are substantially lower than those of leaves. Five- and 12-fold differences in mass-specific transpiration rate confirm this expectation in *E. ramosa* and *E. thunbergii* respectively, thereby providing indirect support for the argument that seasonal leaf loss functions in water conservation. If, as is suggested here, the evolution of leaf deciduousness is functionally linked to the invasion of seasonally arid habitats by *Ehrharta* s. s., this feature must qualify as a potential key innovation. Further tests monitoring plant responses to droughting (cf. Raynal et al. 1985; Boot et al. 1986), as well as additional field data are, however, required to evaluate this suggestion more thoroughly.

Within the *E. ramosa-E. bulbosa* clade, the reversal to a summer-green strategy in *E. longifolia* is perhaps surprising, given the comparatively summer-arid habitats occupied by this species. However, the leaves of this species are highly modified, apparently to minimise water loss. Most notably, leaves of *E. longifolia* are setaceous and lack stomata on the abaxial surface. On the adaxial surface, the stomata are restricted to deep furrows whose walls are covered with interlocking prickles (Gibbs Russell and Ellis 1987).

Adaptive divergence

Figure 3.10 summarises the derivation of alternative growth forms in Cape species of *Ehrharta* s. s. as inferred from parsimony-based optimisation, and indicates the evolution of several growth form novelties subsequent to the occupation of seasonally arid habitats. Three of these, buried culm bases, swollen culm bases and an annual habit, appear to reflect divergence in the strategies employed to survive seasonal drought.

In *Ehrharta* s. s., as in other grasses (White 1973), culms are the major repositories of carbohydrate storage (Watson and Dallwitz 1992; personal observation) and culm base modification in the genus can generally be interpreted as evidence of a geophytic strategy. Except for *E. calycina*, *E. melicoides*, *E. erecta*, *E. ramosa* and *E. rehmannii*, all perennial species of *Ehrharta* s. s. that experience appreciable summer drought possess culms that, at maturity, are generally buried to a depth of 5cm or more below the soil surface. In several of these, (the *E. bulbosa-E. capensis* clade and *E. eburnea*) the basal, subterranean internodes of the culm are long-lived

and swollen to form bulbous, geophytic structures that contain high levels of starch (Fig. 3.2b; Chapter 5). Even in species whose buried culms are typically not basally swollen, such as *E. barbinodis* and *E. thunbergii*, the lower portions of the long-lived culms usually contain appreciable levels of starch (Fig. 3.2c) that exceed concentrations in the aerial culm portions (personal observation). Moreover, in *E. thunbergii* some swelling of the lower culm internodes is occasionally observed (sub-bulbous condition: Gibbs Russell 1987b). These data, plus the significant evolutionary association of culm base burial with bulbous swelling of culm bases, suggest that the former is a precursor to a geophytic habit and is itself implicated in reserve storage. In contrast to species with buried culm bases, *E. melicoides* appears to employ an alternative reserve storage strategy, storing starch in its shallowly buried leaf bases (Fig. 3.2f).

The incidence of geophytic structures in the *E. barbinodis-E. thunbergii* and *E. bulbosa-E. capensis* clades as well as in *E. eburnea* suggests greater vegetative resilience to summer drought. Many plants employ seasonally fluctuating carbohydrate reserves to survive seasonal adversity, especially in the face of foliage die-back (Mooney and Billings 1960; Bloom et al. 1985; Chapin et al. 1986, 1990; Meyer and Hellwig 1997), and geophytic strategies are common in plants exposed to seasonal aridity (e.g. Pate and Dixon 1982; Hocking 1993; Ruiters and McKenzie 1994). Although the origin of subterranean reserve storage in *Ehrharta* s. s. is consistent with an interpretation invoking adaptation to seasonal aridity, adaptation to increased defoliation by either fire (e.g. Pate et al. 1990; Bowen and Pate 1993) or herbivory (e.g. Danckwerts 1993; van der Heyden and Stock 1995) present alternative explanations. Since plants tend to use the same set of traits to escape seasonal aridity and herbivory, the precise adaptive significance of these traits may be difficult to establish (Coughenour 1985). The extremely low herbivore carrying capacity of fynbos vegetation (Cody et al. 1983; Rebelo 1992) suggests that the historical transition from fynbos to succulent karoo and renosterveld shrubland at the base of the *E. erecta-E. eburnea* clade may have entailed increased exposure to herbivory. However, since past and even present herbivore pressure has not been quantified, this scenario remains speculative. Nonetheless, even if accurate, increased herbivory associated with a transition from fynbos to more eutrophic habitats seems an unsuitable explanation for the evolution of a geophytic habit in at least the *E. bulbosa-E. capensis* clade, since this precedes it. While fire survival

cannot be excluded as a force driving the evolution of buried, swollen culm bases in the *E. bulbosa-E. capensis* clade (Linder and Ellis 1990), their evolution in *E. eburnea*, a species that does not experience regular fires in its present-day habitat (succulent karoo), cannot be explained in terms of fire survival. Neither fire- nor herbivore-induced defoliation, therefore, offers a general explanation for the evolution of buried, swollen culm bases in *Ehrharta* s. s. Even if defoliation as a general phenomenon has been influential, this does not preclude a role that these structures may play in facilitating the survival of seasonal drought. In addition, because herbivory may be intensified by low forage availability during dry periods (Dean and Milton 1999), the evolution of these structures may ultimately be attributable to the combined effect of seasonal aridification and herbivory. Indeed, a similar selective suite may also account for the loss of long-lived, branching culms in the *E. bulbosa-E. erecta* clade.

In view of their postulated utility in improving plant resilience to summer drought, the lack of buried or swollen culm bases in some perennial species of *Ehrharta* s. s. (e.g. *E. calycina*, *E. erecta* and *E. ramosa*) suggests that adult plants of these species should suffer a greater mortality risk during periods of seasonal drought. As a result, these species may depend to a greater degree on seed for regeneration (e.g. Kruger 1987) after extremely dry periods (e.g. the facultative annual strategy of *E. erecta*). This alternative apparently exists because a smaller resource investment by seedlings in long-lived culms implies a higher potential growth rate and, therefore, a greater capacity for early flowering (Chapter 4). The total reliance by ephemerals (annuals) on seed as a means of surviving the dry season therefore represents an extreme alternative to a persistence strategy based on reserve storage. Flowering in annuals is known to track rainfall closely (Fox 1989, 1990) and so the short, early-finishing (winter) growing season experienced by most annual *Ehrharta* s. s. species (principally in the Namaqualand area) demands that flowering be completed early in the growing season (October in *E. delicatula*, *E. triandra*, *E. brevifolia* and *E. pusilla*). This then amplifies the need for fast growth and development.

The observation that both origins of an annual habit in *Ehrharta* s. s. follow the evolution of a preference for habitats receiving less than 600mm of rainfall per year suggests that entry into an arid adaptive zone has been critical for the evolution of annualness in the genus. Apparently, this is also true for the evolution of annualness in at least two Cape danthonioid genera (Scott 1994). Four of five annual

(ephemeral) *Ehrharta* s. s. species, compared with only two of 30 perennial species, are restricted to habitats receiving a maximum of 400mm of rainfall annually. Given that there are few barriers to dispersal (excluding historical contingency as an explanation), this suggests that in Ehrharteae an annual habit is better suited to extremely low rainfall conditions and less suited to higher rainfall conditions than a perennial habit. Since a denser cover of perennial species probably excludes annuals from higher-rainfall habitats (van Rooyen 1999), the evolution of annualness may thus reflect adaptation to conditions of extreme and protracted seasonal aridity. Support for this suggestion is provided by a significant association between the evolution of annualness in *Ehrharta* s. s. and the occupation of habitats receiving extremely low rainfall (less than 200mm per year). Indeed, within the *E. calycina-E. brevifolia* clade the evolution of annualness is coincident with the development of an obligate association with habitats receiving less than 400mm of rainfall annually.

Adaptive radiation in *Ehrharta* s. s.

Accelerated diversification in *Ehrharta* s. s. following entry into a summer-arid adaptive zone supports Clayton and Renvoize's (1986) hypothesis that the high species richness of this Cape-centred genus reflects radiation 'following adaptation to the winter rainfall regime' of the region. Specifically, though, it is the seasonal aridity associated with such a rainfall regime that is here considered most critical. The postulated importance of seasonal moisture availability on *Ehrharta* s. s. diversification does not preclude the possible influence of other environmental parameters. Indeed, substrate variation seems to have played an important role, as may other yet-unstudied factors. Nonetheless, the evidence presented does suggest that the occupation of seasonally arid habitats has been critical in stimulating radiation, while the emergence of divergent strategies for coping with such aridity suggests that such radiation may be considered adaptive in the sense described by Simpson (1953), Futuyma (1986), Grant (1986) and Schluter (1996).

Chapter 4. Growth rate, growth form and life history variation in *Ehrharta* s. s.

Introduction

The relationship between plant growth and resource allocation can provide important insights into the evolution of alternative life histories and growth forms (Mooney and Chiariello 1984; Bloom et al. 1985; Begon et al. 1990). The principal aim of this chapter, therefore, is to present experimental data describing relative growth rate (RGR) variation in *Ehrharta* s. s., and to begin to develop an understanding of its underlying causes. In line with the goal of elucidating adaptive radiation, it is ultimately hoped that this approach will facilitate the identification of alternative life-history strategies that represent 'divergence in the kinds of resources exploited and in the morphological traits required to exploit these resources' (Schluter 1996).

RGR variation

Although Grime and Hunt (1975) and Hunt and Cornelissen (1997a) respectively reported 12- and 30-fold differences in RGR across a range of woody and herbaceous plant species, variation in RGR reported by studies using exclusively grasses (to date, such studies have included only pooids) is lower. Garnier (1992) and Garnier and Vancaeyzeele (1994), for example, reported values in the range 0.15-0.33$g.g^{-1}.d^{-1}$ for 14 pooid species, while van Arendonk and Poorter (1994) recorded a similar range (0.11-0.27$g.g^{-1}.d^{-1}$) for a separate set of 14 pooids. Published studies that report RGR variation levels within single grass genera are rare. However, Atkin et al. (1996) reported a 2.3-fold difference among six species in *Poa*, while Villar et al. (1998) found 1.6-fold variation in *Aegilops*. Among the congeneric species examined by Garnier (1992) and Garnier and Vancaeyzeele (1994), the maximum observed intrageneric difference is a 1.8-fold difference in *Bromus* (four species), with lower ranges observed in other genera (generally two species sampled per genus). None of these studies, however, provide details about growth form variation among the grasses studied, except in distinguishing among

perennials and annuals, and so the particular relationship between growth form difference and RGR variation remains speculative.

Plant traits, RGR and life strategies

Inter-individual difference in RGR can be attributed to variation in (a) the degree to which plants invest biomass to maximise assimilatory surface area (leaf area ratio, LAR) and (b) the physiological balance they maintain between photosynthetic carbon gain and respiratory loss, per unit leaf area (net assimilation rate, NAR) (Evans 1972). On current evidence, it is difficult to draw general conclusions regarding the relationship of NAR (equivalent to unit leaf rate, ULR_{area}) to RGR except that it is variable (Hunt and Cornelissen 1997a; Wright and Westoby 1999) and typically much weaker than that between LAR and RGR (Poorter 1989; Hunt and Cornellisen 1997a, b; Saverimuttu and Westoby 1996). Leaf area ratio, by contrast, is identified by most studies as the principal correlate of RGR in early plant growth (Poorter 1989; Poorter and Remkes 1990; Poorter and Pothmann 1992; Lambers and Poorter 1992; Kitajima 1994; Cornelissen et al. 1996; Meerts and Garnier 1996; Hunt and Cornelissen 1997a; Wright and Westoby 1999). In most cases this relationship is thought to be driven principally by variation in specific leaf area (SLA) rather than variation in the other component of LAR, the leaf weight ratio (LWR) (Dijkstra 1989; Poorter and Remkes 1990; Garnier 1992; Lambers and Poorter 1992; van Arendonk and Poorter 1994; Atkin et al. 1996; Cornelissen et al. 1996; Meerts and Garnier 1996; Saverimuttu and Westoby 1996; Hunt and Cornelissen 1997a; Marañón and Grubb 1993; Wright and Westoby 1999).

Although RGR might be expected to rely heavily on the proportion of dry biomass a plant allocates to leaves (LWR) (e.g. Tilman 1988, Körner 1991), most report no relationship between these variables (Garnier 1992; Atkin et al. 1996; Saverimuttu and Westoby 1996; Villar et al. 1998; Wright and Westoby 1999) and those that do generally report a weaker correlation than that between RGR and SLA (Poorter 1989; Poorter and Remkes 1990). Grime and Hunt (1975) nonetheless observed that seedlings of tree species consistently showed low maximum growth rates compared with those of other life forms and argued that this might be due to the 'expenditure of photosynthate on woody tissue, a process concomitant with a slow rate of expansion of leaf area.' Cornelissen et al. (1996) similarly explained a highly significant positive correlation between LWR and RGR in seedlings of 80 woody plant species in terms

of an allocation trade-off between leaves and woody structures, and supported this with evidence for a negative relationship between LWR and the stem weight fraction (SWR). Subsequently, Hunt and Cornelissen (1997a) showed that the relative correlation of LAR with LWR varies taxonomically and with life-form, being lowest for herbaceous monocotyledons and greatest for woody dicotyledons. The failure of some studies (e.g. Garnier 1992; Villar et al. 1998) to identify relationships between LWR and RGR may therefore be due to the particular taxa and/ or life-forms selected for study. Interestingly, a small number of intraspecific studies of herbaceous species have identified relationships between allocation profile and RGR (Rice et al. 1992 [*Bromus tectorum*]; Sugiyama 1995 [*Festuca arundinacea*]; Meerts and Garnier 1996 [*Polygonum aviculare*]).

Apart from wood production, other processes that may incur early growth costs include the allocation of photosynthates to reserve storage (Mooney and Chiariello 1984; Bloom et al. 1985; Chapin et al. 1990; Pate et al. 1990; Suzuki and Hutchings 1997; Lambers et al. 1998) and anti-herbivory defence compounds (Coley et al. 1985; Coley 1986). Quantitative evidence for such costs are, however, sparse. Perhaps the best documented examples of growth costs associated with the formation of storage organs are those described by Mooney and Chiariello (1984) on wild and cultivated radish, and by Chapin et al. (1990) on sugar beet. Several other cases exist but these are mostly anecdotal and lack quantification. For example, massive carbon deposition in underground stems of cabbage palms *Sabal palmetto* may account for the extremely long establishment growth period of seedlings of this species (McPherson and Williams 1996, 1998) but evidence for this is circumstantial.

Some studies have demonstrated a negative correlation between seed mass and seedling RGR (Grime and Hunt 1975; Fenner 1978; Gross 1984; Shipley and Peters 1990; Marañón and Grubb 1993; Swanborough and Westoby 1996; Wright and Westoby 1999) but the causality of such a relationship has been questioned. Thompson (1987), in particular, argued that large seed size and low growth rate represent 'separate solutions to the problem of seedling establishment in a hostile environment', and that the detection of a correlation between these variables is, therefore, incidental. In particular, where slow growth minimises the rate of resource exhaustion, large seededness confers greater initial seedling size and, hence, a competitive benefit. Since any species may adjust either seed size or seedling RGR to maximise seedling fitness the relationship between these traits is not strict.

Therefore, the failure of some studies to detect a relationship among them (Choe et al. 1988; Stock et al. 1990) is unsurprising. In Proteaceae, for example, seedling size is more closely related to seed size than seedling RGR, the latter apparently being relatively homogeneous within the family (Stock et al. 1990).

Because the age at which plants reproduce is often constrained by a minimum size (Weiner 1988; Schmid et al. 1995), ruderal strategies in which plants rapidly attain reproductive maturity are typically associated with high RGR's (Grime 1979; Chapin 1980). Indeed, empirical data suggest that, on average, annual species have higher RGR's than perennials (Garnier 1992; Garnier and Vancaeyzeele 1994). Especially in xeric habitats with a short growing season, fast growth and early flowering may be critical to ensure adequate seed production to guarantee persistence through the unfavourable part of the year (Rice and Mack 1991; Rice et al. 1992). Vegetative persistence, however, provides an alternative strategy for surviving unfavourable periods. Since the formation of storage reserves that facilitate vegetative persistence implies reduced RGR (Bloom et al. 1985, Suzuki and Hutchings 1997), early flowering may not be feasible in species that employ this strategy. Seedlings of *Circaea lutetiana*, a pseudo-annual which reproduces both sexually and vegetatively, show some evidence of a trade-off between reserve storage and flowering (Verburg and Grava 1998). In this species, seed-derived offspring allocate significantly more biomass to storage rhizomes and hibernacles (vegetative propagules) than do clonal offspring, apparently at a cost to flower production in the first growing season (Verburg and Grava 1998). It may be, therefore, that seed- and vegetative persistence strategies are mutually exclusive under extremely tight selection, while mixed strategies are possible under more relaxed selection.

Habitat fertility and RGR

A number of authors (Grime and Hunt 1975; Chapin 1980; Bloom et al. 1985; Poorter 1989; Lambers and Poorter 1992) have noted a positive association between RGR and habitat fertility. Chapin (1980) proposed three possible direct benefits of a low RGR in habitats of low productivity. However, the observation that high-RGR plants outperform low-RGR plants even under low-nutrient conditions, prompted Lambers and Poorter (1992) to suggest that selection in low-fertility habitats might favour a feature linked with low RGR, rather than low RGR itself. In particular, they argued that a low SLA may be advantageous through its link with

higher leaf density (Dijkstra and Lambers 1989; Witkowski and Lamont 1991; Garnier and Laurent 1994; van Arendonk and Poorter 1994; Ryser and Lambers 1995; Wilson et al. 1999) and increased leaf longevity (Reich et al. 1992; Reich 1993; Ackerley and Reich 1999). High leaf density may be important in reducing losses due to herbivory and leaching (Chapin 1980; Chabot and Hicks 1982; Poorter 1989), thereby reducing the need for high leaf turnover.

Objectives of the current study

In *Ehrharta* s. s., change in a series of growth form attributes, specifically plant lifespan, leaf lifespan, culm (stem) lifespan, culm base burial and swelling of the lowest culm internodes, appears to be associated with evolutionary radiation following the occupation of seasonally arid habitats (Chapter 3). Taken together, these modifications account for substantial growth form diversity in *Ehrharta* s. s. The genus includes both annual and perennial species, the latter comprising functionally geophytic, suffrutescent (branching) and caespitose (tufted) forms. Here I examine the variability of seedling RGR across this range of forms and test whether RGR is more strongly related to differences in the resource allocation (LWR, SWR etc.) patterns associated with these forms or to variation in leaf structure (e.g. SLA).

Since the photosynthetic capacity of culms in *Ehrharta* s. s. is considerably less than that of leaves (Chapter 3) and because culms are the major repositories of storage carbohydrates in *Ehrharta* s. s. (Watson and Dallwitz 1992; Chapter 3) as in other grasses (White 1973), high investment in culm tissues represents a potential drain on plant growth. Thus, very low seedling RGR's in geophytic species of *Ehrharta* s. s. (Chapter 3: Fig. 3.2b; Chapter 5) may be expected due to high investment in culm-derived storage structures, while the lack of equivalent structures in annual species should be associated with high seedling RGR's. Even in non-geophytes, burial of culm bases is associated with starch storage and should therefore be associated with some RGR depression.

The possible association of species-specific RGR's with substrates of varying fertility, is also investigated. The species studied are associated with three broad substrate groups: (i) highly leached, shallow sands derived from quartzites and principally associated with the mountains of the Cape Fold belt (Table Mountain Group), (ii) moderately to highly leached gravels derived from granites (Cape Granite Suite, Namaqualand Complex) and representing separate intrusions on the coastal

platform to the south and Namaqualand to the north, and (iii) moderately leached, though nutrient-rich, alluvial soils (clays) derived from shales and mudstones and generally associated with the coastal platform and intermontane valleys (Malmesbury, Witteberg and Nama Groups). In the order listed, these substrate categories represent increasing gradients with respect to both nutrient status and pH (Kruger 1979; Lambrechts 1979; Deacon et al. 1992), and support distinctive vegetation formations (Kruger 1979; Campbell 1983, 1985; Specht and Moll 1983; Cowling 1984; Deacon et al. 1992). In particular, relative to shale-derived clays which support renosterveld and succulent karoo vegetation, quartzite-derived sands show much lower levels of P, N, S, Ca and Mg, and support principally evergreen fynbos vegetation (Specht and Moll 1983). The occurrence of both renosterveld and fynbos on granitic soils in the western Cape confirms its intermediate nutrient status.

In summary, this study uses eight species of *Ehrharta* s. s., representing a range of growth forms, to address five principal questions:

(1) How variable is RGR in *Ehrharta* s. s.?

(2) What are the principal correlates of seedling RGR variation and which of these are potentially causal?

(3) Do differences in RGR and its correlates suggest the existence of alternative life history strategies in *Ehrharta* s. s.?

(4) Are different RGR's associated with habitats of differing fertility (i.e. different substrates)?

(5) Is a high RGR apomorphic in *Ehrharta s. s.* and, if so, has its evolution been prerequisite for the subsequent evolution of annualness?

The role of phylogeny

Since interspecific trait correlations may reflect shared ancestry rather than functional interdependence among traits (Felsenstein 1985b; Harvey and Pagel 1991), the need to accommodate phylogenetic information in comparative analyses of plant function is now widely accepted (Harvey and Pagel 1991; Harvey et al. 1995; Rees 1995; Ackerly and Donoghue 1995; Silvertown and Dodd 1997). For this reason, several studies of plant function now exist which utilise Felsenstein's (1985b) 'phylogenetically independent contrasts (PIC)' method to compare patterns in continuously varying plant traits (e.g. Kelly and Purvis 1993; Ackerly and Donoghue 1998; Villar et al. 1998; Ackerly and Reich 1999; Wilson et al. 1999) while others

draw comparisons exclusively among congeneric or confamilial species pairs (e.g. Garnier 1992; Saverimuttu and Westoby 1996; Swanborough and Westoby 1996; Wright and Westoby 1999). Nonetheless, phylogeny-based comparative methods are not without problems. Most acutely, because the PIC method of Felsenstein (1985b) employs an evolutionary model (a Brownian motion model of trait evolution) to estimate trait values at ancestral nodes, some estimation error is unavoidably introduced. Moreover, because higher node values are calculated as weighted averages of values at lower nodes, estimation error increases with depth in a tree. Alternative methods do exist for estimating nodal values (Kluge and Farris 1969; Huey and Bennett 1987) but these also suffer from estimation error and their relative benefits remain unclear. Regardless of the method used, the reliability of nodal estimations inevitably depends heavily on sampling density and sparse taxonomic sampling is most likely to produce misleading results (Ackerly and Reich 1999), particularly when the traits under investigation are evolutionarily labile (Schluter et al. 1997; Cunningham et al. 1998). Even when sampling of extant taxa is comprehensive, high levels of extinction may result in sampling densities that are effectively low. Therefore, it may be most sensible to restrict the application of PIC-based methods to recent groups (e.g. genera) in which the effect of historical extinction is presumed to be less (Hey 1992) than that in older groups with a broader taxonomic base. The present study employs Felsenstein's (1985b) PIC method, but differs from the bulk of studies using PIC's both in its relatively dense sampling and in its focus on a single genus. In contrast to the approach of Ackerly and Reich (1999), for example, who sampled 108 species across 78 plant genera (from 45 angiosperm families), this study compares eight species from a single genus of 23 species. Thus, the approach employed here more closely matches that of Villar et al. (1998) who investigated RGR variation across 20 out of 23 species of *Aegilops*.

Materials and methods

Seedling growth experiment

Experimental setup and data collection

Seed of eight species, one annual and the remainder perennial (Table 4.1), was collected during the spring and summer of 1996/7. Except for *E. dura*, mean seed

TABLE 4.1. Seed collection vouchers and localities.

Species	Voucher	Collection locality
Ehrharta barbinodis	Verboom 130	Bitterfontein, Namaqualand
E. calycina	Verboom 141	Rondeberg, Western Cape
E. capensis	Verboom 147	Paardeberg, Western Cape
E. dura	Verboom 183	Langeberg, Southern Cape
E. erecta	Verboom 84	Devil's Peak Estate, Western Cape
E. longiflora	Verboom 126	Rondebosch, Western Cape
E. melicoides	Verboom 160	Nieuwoudtville, Western Cape
E. thunbergii	Verboom 170	Porterville, Western Cape

mass was determined for each accession (n=15). Caryopses were removed from diaspores prior to weighing. Seeds were only included if they appeared to be fully developed. Measurements were performed on a balance precise to 0.0001g. Thereafter seeds were treated with an insecticide and stored at 10°C. Vouchers of all accessions are deposited at the Bolus Herbarium, University of Cape Town.

In May 1997 seeds were dusted with a fungicide (Apron C) to prevent decay, sown in sterile potting soil to a depth of about 0.5cm and subsequently irrigated with a fungicide solution of Benlate. Seed trays which showed no evidence of germination after two weeks had elapsed were smoke-treated to stimulate germination. Upon attaining a height of about 5cm, 36 seedlings of each species were transferred to 23l (23 litre) containers containing a modified Hoagland's solution (Poorter and Remkes 1990) and left in a growth room with the following conditions: day - 14hr, 25°C, 400-500µmol.m^{-2}.s^{-1} PPFD (sodium, metal halide and incandescent lamps), 50% relative humidity (r.h.); night - 10hr, 19°C, 50% r.h. For the duration of the experiment, the nutrient solution was renewed weekly to prevent depletion. Seedlings were allowed to adjust to growth room conditions, and harvesting of a given species initiated (day 0) when its seedlings reached the two-leaf developmental stage (corresponding to 500mg dry mass). Subsequent harvests were performed 28 and 56 days following the initial harvest. At each harvest, 12 plants per species were removed, their roots carefully dried with tissue paper, and the whole plant divided into root, stem (including culms, rhizomes and leaf sheaths), leaf (lamina), and (if present) inflorescence (including spikelets and inflorescence branches) fractions. The total leaf area of each harvested plant was measured using a Li-Cor™ Li-3000/3050 portable leaf area meter and the dry mass of each fraction determined on a balance precise to 0.001g, after oven-drying at 90°C (about 48hr).

Seedlings of two additional species, *E. delicatula* (Voucher: Verboom 109) and *E. ramosa* (no voucher), were grown under similar, though non-identical, hydroponic culture conditions during mid-1998. Low seedling availability, however, permitted only two harvests (0d and 28d) and very low replication (four seedlings per harvest in *E. delicatula* and two in *E. ramosa*). For these reasons, and because *E. delicatula* was already at the six-leaf developmental stage when harvesting commenced (0d), data collected for these species were treated separately from those obtained for the main growth experiment. Leaf areas and dry masses of each plant fraction were, however, determined as above.

Data analysis

Growth parameters and seedling traits investigated in this study, along with equations used to calculate each, are listed in Table 4.2. RGR variation is considered in terms of NAR (= ULR_{area}, unit leaf rate) and LAR (Briggs et al. 1920; Evans 1972), the latter being subdivided into SLA and LWR (Poorter 1989; Lambers and Poorter 1992). RGR and NAR were calculated on an interval basis. While RGR was estimated for three time intervals representing early growth (0-28d), late growth (28-56d) and overall growth (0-56d), NAR was only calculated for early and overall growth. In contrast, mean leaf weight ratio (LWR), stem weight ratio (SWR), root weight ratio (RWR), inflorescence weight ratio (FWR), specific leaf area (SLA), leaf

TABLE 4.2. Equations used to calculate growth parameters and seedling attributes, and their units of expression. RGR and NAR were estimated on an interval basis and the remaining parameters on an instantaneous basis.

Variable	Abbrev.	Equation[a]	Unit
Inflorescence weight ratio	FWR	$W_{inflorescence} \times 100/W$	%
Leaf area ratio	LAR	A/W	$cm^2.g^{-1}$
Leaf weight ratio	LWR	$W_{leaf} \times 100/W$	%
Log of inflorescence dry mass	log(FDW)	$\log_{10} W_{inflorescence}$	log(g)
Relative growth rate	RGR	$(W_2-W_1/A_2-A_1)(\ln A_2-\ln A_1/t_2-t_1)$	$g.g^{-1}.d^{-1}$
Root weight ratio	RWR	$W_{root} \times 100/W$	%
Net assimilation rate	NAR	$2(W_2-W_1)/(A_2-A_1)(t_2-t_1)$	$g.cm^{-2}.d^{-1}$
Specific leaf area	SLA	A/W_{leaf}	$cm^2.g^{-1}$
Stem weight ratio	SWR	$W_{stem} \times 100/W$	%

[a]Equation terms: W=plant dry mass (numerical subscripts indicate times flanking interval); t=time; W_{root}=root dry mass; W_{stem}=stem dry mass; W_{leaf}=leaf dry mass; $W_{inflorescence}$=inflorescence dry mass; A=Total leaf area of plant.

area ratio (LAR) and the log (base ten) of inflorescence dry mass (log[FDW]), were calculated on an instantaneous basis, corresponding to 0d, 28d and 56d. Time-specific values of these instantaneous measures are hereafter indicated by a subscript (e.g. LAR_{28} is the LAR at 28d).

Relationships among these traits, as well as seed mass, were tested using linear regression analysis. Three sets of analyses were performed, one using raw species values (ignoring phylogenetic effects) and two using phylogenetically independent contrasts (PIC's) calculated as described by Felsenstein (1985b). Phylogenetic pattern within *Ehrharta* s. s. is well resolved with respect to the species included in this study (Chapter 2) but some uncertainty exists regarding relative branch lengths. Under the assumption that branch lengths correctly estimate the amount of evolution in other attributes, Purvis and Rambaut (1995) argue that branch length differences should be considered, wherever possible, in the estimation of nodal values. Therefore, two sets of analyses using PIC's were performed, which differed in terms of the branch length model used.

In the first (EstBL), branch lengths were set to approximate time intervals as suggested by maximum likelihood analysis of ITS1 data under the assumption of a molecular clock. Although a likelihood analysis including either all species of *Ehrharta* s. s. or, minimally, those species for which growth data were available would have been most suitable for this purpose, this was not possible for two reasons. First, ITS1 sequence information was not available for at least two species included in the growth experiment (*E. capensis* and *E. dura*). Second, topological conflict between the ITS1 tree and that based on total evidence, suggests that such an approach might produce a different set of PIC's as that suggested by total evidence. Instead, the likelihood tree presented in Fig. 3.5a, which contains eight representative *Ehrharta* s. s. species and is fortuitously congruent with the total evidence tree, was used as a framework topology onto which the remaining species were fitted. Fig 4.1a shows the tree that is obtained when additional species are fitted onto this framework in their most likely topological positions (based on total evidence) in such a way that additional internal nodes are evenly spaced and sister species are connected by zero branch length (a multiple speciation event is assumed to described the trichotomy between *E. calycina*, *E. brevifolia* and *E. pusilla*). Pruning the species not included in the present study then produces the tree shown in Fig.

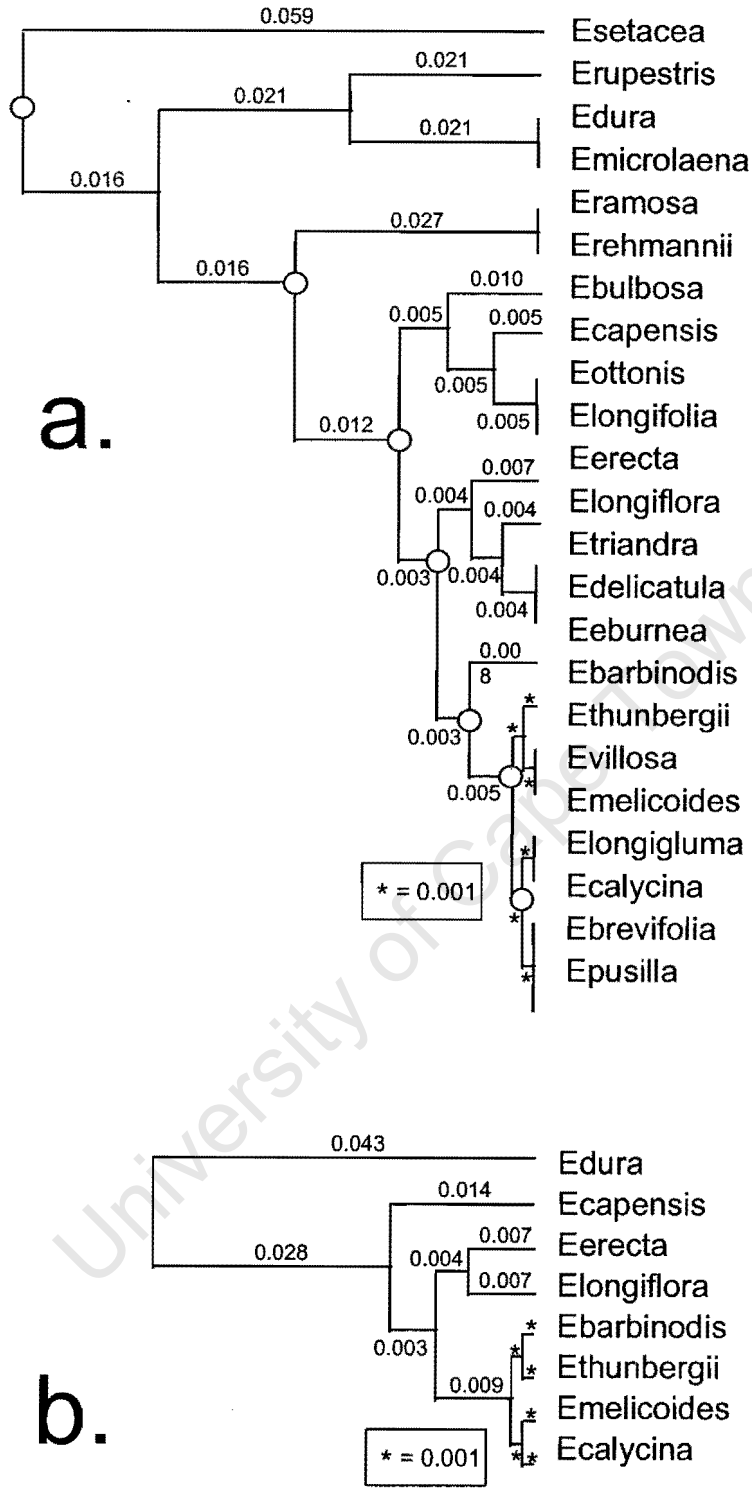


FIGURE 4.1. The EstBL branch length model for (a) all *Ehrharta* s. s. species and (b) for only those species investigated in the current study (remainder pruned). This model is based on the maximum likelihood tree shown in Fig. 3.5a, derived from ITS1 sequence data. Species not included in the original likelihood analysis are inserted in the manner described in the text. Internal nodes present in the original likelihood analysis are marked with open circles. Values above branches are lengths, reflecting the mean number of nucleotide changes per site.

4.1b. The branch length distribution reflected by this tree must be considered, at best, a crude estimate of the 'true' pattern and thus a second branch length model, assuming branch length homogeneity (EquBL), was used as a sensitivity test of branch length uncertainty. Standardised PIC's were calculated using CAIC version 2.0.0 (Purvis and Rambaut 1995). Calculation of contrasts under the EstBL model was done using log (base ten) transformed branch lengths (Garland et al. 1992) because PIC's calculated on the basis of untransformed branch lengths revealed widespread overstandardization (standardization-induced downweighting) of contrasts across deeper nodes, as these involved longer branches.

Linear regression analysis involving both raw species values and PIC's was performed using the multiple regression module in Statistica version 5 (Statsoft 1995). Regressions using PIC's were forced through the origin (Felsenstein 1985b, 1993; Purvis and Rambaut 1995) and the probabilities associated with the resulting correlation coefficients evaluated using degrees of freedom equal to the number of contrasts (Felsenstein 1993).

Adult plant traits

Measurements of herbarium specimens were used to estimate the mean culm burial depths and mean leaf widths of several ehrharteoid grass species. The number of specimens per species varied, in most cases between 10 and 30, depending on the degree of intraspecific variation. Where limited material was available, sample sizes were smaller. Culm burial depths were measured as indicated in Chapter 3, while leaf widths were measured from the broadest leaf, since this was assumed to represent maximum leaf expansion. Both sets of measurements were done using a metal ruler precise to 0.5mm. Leaf thickness for a range of ehrharteoids was measured from transverse leaf sections using a Zeiss™ Standard 25 microscope with an eyepiece graticule having 16µm gradations. In this regard, R. P. Ellis kindly granted permission to make use of his collection of leaf anatomical preparations (and photographs thereof) representing most species of *Ehrharta* s. s.. Correlations of leaf thickness, leaf width and culm burial depth with seedling traits were tested using linear regression analysis, as implemented in Statistica version 5 (Statsoft 1995), using both raw species values and PIC's (see previous section for details).

Trait optimisation onto cladograms

Five quantitative variables (overall RGR, early RGR, mean SLA_{56}, mean leaf thickness and mean leaf width) were traced onto the phylogeny of *Ehrharta* s. s. based on combined data. Species for which data were unavailable were excluded. Squared-change parsimony (Huey and Bennett 1987), as implemented in the 'Trace Continuous' option in MacClade version 3.0 (Maddison and Maddison 1992), was used to estimate values at ancestral nodes.

Substrate preference and RGR

Substrate preferences of the study species were categorised into broad geological groups including sandstone, granite, shale or any combination of these. To test the association between RGR and substrate fertility, two approaches were used. First, box-and-whisker plots were used to check for an obligate association of high overall RGR's with richer substrates (i.e. shales and granites). Second, treating species as observations, Kruskal-Wallis and median tests were used to check, respectively, whether the mean and median RGR's associated with each soil type differed significantly. Both tests were performed using Statistica version 5 (Statsoft 1995).

Growth and flowering of E. calycina on different substrates

In order to test the effect of substrate type on RGR and flowering age of a relatively fast-growing species, seedlings of *E. calycina* were grown under greenhouse conditions on two soils: a quartzitic sand and a granitic loam. In August 1998 seeds of a single accession of this species (Verboom 125: voucher plant growing on loamy soil on Rondebosch Common, Cape Town) were sown in sterile potting soil and germinated as described above. Upon attaining an average height of about 10cm (three-leaf stage), ten similarly-sized seedlings were transplanted separately into large (10l) pots containing quartzitic sand, ten in pots containing granitic loam, and a further ten harvested. Seedlings transplanted to each soil medium were grown for a further 56d before being similarly harvested. Upon harvesting, plants were carefully separated from their substrate taking care not to break the roots, which were then rinsed to remove residual soil particles and dried with tissue paper. Plants were divided into root, stem, leaf and inflorescence fractions as described earlier. The total leaf area of each harvested plant was measured using a Li-Cor™ Li-3000/3050

portable leaf area meter and the dry mass of each fraction determined on a balance precise to 0.001g, after oven-drying at 90°C (about 48hr).

The RGR's (over 56d) of seedlings grown in both media were calculated using the first harvest to determine starting mass. In addition, for each plant the LWR, SWR, RWR, FWR, SLA and LAR at the final harvest were were calculated and the total number of shoots determined. Inter-treatment (soil type) differences in these parameters were tested for significance using Student's t-test, as implemeted in Statistica version 5 (Statsoft 1995), while Fisher's Exact test was used to determine whether the inter-treatment difference in the proportion of plants flowering was significant.

Results

RGR variation

Early RGR (0-28d) is higher than late RGR (28-56d) for all eight species included in the main growth experiment (Table 4.3), although the percent decline between the two phases varies among species. As expected, the linear relationship between early growth and overall growth is highly significant whether raw species values (non-PIC) or PIC's are used (Non-PIC: $r=0.982$, $P<0.001$; EstBL: $r=0.945$, $P<0.001$; EquBL: $r=0.955$, $P<0.001$). For these eight species, overall RGR ranges from 0.067g.g^{-1}.d^{-1} (*E. dura*) to 0.130g.g^{-1}.d^{-1} (*E. longiflora*), while early RGR values range between 0.070g.g^{-1}.d^{-1} (*E. dura*) and 0.183g.g^{-1}.d^{-1} (*E. longiflora*). These ranges represent,

TABLE 4.3. Early, late and overall relative growth rate (RGR) for the eight species included in the main growth experiment. Units are g.g^{-1}.d^{-1}.

Species	Growth form	Overall RGR	Early RGR	Late RGR
E. barbinodis	Suffrutescent perennial	0.090	0.107	0.072
E. calycina	Caespitose perennial	0.119	0.152	0.087
E. capensis	Geophytic perennial	0.084	0.099	0.070
E. dura	Caespitose perennial	0.067	0.070	0.065
E. erecta	Caespitose perennial	0.121	0.152	0.089
E. longiflora	Caespitose annual	0.130	0.183	0.076
E. melicoides	Caespitose perennial	0.106	0.139	0.073
E. thunbergii	Suffrutescent perennial	0.090	0.092	0.088

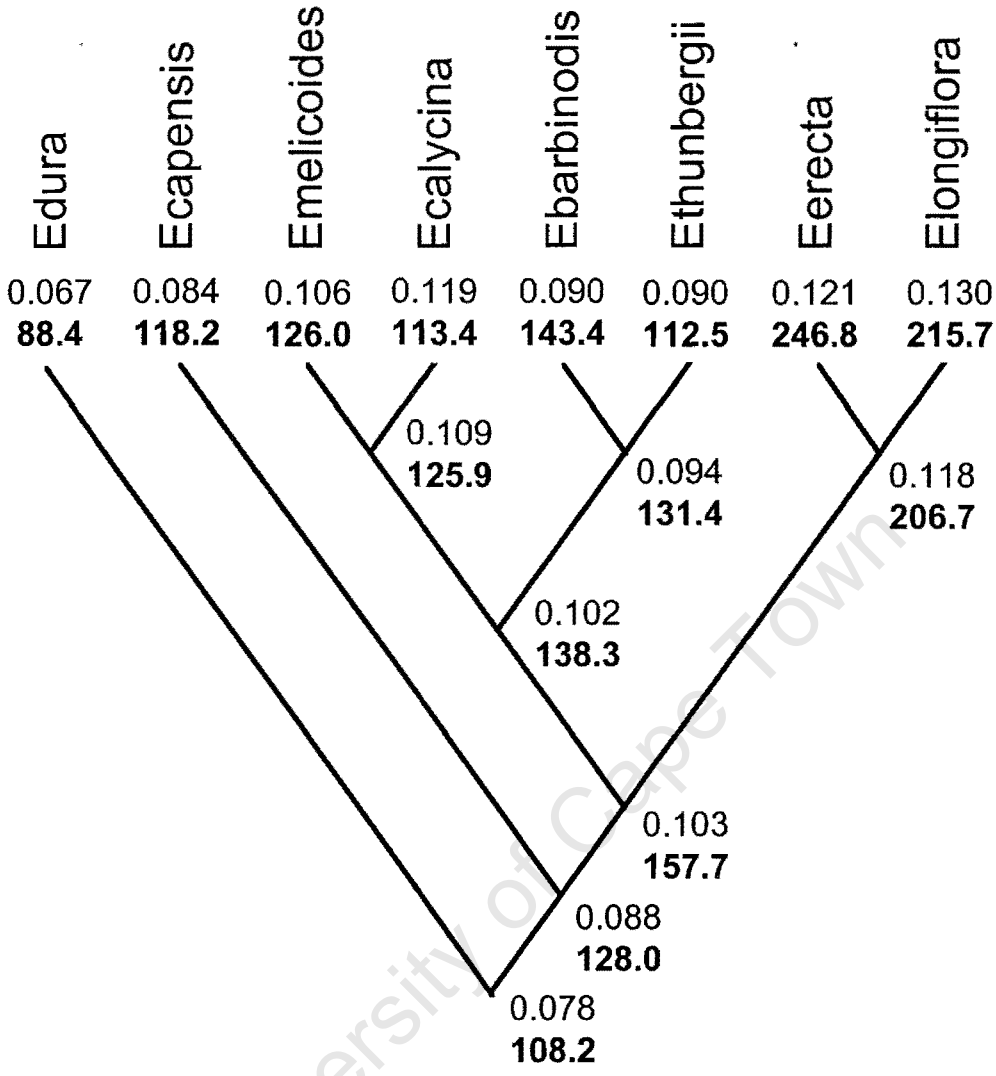


FIGURE 4.2. Tracings of overall RGR (normal type) and mean SLA_{56} (bold type) onto a pruned phylogeny of *Ehrharta* s. s. containing the eight species included in the present growth study. Minimum squared-change parsimony was used to estimate values at internal nodes.

TABLE 4.4. Early RGR, NAR and initial seedling traits (all means, except RGR and NAR) of *E. ramosa* (n=2) and *E. delicatula* (n=4) seedlings grown in hydroponic culture separately from the main growth experiment. Due to differences in culture conditions as well as the advanced developmental state of *E. delicatula* plants at 0d (see methods), these data are not comparable to those obtained in the main growth experiment and are, therefore, omitted from analyses.

Variable[a]	Species	
	E. ramosa	*E. delicatula*
Early RGR ($g.g^{-1}.d^{-1}$)	0.166	0.198
Early NAR ($g.cm^{-2}.d^{-1}$)	0.005	0.005
LWR_0 (%)	58.6	41.1
SWR_0 (%)	19.0	38.9
RWR_0 (%)	22.4	36.2
SLA_0 ($cm^2.g^{-1}$)	349.71	549.08
LAR_0 ($cm^2.g^{-1}$)	205.00	225.57

[a]LAR=leaf area ratio; LWR=leaf weight ratio; NAR=net assimilation rate; RGR=relative growth rate; RWR=root weight ratio; SLA=specific leaf area; SWR=stem weight ratio. Numerical subscripts indicate time of harvest (days after first harvest).

respectively, 1.9-fold and 2.6-fold differences. Preliminary growth data collected for *E. delicatula* and *E. ramosa* in a separate hydroponic experiment indicate, respectively, high early RGR values of $0.198g.g^{-1}.d^{-1}$ (n=4) and $0.166g.g^{-1}.d^{-1}$ (n=2) (Table 4.4), the former increasing recorded early RGR differences in *Ehrharta* s. s. to 2.8-fold. When overall RGR is traced onto a pruned phylogeny of the eight species used in the principal growth experiment a high RGR is interpreted as derived, a low value of $0.078g.g^{-1}.d^{-1}$ being estimated as ancestral (Fig. 4.2). Overall RGR's exceeding $0.100g.g^{-1}.d^{-1}$ occur in two clades, the *E. melicoides-E. calycina* clade and the *E. erecta-E. longiflora* clade. Although these are inferred to have evolved once, at the base of the *E. erecta-E. barbinodis* clade, this is strongly dependent on the optimisation criterion used. Although not shown, early RGR for these species shows the same general pattern. While the high early RGR of *E. delicatula* would not alter this pattern if this species was included in the tree, the high early RGR of *E. ramosa* would produce uncertainty with regard to ancestral RGR in *Ehrharta* s. s.

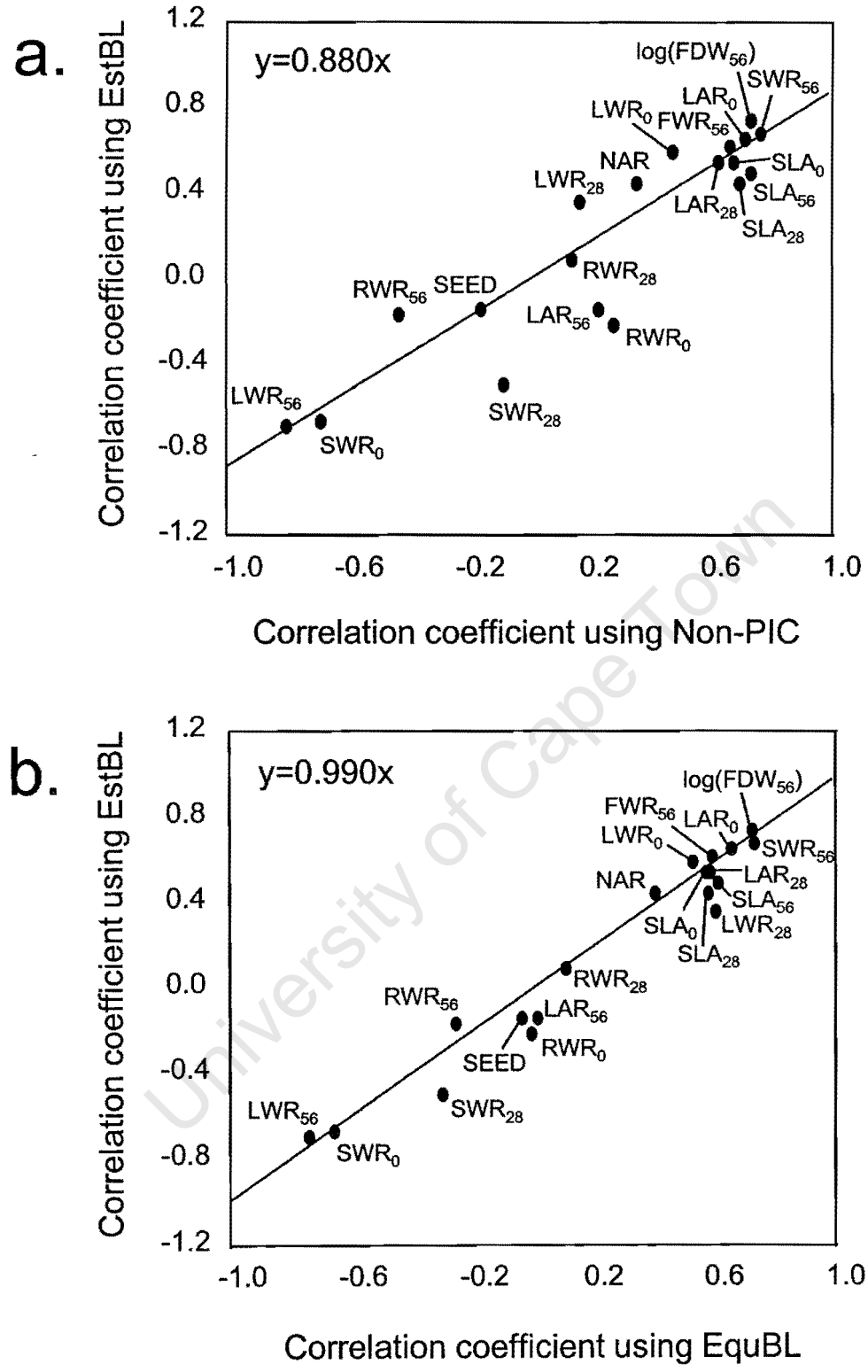


FIGURE 4.3. Comparisons of the correlation coefficients (r) describing the relationships between overall RGR and seed and seedling traits (Table 4.5) calculated on the basis of (a) non-PIC- and EstBL-PIC-based regression analyses, and (b) EquBL PIC- and EstBL-PIC-based regression analyses.

TABLE 4.5. Correlation coefficients describing the linear relationships of overalland early RGR against seed and seedling traits (all means, except NAR), calculated using raw species values (Non-PIC) and PIC's based on two branch length models (EstBL and EquBL). Correlations that are statistically significant at the α=0.05 level are indicated in bold type and marked with an asterisk.

Variable[a]	Overall RGR			Early RGR		
	Non-PIC	EstBL	EquBL	Non-PIC	EstBL	EquBL
LWR_0	0.471	0.603	0.529	0.450	0.451	0.405
LWR_{28}	-0.165	0.367	0.161	0.030	0.279	0.115
LWR_{56}	**-0.802***	**-0.690***	**-0.739***	**-0.734***	-0.611	-0.657
SWR_0	-0.692	-0.660	-0.660	-0.667	-0.506	-0.554
SWR_{28}	-0.089	-0.489	-0.303	-0.153	-0.439	-0.295
SWR_{56}	**0.762***	**0.683***	**0.731***	0.646	0.572	0.630
RWR_0	0.280	-0.202	-0.005	0.276	-0.136	0.062
RWR_{28}	0.138	0.103	0.106	0.144	0.179	0.177
RWR_{56}	-0.434	-0.154	-0.258	-0.321	0.033	-0.085
SLA_0	0.673	0.584	0.575	0.623	0.386	0.431
SLA_{28}	0.690	0.456	0.578	**0.713***	0.477	0.597
SLA_{56}	**0.727***	0.500	0.609	**0.720***	0.527	0.595
LAR_0	**0.710***	0.656	0.656	0.660	0.477	0.497
LAR_{28}	0.622	0.553	0.583	0.660	0.549	0.592
LAR_{56}	0.227	-0.129	0.012	0.280	-0.017	0.088
Overall NAR	0.353	0.454	0.400	-	-	-
Early NAR	-	-	-	0.293	0.355	0.346
$Log(FDW_{56})$	**0.730***	**0.749***	**0.725***	**0.756***	0.595	0.585
FWR_{56}	0.659	0.630	0.589	0.573	0.437	0.405
Seed mass	-0.164	-0.140	-0.040	0.025	-0.109	0.036

[a]FWR=inflorescence weight ratio; LAR=leaf area ratio; log(FDW)=log (base ten) of inflorescence dry mass; LWR=leaf weight ratio; NAR=net assimilation rate; RWR=root weight ratio; SLA=specific leaf area; SWR=stem weight ratio. Numerical subscripts indicate time of harvest (days after first harvest).

Comparative techniques

Correlation coefficients describing the relationships between overall RGR and a series of seed and seedling traits (Table 4.5) are generally higher when phylogeny is ignored (Non-PIC comparisons), and the use of PIC's may reduce statistical significance. Therefore, a regression comparing r-values calculated without PIC's and those calculated using EstBL PIC's has a slope well below unity (Fig. 4.3a: r=0.914, P<0.001). However, a broad scatter of points about the resulting line indicates that the impact of using PIC's varies, and that some comparisons improve with the use of PIC's (e.g. LWR1). In contrast, correlation coefficients calculated using PIC's under different branch length models appear to show broad correspondence (Table 4.5) and exhibit a tight linear relationship (r=0.978, P<0.001)

with a slope approximating unity (Fig. 4.3b). This result, plus the observation that rejection or acceptance of the null models in this study are the same under both branch length models, suggests that the effect of branch length uncertainty is insignificant.

Correlates of seedling RGR variation

NAR

Linear regression analysis does not identify any significant correlation, or even moderately strong association, of early and overall RGR with NAR, whether raw species values or PIC's are used. (Table 4.5).

SLA and LAR

When raw species values are used, linear regression analysis identifies significant positive relationships of overall RGR with LAR_0 and SLA_{56}, and strong (though non-significant at the α=0.05 level) positive associations with SLA_0, SLA_{28} and LAR_{28} (Table 4.5). Highly significant positive linear relationships between SLA and LAR (Table 4.6), plus the much weaker relationships between LWR and LAR, indicate that at these times SLA is the principal correlate of LAR. Thus, the covariance of these two traits in relation to overall RGR at 0d and 28d is not surprising. However, a weak but negative association between SLA and LWR at 56d (Non-PIC: r=-0.580, P>0.1; EstBL: r=-0.446, P>0.2; EquBL: r=-0.531, P>0.1) probably accounts for the lack of a relationship between SLA and LAR at this point and hence explains why LAR_{56} shows no positive association with overall RGR, despite the significant relationship between SLA_{56} and overall RGR.

TABLE 4.6. Correlation coefficients describing the relationships of LAR with SLA and LWR at 0d, 28d and 56d.

	Non-PIC	EstBL	EquBL
SLA_0 and LAR_0	r=0.976, P<0.001	r=0.950, P<0.001	r=0.957, P<0.001
SLA_{28} and LAR_{28}	r=0.953, P<0.001	r=0.937, P<0.001	r=0.939, P<0.001
SLA_{56} and LAR_{56}	r=0.704, P>0.05	r=0.532, P>0.1	r=0.575, P>0.1
LWR_0 and LAR_0	r=0.623, P>0.05	r=0.679, P<0.05	r=0.602, P>0.05
LWR_{28} and LAR_{28}	r=0.446, P>0.2	r=0.451, P>0.2	r=0.457, P>0.2
LWR_{56} and LAR_{56}	r=0.154, P>0.5	r=0.509, P>0.1	r=0.370, P>0.2

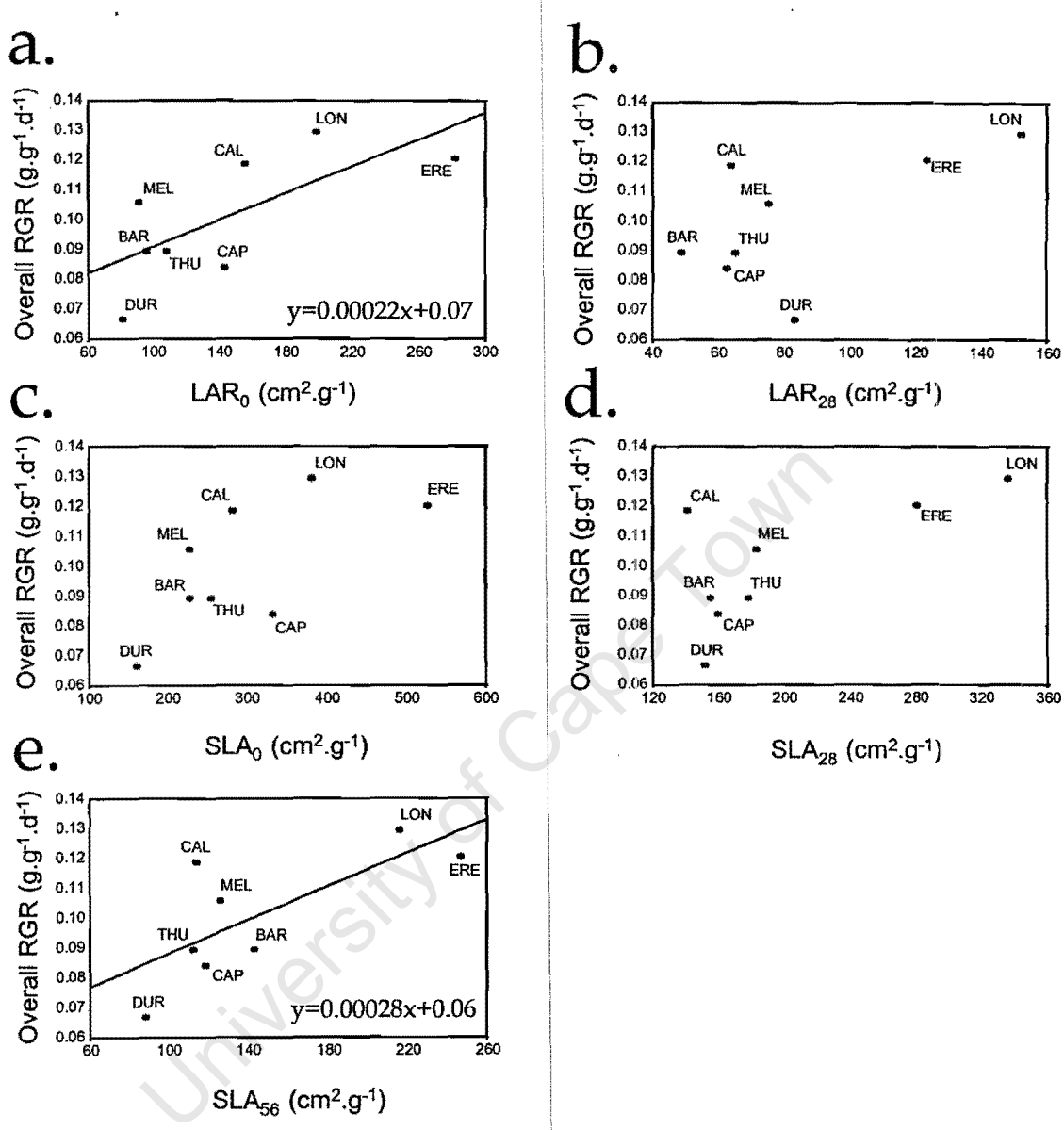


FIGURE 4.4. Raw species value plots of overall RGR against (a) mean LAR_0, (b) mean LAR_{28}, (c) mean SLA_0, (d) mean SLA_{28}, and (e) mean SLA_{56}. Species codes: BAR, *E. barbinodis*; CAL, *E. calycina*; CAP, *E. capensis*; DUR, *E. dura*; ERE, *E. erecta*; LON, *E. longiflora*; MEL, *E. melicoides*; THU, *E. thunbergii*.

The use of PIC's instead of raw species values greatly weakens the positive associations between overall RGR and SLA_0, SLA_{28} and LAR_{28}, and renders the relationships of overall RGR with SLA_{56} and LAR_0 non-significant (Table 4.5). Since the relationship between SLA and LAR is generally strong, the concerted weakening of these relationships is unsurprising. The fact that a strong positive association between LAR_0 and overall RGR remains, despite the absence of a corresponding relationship between SLA_0 and overall RGR (with PIC's), probably reflects a concerted strengthening of the positive relationships between LWR_0 and LAR_0 and between LWR_0 and overall RGR, with the use of PIC's (Tables 4.4, 4.5).

Raw-value (non-PIC) plots of overall RGR against SLA_0, SLA_{28}, SLA_{56}, LAR_0 and LAR_{28} (Fig. 4.4) suggest that the coincidence of high overall RGR, SLA and LAR in *E. longiflora* and *E. erecta* is influential in producing the apparent positive relationships among these variables. Similarly influential is the coincidence of low RGR, SLA and LAR in *E. dura*. The weakening of these associations when PIC's are used, is attributable to two principal factors. First, because *E. longiflora* and *E. erecta* are closely related, the correlated possession of high SLA and overall RGR by both is attributable to common ancestry (Fig. 4.2: both overall RGR and SLA_{56} increase on the branch subtending the *E. erecta-E. longiflora* clade), and is thus reduced to a single PIC-based comparison. Secondly, at least two species-level PIC's contradict these relationships (those between *E. longiflora* and *E. erecta* [Fig. 4.4a, c, e], *E. melicoides* and *E. calycina* [Fig. 4.4 b, d, e]), as do some PIC's across deeper nodes.

A significant negative correlation between leaf thickness of mature, field-grown plants and seedling SLA_{56} for the eight species studied here (Non-PIC: $r=-0.810$, $P<0.05$; EstBL: $r=-0.765$, $P<0.05$; EquBL: $r=-0.816$, $P<0.01$) indicates the importance of leaf thickness differences in determining SLA variation (cf. Witkowski and Lamont 1991). The association of high SLA's with the *E. erecta-E. longiflora* clade, which also contains *E. delicatula* and *E. triandra*, can therefore be demonstrated across a broader species sample if leaf thickness of mature field-grown plants is used as a surrogate for SLA (Fig. 4.5: leaf thickness decreases on the branch subtending this clade). Fig. 4.5 illustrates, further, that the acquisition of thin leaves in the *E. erecta-E. longiflora* clade is correlated with an increase in mature leaf width.

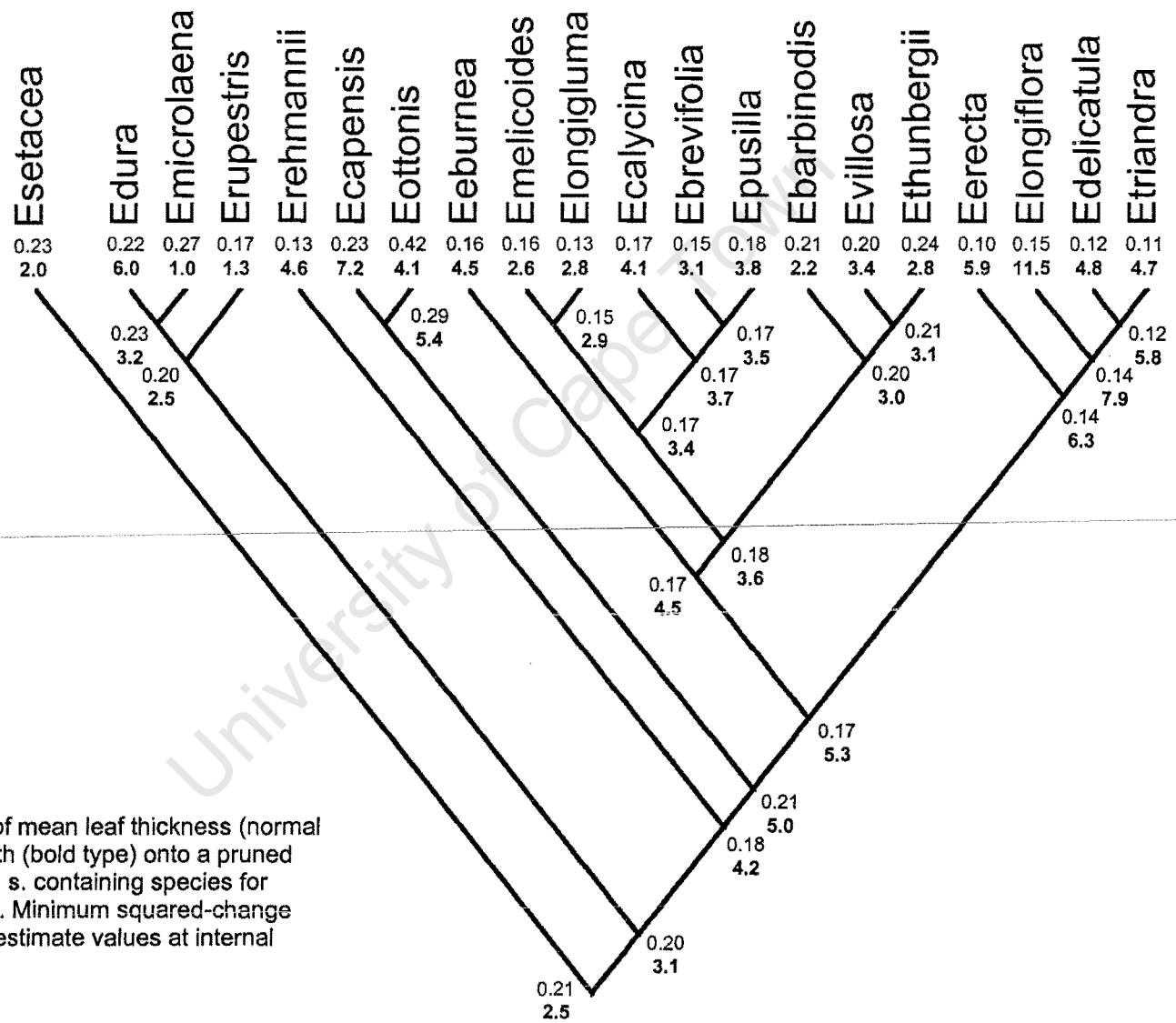


FIGURE 4.5. Tracings of mean leaf thickness (normal type) and mean leaf width (bold type) onto a pruned phylogeny of *Ehrharta* s. s. containing species for which data are available. Minimum squared-change parsimony was used to estimate values at internal nodes.

Comparisons of SLA and LAR against early RGR are similar to those drawn against overall RGR but the observed relationships are somewhat weaker. With phylogeny not taken into account, early RGR displays significant positive linear relationships with SLA_{28} and SLA_{56} as well as non-significant positive associations with SLA_0, LAR_0 and LAR_{28}. However, these associations are weakened substantially when PIC's are used, suggesting again that covariation of these traits with RGR is phylogenetically structured.

The small sample of *E. delicatula* seedlings grown in a later hydroponic experiment had high SLA's (Table 4.4: mean SLA_0=549.1$cm^2.g^{-1}$) confirming the high-SLA status of the *E. erecta-E. longiflora* clade. Seedlings of *E. ramosa* also had comparatively high SLA's (Table 4.4: mean SLA_0=349.7$cm^2.g^{-1}$) though these were lower than those observed in the *E. erecta-E. longiflora* clade.

Early biomass allocation (0d)

Overall RGR shows a strong, though non-significant, negative association with SWR_0, when raw species values are compared (Table 4.5). In *E. capensis* high SWR_0 appears to be associated with swelling of the culm base, while in *E. thunbergii* and *E. barbinodis* it is more strongly linked to culm extension and the basal production of new extravaginal innovation buds which ultimately give rise to new culms. Basal innovation buds in all three species are extravaginal and initially tend to grow side- and downwards. This is in contrast to, for example, those in *E. calycina* and *E. melicoides* which are intravaginal and grow upwards. Despite a significant negative relationship between SWR_0 and LWR_0 (Non-PIC: r=-0.744, P<0.05; EstBL: r=-0.818, P<0.01; EquBL: r=-0.801, P<0.01) the relationship between overall RGR and LWR_0 is surprisingly weak. Overall RGR also shows no association with RWR_0. Where the strength of the associations of overall RGR with SWR_0 and RWR_0 are not markedly altered by the use of PIC's, that with LWR_0 is considerably strengthened (Table 4.5). Inspection of the raw-value plot comparing these variables (Fig. 4.6a) reveals a positive trend. However, this is offset by a single outlier (*E. dura*) that also weakens the negative relationship between overall RGR and SWR_0 (Fig. 4.6b). Whether raw species values or PIC's are used, the exclusion of *E. dura* yields a significant positive linear relationship between overall RGR and LWR_0, (Non-PIC: r=0.809, P<0.05; EstBL: r=0.734, P<0.05; EquBL: r=0.734, P<0.05) and a significant negative linear relationship between overall RGR and SWR_0 (Non-PIC: r=-0.813,

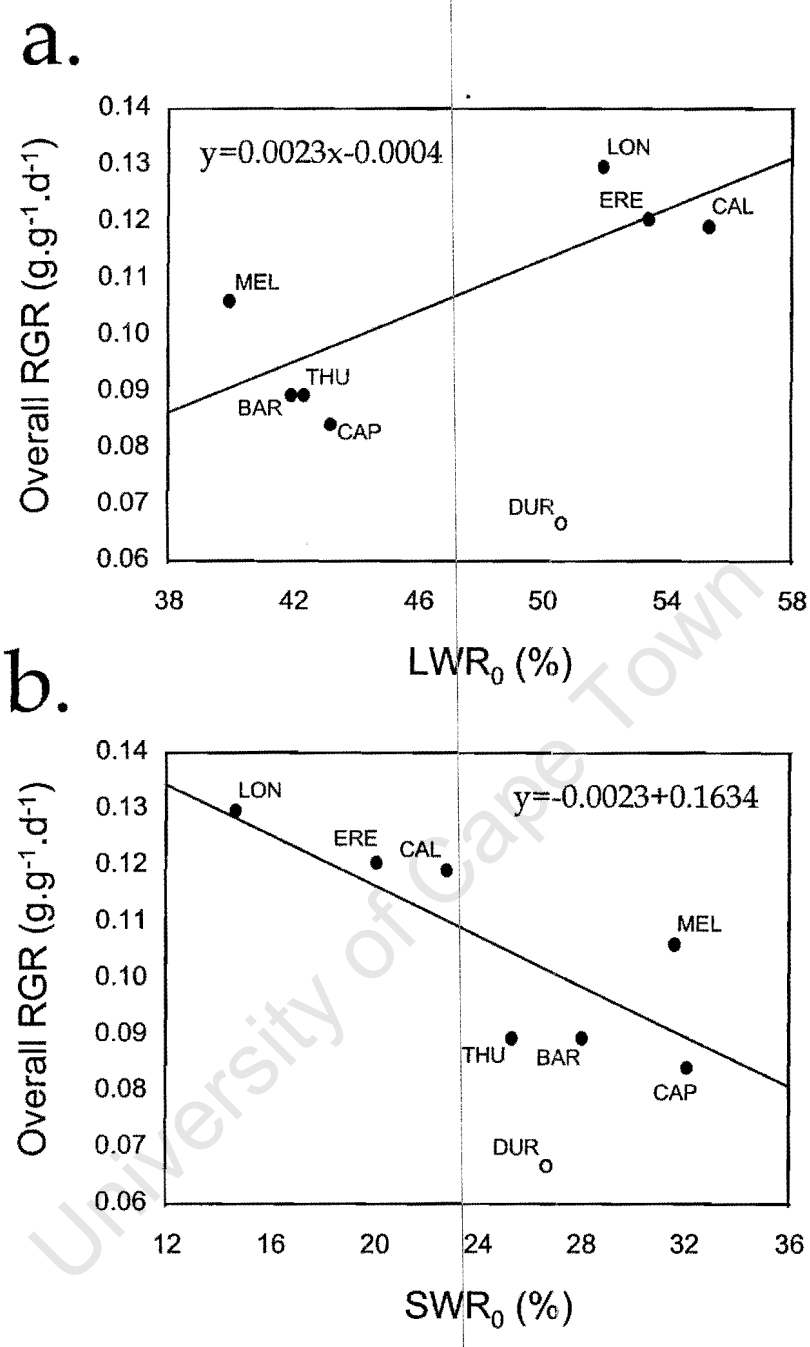


FIGURE 4.6. Raw species value plots of overall RGR against (a) mean LWR_0, and (b) mean SWR_0. Fitted regression lines were calculated with *E. dura* (open circle) excluded. Species codes: BAR, *E. barbinodis*; CAL, *E. calycina*; CAP, *E. capensis*; DUR, *E. dura*; ERE, *E. erecta*; LON, *E. longiflora*; MEL, *E. melicoides*; THU, *E. thunbergii*.

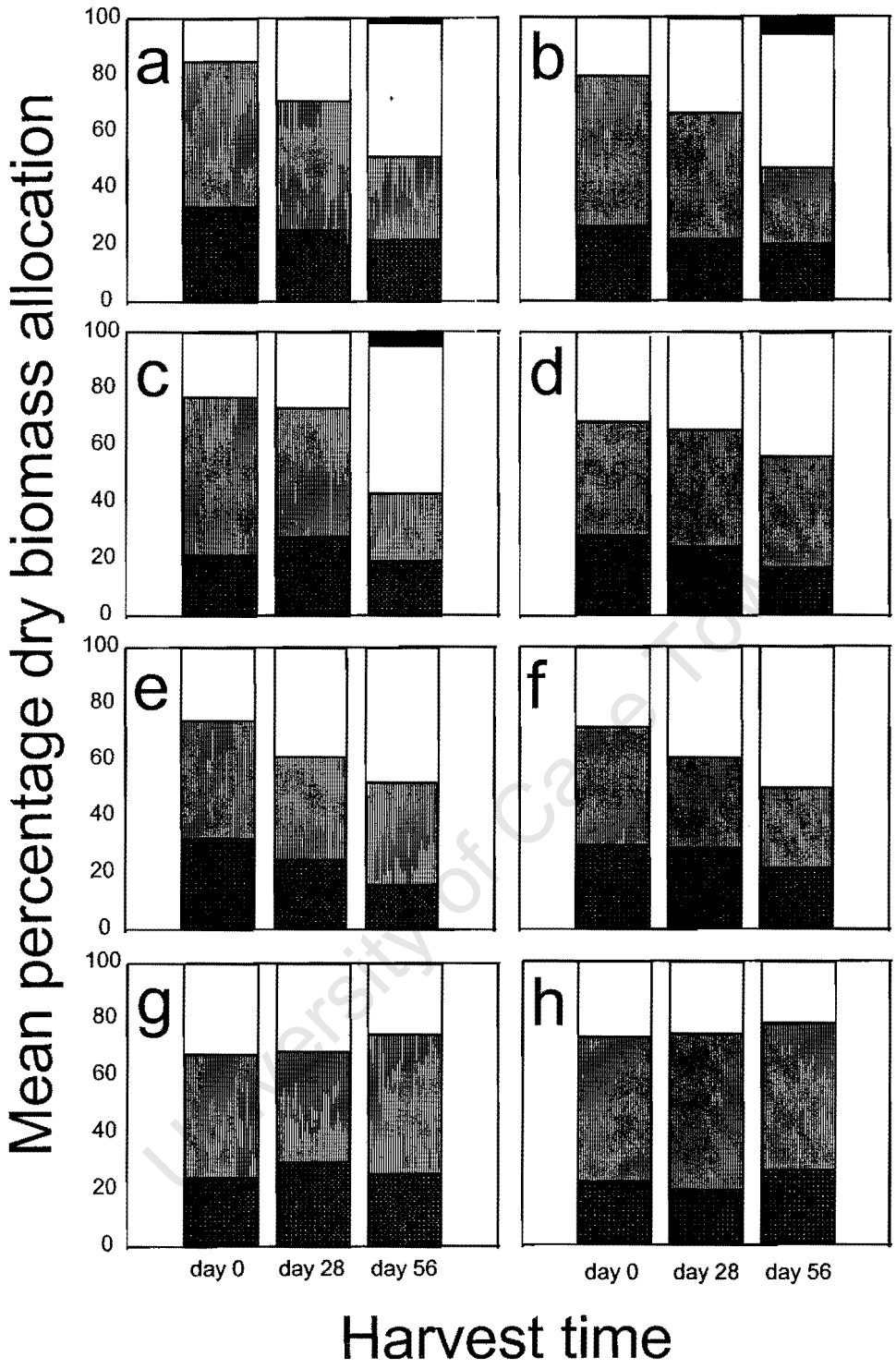


FIGURE 4.7. Mean biomass allocation to root (dark with white stipple), leaf (dense vertical lines), stem (open), and inflorescence (dark solid) fractions, at 0, 28 and 56 days. Species are arranged in order of descending overall RGR: (a) *E. longiflora*, (b) *E. erecta*, (c) *E. calycina*, (d) *E. melicoides*, (e) *E. thunbergii*, (f) *E. barbinodis*, (g) *E. capensis*, and (h) *E. dura*.

P<0.05; EstBL: r=-0.747, P<0.05; EquBL: r=-0.805, P<0.01), despite the loss of a degree of freedom.

The observed associations between overall RGR and biomass allocation patterns at 0d, are also seen with early RGR, though much more weakly. When raw species values are used, early RGR shows a strong but non-significant negative association with SWR_0, a much weaker positive association with LWR_0, and no association with RWR_0 (Table 4.5). Where the association between SWR_0 and early RGR is weakened by the use of PIC's, that between LWR_0 and early RGR is unaffected. As before, both associations are, however, substantially strengthened when *E. dura* is excluded, though both remain non-significant (LWR_0 vs early RGR - Non-PIC: r=0.726; P>0.05; EstBL: r=0.550, P>0.10; EquBL: r=0.569, P>0.10; SWR_0 vs early RGR - Non-PIC: r=-0.748, P>0.05; EstBL: r=-0.565, P>0.10; EquBL: r=-0.667, P>0.05).

Early biomass allocation in *E. delicatula* is not readily comparable to that of species included in the main growth experiment as it was developmentally advanced at the time of first harvest. Allocation patterns change markedly through development (Fig. 4.7), especially in faster growing species (Fig. 4.7a-c). In *E. ramosa* seedlings, high early allocation to leaves (mean LWR_0=0.586) and low early allocation to stems (mean SWR_0=0.397) appears to match expectation based on the high early RGR of this species.

Late biomass allocation (56d)

Plants of five species (*E. calycina*, *E. erecta*, *E. longiflora*, *E. melicoides* and *E. thunbergii*) flowered before the final harvest (56d) (Fig. 4.7), although in *E. thunbergii* this was true of just a single plant (out of twelve). The inception of flowering in these faster-growing species is responsible for a strong positive association between overall RGR and FWR_{56}, as well as a significant positive relationship between overall RGR and $\log(FDW_{56})$ (Table 4.5). Similarly, massive culm production associated with flowering (Fig. 4.7) is directly accountable for the significant positive relationship between overall RGR and SWR_{56} that is observed whether raw species values or PIC's are used (Table 4.5). A highly significant negative relationship between LWR_{56} and SWR_{56} (Non-PIC: r=-0.942, P<0.001; EstBL: r=-0.888, P<0.005; EquBL: r=-0.903, P<0.001) explains the significant negative relationship of overall RGR with

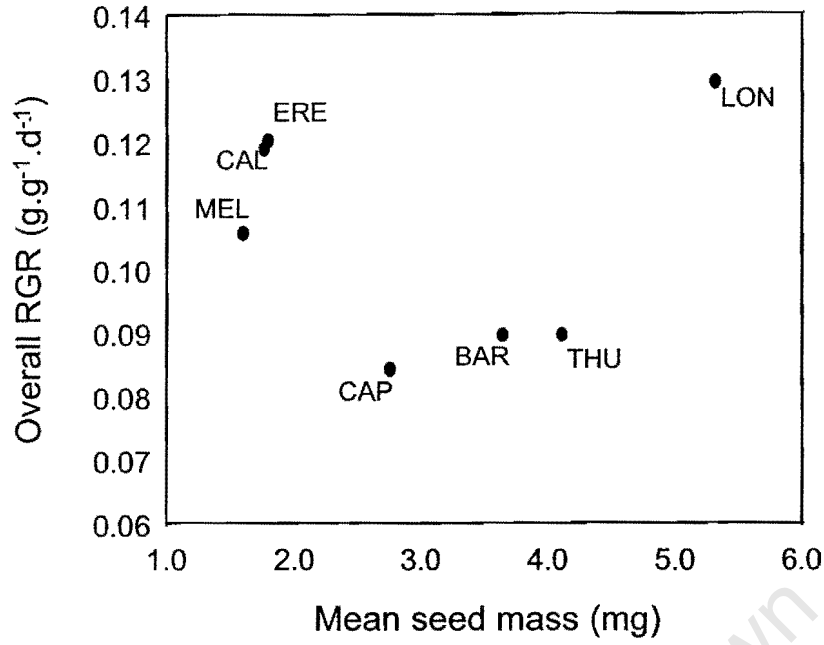


FIGURE 4.8. A raw species value plot of overall RGR against mean seed mass. Species codes: BAR, *E. barbinodis*; CAL, *E. calycina*; CAP, *E. capensis*; DUR, *E. dura*; ERE, *E. erecta*; LON, *E. longiflora*; MEL, *E. melicoides*; THU, *E. thunbergii*.

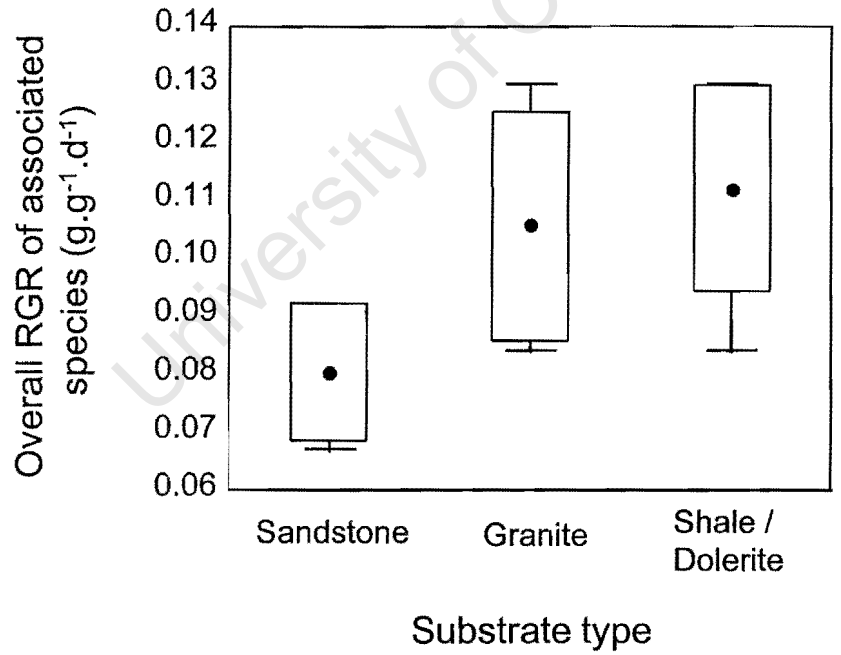


FIGURE 4.9. A comparison of the overall RGR's of species occurring on sandstone-, granite- and shale- or dolerite derived substrates. Solid dots indicate means, boxes standard deviations, and whiskers value ranges.

LWR_{56} that exists whether raw species values or PIC's are compared. No association is apparent between RWR_{56} and overall RGR.

Comparison of early RGR and late biomass allocation patterns reflects a similar though somewhat weaker set of associations than that observed with overall RGR. When raw species values are compared, early RGR shows a significant negative relationship with LWR_{56}, a significant positive relationship with $\log(FDW_{56})$, and positive associations with SWR_{56} (strong) and FWR_{56} (Table 4.5). Although the use of PIC's renders all significant relationships non-significant, the positive and negative associations of SWR_{56} and LWR_{56}, in particular, remain strong.

Seed mass

No apparent associations between seed mass and overall or early RGR exist whether raw species values or PIC's are compared (Table 4.5). A possible negative trend exists (Fig. 4.8) but this is strongly contradicted by a single outlier species, *E. longiflora*. Despite its high RGR, this species has the highest mean seed mass of all the species studied.

Growth form attributes

Of the species included in the main growth experiment, the highest seedling RGR values are associated with the annual *E. longiflora*, the closely-related facultative annual *E. erecta* and the caespitose perennial *E. calycina*. Preliminary data from another annual species, *E. delicatula*, indicate an even higher RGR than that of *E. longiflora*. The lowest RGR values are associated with the two functionally geophytic species, *E. dura* and *E. capensis*, the latter of which has conspicuously swollen basal culm internodes. Two suffrutescent species, *E. thunbergii* and *E. barbinodis*, also display low RGR's, although preliminary data for another suffrutescent species, *E. ramosa*, suggest a high RGR. Due to the small number of species for which RGR data are available, statistical tests of associations of RGR with binary growth form variables (i.e. culm lifespan, culm branching, culm photosynthetic capacity, plant lifespan and culm base swelling) are not viable. However, a remarkably strong association is observed between culm base burial and overall RGR (Non_PIC: r=-0.989, P<0.001; EstBL: r=-0.975, P<0.001; r=-0.980, P<0.001).

TABLE 4.7. Substrate preferences of species of *Ehrharta* s. s. studied. Species are listed in descending order of overall RGR. The natural occurrence of a species on a particular substrate is indicated by '+', non-occurrence by '-'.

Species	Overall RGR ($g.g^{-1}.d^{-1}$)	Substrate parent material		
		Sandstone	Granite	Shale
E. longiflora	0.130	-	+	+
E. erecta	0.121	-	+	+
E. calycina	0.119	-	+	+
E. melicoides	0.106	-	-	+
E. barbinodis	0.090	-	+	-
E. thunbergii	0.090	+	+	-
E. capensis	0.084	+	+	+
E. melicoides	0.067	+	-	-

Substrate preferences

Summarised substrate preferences of the eight species included in the main growth experiment are listed in Table 4.7. Three species (*E. thunbergii*, *E. capensis* and *E. dura*) naturally occur on quartzitic sands, although they are not necessarily restricted to such substrates. Nonetheless, these species were found to have the lowest overall RGR's of all species included in this study. A box-and-whisker plot comparing the mean (± standard deviation) overall RGR associated with each substrate type reveals that the mean RGR of species occurring on sandstone-derived sands is lower than that of those on granite- and shale-derived soils (Fig. 4.9). The difference is non-significant (H=4.070, d. f.= 2, P=0.131) but this may be due to small sample size. A similar comparison among medians also yields a non-significant difference (X^2=4.800, d. f.=2, P=0.091).

Growth and flowering of E. calycina on different substrates

E. calycina seedlings grown on a granitic loam showed substantially higher RGR than those grown on quartzitic sand (Table 4.8), resulting in a three-fold total dry biomass (TDW) difference at the end of the experimental period. Whereas none of the seedlings grown on sand were flowering by the end of the experiment, nine of those grown on granitic loam flowered (Table 4.8). This pattern was correlated with significant differences in infloresence dry mass (FDW), FWR and culm allocation (SWR) at the end of the growth period. Granite-grown plants also averaged three times as many shoots as those grown on sands. Although both SLA and LAR were slightly higher for granite-grown plants, only the difference in LAR was significant.

TABLE 4.8. Growth, allocation and flowering characteristics of *E. calycina* plants after being grown on quartzitic sand and granitic loam for a period of 56 days. Except for RGR and flowering frequencies, data are provided as means ± standard deviations. Statistical significance of inter-treatment differences are indicated in the right column.

Variable	Soil medium		Prob.
	Quartzitic sand	Granitic loam	
RGR ($g.g^{-1}.d^{-1}$)	0.05	0.07	
TDW (g)	0.89 ± 0.21	2.72 ± 0.48	P<0.001
LWR	0.23 ± 0.02	0.27 ± 0.06	P>0.1
SWR	0.32 ± 0.06	0.46 ± 0.09	P<0.001
RWR	0.45 ± 0.06	0.25 ± 0.07	P<0.001
Proportion of plants flowering	0/10	9/10	P<0.001
FDW (g)	0	0.07 ± 0.06	P<0.001
FWR	0	0.03 ± 0.03	P<0.01
SLA ($cm^2.g^{-1}$)	187.55 ± 16.59	206.12 ± 48.37	P>0.2
LAR ($cm^2.g^{-1}$)	43.67 ± 6.83	53.52 ± 11.42	P<0.05
No. of shoots	3.5 ± 1.72	9.9 ± 3.18	P<0.001

Discussion

RGR variation

Data presented in this study suggest that, in comparison with that in other grass genera, RGR variation in *Ehrharta* s. s. is high. Indeed, the 2.6-fold variation in early RGR (2.8-fold if *E. delicatula* is included) reported here exceeds all other published ranges for single genera estimated over a similar early growth window (e.g. Garnier 1992; Garnier and Vancaeyzeele 1994; Atkin et al. 1996; Villar et al. 1998). Since growth form differences reflect different allocation patterns and may thus influence RGR, high RGR variation in *Ehrharta* s. s. may reflect the remarkable growth form diversity of the genus. If so, it should be possible to demonstrate an association between RGR and resource allocation.

Proximal causes of seedling RGR variation

The relationship between growth rate and functional life-history (growth form) traits forms the focus of this study. Since differences in some key traits may only be detectable after a certain period of development, this study considered growth over a longer period (56 days) than that used by the majority of growth studies. Although I

focus here on growth rates calculated over this entire period (overall RGR), it is noteworthy that the relationships of overall RGR with the seedling traits considered in this study are parallelled by those of early RGR. Indeed, a highly significant correlation between overall RGR (0-56d) and early RGR (0-28d) indicates that interspecific RGR differences are entrenched at an early stage of development and may be attributable to traits observed in young seedlings (i.e. at 0d). In general, the associations between overall RGR and seedling traits are slightly stronger than those of early RGR, which may indicate that late growth (28-56d) serves to reinforce or stabilize early RGR differences. However, a general lack of strong statistical support is attributable to small sample size since only eight species are included in this study. In contrast, most previous experimental studies of seedling RGR have included fourteen or more species (e.g. Poorter and Remkes 1990; Garnier 1992; Villar et al 1998).

When phylogenetic covariance is ignored, the data presented in this study suggest that overall RGR (and early RGR) in *Ehrharta* s. s. may be related either to biomass allocation (excepting *E. dura*) or differential investment in leaf area. Although this study identifies positive associations of both SLA and LAR with RGR, these are inconsistently significant and not as strong as is typically the case (e.g. Poorter 1989; Poorter and Remkes 1990; Poorter and Pothmann 1992; Lambers and Poorter 1992; Kitajima 1994; Cornelissen et al. 1996; Meerts and Garnier 1996; Hunt and Cornelissen 1997a; Marañón and Grubb 1993; Wright and Westoby 1999). That both sets of associations are considerably weakened (in some cases with a loss of significance) when PIC's are used instead of raw species values indicates that the number and magnitude of PIC's supporting the apparent covariance between leaf area traits and RGR is insufficient to counter the influence of contradictory PIC's. Indeed, the data presented indicate that the apparent relationship of RGR with both LAR and SLA in *Ehrharta* s. s. relies largely on support from just one or two PIC's (particularly that between the *E. erecta-E. longiflora* clade and the remaining species), while at the same time being contradicted in each case by one or more PIC's.

The remarkably high SLA's observed in the high-RGR *E. erecta-E. longiflora* clade are at least partly attributable to a reduction in leaf thickness on the branch subtending this clade. Since a reduction in leaf thickness and a concomitant increase in lamina width can be expected to reduce the mechanical strength of the leaves of

these species, the evolution of these traits almost certainly accounts for the correlated evolution of the massive parenchymatous midrib that is a prevalent in this clade (Appendices 1, 2: character 951). The fact that large, thin leaves overheat easily under high radiation loads (Parkhurst and Loucks 1972; Chabot and Hicks 1982) may explain why members of the *E. erecta-E. longiflora* clade typically occupy shady microhabitats. Although *E. delicatula*, *E. longiflora* and *E. triandra* all occur in open, arid habitats, within these they show a clear preference for shaded situations, typically beside rocks and beneath trees and shrubs (Gibbs Russell 1990). The association of *E. erecta* with shade is even more marked, this species being most commonly associated with forest margins (Gibbs Russell 1990). These observations, plus the production of lower-SLA leaves under sunny conditions by many plant species (Dijkstra 1989), suggest that high solar radiation in the western Cape may impose limits on SLA variation here. This may, therefore, partly explain the lesser importance of SLA in accounting for RGR variation in *Ehrharta* s. s. than is the case, for example, in European pooid grasses (e.g. Garnier 1992; Garnier and Vancaeyzeele 1994; Atkin et al. 1996). Furthermore, since SLA may be closely tied to leaf-lifespan (Reich et al. 1992; Reich 1993; Ackerly and Reich 1999), the uniform summer deciduousness of leaves in all studied species except *E. dura*, may further explain the lesser influence of SLA in determining observed RGR variation.

Early biomass allocation may provide an alternative explanation of variation in seedling RGR, since a high early (0d) biomass investment in stems (culms) represents a diversion of resources from leaf production and, therefore, growth (Tilman 1988; Cornellisen et al. 1996). In *Ehrharta* s. s., overall RGR is positively associated with LWR_0 and negatively associated with SWR_0, though these trends are consistently non-significant. Interestingly, both associations are equally strong or even stronger when PIC's are used instead of raw species values which suggests that they are not dependent on phylogenetic covariation. This pattern is exceptional across the range of comparisons performed, since the use of PIC's in *Ehrharta* s. s. generally resulted in weakened correlations. The possibility that the relationship of biomass allocation to RGR depends less on phylogenetic covariation than those of leaf area indices, suggests that the former may have greater evolutionary importance in determining seedling RGR in *Ehrharta* s. s. However, additional species data are needed to supplement the data presented here if the relationship of RGR with biomass allocation is to be confirmed.

In the comparison between overall RGR and LWR_0, *Ehrharta dura* emerges as exceptional in having a much higher LWR_0 than expected in terms of its low RGR. At least two explanations may account for this pattern. First, because *E. dura* is the only species studied here which does not belong to the *E. ramosa-E. bulbosa* clade, it may not follow the same physiological 'rules' as the members of that clade. Specifically, a tight association of seedling biomass allocation and RGR may be peculiar to this clade, seedling RGR being governed by other traits elsewhere. Secondly, *E. dura* is exceptional among the species studied in having leaf epidermal cells that contain substantial quantities of tannin (Gibbs Russell and Ellis 1988). Carbon-rich defence compounds such as tannins may incur a cost to growth (Coley et al. 1985; Coley 1986) due to both the direct material (carbon) cost and the construction (energy) cost of producing these substances. Thus, the possession of leaf tannins by *E. dura* may explain the extremely low RGR of this species despite its high initial allocation to leaves. The uniform absence of such substances in the remaining species studied, suggests that RGR differences among these species cannot be accounted for by leaf tannins.

Among species included in the *E. ramosa-E. bulbosa* clade, the morphological manifestation of high early biomass allocation to the stem fraction (in *E. capensis*, *E. barbinodis* and *E. thunbergii*) is variable. In *E. capensis* high SWR_0 primarily reflects early culm base swelling, while in *E. barbinodis* and *E. thunbergii* it reflects individual culm growth as well as the production of new basal innovation buds. These buds ultimately give rise to new culms and so their tendency to grow initially side- and downwards may account for the burial of culm bases in mature plants of these species (Chapter 3). Species of *Ehrharta* s. s., like other grasses (White 1973), store carbohydrate reserves (e.g. starch) principally in their culms and particularly in their culm bases (Watson and Dallwitz 1992; Chapter 3). A high SWR_0 may thus reflect an early investment in reserve storage capacity (Garnier 1992). The coincidence of a high SWR_0 and two adult traits reflecting a geophytic strategy (culm base burial and swelling: Chapter 3) in *E. barbinodis*, *E. capensis* and *E. thunbergii* may therefore have a functional basis, although this requires further evaluation. In view of this trait association the negative relationship between overall RGR and the burial depth of culm bases at maturity is unsurprising although its strength is striking.

Life-history consequences of seedling RGR variation

Overall RGR initially shows a negative relationship with SWR but this switches to a strong, positive relationship at 56d. The significant positive association of high SWR_{56} with high RGR exists because fast-growing species, unlike slow-growing species, entered the reproductive phase prior to the final harvest (56d) and thus invested substantially in inflorescence stalks at this time. Accordingly, there is a significant positive relationship between overall RGR and $\log(FDW_{56})$ as well as a strong positive association of overall RGR with FWR_{56}. Together, these indicate that the high seedling RGR in *Ehrharta* s. s. is linked to early reproductive culm production and early flowering. The negative relationship between SWR_{56} and LWR_{56} indicates that the significant negative correlation of LWR_{56} with overall RGR may be incidental.

All four species having an overall RGR greater than $0.100 g.g^{-1}.d^{-1}$ produced flowers before the end of the growth experiment, suggesting a minimum RGR requirement for early flowering. High RGR is commonly thought to be particularly important in enabling annual and ephemeral species to complete their life-cycles within a single growing season (Grime 1979; Chapin 1980; Poorter 1989). It is, therefore, not surprising that the highest RGR's recorded in *Ehrharta* s. s., are observed in the annuals *E. longiflora* and *E. delicatula*. High RGR's probably also facilitate first-season flowering in *E. erecta* and *E. calycina*. *Ehrharta erecta* is a facultative annual (Gibbs Russell and Ellis 1987: Table 2), implying that seed-to-seed turnover in this species is sufficiently rapid to permit life-cycle completion within a single growing season. Similarly, the existence of a few *E. calycina* specimens (personal observation) from the more arid parts of the northwestern Cape that lack woody bases, suggests that this species may locally adopt an annual strategy.

When RGR variation is traced onto a pruned phylogeny including only the eight species used in the principal growth experiment, high RGR is interpreted as apomorphic, being derived in two clades: the *E. melicoides-E. calycina* clade, and the *E. erecta-E. longiflora* clade. Because these clades contain all the annual *Ehrharta* s. s. species (*E. brevifolia* and *E. pusilla* in the former; *E. delicatula*, *E. longiflora* and *E. triandra* in the latter), an evolutionary association of high RGR with annualness is supported. The derivation of an annual habit plus the possession of high RGR's by perennial members of each clade (especially *E. erecta* and *E.*

calycina) further implies that the evolution of a high RGR in *Ehrharta* s. s. preceded that of annualness and may have been a prerequisite for the evolution of an annual habit. While rigorous assessment of these conclusions requires a more complete RGR data set, the evolution of annualness from low-RGR forms resembling *E. barbinodis*, *E. capensis*, *E. dura*, or *E. thunbergii* is considered unlikely. The observation of a high RGR in *E. ramosa*, interestingly, produces uncertainty with regard to ancestral RGR in *Ehrharta* s. s.. Even with high RGR ancestral, however, the association of annualness with high RGR remains.

In *Ehrharta* s. s., a high-RGR, ephemeral strategy provides an important means of surviving dry summer conditions that prohibit active plant growth (Chapter 3). Seedling traits favouring a high seedling RGR promote early flowering and hence create the possibility of an ephemeral strategy. This option, however, appears to be unavailable to slow-growing species. Low RGR species probably fail to flower in their first growing-season and instead depend on vegetative persistence through the first non-growing season. In low-RGR members of the *E. ramosa-E. bulbosa* clade a high early biomass allocation to stem (culm) tissues may increase reserve storage capacity and, hence, the ability to persist vegetatively through periods of seasonal adversity. In general, periodic non-availability of resources is thought to favour the evolution of seasonally-fluctuating carbohydrate reserves, especially in the face of foliage die-back (Mooney and Billings 1960; Bloom et al. 1985; Chapin et al. 1986; 1990; Meyer and Hellwig 1997). Carbohydrate reserves are thought to be important in initiating regrowth following defoliation in many species (e.g. Davidson and Milthorpe 1966; Kausch et al. 1981; Bloom et al. 1985; Danckwerts and Gordon 1987, 1989; Culvenor et al. 1989; Chapin et al. 1990; Danckwerts 1993; van der Heyden and Stock 1995; McPherson and Williams 1998) and may also be important in facilitating plant recovery following drought-induced foliage loss. In addition, it has been suggested that such stores may fuel respiration during the non-growing period (Meyer and Hellwig 1997).

The low SWR_0 of *E. dura* reflects a relatively low initial investment by this species in stem biomass and indicates a different life-strategy from that observed in slow-growing species within the *E. ramosa-E. bulbosa* clade. Contrary to suggestions by Linder and Ellis (1990) the lowest culm internodes of *E. dura* are not swollen (Gibbs Russell and Ellis 1987, 1988; personal observation), suggesting either that this species is not functionally geophytic (cf. Linder and Ellis 1990) or that culm bases

are not its principal sites of reserve storage. In *E. melicoides*, for example, substantial quantities of starch are stored in the leaf sheath bases (Chapter 3: Fig. 3.2e). The absence of noticeably swollen rhizomes or leaf sheaths in *E. dura* and *E. microlaena* argue against alternative storage sites in these closely related species but more substantive data are required to verify this. Foliage retention through the drier part of the year could obviate the need for a geophytic habit, at least with respect to seasonal drought survival. Data describing the foliage phenology of these closely related species are unfortunately sparse, although Gibbs Russell and Ellis (1988) describe the leaf blades of both *E. dura* and *E. microlaena* as 'persistent'. Moreover, despite the 'mesic' leaf type found in these species (Linder and Ellis 1990), their unique possession of large amounts of epidermal tannin argues for increased leaf longevity (cf. Lambers and Poorter 1992). That both of these species occupy moist habitats (Gibbs Russell and Ellis 1988) that receive high, less-strongly seasonal rainfall (Chapter 3) suggests the increased viability of an evergreen phenology.

In summary, *Ehrharta* s. s. appears to employ two principal strategies to cope with a strongly seasonal growth environment. These correspond broadly to the 'seeder' and 'resprouter' strategies described by others (e.g. Gill 1981; Pate et al. 1990, 1991; le Maitre and Midgley 1992; Bell and Pate 1993; Schutte et al. 1995; van Wilgen and Bond 1996). Species with a low seedling RGR appear to commit high early biomass investment to stems (culms) and fail to flower during their first growing season. Since culms and/or culm bases in these species perennate and contain some starch reserves, high early investment in stem tissues may instead reflect a vegetative persistence strategy. In contrast, species with a high seedling RGR invest maximally in leaves from an early age, reaching reproductive maturity and setting seed within their first growing season. Such species may be annual or perennial, although a smaller early investment in perennating structures plus the non-burial of culm bases may be linked to increased drought-susceptibility of adults. High biomass allocation to leaves and fast seedling growth may equip plants with enhanced competitive ability and hence a greater capacity to establish outside their natural ranges (Baruch and Bilbao 1999). Together with accelerated reproductive maturation and abundant seed production (personal observation), this may explain why the majority of *Ehrharta* s. s. species that are naturalized outside southern Africa are strongly seeding. *Ehrharta calycina* and *E. erecta* are especially weedy, being reported from

both North America and Australia (e.g. Munz 1974; Vickery 1975; Marchant et al. 1987).

Although the distinction between seeders and resprouters is most often discussed in the context of post-fire regeneration (e.g. Gill 1981; Pate et al. 1990, 1991; Bond and van Wilgen 1996), this study highlights the roles that these alternative strategies play in facilitating survival in a seasonally arid environment. Whether or not these represent adaptations to seasonal drought remains unclear, but whatever its cause, canalisation into two strategies appears to determine the regenerative responses of *Ehrharta* s. s. species to fire. Thus, where Kruger (1987) assigned members of the *E. bulbosa-E. capensis* clade to the autoregenerative long-lived sprouter (ALS) category of Bell et al. (1984), he classified *E. ramosa* and *E. calycina* as having facultative seeder sprouter (FSS) and obligate seeder (OS) strategies, respectively.

Substrate dependence

Of the eight species included in the main growth experiment, only three - each with an overall RGR less than $0.100 g.g^{-1}.d^{-1}$ - commonly occur on oligotrophic, sandstone-derived substrates. These species all possess relatively large seeds (seed mass data for *E. dura* not provided in this study), which supports the suggestion that low seedling RGR's and large seededness are alternative but potentially complementary strategies used by plants to aid seedling establishment in hostile environments (Thompson 1987; Stock et al. 1990). However, the combination of large seededness and high RGR in *E. longiflora* precludes a significant relationship between RGR and seed mass and confirms the lack of a strict association between these strategies (Thompson 1987). The general absence of fast-growing species from sandstone-derived substrates, plus the association of a lower mean RGR with such substrates, provides some support for the contention of Chapin (1980) and Poorter (1989) that high RGR's are sustainable only in fertile habitats. Since a minimum RGR may be required to support early flowering, this dependence accounts for the strict association of annual *Ehrharta* s. s. species with substrates derived from granites, shales and dolerites, as well as Quaternary sands, and suggests that access to such substrates may have been critical for the evolution of an annual habit. Indeed, this may be true for Cape grasses in general (Linder and Ellis 1990; Ellis and Linder 1992). The dependence of early flowering on high RGR and high substrate fertility is supported by experimental data describing the growth of *E. calycina* on different

soils. This species grows more quickly and flowers sooner when grown on granitic loam, than when grown on quartzitic sand.

In view of its obligate association with the oligotophic sandstones of the Cape Fold mountains, the high seedling RGR of *E. ramosa* seems paradoxical. Apparently, however, this species recruits exclusively after fire (Kruger 1987) when soil nutrient availability is temporarily increased (Brown and Mitchell 1985; Stock and Lewis 1986) and faster growth may therefore be possible. Field data indicate that leaf production and growth in adult plants of this species is greater after fire than is otherwise the case (Chapter 5). Presumably, increased nutrient availability at such times temporarily reduces the nutrient cost of producing ephemeral leaf material and hence facilitates peaked growth. Later, as nutrients become more limiting, reliance on culm photosynthesis presumably increases, although even then some leaf material continues to be produced annually.

Lambers and Poorter (1992) argued that the predominance of low-RGR species in low-fertility habitats reflects selection on a trait linked with RGR rather than selection on RGR itself and in this regard highlighted a potential role for SLA. In *Ehrharta* s. s., however, SLA is not significantly correlated with RGR once phylogeny is taken into account, which suggests that SLA is not evolutionarily important in this group. Instead, the low RGR's of *E. barbinodis*, *E. capensis* and *E. thunbergii* may be explained at least as well (if not better) by high early biomass investment in stems. Thus SWR is an alternative object of selection. Given the presumed inability of low-fertility habitats to support a high RGR, two growth strategies are available to plants occupying such habitats. An evergreen strategy, characterised by long-lived, low-SLA foliage (Chabot and Hicks 1982; Poorter 1989) is possible if conditions during the non-growing season are not excessively severe. If the non-growing season is too severe to permit leaf perennation, reserve storage and a greater production of perennating structures may compensate for the losses incurred by leaf deciduousness (Bloom et al. 1985; Chapin et al. 1990). Low-RGR species in the *E. ramosa-E. bulbosa* clade (note that, on current evidence, *E. ramosa* is probably exceptional) appear to use the latter strategy, while *E. dura* and *E. microlaena*, at least, probably employ the first. Additional phenological data pertaining to the latter two species are, however, required.

Chapter 5. Post-fire growth and flowering in *E. ramosa* and *E. capensis*

Introduction

In fynbos, the principal vegetation formation in the mountains of the southwestern Cape, wildfires are frequent and are believed to play a central role in nutrient cycling and vegetation dynamics (Kruger and Bigalke 1984; Kruger 1987; Stock and Allsopp 1992). Resource availability in nutrient-stressed shrublands such as fynbos and kwongan is temporarily elevated by fire (Pate and Dell 1984; Stock and Allsopp 1992), with the result that many plant species show pulsed growth and reproduction immediately after fire (Bond and van Wilgen 1996). Recruitment opportunities at such times are improved for most species but low-growing plants (therophytes, cryptophytes, hemicryptophytes and nanerophytes) with high light and nutrient requirements may be particularly dependent on the post-fire period for growth and reproduction. Among perennials, geophytes and graminoids appear to be especially capable of exploiting post-fire opportunities and typically dominate the early stages of post-fire succession (Kruger and Bigalke 1984; van Wilgen and Forsyth 1992). Annuals (ephemerals), moreover, are typically evident only immediately after fire (Le Maitre and Midgley 1992). An increase in the cover of annuals and graminoids can be similarly induced by fertilizer addition (Witkowski 1989) which suggests that this 'fire' response is at least partly attributable to increased resource availability.

Fire results in the removal of competitors as well as a temporary reduction in predation levels (e.g. Bond 1984; Le Maitre 1984). In addition, the post-fire environment is characterised by higher light, temperature, moisture and nutrient availability (Knapp 1984; Kruger and Bigalke 1984; Pate and Dell 1984; Brown and Mitchell 1986; Stock and Lewis 1986; Kruger 1987; Hulbert 1988;) than that in the pre-burn environment. Canopy removal is important in permitting the penetration of sunlight to the soil surface (Kruger 1987; Hulbert 1988) and this, in addition to increasing light availability, may cause the temperature of the soil surface as well as that of the air above it to be raised (Knapp 1984; Kruger and Bigalke 1984). Even though elevated soil temperatures may cause the surface soil to dry out more

rapidly, reduced transpiration due to the removal of cover may actually increase water retention and availability (Kruger and Bigalke 1984). In fynbos, as in kwongan (Pate and Dell 1984), there is also evidence for increased soil nutrient availability after fire due to the deposition of ash, although this effect is short-lived (van Wilgen and Le Maitre 1981; Stock and Lewis 1986; Brown and Mitchell 1986; Stock and Allsopp 1992). In particular, brief post-fire pulses of soil N and P have been observed in fynbos (Stock and Lewis 1986; Brown and Mitchell 1986).

'Fire-stimulated' flowering (e.g. Le Maitre and Brown 1992; Lamont and Runciman 1993; Brewer 1995), 'fire-stimulated' seed release (often in association with serotiny) (e.g. Lamont et al. 1991, Bond and van Wilgen 1996) and 'fire-stimulated' germination (e.g. Keeley et al. 1991; Bell et al. 1993) all represent mechanisms by which plants apparently exploit post-fire conditions. However, in many instances it is unclear whether such 'fire-stimulated' responses are triggered by direct fire cues and are therefore strictly fire-dependent, or whether their manifestation is due simply to increased post-fire resource availability, the association with fire being incidental. The demonstration in at least some 'fire-stimulated' species that flowering can be induced by increased light or nutrient availability alone (Old 1969; Le Maitre and Brown 1992; Brewer 1995), suggests a lack of strict fire-dependence. 'Fire' effects may accompany other processes such as herbivory which may, therefore, also be capable of stimulating flowering to some extent. In other cases, however, a dependence on proximal fire-cues such as smoke-bound chemicals (Gill and Ingwersen 1976) and heat pulses (Bean 1962) may indicate genuine fire-induced responses.

In this chapter I examine two fynbos species of *Ehrharta* s. s., in an attempt to determine (i) whether these show marked post-fire responses, (ii) whether such responses reflect utilisation of post-fire conditions and (iii) how such responses relate to the particular growth form/ life strategy of either species. In particular, this study investigates post-fire growth in *E. ramosa* and post-fire flowering in *E. capensis*. For the latter, the specific dependence of flowering on fire is tested.

Post-fire growth in *E. ramosa*

E. ramosa is a suffrutescent species restricted to sandstone-derived substrates of the Cape Fold mountain belt. Mature plants are characterised by long-lived culms in which the chlorenchyma is well developed and functions in carbon assimilation

(Linder and Ellis 1990; Chapter 3) as in the Restionaceae (Cutler 1969; Linder 1991b). Leaves, by contrast, are infrequently produced and extremely short-lived (Gibbs Russell and Ellis 1987), so that plants often appear leafless.

Given its preference for nutrient-stressed habitats, plus its 'leafless' adult morphology, both the leafiness and high relative growth rate (RGR) of seedlings grown in hydroponic culture (Chapter 4) are unexpected. High growth strategies are typically associated with plants from productive habitats (Grime and Hunt 1975; Chapin 1980; Poorter 1989). Kruger (1987), however, indicates that *E. ramosa* recruits exclusively after fire, so that seedling growth occurs only when the productivity of the fynbos environment is exceptionally high compared with that during the inter-fire period. At this time, high seedling leafiness and RGR may be possible. Similarly, adult plants, resprouting after fire, may also maximise leaf production and growth at this time. To test this suggestion, this study investigates differences in mean shoot mass and shoot-specific leaf mass and leaf area, between adult plants growing in unburnt vegetation, and vegetation burnt six months previously. Flowering levels in burnt and unburnt plants are also compared.

Post-fire flowering in *E. capensis*

E. capensis is one of a clade of four geophytic species whose members occur on a diverse range of substrates, from relatively eutrophic shale-derived clays to highly leached sandstone-derived sands. As with other geophytic grasses of the Cape region, mass flowering in these species is apparently stimulated immediately after fire (Linder and Ellis 1990), soon dropping off to insignificant levels. Between fires, survival from one season to the next is largely vegetative, relying on the starch-filled, swollen culm bases which are replenished through the seasonal production of new foliage.

Post-fire mass flowering, in which flowering is either concentrated in or restricted to (e.g. *Drosera erythrorhiza*: Dixon and Pate 1978; Pate and Dixon 1982) the period immediately after fire, has been reported from a broad range of plants, most notably monocotyledons. In monocotyledons it occurs in an array of families, including Poaceae (Linder and Ellis 1990), Orchidaceae (Linder 1981; Pate and Dixon 1982; Linder and Kurzweil 1999), Iridaceae (Kruger and Bigalke 1984; Le Maitre and Brown 1992), Haemodoraceae (Lamont and Runciman 1993), Amaryllidaceae (Le Maitre and Brown 1992; Keeley 1993), Hypoxidaceae (Herndon 1988), Arecaceae

(Abrahamson 1984; 1999) and Xanthorrhoeaceae (Gill and Ingwersen 1976; Pate and Dixon 1982). With the exception of fire-ephemerals, the post-fire mass flowering strategy in fynbos communities is most marked in geophytes (Levyns 1929; Le Maitre and Brown 1992; Le Maitre and Midgley 1992).

Although post-fire mass flowering is commonly observed and may have clear selective benefits (Bond and van Wilgen 1996), it is not clear that such responses are directly induced by, and thus strictly dependent on, fire. Several studies have attempted to identify proximal cues for 'fire-stimulated' flowering (e.g. Bean 1962; Gill and Ingwersen 1976; Le Maitre and Brown 1992; Lamont and Runciman 1993; Abrahamson 1999). Such cues are thought to include smoke-bound chemicals (Gill and Ingwersen 1976; Keeley 1993) and heating of subterranean storage organs (Bean 1962). However, a lack of clear evidence for simple flowering cues led Le Maitre and Brown (1992) to propose that apparent fire-stimulated flowering in at least two iridaceous species is 'not a direct effect of fire, but an indirect effect linked to changes in the environment.' This perspective is supported by the observation that enhanced productivity and flowering can be experimentally induced by the combined application of simulated 'fire effects' (e.g. Hulbert 1988). Foremost among these are increased light and nutrient availability, neither of which is, however, unique to post-fire environments. In clonal plants, a plastic allocation response to fluctuating resource availability may thus account for mass flowering after fire (Brewer 1995). Specifically, given a trade-off between sexual reproduction (flowering) and continued persistence of the parent clone (e.g. Loehle 1987; Lovett Doust 1989; Hartnett 1990; Schmid et al. 1995), clonal species may flower only when resource availability is sufficient. As a result, flowering does not compromise continued survival of the parent clone. In closed, nutrient-deficient habitats resources are abundant only in the wake of fire and post-fire flowering responses are, therefore, expected to be strongest in populations/ species from such habitats (Brewer 1995).

Here I use a field-based experiment in which two indirect fire effects are simulated to explore the basis of apparent fire-stimulated flowering in *E. capensis*. I focus on two effects, defoliation and fertilization, because these have repeatedly been identified as influential in stimulating flowering effort in other taxa (Hulbert 1988; Lamont and Runciman 1993; Brewer 1995; Abrahamson 1999). However, neither of these effects is unique to the post-fire environment and so a positive flowering response to either or both would suggest that flowering is not strictly dependent on fire. Conversely, no

response might indicate dependence on a proximal fire cue. For comparative purposes, treatment responses of two other non-geophytic species are examined: *E. calycina* is a high-RGR seeder, typically associated with fertile substrates, while *E. thunbergii* is a lower-RGR suffrutescent species typically associated with granite- and sandstone-derived soils.

Four specific questions are addressed:
(1) Does *E. capensis* show a positive flowering response to indirect fire-effects, and is this more marked than in the other non-geophytic species examined?
(2) If so, do fire effects operate singly or in combination to stimulate flowering?
(3) Does flowering occur at a cost to clonal persistence, or is there evidence to suggest that plants allocate resources to flowering only above a minimum vegetative mass and, more specifically, once a minimum investment in clonal persistence is assured? This question is addressed at the level of the individual ramet, as this is suggested to be most appropriate for clonal plants (Hartnett 1990).
(4) Is there evidence to indicate that reserves in previous years' corms are utilised to promote rapid and/ or mass flowering, as has been previously suggested (e.g. Linder and Ellis 1990)?

Materials and methods

Post-fire growth in *E. ramosa*

Sampling

Two sites that had been burnt during the late summer/ autumn season preceding sampling were selected to compare post-fire growth responses in *E. ramosa*. The first of these, situated on the upper slopes of the Helderberg (Fig. 5.1: 34°02'15"S, 18°52'12"E) at an altitude of about 870m, was sampled in mid-October (spring) 1998, while the second, located on Devil's Peak (Fig. 5.1: 33°57'30"S, 18°26'05"E) at an altitude of about 700m, was sampled in mid-October 1999. The Helderberg site had been burnt in March 1998 and the Devil's Peak site in February 1999. Both sites are on Table Mountain group sandstones with mountain fynbos vegetation.

At each site, representative above-ground material of mature *E. ramosa* plants was taken both from vegetation which had been recently burnt (treatment) as well as from

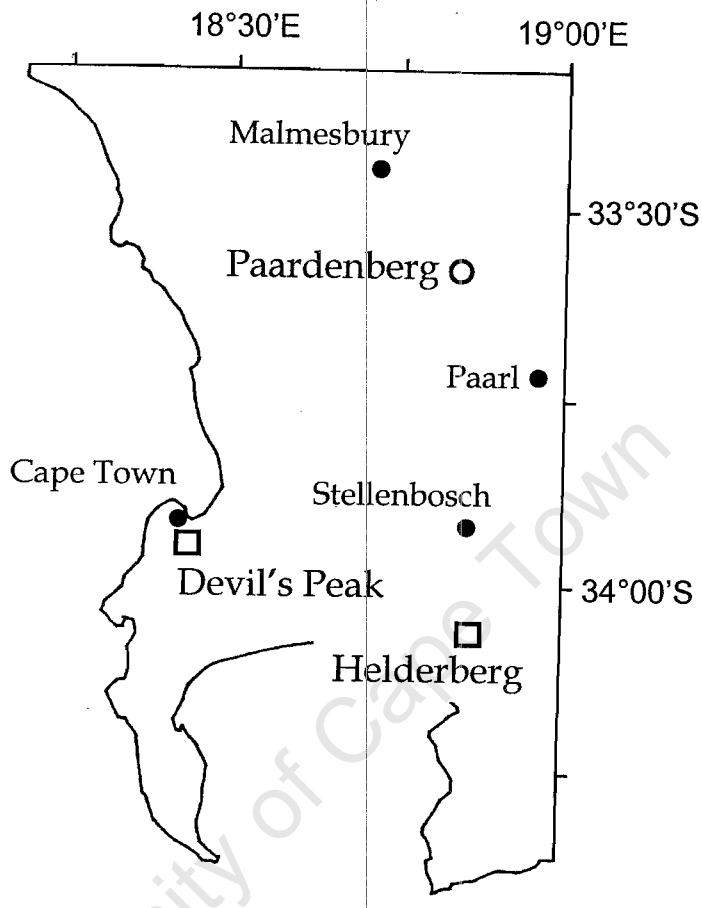


FIGURE 5.1. Map showing the geographical localities of the study sites used to study post-fire growth in *E. ramosa* (open squares) and fire-stimulated flowering in *E. capensis* (open circle).

adjacent, unburnt patches (controls). Care was taken to ensure that separate samples represented distinct individuals. At Helderberg, 15 and 13 individuals were sampled from burnt and unburnt vegetation, respectively, while at Devil's Peak, 11 burnt and unburnt plants were sampled.

Data collection and statistical analysis

Depending on whether plants had been burnt or not, shoots produced during the 1998 growing season were either initiated at ground level (burnt plants) or produced chiefly from elevated buds situated on the perennating culms (unburnt plants). Sixteen new shoots were randomly selected from each *E. ramosa* individual sampled on the Helderberg and divided into leaf, culm (including leaf bases) and inflorescence fractions. Material produced in the previous years (persistent culm material) was discarded. Shoot-specific leaf areas were determined using a Li-Cor™ Li-3000/3050 portable leaf area meter, after which each plant fraction was dried at 80°C. The dry mass of each fraction was determined on a balance precise to 0.001g. Sample plants from Devil's Peak were similarly treated except that 20 new shoots were sampled per individual and leaf areas were not determined.

Mean shoot-specific leaf, culm and inflorescence dry mass (both sites) and mean shoot-specific leaf area (Helderberg only) were determined and compared between burnt and unburnt plants at both sites. Student's t-test, as implemented in Statistica version 5 (Statsoft 1995), was used to assess the significance of observed differences. Wherever an F-test indicated significant inequality of variances, an approximate t-test using separate variance estimates and approximate degrees of freedom was employed. Differences in flowering frequency among the sampled burnt and unburnt plants were assessed using a two-tailed Fisher exact test.

Post-fire flowering in *E. capensis*

Study site and experimental setup

Fire-stimulated flowering in *E. capensis* was experimentally studied at a field-based site situated on Weltevreden farm on the south-eastern slopes of the Paardenberg (Fig. 5.1: 33°37'10"S, 18°50'10"E). The latter is an isolated but sizeable (4000ha) granitic outcrop situated on the shale-underlain Swartland coastal platform. Vegetation on the Paardenberg is principally mountain fynbos with a circumbasal

transition zone to west coast renosterveld (Campbell 1985) and it is in the latter that the study site was located. All three study species, *E. capensis*, *E. calycina* and *E. thunbergii*, co-occur on the study site. A large portion of the southern and eastern slopes, including the study site, experienced an intense bush fire about a year prior to experimental setup.

In early April 1997 (autumn), prior to the wet growing season, 56 individuals of each study species were subjected to one of four treatments simulating fire effects (14 plants per treatment): defoliation only, fertilization only, defoliation plus fertilization, and neither defoliation nor fertilization (control treatment). In order to accommodate fine-scale environmental heterogeneity, care was taken to ensure that different treatments were spatially interspersed. Sample plants were located along four linear transects and treatments applied more or less sequentially along these so that, on average, every fourth plant along any transect was identically treated. To minimise the risk of unwanted fertilization due to runoff non-fertilization treatment plants were consistently located upslope of fertilization treatment plants. Defoliation entailed removal of all above ground plant material (living and senescent) within a 1m radius of each study plant by means of secateurs and a petrol-fuelled brush-cutter (strimmer/ weed-eater). In the suffrutescent *E. thunbergii* this resulted in the removal of some live biomass (i.e. the persistent culms). However, defoliation did not result in the removal of living material from either *E. capensis* or *E. calycina*, as both survive the dry season by means of structures that are situated below-ground (swollen culm bases in the former, shallowly-buried rhizomes in the latter). The principal effect of defoliation, therefore, was canopy removal. Fertilization entailed addition of a commercial lawn and evergreen plant fertilizer produced by Wonder™ (176g.kg^{-1} N, 22g.kg^{-1} P, 22g.kg^{-1} K). Estimated post-fire N- and P-inputs of 66kg.ha^{-1} and 0.6-6.5kg.ha^{-1}, respectively, in fynbos systems (van Wilgen and Le Maitre 1981; Stock and Lewis 1986) were used to guide fertilizer choice and dosage. Approximately 100g of fertilizer was sprinkled over the soil surface within 1m of each study plant, this representing N- and P- inputs of about 60kg.ha^{-1} and 6.5kg.ha^{-1} respectively. The soil surface was lightly scarified to ensure penetration of the fertilizer granules and to minimise runoff. Following treatment application, each experimental plant was marked with a 1.5m iron stake which was colour coded with spray paint to indicate the treatment applied. The first autumn rains fell within a week of treatment application.

TABLE 5.1. Final sample sizes used in the analysis of data from a field-based experiment monitoring fire-stimulated flowering responses in three *Ehrharta* species. Reductions from an original replication of 14 reflect plant death and loss of samples.

Species	Treatment			
	Defoliated only	Fertilized only	Defoliated and fertilized	Control
E. capensis	14	14	14	14
E. calycina	14	13	14	11
E. thunbergii	14	11	12	13

Data collection

In mid-October 1997, six months after the experiment was set up, portions of each treated and marked individual were harvested. Plant death and loss of material during harvesting and/ or transfer of material to the lab resulted in some sample size reductions (Table 5.1) for the control species. Except where insufficient material was available, five new shoots or tillers (produced in the 1997 growing season, subsequent to treatment application) per sampled individual of *E. capensis*, and 30 per individual of both *E. calycina* and *E. thunbergii*, were randomly selected and divided into leaf, culm (including leaf bases) and inflorescence fractions. Culm material of *E. capensis* was further divided into the swollen culm base portion and the remaining (upper) culm fraction. In addition, for each *E. capensis* individual sampled, all swollen culm bases persisting from previous years but directly connected to the five sampled shoots were harvested (referred to as the 'old' swollen culm base fraction). For each sample plant, the proportion of shoots having inflorescences as well as the size (number of spikelets) of the largest inflorescence were noted. After drying at 80°C, the dry mass of each plant fraction was determined using a balance precise to 0.001g.

Starch and phosphorus concentrations in old and new swollen culm bases of *E. capensis* were assayed using the colorimetric methods described by Buysse and Merckx (1993) and Murphy and Riley (1962), respectively. For this purpose, seven individuals were selected per treatment. Prior to digestion/ hydrolysis, dried culm base material was finely ground using a Wiley mill fitted with a 0.1mm mesh. For starch analysis, 0.05g aliquots of ground plant material were hydrolysed in a 3%HCl solution (5ml) in a boiling water bath for 3hr. Three blank tubes (water only) were

included. Hydrolysis products were centrifuged, and the supernatant decanted and made up to 50ml with 80% ethanol. Because sugar concentrations in this solution yielded absorbance readings above the linear portion of a standard curve produced using a glucose dilution series, an additional 1/20 dilution (in 80% ethanol) was necessary. Tubes containing 1ml of diluted sugar solution, 1ml of phenol solution (28%v/v, diluted in 80% ethanol) and 5ml of concentrated H_2SO_4 were allowed to stand for 15min before their absorbances were measured at 490nm, using a Bausch and Lomb™ Spectronic 21 spectrophotometer. Absorbances were converted to concentrations using the standard curve described above.

For determination of phosphorus concentration, 0.1g aliquots of ground culm base material were pre-digested in 1ml concentrated HNO_3 on a heating block at 180°C until almost dry. Three blank tubes (HNO_3 only) were included. Following cooling, samples were digested in 1ml triacid mix (10 HNO_3:1 $HClO_4$:1 H_2SO_4, by volume) for a further 60min at 180°C. Products were allowed to cool before being made up to a total volume of 25ml with water. Murphy and Riley (1962) reagent (8ml) was added to flasks containing 5ml of digestion product diluted in about 25ml of distilled water, which were then made up to a volume of 50ml, and allowed to develop colour for 1hr. Absorbances were measured at 882nm using a Bausch and Lomb™ Spectronic 21 spectrophotometer and converted to concentrations using a standard curve based on a KH_2PO_4 dilution series.

Statistical analysis

For each experimental plant, mean tiller dry mass was calculated as the total dry mass of the sampled shoots, divided by the number of shoots sampled. Shoot-specific dry masses of culm, leaf and inflorescence fractions were similarly determined (i.e. through division by the total number of shoots sampled) and are hereafter simply referred to as the mean culm, leaf, and inflorescence dry mass. For *E. capensis*, the mean shoot-specific dry masses of both current and 'old' swollen culm bases as well as the upper culm fraction were also determined and are referred to as the mean dry masses of swollen culm bases, 'old' swollen culm bases and upper culms.

In all three study species, the effects of experimental treatment on mean tiller dry mass as well as the mean dry mass of culm, leaf and inflorescence fractions, the proportion of tillers flowering and the maximum inflorescence size, were assessed as

far as possible using one-way ANOVA as implemented in Statistica version 5 (Statsoft 1995). Post hoc pairwise comparisons were done using the LSD test. Wherever Levene's test indicated significant inequality of variances, the data were appropriately transformed prior to analysis. If transformation failed to equalize variances, a Kruskal Wallis ANOVA by ranks was used instead. For *E. capensis*, inter-treatment differences in the mean dry mass of both the upper and swollen basal portions of culms were also assessed in this manner, as were inter-treatment differences in the starch and P concentrations of both current year's and persistent, old swollen culm bases. The mean dry mass of swollen culm bases as well as their starch and P concentrations were also compared between flowering and non-flowering plants, using Student's t-test to assess the significance of observed differences. Wherever an F-test indicated significant inequality of variances, an approximate t-test using separate variance estimates and approximate degrees of freedom was employed.

Linear regression analysis, as implemented in Statistica version 5 multiple regression module (Statsoft 1995) was used to evaluate the relationship of mean inflorescence dry mass to mean upper culm dry mass in flowering plants of *E. capensis*. The relationship of the mean dry mass of the upper and swollen basal portions of culms to mean leaf dry mass was also evaluated using regression analysis, both on a treatment-specific basis, and combining data for all treatments.

In order to test whether flowering in *E. capensis* occurs at a cost to clonal persistence, it was necessary to quantify reproductive (sexual) investment. While inflorescence mass may be used as a surrogate for reproductive investment, the role of culms (upper portion) in raising the inflorescence suggests that culm mass represents, at least in part, an additional reproductive investment. However, because upper culms also perform a vegetative function in supporting the leaves, allocation to culms also involves a vegetative investment. In order to distinguish the mass allocations to reproductive and vegetative function in upper culms of *E. capensis*, regression analysis was first used to estimate the relationship between the mean dry mass of upper culms and that of leaves in non-flowering plants only. Reproductive investment in non-flowering plants was assumed to be nil, so that the upper culm mass values predicted by this regression (on the basis of mean leaf dry mass) were inferred to estimate the mass allocation to vegetative function. Upper culm production in flowering plants showed marked deviation from this relationship and

this was attributed to an investment in reproductive function. In these plants the mass investment in reproduction was estimated using their residual values from the regression described above.

The mean reproductive dry mass of tillers in flowering plants was then calculated as the summed mean dry masses of the inflorescence and the reproductive (estimated) upper culm portion, while the mean vegetative dry mass was calculated as the summed mean dry masses of the swollen culm base, the vegetative (estimated) upper culm portion and the leaves. In contrast, the entire tiller mass of non-flowering plants was assumed to be vegetative. The relationships of reproductive investment (estimated both as mean inflorescence dry mass and mean reproductive dry mass) with the mean dry mass of swollen culm bases, as well as the mean vegetative dry mass, were then evaluated using linear regression analysis.

Results

Post-fire growth in *E. ramosa*

Spring leaf production in *E. ramosa* at both Helderberg and Devil's Peak was substantially and significantly higher in plants resprouting after fire than in those growing in unburnt (senescent) vegetation (Table 5.2). While unburnt plants at Helderberg produced a negligible quantity of leaf material (about 1mg.shoot^{-1}), those at Devil's Peak were entirely leafless. Unsurprisingly, the significantly higher leaf

TABLE 5.2. Comparison of mean shoot dry mass, mean shoot-specific leaf area, and mean shoot-specific leaf, culm, and inflorescence dry mass, between burnt and unburnt plants of *E. ramosa*. Asterisks indicate t-statistics based on separate variance estimates and evaluated using approximate degrees of freedom.

Study site	Trait (shoot-specific)	Exposure to fire		t-value	d.f.	Prob.
		Burnt	Unburnt			
Helderberg	Leaf surface area (cm^2)	4.464 ± 1.339	0.209 ± 0.272	12.023*	15	P<0.0001
	Leaf dry mass (g)	0.015 ± 0.004	0.001 ± 0.002	12.400*	19	P<0.0001
	Culm dry mass (g)	0.064 ± 0.026	0.009 ± 0.004	8.195*	14	P<0.0001
	Inflorescence dry mass (g)	-	0.039 ± 0.012	-	-	-
	Total shoot dry mass (g)	0.080 ± 0.028	0.048 ± 0.013	3.888*	20	P=0.0009
Devil's Pk	Leaf dry mass (g)	0.015 ± 0.007	-	-	-	-
	Culm dry mass (g)	0.092 ± 0.027	0.049 ± 0.014	4.623	20	P=0.0002
	Inflorescence dry mass (g)	0.001 ± 0.001	0.123 ± 0.049	-8.308*	10	P<0.0001
	Total shoot dry mass (g)	0.107 ± 0.030	0.173 ± 0.053	-3.592	20	P=0.0018

mass produced by burnt plants at Helderberg translates into a significantly higher leaf area. Culm dry mass is also significantly higher for burnt than unburnt plants at both sites (Table 5.2). However, the magnitude of this difference varies: while the mean culm fraction of shoots on burnt plants has a mass about seven times that on unburnt plants at Helderberg, at Devil's Peak this difference is barely two-fold.

In contrast to leaf and culm production, the proportion of plants flowering at both sites were much lower among burnt plants than among plants growing in unburnt vegetation. At Helderberg, none of the burnt sample plants flowered, while nine of the 13 unburnt sample plants had inflorescences, these proportions being significantly different (Fisher exact test, $P<0.0001$). Similarly, at Devil's Peak, four out of the 11 burnt sample plants were flowering, compared with 11 out 11 unburnt plants (Fisher exact test, $P=0.004$). At both sites these differences resulted in unburnt plants having a significantly higher shoot-specific mean inflorescence dry mass than unburnt plants (Table 5.2), that of the latter being either negligible (Devil's Peak) or zero (Helderberg). Depending on the amount of inflorescence material produced, the mean total shoot dry mass can be either higher or lower in unburnt than in burnt plants (Table 5.2).

Post-fire flowering in *E. capensis*

Treatment effects on dry mass production and flowering

The effects of defoliation and fertilization on dry mass production and flowering in *E. capensis*, *E. calycina* and *E. thunbergii* are shown in Fig. 5.2. While combined application of fertilization and defoliation resulted in a substantial and significant increase in the mean dry mass of tillers produced by *E. capensis*, when applied individually these treatments did not produce a response significantly different from that observed in control (untreated) plants (Fig. 5.2a). Comparable response patterns are not evident in either control species, inter-treatment differences in mean tiller size being relatively minor in *E. thunbergii* and absent in *E. calycina* (Fig. 5.2b, c). Although the mean dry masses of tillers produced by *E. thunbergii* under both fertilization and fertilization + defoliation treatments were higher than in the absence of fertilization (Fig. 5.2b), these differences are non-significant. The markedly higher dry mass of *E. capensis* tillers observed following fertilization + defoliation is primarily attributable to differences in mean culm dry mass (Fig. 5.2d), this representing the largest proportion of tiller dry mass. The markedly higher culm dry mass of fertilized

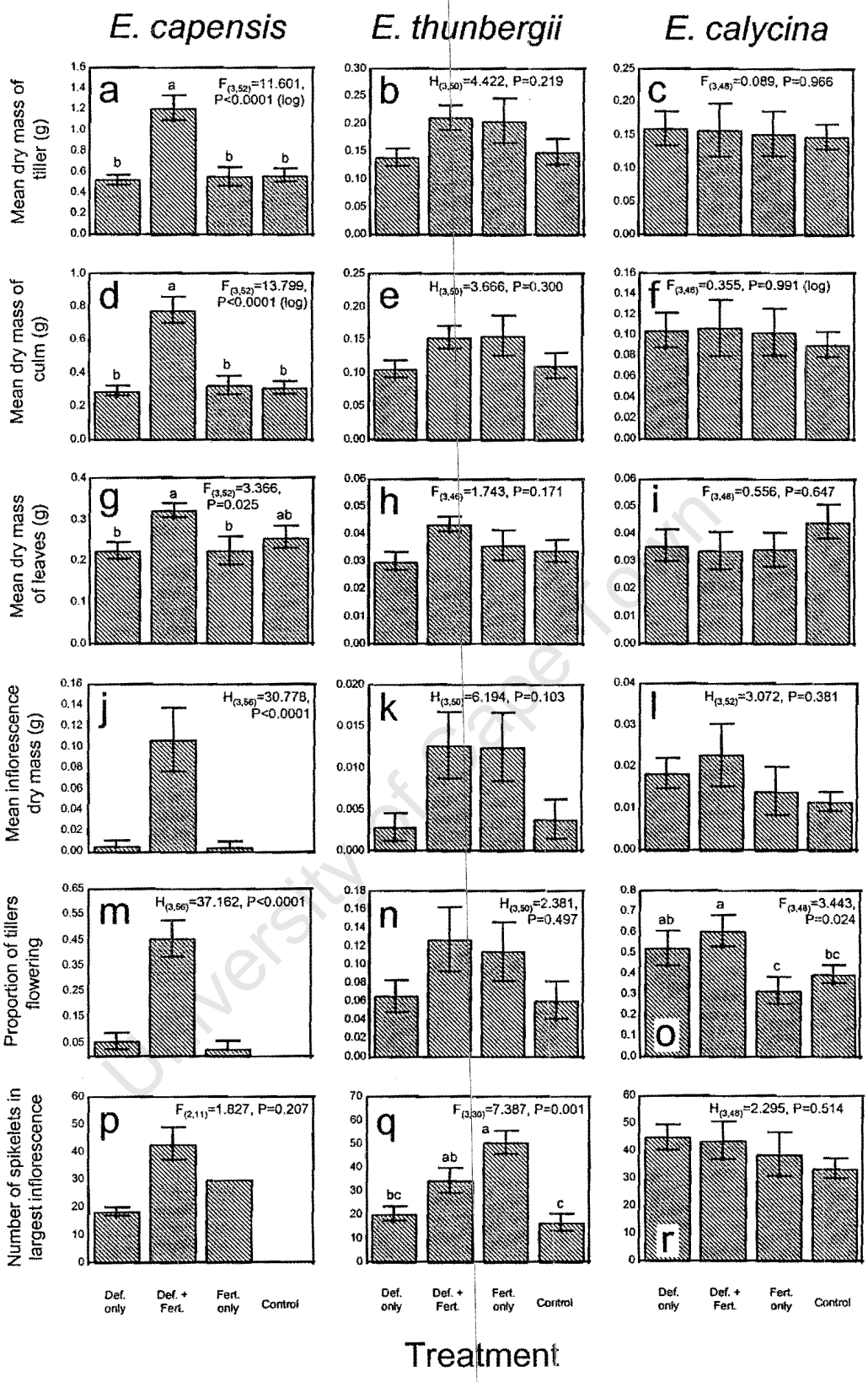


FIGURE 5.2. Comparison of mean tiller, culm, leaf and inflorescence dry mass, proportion of tillers flowering, and maximum inflorescence size, in field-grown plants of *E. capensis*, *E. calycina* and *E. thunbergii* subjected to different combinations of fertilization (fert.) and defoliation (def.). Control plants were neither fertilized nor defoliated. Results of ANOVA or Kruskal-Wallis ANOVA by ranks, are provided, transformations being indicated in parentheses where relevant.

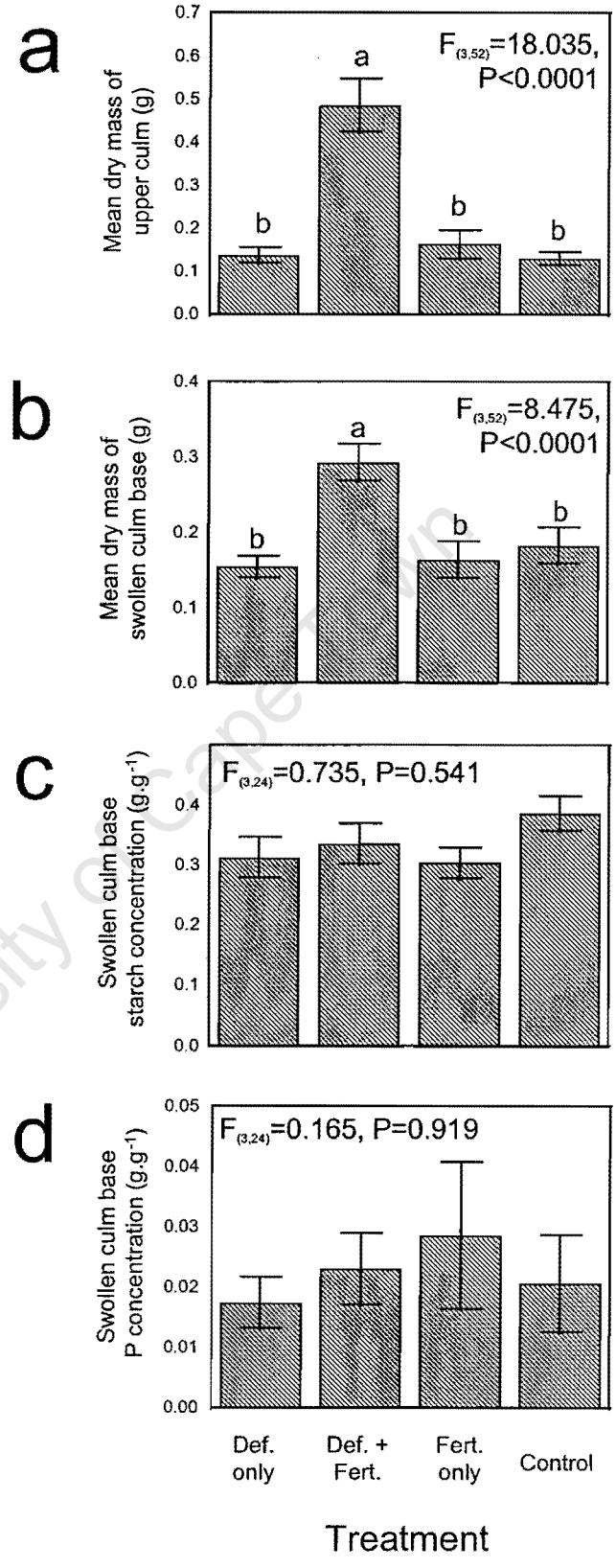


FIGURE 5.3. Comparison of mean dry mass of the (a) upper and (b) swollen basal portions of culms, as well as (c) starch and (d) P concentrations in the latter, in field-grown plants of *E. capensis* subjected to different combinations of fertilization (fert.) and defoliation (def.). Control plants were neither fertilized nor defoliated. Results of ANOVA are provided.

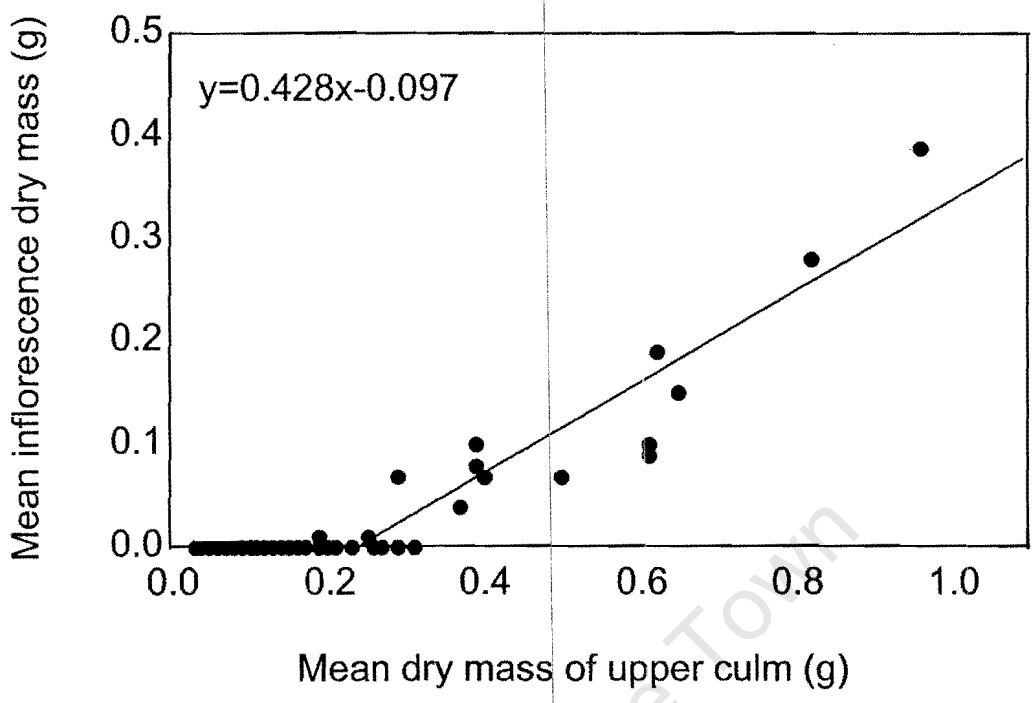


FIGURE 5.4. The relationship between mean inflorescence and upper culm dry mass in experimental plants of *E. capensis*, disregarding treatment. The regression line shown is based only on points with a non-zero inflorescence mass.

+ defoliated plants in turn reflects mass differences in both the upper (Fig. 5.3a) and basally swollen culm portions (Fig. 5.3b). Although leaf dry mass in *E. capensis* is significantly higher in fertilized + defoliated plants, than in plants receiving other treatments, this pattern is comparatively weak (Fig. 5.2g). Nonetheless, some of the differences are statistically significant. The absence of significant inter-treatment differences in mean tiller dry mass in *E. calycina* and *E. thunbergii* mirrors a lack of significant inter-treatment differences in both culm and leaf dry mass production in these species (Fig. 5.2e, f, h, i).

In *E. capensis*, mean inflorescence mass is tightly related to the mean dry mass of the upper portion of the culm (Fig. 5.4: $r=0.925$, d. f.=15, $P<0.0001$). The positive x-intercept of this relationship indicates that flowering is expected to occur only in plants having a mean upper culm dry mass exceeding 0.2g (Fig. 5.4: x-intercept value). Marked inter-treatment differences in the mean dry mass of upper culms of *E. capensis* (Fig. 5.3a) are, therefore, mirrored by differences in flowering effort. Inflorescence dry mass production in fertilized + defoliated plants is substantially higher than in plants receiving other treatments (Fig. 5.2j), being negligible in plants that were either fertilized or defoliated (but not both) and zero in control plants. This pattern is highly significant when analysed non-parametrically and appears to reflect, at least in part, inter-treatment differences in the proportion of tillers flowering (Fig. 5.2m). For the most part, plants not receiving fertilization + defoliation failed to flower at all. Plants that did flower, however, had smaller inflorescences than fertilized + defoliated plants.

Inter-treatment variation in the flowering response of both control species is much less stark than in *E. capensis*. As in *E. capensis*, positive relationships between inflorescence and culm dry mass exist in *E. thunbergii* ($r=0.792$, d. f.=29, $P<0.0001$) and *E. calycina* ($r=0.879$, d. f.=46, $P<0.0001$) but the minimum culm mass associated with the inception of flowering is lower (about 0.1g and 0.03g respectively). Compared with *E. capensis*, these species show a more even inter-treatment flowering response because the mean culm mass associated with all four treatments exceeds these thresholds and because inter-treatment culm mass differences are less (Figs. 5.2e,f). Although mean inflorescence dry mass and the proportion of tillers flowering in *E. thunbergii* is higher in fertilized than unfertilized plants (irrespective of defoliation), these patterns are non-significant (Fig. 5.2k, n). Variation in maximum inflorescence size follows a similar trend, but here some

significant differences are evident (Fig. 5.2q). Although mean inflorescence mass in *E. calycina* is highest in fertilized + defoliated plants, this pattern is weak and all differences non-significant (Fig. 5.2l). Fertilized + defoliated plants of this species do, however, show a significantly higher proportion of flowering tillers than do fertilized and control plants, but not defoliated plants (Fig. 5.2o). Maximum inflorescence size shows no clear inter-treatment differences in *E. calycina* (Fig. 5.2r).

Effects of treatment and flowering on swollen culm bases of *E. capensis*

Swollen culm bases of *E. capensis* tillers formed during the 1997 growing season (i.e. during the experiment) were substantially and significantly larger (mean dry mass) in fertilized + defoliated plants than in plants receiving other treatments (Fig. 5.3b). Although neither the starch nor P concentration in these culm bases differ significantly among treatments (Fig. 5.3c, d), the swollen culm base P concentration of fertilized plants is, on average, higher than that of unfertilized plants. The mean dry mass of swollen culm bases is also significantly higher for flowering than non-flowering plants, but differences in starch and P concentration are non-significant (Table 5.3).

The mean dry mass of 'old' culm bases (persisting from previous seasons) does not differ significantly among treatments (Fig. 5.5a). In addition, neither the starch nor P concentration in old culm bases show any significant inter-treatment differences (Fig.

TABLE 5.3. Comparison of mean dry mass, and starch and P concentration of current year's and 'old' swollen culm bases in flowering and non-flowering plants of *E. capensis*. Asterisks indicate t-statistics based on separate variance estimates and evaluated using approximate degrees of freedom.

Trait (unit)	Flowering status		t-value	d.f.	Prob.
	Flowering	Non-flowering			
Mean dry mass of swollen culm base (g)	0.276 ± 0.098	0.164 ± 0.077	4.620	54	P<0.0001
Swollen culm base starch concentration ($g.g^{-1}$)	0.345 ± 0.107	0.329 ± 0.119	1.747	26	P=0.092
Swollen culm base P concentration ($g.g^{-1}$)	0.020 ± 0.019	0.023 ± 0.034	-0.371	26	P=0.714
Mean dry mass of old swollen culm bases (g)	0.390 ± 0.219	0.364 ± 0.143	0.522	54	P=0.604
Old swollen culm base starch concentration ($g.g^{-1}$)	0.352 ± 0.120	0.407 ± 0.090	-1.362	26	P=0.185
Old swollen culm base P concentration ($g.g^{-1}$)	0.070 ± 0.032	0.058 ± 0.017	1.164*	12	P=0.267

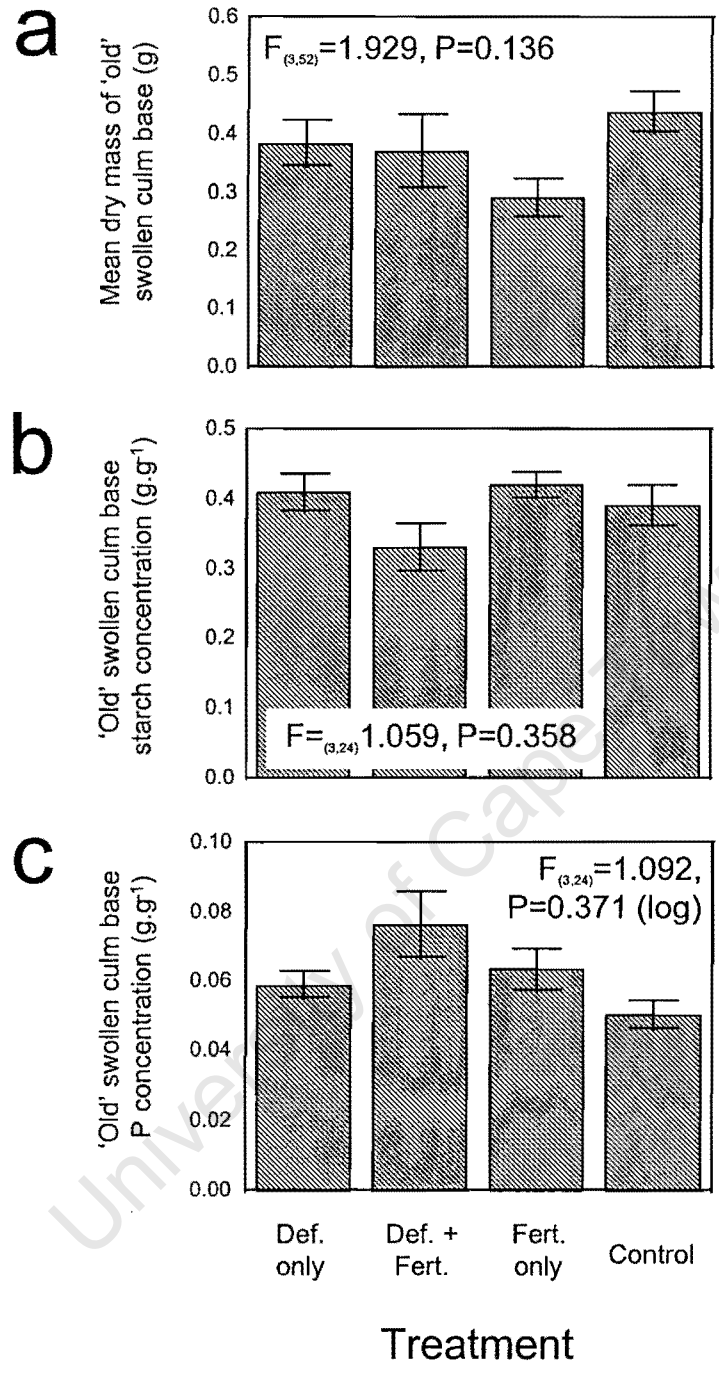


FIGURE 5.5. Comparison of (a) mean dry mass, (b)starch concentration and (c) P concentration of 'old' swollen culm bases, in field-grown plants of *E. capensis* subjected to different combinations of fertilization (fert.) and defoliation (def.). Control plants were neither fertilized nor defoliated. Results of ANOVA are provided, transformations being indicated in parentheses where relevant.

TABLE 5.4. Correlation and regression statistics describing the relationships between mean culm dry mass and mean leaf dry mass in *E. capensis*, *E. thunbergii* and *E. calycina*. All statistics are based on cross-treatment comparisons.

Species	Correlation coefficient	d.f.	Prob.	Regression equation
E. capensis	0.750	54	P<0.0002	y = 2.142x - 0.123
E. thunbergii	0.790	48	P<0.0002	y = 4.037x - 0.013
E. calycina	0.767	50	P<0.0001	y = 2.548x - 0.010

5.5b, c). Starch and P levels are, however, respectively lowest and highest in fertilized + defoliated plants. Neither the mean dry mass nor the starch or P concentration of old swollen culm bases show significant differences between flowering and non-flowering plants (Table 5.3).

Relationship between culm mass, leaf mass and treatment

In all three study species, mean culm dry mass shows strong, positive linear relationships with mean leaf dry mass (Table 5.4). In addition, in *E. capensis* the mean dry masses of both the upper culm portion and the swollen basal culm portions are positively related to leaf mass (Table 5.5).

While the relationship between upper culm mass and leaf mass in *E. capensis* is highly significant both when based on treatment-specific comparisons or on a single

TABLE 5.5. Correlation and regression statistics describing the relationships of the mean dry mass of the upper and swollen basal portions of culms against the mean dry mass of leaves, in *E. capensis* (plotted in Fig. 5.6). Data for cross-treatment (treatment = all) and treatment-specific comparisons are presented.

Variable	Treatment	Correlation coefficient	d.f.	Prob.	Regression equation
Upper culm dry mass	all	0.657	54	P<0.0001	y = 1.324x - 0.111
	def. only	0.715	12	P=0.004	y = 0.643x - 0.006
	fert. + def.	0.722	12	P=0.003	y = 2.635x - 0.363
	fert. only	0.832	12	P=0.0002	y = 0.809x - 0.019
	control	0.943	12	P<0.0001	y = 0.534x - 0.008
Swollen culm base dry mass	all	0.837	54	P<0.0001	y = 0.818x - 0.012
	def. only	0.813	12	P=0.0004	y = 0.585x + 0.023
	fert. + def.	0.842	12	P=0.0002	y = 1.206x - 0.096
	fert. only	0.840	12	P=0.0002	y = 0.599x + 0.029
	control	0.853	12	P=0.0001	y = 0.769x - 0.015

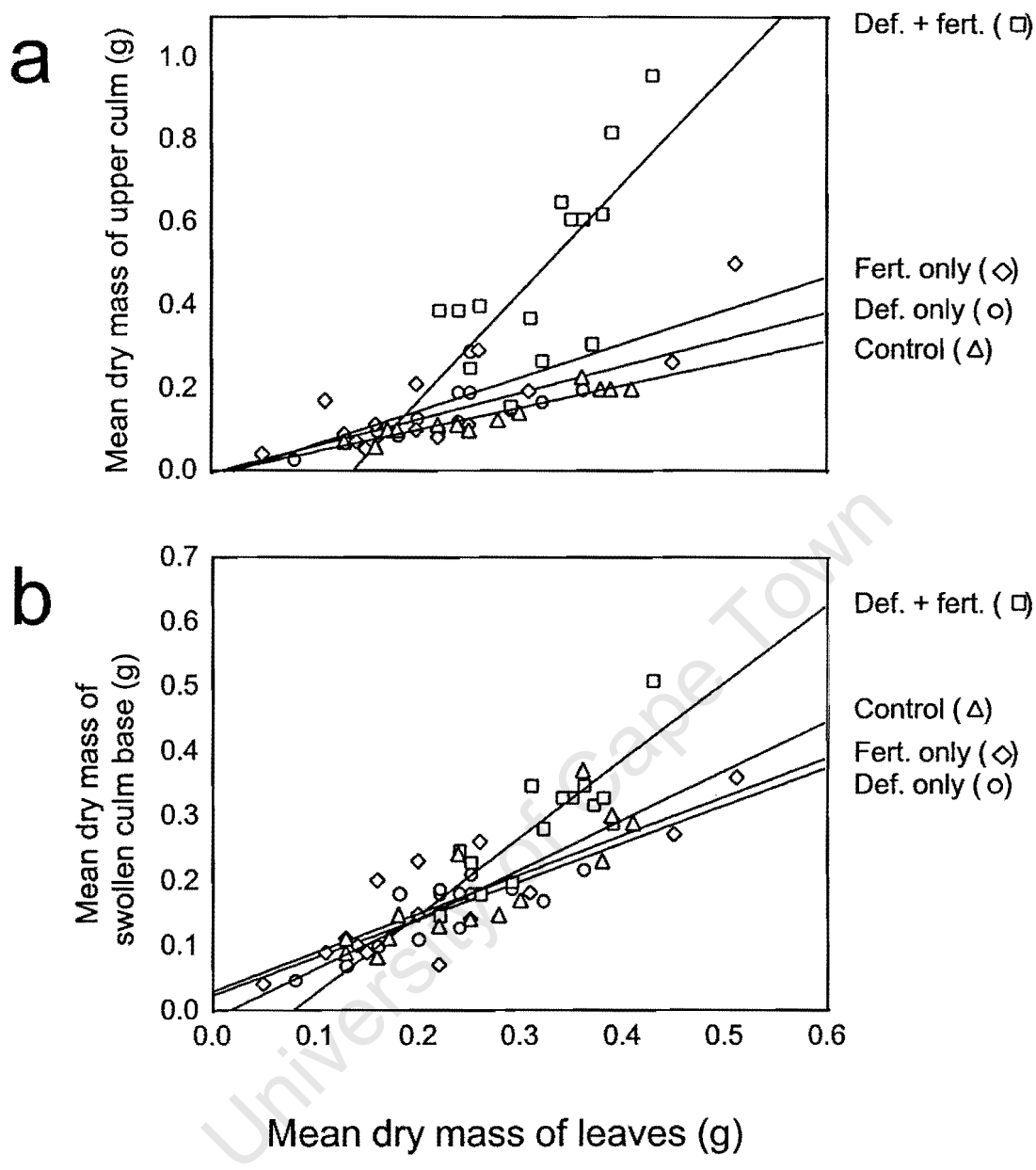


FIGURE 5.6. Relationships between (a) mean upper culm and leaf dry mass, and (b) mean swollen culm base and leaf dry mass, in plants of *E. capensis* grown under different experimental treatments. The regressions shown are calculated on a treatment-specific basis, details being listed in Table 5.5.

cross-treatment comparison (Table 5.5), treatment-specific plots (Fig. 5.6a) indicate that this relationship is substantially different in fertilized + defoliated plants from those in plants subjected to the other three treatments. As a result the correlation coefficients calculated for treatment-specific comparisons are consistently better than that calculated for the single cross-treatment comparison (Table 5.5). In particular, the curve described by fertilized + defoliated plants is roughly three to five times steeper than those for plants experiencing other treatments (Fig. 5.6a, Table 5.5), intersecting the latter curves at a leaf dry mass value of about 0.15g (Fig. 5.6a). Thus for a given leaf mass, the expected culm mass for fertilized + defoliated plants is substantially greater than that for plants otherwise treated. Since the dry masses of upper culms and inflorescences are closely related, this difference in turn relates to the higher incidence of flowering in fertilized + defoliated plants (Fig. 5.2j, m). In contrast to data for *E. capensis*, culm mass - leaf mass curves of *E. thunbergii* and *E. calycina* do not show strong inter-treatment differences.

Compared with the relationship between upper culm and leaf dry mass, the relationship between swollen culm base mass and leaf mass shows a much weaker treatment interaction. Although the curve described by fertilized + defoliated plants is steeper than that described by plants receiving other treatments (Fig. 5.6b), this slope difference is comparatively small (1.5 to 2 times greater). Thus, although the masses of both the upper and swollen basal culm portions show positive relationships with leaf mass, these relationships are differentially influenced by experimental treatment. In particular, while the combination of fertilization and defoliation has a major influence on upper culm mass, its influence on the mass of swollen culm bases is much less.

Investment in reproduction (flowering) versus vegetative persistence

The strong relationship between inflorescence mass and upper culm mass (Fig. 5.4) confirms that the latter represents, partially at least, a reproductive investment that is additional to inflorescence mass. However, the possession of appreciable upper culm mass by non-flowering plants indicates that upper culms also serve a vegetative function, specifically leaf support. In *E. capensis*, mean upper culm dry mass shows a strong linear relationship with the mean dry mass of leaves (Table 5.5). However, as is the case among treatments (Fig. 5.6b), this relationship differs markedly between flowering and non-flowering plants (Fig. 5.7). Indeed, the leaf-

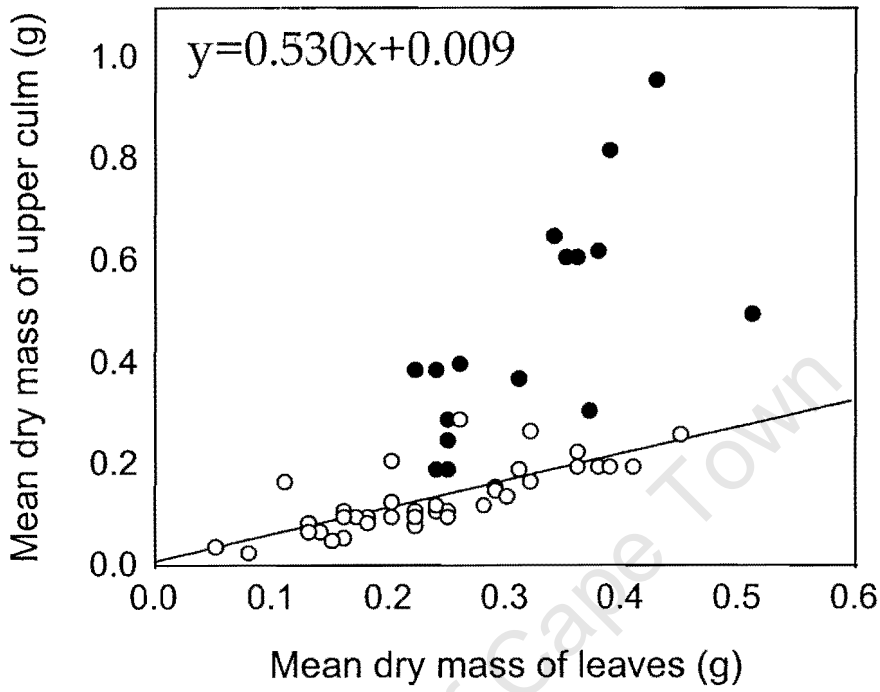


FIGURE 5.7. The relationship between mean upper culm and leaf dry mass among flowering (solid circles) and non-flowering (open circles) experimental plants of *E. capensis*, disregarding treatment. The regression line shown is based only non-flowering plants.

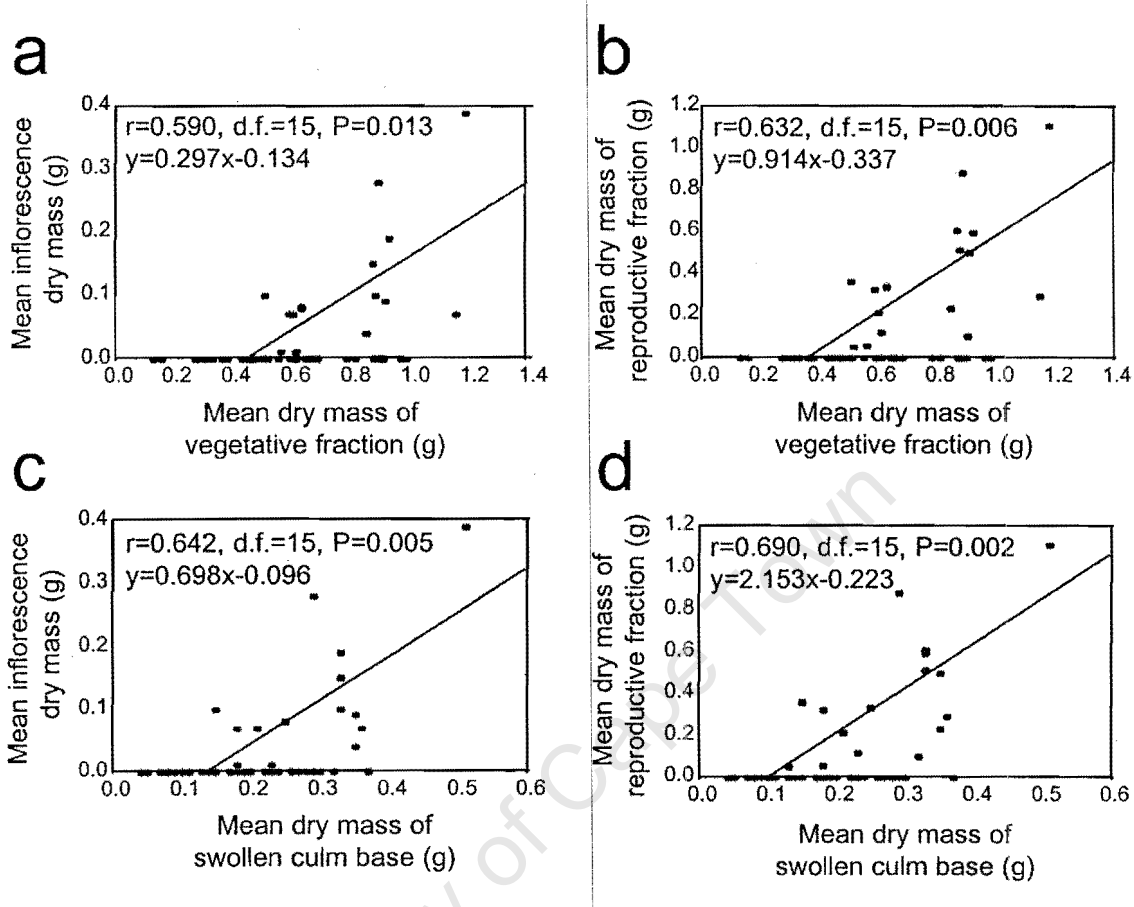


FIGURE 5.8. Relationships between (a) mean inflorescence and vegetative dry mass, (b) mean reproductive and vegetative dry mass, (c) mean inflorescence and swollen culm base dry mass, and (d) mean reproductive and swollen culm base dry mass, in experimental plants of *E. capensis*, disregarding treatment. In each case, the regression line shown is based only on the points with a non-zero inflorescence or reproductive mass.

upper culm mass relationship for non-flowering individuals is particularly strong ($r=0.775$, d.f.=37, $P<0.0001$). The conspicuous and positive deviation from this relationship by flowering plants (Fig. 5.7) appears to validate the use of this curve to estimate the relative investments of upper culm dry mass in vegetative (predicted) and reproductive (residual) function.

For flowering plants, the mean dry mass of the upper culms thus attributed to reproductive function is calculated to be 0.28±0.20g (mean ± standard deviation), this representing 52±21% of total upper culm mass. With inflorescences included, this results in a total reproductive dry mass of 0.37±0.30g per tiller, which represents 28±15% of mean tiller dry mass. Both mean inflorescence dry mass and total reproductive dry mass show significant positive linear relationships with mean vegetative dry mass (Fig. 5.8a, b) and the mean dry mass of swollen culm bases (Fig. 5.8c, d). In each case, the relationship has a positive x-intercept indicating that inflorescence production and reproductive investment is expected only in tillers whose vegetative and swollen culm base dry masses exceed threshold values, these being about 0.4g and 0.1-0.15g, respectively. Among flowering tillers, inflorescence and total reproductive mass increase with both vegetative mass and the mass of swollen culm bases. As a result, the swollen culm bases produced by flowering plants have a significantly greater mean mass than those produced by non-flowering plants (Table 5.3).

Discussion

Post-fire growth in *E. ramosa*

Short leaf lifespans plus an apparent lack of traits to prevent nutrient leaching from leaves (leaves in *E. ramosa* have thin cuticles and are weakly sclerified [personal observation; also see Gibbs Russell and Ellis 1987]) suggest that leaf production in *E. ramosa* incurs a nutrient cost (e.g. Chapin 1980; Bloom et al. 1985). In a nutrient-limited fynbos system, brief increases in nutrient availability following episodic fire events may, therefore, present opportunities for increased leaf production. Indeed, data presented here confirm that mature plants of *E. ramosa* resprouting in the growing season immediately following fire produce substantially more leaf material than in subsequent growing seasons. During the inter-fire period, leaf production in

this species is very low or negligible. Since *E. ramosa* recruits exclusively after fire (Kruger 1987), the opportunity to allocate appreciably to leaves is also afforded to seedlings, which accordingly show a comparatively high early leaf weight ratio (Chapter 4: Table 4.4).

Carbon assimilation rates in *E. ramosa* leaves are substantially higher than those in the photosynthetic culms (Chapter 3: Table 3.4) suggesting that the former are important for accelerated growth. Thus the high RGR of *E. ramosa* seedlings (Chapter 4) may be accounted for by a high biomass allocation to leaves. Increased leaf production may also explain the greater shoot-specific culm mass observed in plants resprouting after fire at both Helderberg and Devil's Peak. Increased post-fire culm growth in *E. ramosa* does not, however, appear to translate into increased flowering effort in the season immediately after fire. Burnt plants at both Helderberg and Devil's Peak displayed significantly lower flowering frequency as well as significantly lower mean shoot-specific inflorescence mass, than did unburnt plants. This observation accords with Kruger's (1987) observation that, in contrast to geophytic grass species (e.g. *Merxumellera rufa* and *Pentaschistis viscidula*) which dominate fynbos vegetation immediately after fire, *E. ramosa* becomes dominant only in the second season after fire.

Given that *E. ramosa* has a seedling RGR comparable to those of rapidly-flowering seeder species (Chapter 4), its delayed flowering response as well as its 18-month seedling maturation period (Kruger 1987) are unexpected. One possibility is that *E. ramosa* utilises the improved growth conditions of the first post-fire season to maximise culm growth, simultaneously capitalising on the elevated availability of nutrients by taking these up and storing them in the newly produced culm tissues. These reserves are then available to support flowering in subsequent seasons. In addition, because *E. ramosa* is suffrutescent, maximum culm extension during the first growing season implies a reduction in the amount of subsequent culm growth required to elevate inflorescences. This strategy may therefore allow *E. ramosa* to flower through the inter-fire period, despite resource limitation at this time. In contrast to the situation in obligate resprouters, prolonged flowering may be particularly important in *E. ramosa* as it is apparently killed by severe fire and then relies on soil-stored seed for regeneration (Kruger and Bigalke 1984; Kruger 1987).

In the restioid *Thamnochortus punctatus*, rhizome, culm and inflorescence development is staggered to match seasonal shifts in the availability of different resources (Stock et al. 1987) which maximises resource utilization in an edaphically and climatically constrained environment (Stock et al. 1987). Although further supporting data are required, it is suggested here that decoupled culm and inflorescence production in *E. ramosa* (between growing seasons) allows post-fire resources to be most efficiently channelled into seed production during the inter-fire period. It is possible that some Restionaceae employ a similar strategy to utilise post-fire conditions but this requires testing. Linder (1984) noted that many Restionaceae produce profusely branched, sterile culms immediately following fire. If these show greater photosynthetic activity than the reproductive culms produced during inter-fire periods, then their production may be linked to maximising resource capture at these times.

Post-fire flowering in *E. capensis*

Evidence for flowering cues in *E. capensis*

Despite anecdotal reports of a high incidence of mass post-fire flowering in geophytic fynbos grasses (e.g. Kruger 1983; Linder and Ellis 1990), experimental evidence for fire induced flowering in a Cape grass has hitherto been lacking. In demonstrating that *E. capensis* flowers appreciably only after the application of a combination of experimental treatments designed to simulate some important fire effects, this study provides the first such evidence for a Cape grass species. Since a comparably strict dependence of flowering on 'fire effects' was not observed in two non-geophytic species, *E. thunbergii* and *E. calycina*, the association of apparent fire-stimulated flowering with a geophytic habit is confirmed.

The results also indicate that flowering in *E. capensis* does not depend on direct fire cues such as increases in levels of smoke-bound chemicals and raised soil temperatures. Rather, a combination of increased nutrient (fertilizer addition) and light (canopy removal) availability, both indirect effects of fire on the fynbos environment, is sufficient to stimulate flowering. However, the extent to which the resultant flowering effort matches that after fire remains unknown. Nonetheless, since neither of these effects is unique to the post-fire environment but may result from other processes (e.g. herbivory, vegetation clearing) (e.g. Holland and Detling 1990), this suggests that flowering in *E. capensis* is not strictly fire-dependent.

Rather, the fact that both treatments mimic important ways in which fire increases resource availability suggests that flowering is dependent on improved plant performance associated with reduced resource limitation. In tallgrass prairie, fires are thought to effect a transient release from light- and nitrogen limitation and this has been used to explain observed post-fire productivity increases (Seastedt and Knapp 1993; Blair 1997). Experimental data for other species suggest that in other species as in *E. capensis* a release from limitation in one or more resources may be responsible for producing strong flowering responses (Hulbert 1988; Brewer 1995; Abrahamson 1999). The possibility that either increased light or nutrient availability act as simple flowering cues in *E. capensis* is refuted by the necessity of combined experimental treatments to stimulate flowering.

In contrast to *E. capensis*, inter-treatment differences in flowering in both *E. thunbergii* and *E. calycina* were found to be subtle and, for the most part, non-significant. Nonetheless, flowering effort in *E. thunbergii* and *E. calycina* did increase marginally in response to fertilization and to defoliation, respectively. The lack of flowering response by *E. thunbergii* to defoliation supports the suggestion that the suffrutescent habit of this species allows the foliage to be elevated to the height of the surrounding vegetation and thus obviates shading problems in unburnt vegetation. Linder and Ellis (1990) considered the branching culms of suffrutescent fynbos grasses to be an important device promoting above-ground persistence in mature vegetation. Conversely, the positive flowering response of *E. calycina* to defoliation probably follows from the low, tufted growth form of this species.

Flowering versus vegetative persistence in *E. capensis*

Sexual reproductive effort in many clonal species is strongly size-dependent (Hartnett 1990; Schmid and Weiner 1993; Schmid et al. 1995; Mulder and Ruess 1998) whereas vegetative reproductive effort is less so (Hartnett 1990). A similar pattern is true for *E. capensis*. Basal swelling of culms was observed in all studied tillers of *E. capensis* but investment in reproduction was observed only in tillers attaining a threshold vegetative dry mass (here calculated as the summed masses of the leaves, the swollen culm base and the vegetative portion of the upper culm) of about 0.4g. Because a portion of this threshold mass is attributable to swollen culm bases, a minimum investment in vegetative persistence structures prior to the onset of flowering is implied. Indeed, flowering does not occur in plants having a swollen

culm base mass less than about 0.1g. These data, plus the positive linear relationships of culm base mass with both inflorescence and total reproductive mass suggest that reproductive allocation in *E. capensis* does not occur at a cost to vegetative persistence. Instead, flowering in *E. capensis* is associated with the production of larger swollen culm bases than those observed in non-flowering plants. Since starch and phosphorus concentrations in the swollen culm bases of flowering and non-flowering plants do not differ significantly, this mass difference can be assumed to reflect differences in the absolute sizes of reserve pools.

Since both flowering and the production of larger swollen culm bases are observed in plants that experienced combined fertilization and canopy removal, their co-incidence is presumably attributable to the greater resource availability associated with this treatment. Both upper culm mass and the mass of swollen culm bases are positively related to leaf mass, reflecting either a dependence on primary production or, alternatively, allometry (since the leaves are borne on the upper culm). However, the much weaker influence of experimental treatment on the form of the latter relationship (i.e. between leaf mass and culm base mass) suggests that the size of swollen culm bases produced is ultimately less dependent on resource availability than is that of the upper culms. Upper culm mass is closely related to inflorescence production and size (culms with a mass less than about 0.2g are not expected to flower) which, therefore, implies that fluctuations in environmental resource availability influence flowering in *E. capensis* much more strongly than the mass of swollen culm bases.

Taken together, these data suggest a 'save first' strategy in the geophytic *E. capensis*, in which plants invest exclusively in storage when resources are sparse but in both storage and flowering when resources are abundant. Periodic fires may drive massive fluctuation in resource availability especially in resource-limited habitats and so it is here that apparent 'fire-stimulated' flowering responses are expected to be strongest (Brewer 1995). Thus, an association with oligotrophic fynbos habitats probably explains the prevalence of apparent fire-stimulated among members of the geophytic *E. bulbosa-E. capensis* clade. Fire-induced flowering is not evident in another geophytic species, *E. eburnea*, which appears instead to flower annually (personal observation). In contrast to members of the *E. bulbosa-E. capensis* clade, *E. eburnea* occurs in open, arid habitats underlain by shale-derived clays and dolerite-derived loams. Since these substrates are more eutrophic than

those associated with fynbos, they probably provide sufficient nutrients to support flowering in *E. eburnea* in the absence of fire.

Since reserve storage is most strongly developed in geophytes, a strong association of post-fire mass flowering with this habit (e.g. Le Maitre and Brown 1992) is unsurprising. The major benefit of such storage may be to facilitate resprouting (Davidson and Milthorpe 1966; Kausch et al. 1981; Bloom et al. 1985; Danckwerts and Gordon 1987, 1989; Culvenor et al. 1989; Chapin et al. 1990; Danckwerts 1993; van der Heyden and Stock 1995; McPherson and Williams 1998), this being an integral part of a life-history strategy centred on vegetative persistence. An alternative explanation for the association between post-fire flowering and a geophytic habit is that the storage reserves of geophytes are used to fuel rapid and/ or mass post-fire flowering in these plants (e.g. Linder and Ellis 1990). This, however, is not supported by data from *E. capensis*. Swollen culm bases persisting from previous seasons ('old' culm bases) showed no significant differences in dry mass, starch concentration or P concentration, whether flowering and non-flowering plants were compared or whether plants experiencing different treatments were compared.

Post-fire responses of *Ehrharta* s. s.

Both species investigated in this study show marked post-fire responses. In *E. capensis* at least, the response investigated (mass flowering) is not strictly fire-dependent, being driven instead by increased resource availability. Although not tested experimentally, it seems likely that the post-fire growth response of *E. ramosa* is also resource-dependent.

The different responses of the two study species reflect differences in persistence strategy. *E. capensis* (and other members of the *E. bulbosa-E. capensis* clade) is an obligate resprouter (Kruger 1987) and persists principally by vegetative means. In contrast, *E. ramosa* is a facultative seeder (Kruger 1987) and therefore relies more heavily on seed for regeneration. Thus, regular flowering through the inter-fire period may be more important for *E. ramosa*. Although current evidence does not exclude fire as a force driving the evolution of these strategies in *Ehrharta* s. s., the possibility that they represent adaptations for persistence through seasonal drought (Chapter 4) suggests that their utility as fire-survival mechanisms may be preadaptive (cf. Lloret 1999).

Chapter 6. Synthesis

This study uses a phylogenetic approach to evaluate the evolutionary signficance of life history attributes in *Ehrharta* s. s. Since the set of ways in which any trait can evolve is logically constrained by its initial state, the study of trait evolution demands cognizance of the phylogenetic context in which such trait evolution has occurred (e.g. Coddington 1988, 1994; Carpenter 1989; Baum and Larson 1991; Brooks and McLennan 1991; Harvey and Pagel 1991; Pagel 1994; Wenzel and Carpenter 1994; Ackerly and Donoghue 1995; Andersen 1995; Harvey et al. 1995; Rees 1995; Larson and Losos 1996). Data presented in this thesis suggest, for example, that an annual (ephemeral) life history is more likely to evolve in clades that have previously acquired high seedling growth rates than in those that have not. In the study of trait variation, subtle historical effects can be accounted for only through the accommodation of phylogenetic history. Therefore, the phylogeny estimated in Chapter 2 of this thesis, besides being useful in guiding taxonomic decisions, is fundamental to the comparative interpretations drawn in Chapters 3 and 4. In addition, although the conclusions drawn in Chapter 5 are not strictly phylogeny-dependent, I suggest that they certainly benefit from the broader phylogenetic context provided by the preceding chapters.

In the absence of clear support for ehrharteoid monophyly, evidence for the monophyly of four major clades within the tribe that approximate the genera *Ehrharta* s. s., *Microlaena*, *Tetrarrhena* and *Zotovia* tentatively tentatively favours the recognition of four genera (Watson and Dallwitz 1992, 1994; Edgar and Connor 1998) instead of one (Willemse 1982; Clayton and Renvoize 1986). Ancillary reasons supporting this decision include (i) the possession of distinctive morphological synapomorphies by each of the genera thus recognised, (ii) the existence of marked intergeneric ecological and distributional differences, and (iii) current user satisfaction with the four-genus classification (Watson and Dallwitz 1992, 1994; Edgar and Connor 1998). Although some taxonomic changes are required to ensure generic monophyly (involving *E. avenacea*, *M. polynoda* and *M. stipoides*), it is necessary to fill critical holes in the molecular data before these are effected. In

addition, the monophyly of some species (e.g. *M. stipoides*) requires confirmation through the inclusion of multiple accessions in molecular analyses.

On current evidence, generic status may be accorded to four clades as follows:
(1) The *Ehrharta* s. s. clade - This includes all species of *Ehrharta* s. s. except *E. avenacea* and is characterized by the possession of a conspicuous rachilla extension. The group is restricted to Africa and Madagascar, where it occupies a broad range of habitats.
(2) The *Tetrarrhena* clade - This includes *M. stipoides*, *M. polynoda* and species of *Tetrarrhena* and is recognisable by its four-staminate flowers. Members of the group typically inhabit the understorey of dry forests and woodlands and occur in Malesia, New Guinea, Australia and New Zealand.
(3) The *Microlaena* clade - This includes *E. avenacea*, *M. avenacea* and *M. tasmanica*. The clade shares two-staminate flowers with *Zotovia* but lacks the cushion-like habit of that genus. The natural distribution range of the group includes Réunion, Malesia, New Zealand, Fiji, Tahiti and Tasmania. Throughout this range, its members typically occur in the understorey of forests.
(4) The *Zotovia* clade - This includes the three species of *Zotovia* and is distinguishable from the *Microlaena* clade (with which it shares two stamens per flower) by its smaller flowers and cushion-like habit. The group is endemic to New Zealand where it occurs in alpine grassland bogs.

Support for the monophyly of *Ehrharta* s. s. validates the focus of the current study on evolutionary patterns within the genus. Had the group been paraphyletic, for example, greater cognizance of other, nested genera would have been necessary. Consistently weak support for phylogenetic structure in the *Ehrharta* s. s. clade, especially at the base of the *E. erecta-E. eburnea* clade, may be interpretable as evidence of rapid diversification (Baldwin 1997; Springer et al. 1997; Jackman et al. 1999). Under the assumption that character change tracks time, a general 2.7- to 3.6-fold decrease in branch lengths on the node subtending the *E. erecta-E. eburnea* clade supports this inference, suggesting phylogenetic radiation at this point. Potential species paraphyly (Crisp and Chandler 1996) leaves open the possibility that some branching events have been missed but this can only be evaluated by the inclusion of multiple accessions per species.

Ancestral habitat reconstruction suggests that the evolution of a tolerance of more intense and protracted seasonal aridity on the branch subtending the *E. ramosa-E. bulbosa* clade has been influential in permitting *Ehrharta* s. s. subsequent access to eutrophic substrates (most notably shale-derived clays and granitic gravels) associated with the Cape arid zone. Since radiation in *Ehrharta* s. s. is coincident with an inferred transition from oligotrophic, quartzitic sands to these more eutrophic substrates, the acquisition of increased aridity-tolerance is inferred to be a key step in facilitating radiation. This study therefore supports the contention of Clayton and Renvoize (1986) that the remarkable diversity of *Ehrharta* s. s. in the Cape region reflects radiation following 'adaptation to the winter rainfall regime of the South African Cape.' However, it is the summer-drought aspect of such a rainfall regime that is here considered critical. Since the inception of strongly summer-arid climate in the Cape region is inferred to have taken place around the end of the Tertiary (Deacon et al. 1992; Linder et al. 1992; Meadows and Watkeys 1999), radiation in *Ehrharta* s. s. is presumed to postdate this. The inference that the evolution of summer-deciduous foliage in *Ehrharta* s. s. was coincident with its entry into seasonally arid habitats suggests that the latter may be interpreted as a 'key innovation' *sensu* Simpson (1953). Markedly higher transpiration rates in leaves than in culms (which are long-lived in some species) of *Ehrharta* s. s. support the suggestion that their seasonal loss is a water conservation device (Orians and Solbrig 1977; Chabot and Hicks 1982). Droughting experiments comparing survival responses in summer-green species such as *E. setacea* and *E. rupestris* and their closest summer-deciduous relatives (e.g. *E. ramosa* and *E. capensis*) are, however, required to test this hypothesis further. If the principal benefit of seasonal foliage-deciduousness is water conservation then the summer-greenness of the former species should be interpreted as a nutrient-stress and not a moisture-stress avoidance mechanism (see Stock 1988; Stock et al. 1992). *E.setacea*, *E. rupestris*, *E.dura* and *E. microlaena* all occupy nutrient-limited fynbos habitats. Perhaps surprisingly, the evolution of leaf deciduousness in *Ehrharta* s. s. precedes the shift from oligotrophic sandstones to more fertile substrates. However, this may be explained by the existence of strategies that are suited to exploit episodic, fire-induced nutrient augmentation (e.g. as in *E. ramosa*).

The evolution of several growth form novelties (e.g. swollen and buried culm bases, annual habit) in *Ehrharta* s. s. is consistent with an interpretation invoking adaptation

to seasonal-aridity but the data presented in this thesis do not exclude alternative adaptive hypotheses. Regardless of the circumstances surrounding their origin, these traits appear to be implicated in the survival of seasonal drought. Within the aridity-tolerant *E. ramosa-E. bulbosa* clade drought survival involves reliance either on seed banks (seeders) or vegetative resilience (persisters or sprouters). Morphological divergence associated with such strategic differentiation in *Ehrharta* s. s. is mirrored by allocation differences at the seedling level. If these are causally linked, then the wide variation in seedling RGR shown by *Ehrharta* s. s. may reflect its high growth form diversity. In *Ehrharta* s. s. a seeder strategy (facultative or obligate) is characterized by high seedling RGR and early flowering and is prevalent in the *E. calycina-E. melicoides* and *E.erecta-E. longiflora* clades. Because seeding is the basis of an ephemeral (annual) strategy, the evolution of annualness is restricted to these clades. In contrast, a persistence (sprouting) strategy in *Ehrharta* s. s. is characterized by a low seedling RGR, a longer time to flowering and the possession of 'persistence traits' at maturity. The latter include geophytic swollen culm bases and the evolutionarily associated trait of culm base burial. A vegetative persistence strategy is prevalent in the geophytic *E. bulbosa-E. capensis* clade and in *E. eburnea*, as well as in the suffrutescent *E. barbinodis-E. thunbergii* clade.

Many species in resource-limited fynbos habitats show marked post-fire responses that reflect an apparent ability to exploit increased resource availability following fire (Bond and van Wilgen 1996). At least two fynbos *Ehrharta* species show marked post-fire responses but these differ, apparently due to differences in the basic regeneration strategy (i.e. seeding or sprouting) employed by each. In *E. capensis*, a geophytic morphology reflects a particularly strong reliance on vegetative persistence and this species is a strong sprouter. In resource-limited habitats, the 'save first' strategy employed by geophytic species may prohibit flowering in years of low resource availability (i.e. between fires) and inflorescence production is supported only after fire, when resources are more abundant. Like most geophytic fynbos grasses (Linder and Ellis 1990), flowering in *E. capensis* is, therefore, episodic and apparently 'fire-stimulated'. By contrast, the suffrutescent *E. ramosa* is a weak sprouter which apparently relies heavily on seed for regeneration (Kruger 1987). Flowering in this species is not 'fire-stimulated' and, instead, elevated post-fire investment in the production of long-lived culms may facilitate regular flowering during the subsequent inter-fire period. This may produce larger and more

frequently-renewed seed banks than would be observed in species having strictly 'fire-stimulated' flowering.

The evolution of divergent functional morphologies in *Ehrharta* s. s. may be closely linked to the rapid diversification and comparatively high species richness of the genus in the Cape arid zone. Because plants of different growth form utilise resources differently or are limited by different resources they may be competitively superior in different habitats (Chapin 1980; Cody 1986, 1989; Tilman 1988). For example, this study tentatively suggests that annual *Ehrharta* s. s. species are better adapted to extreme seasonal aridity than perennial persisters (e.g. geophytes), but that the latter are more tolerant of substrate infertility. In spatially heterogeneous environments, growth form diversification may therefore facilitate species coexistence at a variety of spatial scales (Cody 1986, 1989; Cowling et al. 1992; Tilman and Pacala 1993) and thus favour speciation (Schluter 1996). The potential importance of environmental heterogeneity in facilitating coexistence in *Ehrharta* s. s. supports the perspective that high speciation in many Cape taxa is attributable to ecological diversification along steep environmental gradients (Linder 1985; Linder and Vlok 1991; Goldblatt 1991; Cowling et al. 1992).

In choosing to incorporate phylogenetic data in the investigation of evolutionary pattern in the Cape grasses, I have in this study opted to focus on a single taxonomic group (Ehrharteae, *Ehrharta* s. s.). While the principal benefit of such an approach is scope for a better and more detailed understanding of change within this group (cf. Coddington 1994; Wenzel and Carpenter 1994) its major cost is restricted generality of the conclusions drawn. Specifically, because all conclusions are based on data from *Ehrharta* s. s. alone, it is impossible to draw generalizations regarding the Cape grasses as a whole. However, since historical events are unique (Coddington 1988; 1994; Carpenter 1989; Wenzel and Carpenter 1994) it is possible that the factors governing the evolution of specific traits in different taxa vary and, hence, that general explanations may not actually exist. The best way, then, to achieve a more general understanding of evolutionary pattern may be through an accumulation of detailed taxon-based case studies, such as this one, whose results can then be synthesised to infer a broader perspective (cf. Coddington 1994). From this it should then be possible to determine which patterns are truly general and which not.

In this study, I argue that high species diversity in *Ehrharta* s. s. reflects adaptive radiation following the relatively recent inception of a summer-arid climate in the western Cape region of South Africa. This pattern matches the expectation of Linder et al. (1992), who suggested that such radiation may be a general feature of the Cape flora. Specifically, these authors argued that forest contraction associated with late-Tertiary aridification would have provided new habitats which would in turn have promoted 'rampant speciation of the few taxa that survived the crisis'. Certainly, Cowling and Hilton-Taylor (1999) noted that 'there is no evidence to suggest that the massive diversification of the succulent karoo flora is the result of an unusually strong and stable history.' Thus, the high taxonomic diversity of this aridland flora is likely a product of recent processes. The demonstration that a preference for arid, succulent karoo habitats is derived in both *Ehrharta* s. s. and the danthonioid genus *Tribolium* (Linder and Davidse 1997) is consistent with this view. However, further detailed phylogenetic studies are required to demonstrate that the radiation of Cape taxa in response to the occupation of summer-arid habitats is a recurring pattern. These would also provide an indication of the extent to which radiation in the Cape flora is associated with functional diversification along ecological gradients as observed in *Ehrharta* s. s. (cf. Johnson 1996).

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Appendices

APPENDIX 1. List of morphological and anatomical characters used in phylogenetic analysis.

920. Leaf base: constricted (0), not constricted (1).
The majority of species have a broad-based leaf of the type typical in most higher grasses. The bases of such leaves, particularly when these are broad, are furnished with auricles for support. This is contrasted with the condition in some species of *Ehrharta s. s.* and *Microlaena* in which the leaf base is constricted, resulting in an almost pseudopetiolate leaf base that lacks auricles altogether.

921. Inflorescence type: panicle (0), spike or raceme (1).
Inflorescences vary from branched (paniculate) to linear (spicate or racemose). Panicle form varies from being contracted to open and in some species is somewhat verticillate. However, the prevalence of intermediate types prevents cladistic coding of this variation.

922. Spikelets: sessile (0), pedicellate (1).
This character has an overtly quantitative basis, reflecting spikelet pedicel length variation. A break in the variation in this character separates four species of *Tetrarrhena*, which have pedicels shorter than 1mm, from the remaining species.

923.Lower glume length: less than 0.33x spikelet length (0), 0.33-0.72x spikelet length (1), more than 0.72x spikelet length (2).
An overtly quantitative charactershowing substantial variation (Fig. A1.1a-e) which can be shown graphically (Fig. 2.2) to be divisible into three or four states. A three-state delimitation is selected here to minimise heterogeneity in the number of states among characters, the majority of characters being binary.The state delimitation thus justified is comparable to that implied by Gibbs Russell and Ellis (1987).

924. Upper glume apex: rounded, truncate or irregular (0), acute (1).

925. Sterile lemmas: glabrous (0), villous (1).
This character refers to the presence or absence of macrohairs on the back of the lemma, excluding the callus. These vary, somewhat continuously, from being scattered over the entire lemma back (e.g. *E. thunbergii* Fig. A1.1j) to being concentrated along the keel and sometimes also submarginally (e.g. *E. barbinodis* Fig. A1.1h). Gibbs Russell and Ellis (1987) do not report the lemma in *E. eburnea* and *E. barbinodis* as hairy because the hair in these species is restricted to the lemma keel.

926. Sterile lemmas: smooth (0), corrugated (1).
This conspicuous feature (Fig. A1.1g, h, m), restricted to *Ehrharta s. s.*, was rejected by Willemse (1982) as a generic character. Occasional specimens of *E. setacea* and *E. rupestris* have lemmas that are coarsely bumpy (Fig. A1.1n), a condition that may be homologous with lemma corrugation. However, both because this is uncertain and because the presence of lemma bumpiness in these species is inconsist, this is not coded as such.

927. Lower sterile lemma callus: glabrous (0), two-tufted (1), diffuse-villous (2).
This character distinguishes between the diffuse-villous callus found in species of *Microlaena* (Fig. A1.1p) and the condition in most species of *Ehrharta s. s.* (Fig. A1.1o) which possess a dorsal-ventral pair of distinct tufts.

928. Lower sterile lemma microhairs: absent (0), present (1).
In several species of *Ehrharta s. s.* microhairs occur basally on the lateral margins of either the fertile lemma alone or the fertile lemma and the upper sterile lemma or all three lemmas, thus producing a set of nested distributions. The occurrence of microhairs on the lemmas is not, therefore, correlated and their occurrence on each is treated as an independent character (see also characters 933 and 938).

929. Upper sterile lemma callus: glabrous (0), diffuse-villous (1), two-tufted (2).
As for character 927, the two-tufted condition described here is restricted to *Ehrharta s. s.* In this case, however, both tufts are dorsal and flank the midrib (Fig. A1.1g, h, j, m). Although these tufts appear to be situated on the lemma back near its base rather than on the callus, careful examination shows that the elevation of these tufts is due to dorsal callus elongation. Callus hairs in *Microlaena* and *Zotovia* are typically not tufted (Fig. A1.1i, k)

930. Upper sterile lemma base: unconstricted (0), constricted (1), stipitate (2).
This character is probably related to the preceding one in that dorsal elongation of the lemma callus seems to be responsible for producing the constricted lemma base found in several species of *Ehrharta s. s.* (Fig. A1.1g, h, j). Further elaboration results in the formation of a substantial stipe as in *E. longifolia* (Fig. A1.1m). The distinction between the states 'constricted' and 'stipitate' employed here differs slightly from that used by Gibbs Russell and Ellis (1987).

931. Upper sterile lemma ear-like appendages: absent (0), present (1).
This curious feature, restricted to *Ehrharta s. s.* (Fig. A1.1g, l), appears to be derived from the basal margins of the upper sterile lemma, resembling the auricle of a foliage leaf. This derivation is apparent in *E. erecta* in which the structure lacks elaboration.

932. Upper sterile lemma ear-like appendages: calycina-type (0), melicoides-type (1).
These appendages are typically somewhat U-shaped with a membranous edge (Fig. A1.1g; see also Gibbs Russell and Ellis 1987), a morphology that is here referred to as the calycina-type. *E. melicoides* and *E. longigluma* have distinctively shaped ear-like appendages in which the 'U' is upside down (Fig. A1.1l). This type, not seen elsewhere, is termed melicoides-type.

933. Upper sterile lemma lateral microhairs: absent (0), present (1).
Refer to character 928.

934. Upper sterile lemma apex: unhooded (0), hooded (1).
The hooded condition described here (Fig. A1.1l, n), found in four species of *Ehrharta s. s.*, is equivalent to the 'canoe-shaped' lemma tips described by Gibbs Russell (1987a).

935. Upper sterile lemma apex: rounded to truncate (0), tapering into an awn (1), mucronate to long-mucronate (2).
The distinction between first state of this character and the other two states is justified graphically and distinguishes an awnless condition (Fig. A1.1f, l, n) from the possession of an awn of some sort. Recognition of two awn types forms the basis for distinguishing between the latter two states. The first awn type is typical of *Microlaena* and *Zotovia* and emerges gradually from the lemma apex (Fig. A1.1i, k) while the second type, which is more typical of *Ehrharta s. s.*, emerges abruptly (mucro-like) and is usually flanked by distinct shoulders (Fig. A1.1h, j, m). Gibbs Russell and Ellis (1987) appear to utilise a similar state distinction although they code *E. pusilla* and *E. longiflora* as awned.

936. Fertile lemma: glabrous (0), villous (1).

937. Fertile lemma lateral swellings: absent (0), small (1), large to massive (2).
These structures are situated marginally near the fertile lemma base in a number of *Ehrharta s. s.* species (Fig. A1.1q, r) and fall into two fairly clear size categories. In *E. ramosa* and *E. rehmannii* they remain attached to the lemma callus when the fertile lemma is dissected out, while in the remaining species they come away with the lemma. When large, they are visible prior to dissection (Fig. A1.1q).

938. Fertile lemma lateral microhairs: absent (0), present (1).
Refer to character 928.

939. Fertile lemma lateral microhairs: diffuse (0), clustered (1).
In five species the microhairs are tightly clustered to form a small tuft, while in the majority they tend to be rather more diffusely distributed, either along the basal margins or over the entire lemma base.

940. Fertile lemma microhair apical cells: as wide as basal cells (0), much wider than basal cells (1).
This character distinguishes a peculiar, mushroom-like microhair type, found in three *Ehrharta s. s.* species (Fig. A1.2h) with the more common condition in which the width of the apical and basal cells is similar (Fig. A1.2i, j, k).

941. Fertile lemma microhair apical cells: isodiametric to slightly longer than wide (0), much longer than wide (1).
This character distinguishes a microhair type having elongated, thin-walled apical cells, and occurring in three species of *Ehrharta s. s.* (Fig. A1.2j).

942. Fertile lemma keel: glabrous (0), with a row of stout, glassy bristles (1).
This character distinguishes the outgroup taxa (*Oryza* and *Leersia*). Note that this character doesn not refer to the small prickles found on the fertile lemma keels of some ingroup species.

943. Palea texture: membranous to thinly chartaceous (0), coriaceous (1).
Paleas in the outgroup taxa are distinctly leathery to coriaceous, having the same texture as the fertile lemmas.

944. Palea nerve number: two (0), one (1).
This character was considered potentially useful as for generic delimitation by Willemse (1982). The distribution of this character and that of character 949 (presence/ absence of a rachilla process) suggests that these two characters may be developmentally related, although this would be difficult to substantiate.

945. Lodicules: one-parted (0), two-parted (1).
Lodicules in Ehrharteae are quite variable, being lobed in several species and frequently bilobed in *Ehrharta s. s.* (Fig. A1.2d-g). This variation is difficult to code cladistically, however, as these states intergrade. In three species of *Ehrharta s. s.*, however, the bi-lobing is strongly developed and the fleshy lower part of the lodicule divided into two distinct segments connected by a thin membrane (Fig. A1.2e) which tears easily. These are here termed bi-parted.

946. Lodicules: glabrous (0), with apical bristles (1).
This character refers to the presence of bristles on the distal margins of the lodicules (Fig. A1.2d, g). In some species (e.g. *E. capensis*) these are numerous while in others they are sparse (e.g. *E. ramosa*).

947. Stamen number: two (0), three (1), four (2), six (3).
This character was also considered by Willemse (1982) to be a potential generic character. Two species show variation in stamen number: *M. stipoides* typically has two or four stamens, while *E. dura* has four or six (Gibbs Russell and Ellis 1988). Although the former also rarely has one or six stamens (Willemse 1982), this is unusual and is not coded here.

948. Stigmatic branches: elongated (0), contracted (1).
Henry Connor (pers. comm.) has pointed out that, whereas in *Microlaena*, *Tetrarrhena* and *Zotovia* each stigma comprises an elongated central axis with short lateral branchlets (Fig. A1.2b, c), in most species of *Ehrharta s. s.* the central axis is greatly contracted and the basal lateral branches greatly elongated, to produce a brush-like structure in which the branches radiate out from a more or less single point (Fig. A1.2a).

949. Rachilla process: absent (0), small (1), large (2).
In *Ehrharta s. s.* the rachilla process is typically conspicuous, being large (exceeding 400µm in length) and often inflated (Fig. A1.2l, o), while in *M. avenacea*, *M. tasmanica* and *E. avenacea* it is small and sometimes even apparently absent (Fig. A1.2m). Species of *Zotovia* show a somewhat intermediate condition (Fig. A1.2n) although assessment of this condition is made difficult by a paucity of study material. Although the rachilla process in *Zotovia* is here coded as small, it is worth noting that a large' coding does not significantly alter the results of cladistic analysis. Rachilla processes are also shape-variable but this variation is not readily amenable to discrete state delimitation.

950. Rachilla process: glabrous (0), villous or prickly (1).
Rachilla processes may be glabrous (Fig. A1.2l, o) or prickly to shortly hairy (Fig. A1.2m, n). The existence of intermediates prohibits the recognition of short hairs and prickles as distinct.

951. Midrib parenchyma: absent to slight (0), massively developed (1).
Parenchyma proliferation about the median leaf trace results in the production of a distinct midrib. In several species this is absent (Fig. A1.2q) while in others it is just weakly developed (Fig. A1.2p) and quite indistinct. In four species, this parenchyma is, however, massively developed forming a large, distinct midrib that is triangular in section (Fig. A1.2r).

952. Midrib anatomy: simple type (0), oryzoid type (1).
Both Tateoka (1963) and Gibbs Russell and Ellis (1987) pointed out the absence of complex oryzoid vasculature in Ehrharteae and this character therefore segregates the two oryzoid outgroups.

953. Midrib lateral air channels: absent (0), present (1).
This is another oryzoid character that distinguishes the outgroups from the ingroup.

954. Leaf mesophyll: tearing (0), not tearing (1).
This character describes a peculiar artifact in which the leaf mesophyll tears during sectioning (Fig. A1.2r). The feature appears to be phylogenetically informative, being consistently noted in sections of three closely related species of *Ehrharta s. s.*, but absent elsewhere.

955. Abaxial epidermal intercostal stomata: absent (0), present (1).

956. Abaxial epidermal intercostal stomata: with flanges (0), lacking flanges (1).
This character describes the occurrence of peculiar cuticular flanges (Fig. A1.2s) that overlap the stomatal guard cells in *E. thunbergii* and *E. villosa* (Ellis 1987b).

957. Abaxial epidermal intercostal silica bodies: absent (0), present (1).

958. Abaxial epidermal cells: clear (0), containing tannin crystals (1).

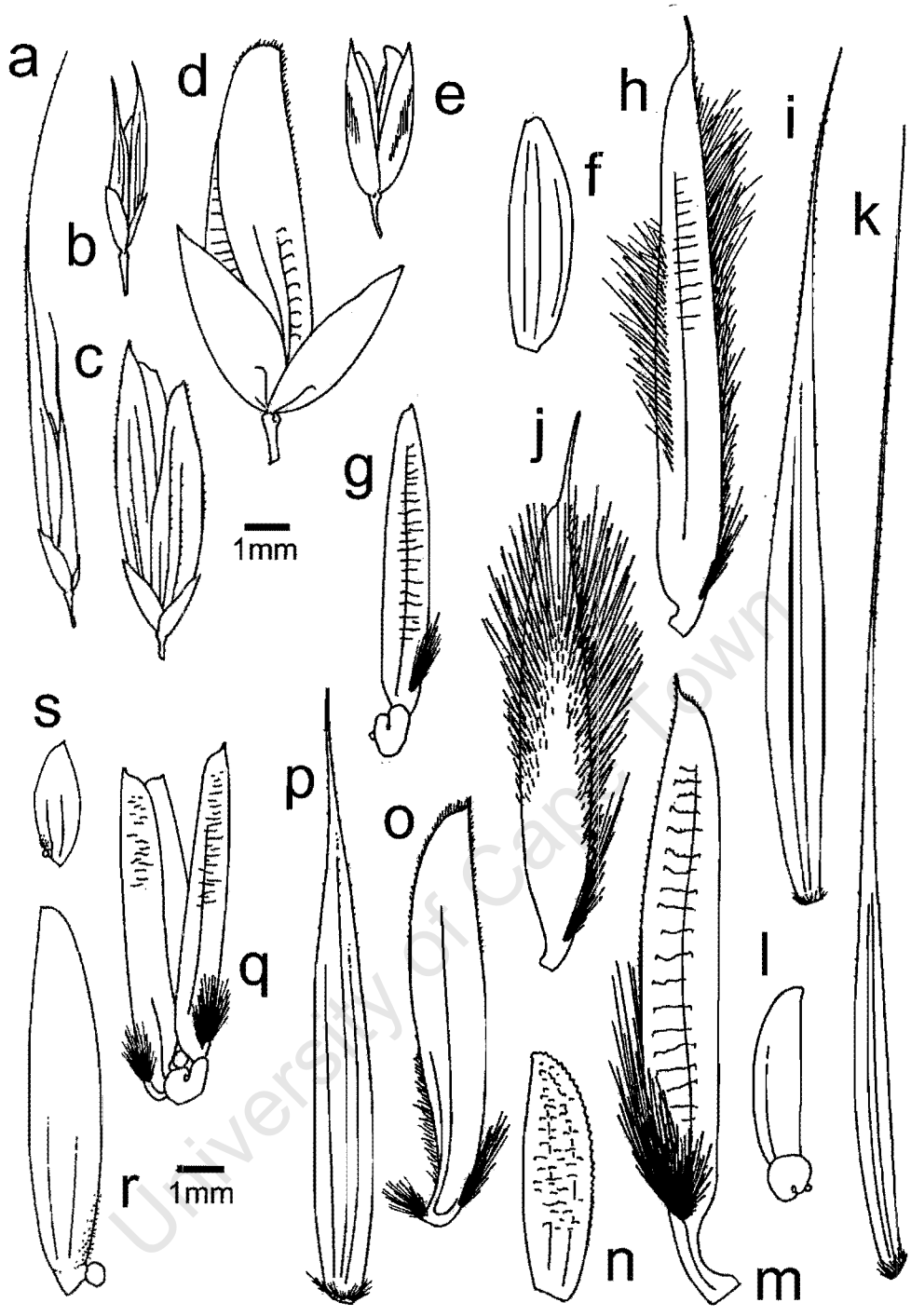

FIGURE A1.1. Spikelet and lemma variation in Ehrharteae (vouchers in square parentheses). **(a)-(e):** Whole spikelets of (a) *M. avenacea* [WELT 68449], (b) *Z. thomsonii* [WELT 76146], (c) *T. acuminata* [NSW 389303], (d) *E. capensis* [Ellis 645], and (e) *E. melicoides* [Ellis 4645]. **(f)-(n):** Upper sterile lemmas of (f) *T. turfosa* [NSW 389310], (g) *E. ramosa* subsp. *aphylla* [Smook 3697], (h) *E. barbinodis* [Davidse 33373], (i) *M. tasmanica* [NSW389307], (j) *E. thunbergii* [Spies 3696], (k) *M. avenacea* [WELT 78904], (l) *E. melicoides* [Verboom 153], (m) *E. longifolia* [Hanekom 2698], and (n) *E. setacea* subsp. *setacea* [Kruger KR521]. **(o)-(p):** Lower sterile lemmas of (o) *E. ottonis* [Gibbs Russell 5656], and (p) *M. tasmanica* [NSW 389307]. **(q)** Spikelet of *E. ramosa* with glumes removed [Smook 3697]. **(r)-(s):** Fertile lemmas of (r) *E. ottonis* [Verboom 150], and (s) *E. delicatula* [Mittendorf 21].

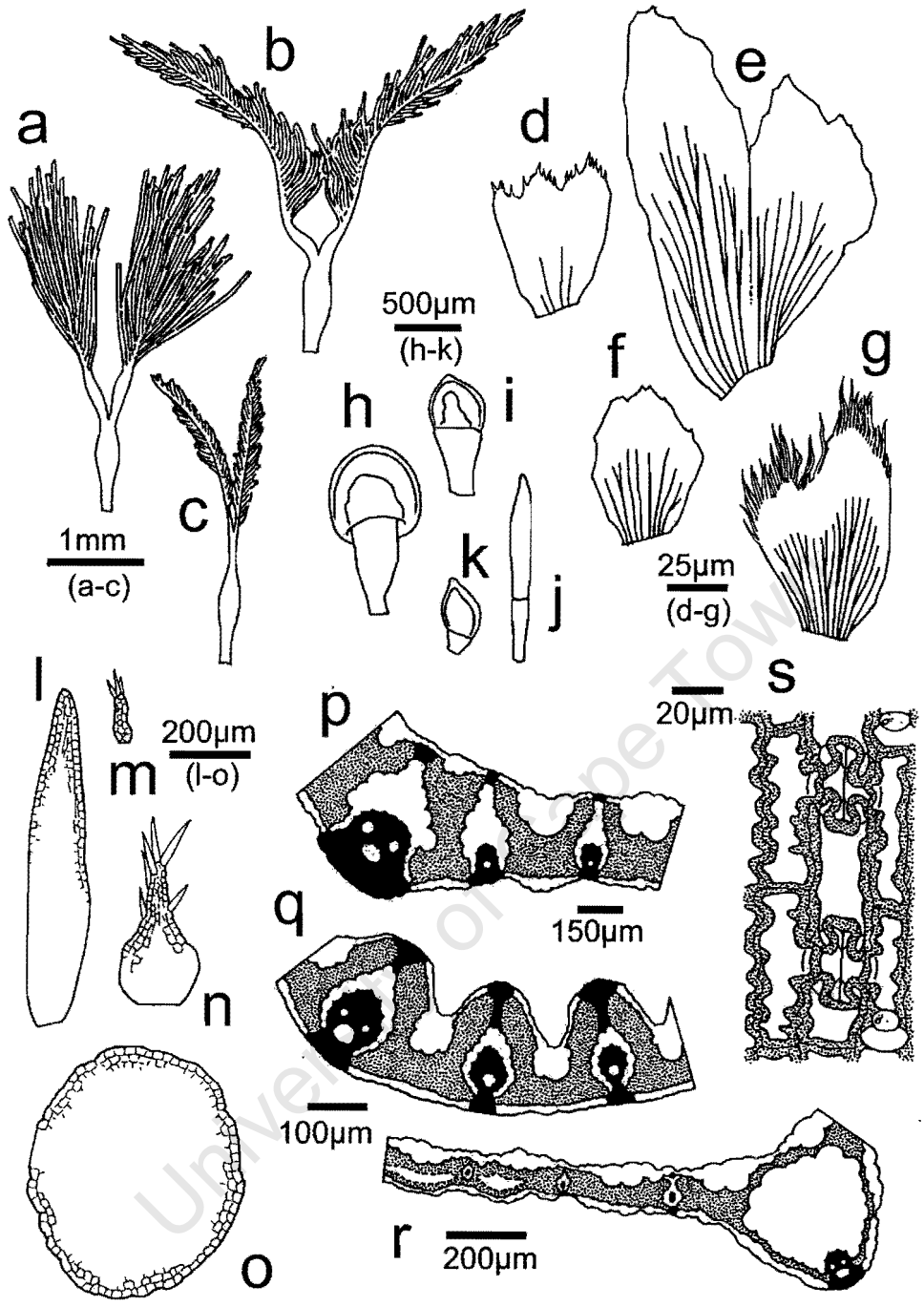

FIGURE A1.2. Micromorphological and anatomical variation in Ehrharteae (vouchers in square parentheses). **(a)-(c):** Ovaries of (a) *E. calycina* [Smook 1284], (b) *E. setacea* subsp. *setacea* [Gibbs Russell 5634], and (c) *E. polynoda* [WELT 68434]. **(d)-(g):** Lodicules of (d) *M. tasmanica* [NSW 389307], (e) *E. villosa* subsp. *villosa* [Verboom 166], (f) *T. acuminata* [NSW 389302], (g) *E. capensis* [Gibbs Russell 5691]. **(h)-(k):** Fertile lemma microhairs of (h) *E. triandra* [Oliver, Tölken and Venter 561] , (i) *E. ottonis* [Gibbs Russell 5656], (j) *E. pusilla* [Gloss 13085], and (k) *E. melicoides* [Verboom 153]. **(l)-(o):** Rachilla processes of (l) *E. barbinodis* [Davidse 33373], *M. tasmanica* [NSW 389307], (n) *Z. colensoi* [WELT 76406], and (o) *E. capensis* [Gibbs Russell 5691]. **(p)-(q):** Partial transverse sections, showing midvein, through leaves of (p) *E. capensis* [Ellis 5219], (q) *E. rupestris* subsp. *tricostata* [Ellis 5564], and (r) *E. delicatula* [Ellis 2147]. **(s)** Abaxial epidermis of leaf of *E. thunbergii* [Ellis 4648].

APPENDIX 2. Nexus file used in phylogenetic analyses. The matrix block contains trnL-F and ITS1 sequence data, binary insertion/ deletion data for trnL-F and ITS1, and morphological data.

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format datatype=dna missing=? gap=- interleave symbols="0123";
options ignore=uninform;
matrix

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Zthomson    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace    ATCCGTGGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TTTCTTTTAT
Mpolynod    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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Tacumina    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
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Tjuncea     NNNNNNNNNN NNNNNNNNNN NNNNNNNNNC AAGTCCCTNT ATCCCCAAAN TATCTTTTAT
Tturfosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNT ATCCCCAAAC TATCTTTTAT
Edura       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emicrola    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Esetacea    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TNTCTTTTAT
Eramosa     ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Erehmann    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Ecapensi    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Ebulbosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Elongifo    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emelicoi    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Elongigl    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eerecta     ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Elongifl    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Edelicat    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TAGCTTTTAT
Etriandr    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Ebarbino    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Evillosa    NNNNNNNNNN NNNNNNNNTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Ethunber    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TATCTTTTAT
Ecalycin    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TTTCTTTTAT
Ebrevifo    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TTTCTTTTAT
Epusilla    ATCCGTCGAC TTTATAAGTT GTGAGGGTTC AAGTCCCTCT ATCCCCAAAC TTTCTTTTAT
ORYZA       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNTTTAT
LEERSIA     NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
AEGILOPS    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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Eavenace    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace    TCCCTAAC-- ---TATAGTA TTTATCCTCT TTTTTTATTT TT---ATTAG T-----GGGT
Mpolynod    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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Ebulbosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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ORYZOID     T-CCTAAC-- ---TCTAGTA TTTATCCTGT TTTTTT---- -----ATTAA T-----AGGT

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Eavenace    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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Mtasmani    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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Tlaevis     ---------- ---------- ---------- ---------- ---------- ----------
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Eottonis    ---------- ---------- ---------- ---------- ---------- ----------
Elongifo    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea    ---------- ---------- ---------- ---------- ---------- ----------
Emelicoi    ---------- ---------- ---------- ---------- ---------- ----------
Elongigl    ---------- ---------- ---------- ---------- ---------- ----------
Eerecta     ---------- ---------- ---------- ---------- ---------- ----------
Elongifl    ---------- ---------- ---------- ---------- ---------- ----------
Edelicat    ---------- ---------- ---------- ---------- ---------- ----------
Etriandr    ---------- ---------- ---------- ---------- ---------- ----------
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Epusilla    ---------- ---------- ---------- ---------- ---------- ----------
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Tdistich   TAGATCTTAT GTTATTCATT GAATACATTT CCTTTTTATT AGAGTATCG- --GCAAGGAA
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Erupestr   TAGATCTTAT GTTATTCATT GAATACATTT CCTTTTTATT AGAGTATCG- --GCAAGGAA
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Eramosa    ---------- ---------- ---------- CCTTTTTATT AGAGTATCA- --GCAAGGAA
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Ecapensi   ---------- ---------- ---------- CCTTTTTATT AGAGTATCC- --GCAAGGAA
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Eottonis   ---------- ---------- ---------- CCTTTTTATT AGAGTATCC- --GCAAGGAA
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
Emelicoi   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
Elongigl   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
Eerecta    ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
Elongifl   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
Edelicat   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
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Ebarbino   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
Evillosa   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
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Epusilla   ---------- ---------- ---------- CCTTTTTATT AGAGTATCAG CAGCAAGGAA
ORYZA      ---------- -------ATG GAATACATTT CTTTTTTATT ATAGTATCG- --GCAAGGAA
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ORYZOID    ---------- -------ATG GAATACATTT CTTTTTTATT ATAGTATCG- --GCAAGGAA

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Zthomson   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Mpolynod   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid   TCTCGATTAT TACCTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
Tlaevis    ---------- ----------AAAGTATT A-------TT AAGTAAGCCC T----GTACA
Tacumina   TCTCGATTAT TACCTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
Tdistich   TCTCGATTAT TACCTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
Tjuncea    TCTCGATTAT TACCTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
Tturfosa   TCTCGATTAT TACCTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
Edura      TCTCGATTAT TACCCCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Emicrola   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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Esetacea   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Eramosa    TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Erehmann   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Ecapensi   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCT T----GTACA
Ebulbosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCT T----GTACA
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea   TCTCGATTAT TACCTCGATT TTGAAGTATT A-------TT AAGTAAGCCC T----GTACA
Emelicoi   TCTCGATTAT TACCTCGATT TTTAAGTATT ATTAATTATT AAGTAAGCCC T----GTACA
Elongigl   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Eerecta    TCCCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Elongifl   TCCCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Edelicat   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Etriandr   TCTCGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Ebarbino   TC-CGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Evillosa   TC-CGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Ethunber   TC-CGATTAT TACCTCGATT TTTAAGTATT A-------TT AAGTAAGCCC T----GTACA
Ecalycin   TCCCGATTAT TAACTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
Ebrevifo   TCCCGATTAT TAACTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
Epusilla   TCCCGATTAT TAACTCGATT TTAAAGTATT A-------TT AAGTAAGCCC T----GTACA
ORYZA      TGTCGATTAT TAACTCGATA TTTAAATATT A-------TT AAATAGGCTT TCTTTGTACA
LEERSIA    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
AEGILOPS   TCTTGGTTAT TCACTTTATT TTTAAGTTTT AT------TT AAGTAAACCA T----GCACA
ORYZOID    TGTCGATTAT TAACTCGATA TTTAAATATT A-------TT AAATAGGCTT TCTTTGTACA
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Zcolenso   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTGGAATAT TTTA-TTCCT
Zthomson   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGTA TTTGGAATAT TTTA-TTCAT
Mpolynod   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid   ATGCGTAGGA CTAC----TC CTCACTTCC- AAATTTGGAA TTTGGAATAT TTTA-TTAAT
Tlaevis    ATGCGTAGGA CTAC----TC CTCACTTCC- AAATTTGGAA TTTGGAATAT TTTA-TTAAT
Tacumina   ATGCGTAGGA CTAC----TC CTCACTTAC- AAATTTGTAA TTTGGAATAT TTTA-TTAAT
Tdistich   ATGCGTAGGA CTAC----TC CTCACTTAC- AAATTTGTAA TTTGGAATAT TTTA-TTAAT
Tjuncea    ATGCGTAGGA CTAC----TC CTCACTTAC- AAATTTGTAA TTTGGAATAT TTTA-TTAAT
Tturfosa   ATGCGTAGGA CTAC----TC CTCACTTAC- AAATTTGTAA TTTGGAATAT TTTA-TTAAT
Edura      ATGCGTAGGA CTAC----TC CCCACTTAC- AAA------- ---------- ---------T
Emicrola   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr   ATGCGTAGGA CTAC----TC CCCACTTAC- AAA------- ---------- ---------T
Esetacea   ATGCGTAGGA CTAC----TC CCCACTTCA- AAATTTTGAG TTTGGAATAT TTTA-TTAAT
Eramosa    ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTGGAA TTTGGAATAT TTTA-TTAAT
Erehmann   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTGGAA TTTGGAATAT TTTA-TTAAT
Ecapensi   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTGGAATTT TTTA-TTAAT
Ebulbosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTGGAA TTTGGAATTT TTTA-TTAAT
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTGGAATAT TTTA-TTAAT
Emelicoi   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTGGAATAT TTTA-TTAAT
Elongigl   ATGCGTAGGA CTAC----TC CCCACTTACC AAATTTTGAA TTTGGAATAT TTTA-TTAAT
Eerecta    ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTGGAATAT TTTA-TTAAT
Elongifl   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTTGA---T TTTA-TTAAT
Edelicat   ATGCGTAGGA CTAC----TC CCCACTTAC- AAA------- ---------- ----------
Etriandr   ATGCGTAGGA CTAC----TC CCCACTTAC- AAA------- ---------- ----------
Ebarbino   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTTGAATAT TTTA-TTAAT
Evillosa   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTTGAATAT TTTC-TTAAT
Ethunber   ATGCGTAGGA CTAC----TC CCCACTTAC- AAATTTTGAA TTTTGAATAT TTTC-TTAAT
Ecalycin   ATGCGTAAGA CTAC----GC CCCACTTCC- AAATTTTGAA TTTGGAATAT TTTA-TTAAT
Ebrevifo   ATGCGTAAGA CTAT----GC CCCACTTCC- AAATTTTGAA TTTGGAATAT TTTA-TTAAT
Epusilla   ATGCGTAAGA CTAC----GC CCCACTTTC- AAATTTTGAA TTTGGAATAT TTTA-TTAAT
ORYZA      ATGCATAGGA CTGCCCCCTC CCCATTTCC- AAATTTTGGA T--------- ----------
LEERSIA    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
AEGILOPS   ATGCATAGGA CTAC----CC CCCATTTTC- AAATTTAAAA TTTGAAATAC TTTAATTAAT
ORYZOID    ATGCATAGGA CTGCCCCCTC CCCATTTCC- AAATTTTGGA T--------- ----------

Zcolenso   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Zthomson   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Mpolynod   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTGC TAGGATGATA CACAAGAAAA
Tlaevis    TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTGC TAGGATGATA CACAAGAAAA
Tacumina   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTGC TAGGATGATA CACAAGAAAA
Tdistich   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTGC TAGGATGATA CACAAGAAAA
Tjuncea    TTTTTAGTCC CTNTAATTGA CATAGATACA AATACTCTGC TAGGATGATA CACAAGAAAA
Tturfosa   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTGC TAGGATGATA CACAAGAAAA
Edura      TTTTTAGTGT CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Emicrola   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr   TTTTTAGTGT CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Esetacea   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Eramosa    TTT-------- -----ATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Erehmann   TTT-------- -----ATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Ecapensi   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Ebulbosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Emelicoi   TTTTGAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Elongigl   TTTTGAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Eerecta    TTTT-AGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Elongifl   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Edelicat   ---------- ---------- ---------- ---------- TAGGATGATA CACAAGAAAA
Etriandr   ---------- ---------- ---------- ---------- TAGGATGATA CACAAGAAAA
Ebarbino   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Evillosa   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Ethunber   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Ecalycin   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Ebrevifo   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
Epusilla   TTTTTAGTCC CTTTAATTGA CATAGATACA AATACTCTAC TAGGATGATA CACAAGAAAA
ORYZA      ---------- -----ATTGA CATAGATACA AATACTCTAC TAGGATGATG CACAAGAAAA
LEERSIA    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
AEGILOPS   TTTT-AGTCC TTTTAATTGA CATAGCTACA AANNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZOID    ---------- -----ATTGA CATAGATACA AATACTCTAC TAGGATGATG CACAAGAAAA
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Zcolenso   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Zthomson   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
Eavenace   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
Mavenace   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTNNNNNN NNNNNNNNNN NN
Mpolynod   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
Mtasmani   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
Mstipoid   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTNNNNNN NNNNNNNNNN NN
Tlaevis    GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Tacumina   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAANNN NNNNNNNNNN NN
Tdistich   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAN NNNNNNNNNN NN
Tjuncea    GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAN NNNNNNNNNN NN
Tturfosa   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCNNNNNNNN NN
Edura      GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Emicrola   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
Erupestr   GGTCAGGATA GCTCAGTTGG TAGAGCANNN NNNNNNNNNN NNNNNNNNNN NN
Esetacea   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTNNNNNNN NN
Eramosa    GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Erehmann   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Ecapensi   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Ebulbosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
Eottonis   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CNNNNNNNNN NN
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
Eeburnea   GGTCAGGATA GCTCAGTTGG TAGAGCAGAN GACTGAAAAT CCTCGTGTCA CC
Emelicoi   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCTNNNNN NN
Elongigl   GGTCAGGATN GCTCAGTTGG TAGAGCAGAN GACTGAAAAT CCTCNNNNNN NN
Eerecta    GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Elongifl   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Edelicat   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Etriandr   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Ebarbino   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CN
Evillosa   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Ethunber   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Ecalycin   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Ebrevifo   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
Epusilla   GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGNNNNN NNNNNNNNNN NN
ORYZA      GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC
LEERSIA    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
AEGILOPS   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NN
ORYZOID    GGTCAGGATA GCTCAGTTGG TAGAGCAGAG GACTGAAAAT CCTCGTGTCA CC

[its data]
Zcolenso   NNNNNNNNNN TGCGGAAGGA TCATTGTCGT GACCC-TGAC CAAAACAGAC CGCGAACGCG
Zthomson   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace   NNNNNNNNNN NNNNGAAGGA TCATTGTCGT GACC--TGAC CAAAACAGAC CGCGAACGCG
Mpolynod   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid   NNNNNNNNNN NNNNNNNNNN NNNNNNNNGA GACCC-TGAC CAAAACAGAC CGCGAACTCG
Tlaevis    NNNNNNNNNN NNNNNNNNNN NNNNNGTCGT NACC--TGAC CAAAACAGAC CGCGAACGCG
Tacumina   NNNNNNNNNN NNNNNAAGGA TCATTGTCGT GACC--TGAC CAAAACAGAC NGCGAACGCG
Tdistich   NNNNNNNNNN NNNNNNNGGA TCATTGTCGT GANNNNNNNN NAAAACAGAC CGCGAACGCG
Tjuncea    NNNNNNNNNN NNNNNNNNNN NNNNNGTCGT GACC--TGAC CAAAACAGAC CGCNAACGCG
Tturfosa   NNNNNNNNNN NGCGGAAGGA TCATTGTCGT GACC--TGAC CAAAACAGAC CGCGAACACG
Edura      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emicrola   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr   TAGGTG-ANC TGCGGAAGGN TNATNGTCGT GACC-GAAAC CNAAACCGAC NGTGAACAAG
Esetacea   NNNNNNNNNN NNNNNAAGGA TCATTGTCGN GACC-GAAAC CAAAACCGAC CGTNAACACG
Eramosa    TAGGTG-ANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Erehmann   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ecapensi   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ebulbosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis   TAGGTG-ANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea   NNNNNNNNNN NNNGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Emelicoi   TAGGTGTANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Elongigl   TAGGTGTANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Eerecta    TAGGTG-ANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Elongifl   TAGGTG-ANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Edelicat   TAGGTG-ANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Etriandr   TAGGTG-ANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Ebarbino   TANGTNNANC TGCGGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Evillosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ethunber   TAGGTG-ANC TGCGGAAGGA TCANTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Ecalycin   TANGTNNANC TNNNGAAGGA TCATTGTCGT GACC-GAAAC CAAAACCGAC CGTGAACAAG
Ebrevifo   TAGGTG-ANC TGCGGAAGGA TCATTGTCGT GACCCGAAAC CAAAACCGAC CGTGAACAAG
Epusilla   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZA      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
LEERSIA    NNNNNNNNNN NNNNNNNNNN NNNNNNNTCGT GACCC-TGAC CAAAACAGAC CGCGAACGCG
AEGILOPS   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNT CACCC-TCAC CAAAACAGAC CGCGCACGCG
ORYZOID    NNNNNNNNNN NNNNNNNNNN NNNNNNTCGT GACCC-TGAC CAAAACAGAC CGCGAACGCG
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Zcolenso   TCACCCGCCC GGCCGGG--- ------TAAC --CC-----G GCCGCCC--- -GGCCACCGG
Zthomson   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace   TCACCCGCCC GGCTGGG-CG ACGGGCTAAC --CC----CG GCCGCCC--- -GGCCACCGG
Mpolynod   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid   TTACCCGCCC GGCCGGG-CG GCGTGCTAAG CACCTAGCCA GCCACC---- -GGCCACCGG
Tlaevis    TCACCCGCCC GGCCGGG-CG GCGGGCTAAA --CCTAGCCA GCCTCCC--- -GGCCACCTG
Tacumina   TCATCCGCCC GGCCGGG-CG GCGGGCTAAC --CCTAGCCA GCCTCCC--- -GGCCACCTG
Tdistich   TCACCCGCCC GGCCGGG-AG GAGGGCTAAC --CCTANNNN NNNTCCC--- -GGCCACCGG
Tjuncea    TCACCCGCCC GGCCGGG-CG GAGGGCTAAC --CCTANNNN NCCTCCC--- -GGCCACCGG
Tturfosa   TCACCCGCCC GGCCGGGGAG GCGGGCTAAC --CCTAGCCA GCCTCCC--- -GGCCACCGG
Edura      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emicrola   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr   TCACCCGCCC GGCCATG--- -CGCGCTAAG --GCTCGCGC CCCACCCGCA TGGCCACCGG
Esetacea   TCACCCGCCC GGNCGTG--- -CGCCC---- -------CGC CTCAC----- -GGCTACAGG
Eramosa    TCACCCGCCC GGCCACG--- -CGCGCTAAG --GCTCACGC CCGACCCGCG TGGCCACCGG
Erehmann   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ecapensi   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ebulbosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis   TCACCCGCCC GGCCACNNNN NCNCGCTAAG --GCTCACGC CCCACCCGCG TGGCCACCGG
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea   TCACCCGCCC GGCCACG--- -CGCGCTAAG --GCTCACGC CCCACCCGCG TGGCCACCGG
Emelicoi   TCACCCGCCC GGCCATG--- -CGCGCTAAN NNGCTCGCGC CCCACCNGTG TGGCCACCGG
Elongigl   TCACCCGCCC GGCCACG--- -CGTGCTAAG --GCTAACGC CCCACCCGTG TGGCCACCGG
Eerecta    TCACCCGCCC GGCCACG--- -CGTGCTAAG --GCTCACGC CCCACCCGCG TGGCCACCGG
Elongifl   TCACCCGCCC GGCCACG--- -CGTGCTAAG --GCTCACGC CCCACCCGTG TGGCCACCGG
Edelicat   TCACCCGCCC GGCCACG--- -CGTGCTAAG --GCTAACGC CCCACCCGTG TGGCCACCGG
Etriandr   TCACCCGCCC GGCCACG--- -CGTGCTAAG --GCTAACGC CCCACCCGTG TGGCCACCGG
Ebarbino   TCACCCACCC GGCCACG--- ---CGCTAAG --GCTCACGC CCCACCCGTG TGGCCACCGG
Evillosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ethunber   TCACCCACCC GGCCACG--- ---CGCTAAG --GCTCACGC CCCACCCGTG TGGCCACCGG
Ecalycin   TCACCCGCCC GGCCACG--- ---CGCTAAG --GCTCACGC CCCACCCGTG TGGCCACCGG
Ebrevifo   TCACCCGCCC GGCCACG--- ---CGCTAAG --GCTCACGC CCCACCCGCG TGGCCACCGG
Epusilla   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZA      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
LEERSIA    TCACCCNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
AEGILOPS   TCATCCNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZOID    TCACCCNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN

Zcolenso   CAATGCC-TT CT-------- T-GGG----- GGGCAGAGCC ACAAAAGAAC CCACGGCGCC
Zthomson   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace   CAATGCCCTC CT-------- T-GGG----- GGGCAGAGCC ACAAAAGAAC CCACGGCGCC
Mpolynod   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid   CGACGCCCTC GTCTCCCAGC T-GGGGGGCA GGGCGGAGCC ACAAAAGAAC CCACGGCGCC
Tlaevis    CGACGCCCTC GTCTCCCC-- TCGGGGGGCA GGGCGGAGCC ACAAAAGAAC CCACGGCGCC
Tacumina   CGACGCCCTC GTCTCCCC-- TCGGGAG-CA GGGCGGANCC ACAAAAGAAC CCACGGCGCC
Tdistich   CGACGCCNTN GTCTCCCT-- TCGGGAG-CA GGGCGGAGCC ACAAAAGAAC CCACGGCGCC
Tjuncea    CAACNCCCTC NTCTCCCA-- TCGGGAG-CA GGGCGGANCC ACAAAAGAAC CCACGGCGCC
Tturfosa   CGACGCCNTC GTCTCCCA-- TCGGGAG-CA GGGCAGAGCC ACAAAAGAAC CCACGGCGCC
Edura      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emicrola   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr   TGAAGCCCTC C--------- TCGGG----- GGGCCGACCC ACAAAAGAAC CCACGGCGCC
Esetacea   CCCTGCCCTC CCTACC---- -CGG----TT GGGCAGAGAT ACAAAAGAAC CCATGGCGCC
Eramosa    CAACGCCCTC C--------- -CGG------ GGGCGGAGCC ACAAAAGAAC CCACGGCGCC
Erehmann   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ecapensi   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ebulbosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis   CGAAGCCCTC C--------- CCGGG----- GGGCCGAGCC ACAAAAGAAC CCACGGCGCA
Elongifo   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea   TGAAGCCCTC C--------- TCGGG----- GGGCCGAGCC ACAAAAGAAC CCACGGCGCC
Emelicoi   TGAAGCCCTC C--------- TCGGG----- GGGCCGACCC ACAAAAGAAC CCACGGCGCC
Elongigl   TGAAGCCCTC C--------- TCGGG----- GGGCCGAGCC ACAAAAGAAC CCACGGCGCC
Eerecta    TGAAGCCCTC C--------- TCGGG----- GGGCCTAGCC ACAAAAGAAC CCACGGCGCC
Elongifl   TGAAGCCCTC C--------- TCGGG----- GGGCCGANCC ACAAAAGAAC CCACGGCGCC
Edelicat   TGAAGCCCTC C--------- TCGGG----- GGGCCGAGCC ACAAAAGAAC CCACGGCGCC
Etriandr   TGAAGCCCTC C--------- TCGGG----- GGGCCGAGCC ACAAAAGAAC CCACGGCGCC
Ebarbino   TGAAGCCCTC C--------- TCGGG----- GGGCTGAGCC ACAAAAGAAC CCACGGCGCC
Evillosa   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ethunber   TGAAGCCCTC C--------- TCGGG----- GGGCCGAGCC ACAAAAGAAC CCACGGCGCC
Ecalycin   TGAAGCCCTC C--------- TCGGG----- GGGCNGAGCC ACAAAAGAAC CCACGGCGCC
Ebrevifo   TGAAGCCCTC C--------- TCGGG----- GGGCCGAGCC ACAAAAGAAC CCACGGCGCC
Epusilla   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZA      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
LEERSIA    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN GGGAGGGGCC GCAAAAGAAC CCACGGCGCC
AEGILOPS   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN GGGCTC-GGG GTANAAGAAC CCACGGCGCC
ORYZOID    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN GGGAGGGGCC GCAAAAGAAC CCACGGCGCC
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```
Zcolenso    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TAGCT-AA C-CGGCG-GG AGCTACCGGC
Zthomson    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TAGCT-AA C-CGGCG-GG AGCTGCCGGC
Mpolynod    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid    GA-AGGCGTC AAGGAACAC- TGTGT-A--- --TAGCG-AA C-CGGGG-GG CGCTGCCGGC
Tlaevis     GA-TGGCGTC AAGGAACAC- TGTGTCA--- --TAGCG-AA C-CGGGG-GG CG--GCCGGC
Tacumina    GA-CGGCGTC AAGGAACAC- TGTG-CA--- --TAGCG-AA C-CGGGG-GG CA--GCCGGC
Tdistich    GA-CGGCGTC AAGGAACAC- TGTG-CATAG CATAGCG-AA C-CGGGG-GG CA--GCCGGC
Tjuncea     GA-CGGCGTC AAGGAACAC- TGTG-CA--- --TANCG-AA C-CGGGG-GG CA--GCCGGC
Tturfosa    GA-CGGCGTC AAGGAACAC- TGTG-CA--- --TAGCG-AA C-CAGGG-GT CA--GCCGGC
Edura       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emicrola    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr    GA-TGGCGTC AAGGAACAC- TGTG-CC--- --TACCA--A C-CAGGG-G- TGTGACCGGC
Esetacea    NA-CGCCGTC AAGGAACAC- TGTG-CC--- --TAGCA-GA C-CGGGTGGG CGTGGCCGGC
Eramosa     GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TAGCTTAA C-CAGGG-G- CGTGGCTGGC
Erehmann    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ecapensi    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ebulbosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- CGTGACCGGC
Elongifo    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A CAGANGG-A- TGTGACCGGC
Emelicoi    GA-TGGCGTC AAGGAACAC- TGTG-CN--- --TACCG--A C-CAGGG-G- TGTGACCGGC
Elongigl    GA-AGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTGACCGGC
Eerecta     GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTGACCGGC
Elongifl    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TACCNNNA C-CAGGG-G- TGTGACCGGC
Edelicat    GA-AGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTTACCGGC
Etriandr    GA-AGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTTACCGGC
Ebarbino    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTGACCGGC
Evillosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ethunber    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTGACCGGC
Ecalycin    GA-CGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTGACCGGC
Ebrevifo    GA-TGGCGTC AAGGAACAC- TGTG-CC--- --TACCG--A C-CAGGG-G- TGTGACCGGC
Epusilla    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZA       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
LEERSIA     GA-GGGCGTC AAGGAACACA TGTANNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
AEGILOPS    GA-AGGCGTC AAGGAACAC- TGTGNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZOID     GA-GGGCGTC AAGGAACACA TGTANNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN

Zcolenso    TTGCCGGCAG CACCCCC--G TGTTGCGATG CAACATCTAA AAGTCCACAC GACTCTCGGC
Zthomson    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace    TTGCCGGCAG CACCCCC--G TGTTGCGATG CAATATCTAA AATTCCACAC GACTCTCGGC
Mpolynod    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid    CTGCCGGAAG CACCCCC--G CGCCGGGACG CGATATCTAA AAATCCACAC GACTCTCGGC
Tlaevis     NTGCCGGCAG CACCCCT--G TGCCGCGACG CGATATCTAA AAATCCACAC GACTCTCGGC
Tacumina    CTGCCGGCAG CACCCCC--G NGACGNGACG CGACGTCTAA AAATCCACAT GACTCTCGGC
Tdistich    NTGCCNGCAG CACCCNC--G CGAC-----G CGACGTCTAA AAATCCACAT GACTCTCGGC
Tjuncea     CTGCCNGCAN CACCCCC--G CGACGCGACG CGACGTCTAA AAATCCACAT GACTCTCGGC
Tturfosa    CTGCTGGCAG CACCCCC--G CGAC-----G CGACGTCTAA AAATCCACAT GACTCTCGGC
Edura       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emicrola    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr    TTGCTGGCCG CTCCCCC--G TGTTGTGATG CAATATCTTT AAATCCACAT GACTCTCGGC
Esetacea    TTGCCGGAAG TGCCNCCNCG TGTCGCGACG CAATATCTAA GAATCCACAT GACTCTCGGC
Eramosa     TTGCCGGCCG CTCCCCT--G TGTTGCGATG CAATATCTTA AAATCCACAC GACTCTCGGC
Erehmann    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ecapensi    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ebulbosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis    TTGCCGGCCG CTCCCCC--G TGTCGCGATG CAATATCTTT AAATCCACAC NACTCTCGGC
Elongifo    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea    TTGCCGGCCG CTCCCCC--T TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Emelicoi    TTGCNGGCCG CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Elongigl    TTGCCGGCCA CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Eerecta     TTGCCGGCCG CTCCCCT--G TGTCGCGATG CAATCTATTT AAAACCACAT GACTCTCGGC
Elongifl    TTGCCGGCCG CTCCCCC--G TGTCGCGATG CAATATNTTT AAATCCACAT GACTCTCGGC
Edelicat    TTGCCGGCCA CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Etriandr    TTGCCGGCCA CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Ebarbino    TTGCCGGCTG CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Evillosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ethunber    TTGCCGGCTG CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Ecalycin    TTGCCGGCTG CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAN GACTCTCGGC
Ebrevifo    TTGCCGGCCG CTCCCCC--G TGTCGTGATG CAATATCTTT AAATCCACAC GACTCTCGGC
Epusilla    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZA       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
LEERSIA     NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NAATCCACAC GACTCTCGGC
AEGILOPS    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NAATCCACAC GACTCTCGGC
ORYZOID     NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NAATCCACAC GACTCTCGGC
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Zcolenso    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA NNNNGTGTGA
Zthomson    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eavenace    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mavenace    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Mpolynod    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mtasmani    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Mstipoid    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Tlaevis     AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCNNNNNNNN
Tacumina    AACGGATATC TCGGCTCTCG CATCNATGAA GAACGTAGCG AAATGCGATA CCNNNNNNNN
Tdistich    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAANNNGATA CCNNNNNNNN
Tjuncea     AACGGATATC TCGGCTCTCG CATCGATNAA GAACNTAGCG AAATGCGATA CCNNNNNNNN
Tturfosa    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGNAGCG AAATGCGATA CCTGGTGTGA
Edura       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Emicrola    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Erupestr    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Esetacea    AACGGATATC TCGGCTCTCG AATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTATGA
Eramosa     AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Erehmann    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ecapensi    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ebulbosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eottonis    AACGGATATC TCGGCTCTCG CATCGATNAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Elongifo    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Eeburnea    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Emelicoi    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAANNNNNNN NNNNNNNNNN
Elongigl    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Eerecta     AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Elongifl    AACGGATATC TCGGCTCTCG CATCGATNAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Edelicat    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Etriandr    AACGGATATC TCGGCTCTCG CATCGATNAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Ebarbino    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTNTGA
Evillosa    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
Ethunber    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Ecalycin    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Ebrevifo    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
Epusilla    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
ORYZA       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
LEERSIA     AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
AEGILOPS    AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA
ORYZOID     AACGGATATC TCGGCTCTCG CATCGATGAA GAACGTAGCG AAATGCGATA CCTGGTGTGA

Zcolenso    ATTGCAGAAT CCNNNNNNNN NNNNNN
Zthomson    NNNNNNNNNN NNNNNNNNNN NNNNNN
Eavenace    NNNNNNNNNN NNNNNNNNNN NNNNNN
Mavenace    ATTGCAGAAT CCCGTGAACN NNNNNN
Mpolynod    NNNNNNNNNN NNNNNNNNNN NNNNNN
Mtasmani    NNNNNNNNNN NNNNNNNNNN NNNNNN
Mstipoid    ATTGCAGAAT CCCGTGAACC ATCGAG
Tlaevis     NNNNNNNNNN NNNNNNNNNN NNNNNN
Tacumina    NNNNNNNNNN NNNNNNNNNN NNNNNN
Tdistich    NNNNNNNNNN NNNNNNNNNN NNNNNN
Tjuncea     NNNNNNNNNN NNNNNNNNNN NNNNNN
Tturfosa    ATTGCAGAAT CCCGTGAACC ATCGAG
Edura       NNNNNNNNNN NNNNNNNNNN NNNNNN
Emicrola    NNNNNNNNNN NNNNNNNNNN NNNNNN
Erupestr    ATTGCAGAAT CCCGTNAACC NNNNNN
Esetacea    ATTGCANAAT CCCGTGAACC ATCGAG
Eramosa     ATTGCAGAAT CCCGTGAACC ATCNNN
Erehmann    NNNNNNNNNN NNNNNNNNNN NNNNNN
Ecapensi    NNNNNNNNNN NNNNNNNNNN NNNNNN
Ebulbosa    NNNNNNNNNN NNNNNNNNNN NNNNNN
Eottonis    ATTGCANAAT CCCGTGAACC ATCGAG
Elongifo    NNNNNNNNNN NNNNNNNNNN NNNNNN
Eeburnea    ATTGCANAAT CCCGTGNNNN NNNNNN
Emelicoi    NNNNNNNNNN NNNNNNNNNN NNNNNN
Elongigl    ATTGCAGAAT CCCGTGAACC ATCNNN
Eerecta     ATTGCAGAAT CCCGTGAACC ATCGAG
Elongifl    ATTGCANAAT CCCGTGAACC ATCGAG
Edelicat    ATTGCANAAT CCCGTGAACC ATCGAG
Etriandr    ATTGCANAAT CCCGTGAACC ATCGAG
Ebarbino    ATTGCAGAAT CCCGTGAACC NNNNNN
Evillosa    NNNNNNNNNN NNNNNNNNNN NNNNNN
Ethunber    ATTGCAGAAT CCCGTGNACC NNNNNN
Ecalycin    ATTGCAGAAT CCCGTGANNN NNNNNN
Ebrevifo    ATTNCAGAAT CCCGTGNACC NNNNNN
Epusilla    NNNNNNNNNN NNNNNNNNNN NNNNNN
ORYZA       NNNNNNNNNN NNNNNNNNNN NNNNNN
LEERSIA     ATTGCAGAAT CCCGTGAACC ATCGAG
AEGILOPS    ATTGCAGAAT CCCGCGAACC ATCGAG
ORYZOID     ATTGCAGAAT CCCGTGAACC ATCGAG
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[indel data]
Zcolenso    1?000010110101?11?000000?011???011?001001O?001?01110101110010
Zthomson    ????????????????????????????????????????????????????????????
Eavenace    ????????????????????????????????????????????????????????????
Mavenace    1?000010110101?11?000000?1100010101001001O?001?01110101110010
Mpolynod    ????????????????????????????????????????????????????????????
Mtasmani    ????????????????????????????????????????????????????????????
Mstipoid    1?000010110101?11?000000?0100010007?01?000000000011011011110010
Tlaevis     1?000011?????1?11?000000?1100010107?0010000100000110010111O110
Tacumina    1?000010100101?11?000000?1100010107?0010000100010111010111O110
Tdistich    1?000010100101?11?000000?1100010107?0010000100010111000111O111
Tjuncea     1?000010100101?11?000000?1100010107?0010000100010111010111O110
Tturfosa    1?000010100101?11?000000?1100000107?0010000100010111010111O111
Edura       0100001011010171170001007?????????????????????????????????????
Emicrola    ????????????????????????????????????????????????????????????
Erupestr    0100001011010171170001001100017010?00001?0?001?0111011?111010
Esetacea    1?000000110101?11?000000?10001?1?0?1??0001?010?011101011000000
Eramosa     1?1????0???101?11?000001110001?010?0001????1???01110100111010
Erehmann    1?1????0???101?11?000001????????????????????????????????????
Ecapensi    1?1????0???101?11?000000????????????????????????????????????
Ebulbosa    ????????????????????????????????????????????????????????????
Eottonis    1?1????0???101?11?000000110001?010?00001?0?001?0111011?111010
Elongifo    ????????????????????????????????????????????????????????????
Eeburnea    1?1????0???001?11?000000?10001?010?00001?0?001?0111011?011010
Emelicoi    1?1????0???000011?000000010001?010?00001?0?001?0111011?111010
Elongigl    1?1????0???001?11?010000010001?010?00001?0?001?0111011?111010
Eerecta     1?1????0???001?11?000000110001?010?00001?0?001?0111011?111010
Elongifl    1?1????0???001?11?000000110001?010?00001?0?001?0111011?111010
Edelicat    1?1????0???001?11?001???110001?010?00001?0?001?0111011?111010
Etriandr    1?1????0???001?11?001???110001?010?00001?0?001?0111011?111010
Ebarbino    1?1????0???011?11?000000?1001??010?00001?0?001?0111011?111010
Evillosa    1?1????0???011?11?000000????????????????????????????????????
Ethunber    1?1????0???011?11?000000110017?010?00001?0?001?0111011?111010
Ecalycin    001????0???001?11?000000?1001??010?00001?0?001?0111011?111010
Ebrevifo    001????0???001?11?000000?10001??010?00001?0?001?0111011?111010
Epusilla    001????0???001?11?000000????????????????????????????????????
ORYZA       1?0001?0111101?00000001?????????????????????????????????????
LEERSIA     ????????????????????????01?????????????????????010???????????
AEGILOPS    ??01?0100101001117000000?01?????????????????????111???????????
ORYZOID     1?0001?0111101?00000001??01?????????????????????010???????????
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[morphology]
Zcolenso    10110002010?001000???0000000110000?10
Zthomson    10110002010?001000???0000000100000?00
Eavenace    00100002010?001000???0010000{01}100001110
Mavenace    00100002010?001000???0010000{01}100001110
Mpolynod    10101002000?001000???0010020?00001110
Mtasmani    00100002010?001000???0010{01}00{01}100001110
Mstipoid    10101002000?001000???00100{02}00?000011{01}0
Tlaevis     1{01}11{01}0000000?000000???0010020?00001110
Tacumina    11000000000?002000???0010020?00001100
Tdistich    11000000000?000000???0010020?00000?10
Tjuncea     11000000000?000000???0010020?00000?10
Tturfosa    11000000000?000000???0010020?00000?10
Edura       00100002010?001000???00001{23}021000011{01}1
Emicrola    00100002000?001000???0000120210000l101
Erupestr    111{01}0000000?010000???0000030210000?10
Esetacea    1{01}1210000000?010000???0000030210000{01}110
Eramosa     1{01}1210{01}10211000002O???0000130200000l110
Erehmann    1{01}12101102110000020???0000{01}302000001110
Ecapensi    10111011022O?10202100000001312000001100
Ebulbosa    10111011022O?10202100000001312000000110{01}
Eottonis    10121011022O?10{02}02100000001312000000{01}110
Elongifo    10111011022O?10202100000001312000001111
Eeburnea    10101101021O?10201100000001312000001100
Emelicoi    10121000001111100011000000003120000011{01}0
Elongigl    10121000001111100011000000003120000011{01}0
Eerecta     101110100{02}1{01}0100011000000013120{01}00{01}1100
Elongifl    1011{01}0{01}11210?10201101000001312010011100
Edelicat    101{12}10101011010001101000000112010011100
Etriandr    10111010101O?10101101000000112010011100
Ebarbino    101111{01}10210?10200100000010312000001100
Evillosa    10121101021O?10210100000010312000001010
Ethunber    10111101021O?10210100000010312000001010
Ecalycin    10121101001l000{02}10110100000312000000l1{01}0
Ebrevifo    10121101001l000{02}1011010000O?12000001100
Epusilla    10121101001l0002101101000003120000011OO
ORYZA       1010????????????000???1110010O?01101100
LEERSIA     1010????????????000???1110010O?01101110
AEGILOPS    ??????????????????????????????????????????
ORYZOID     1010????????????000???1110010O?01101110
;
endblock;

begin assumptions;
options deftype=unord;
charset trn=1-472 859-882;
charset its=473-858 883-919;
charset morphology=920-958;
taxset in_trn=1 4 7-13 15-19 21 23-35;
taxset in_its=1 4 7-12 15-17 21 23-30 32-34;
taxset in_morph=1-35;
taxset most=1 4 7-13 15-19 21 23-35 38 39;

endblock;
```

APPENDIX 3. Morphological character matrix used in phylogenetic analyses. Character numbers are indicated across the top and states are as listed in Appendix 1. Square brackets containing multiple states indicate state polymorphism.

	920										930										940										950								
E.avenacea	0	0	1	0	0	0	0	2	0	1	0	0	?	0	0	1	0	0	0	?	?	?	0	0	1	0	0	0	0	[01]	1	0	0	0	0	1	1	1	0
E.barbinodis	1	0	1	1	1	1	[01]	1	0	2	1	0	?	1	0	2	0	0	1	0	0	0	0	0	1	0	3	1	2		0	0	0	0	0	1	1	0	0
E.brevifolia	1	0	1	2	1	1	0	1	0	0	1	1	0	0	0	[02]	1	0	1	1	0	1	0	0	0	0	?	1	2		0	0	0	0	0	1	1	0	0
E.bulbosa	1	0	1	1	1	0	1	1	0	2	2	0	?	1	0	2	0	2	1	0	0	0	0	0	0	1	3	1	2		0	0	0	0	0	1	1	0	[01]
E.calycina	1	0	1	2	1	1	0	1	0	0	1	1	0	0	0	[02]	1	0	1	1	0	1	0	0	0	0	3	1	2		0	0	0	0	0	1	1	[01]	0
E.capensis	1	0	1	1	1	0	1	1	0	2	2	0	?	1	0	2	0	2	1	0	0	0	0	0	0	1	3	1	2		0	0	0	0	0	1	1	0	0
E.delicatula	1	0	1	[12]	1	0	1	0	1	0	1	1	0	1	0	0	0	1	1	0	1	0	0	0	0	0	1	1	2		0	1	0	0	1	1	1	0	0
E.dura	0	0	1	0	0	0	0	2	0	1	0	0	?	0	0	1	0	0	0	?	?	?	0	0	0	0	1	[23]	0	2		1	0	0	0	0	1	1	[01]	1
E.eburnea	1	0	1	0	1	1	0	1	0	2	1	0	?	1	0	2	0	1	1	0	0	0	0	0	0	1	3	1	2		0	0	0	0	0	1	1	0	0
E.erecta	1	0	1	1	1	0	1	0	0	[02]	1	[01]	0	1	0	0	0	1	1	0	0	0	0	0	0	1	3	1	2		0	[01]	0	0	[01]	1	1	0	0
E.longiflora	1	0	1	1	[01]	0	[01]	1	1	2	1	0	?	1	0	2	0	1	1	0	1	0	0	0	0	1	3	1	2		0	1	0	0	1	1	1	0	0
E.longifolia	1	0	1	1	1	0	1	1	0	2	2	0	?	1	0	2	0	2	1	0	0	0	0	0	0	1	3	1	2		0	0	0	0	0	1	1	1	1
E.longigluma	1	0	1	2	1	0	0	0	0	0	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	3	1	2		0	0	0	0	0	1	1	[01]	0
E.melicoides	1	0	1	2	1	0	0	0	0	0	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	3	1	2		0	0	0	0	0	1	1	[01]	0
E.microlaena	0	0	1	0	0	0	0	2	0	0	0	0	?	0	0	1	0	0	0	?	?	?	0	0	0	0	1	2	0	2		1	0	0	0	0	1	1	0	1
E.ottonis	1	0	1	2	1	0	1	1	0	2	2	0	?	1	0	[02]	0	2	1	0	0	0	0	0	0	1	3	1	2		0	0	0	0	0	[01]	1	1	0
E.pusilla	1	0	1	2	1	1	0	1	0	0	1	1	0	0	0	2	1	0	1	1	0	1	0	0	0	0	0	3	1	2		0	0	0	0	0	1	1	0	0
E.ramosa	1	[01]	1	2	1	0	[01]	1	0	2	1	1	0	0	0	0	0	2	0	?	?	?	0	0	0	0	1	3	0	2		0	0	0	0	0	1	1	1	0
E.rehmannii	1	[01]	1	2	1	0	1	1	0	2	1	1	0	0	0	0	0	2	0	?	?	?	0	0	0	0	[01]	3	0	2		0	0	0	0	0	1	1	1	0
E.rupestris	1	1	1	[01]	0	0	0	0	0	0	0	0	?	0	1	0	0	0	0	?	?	?	0	0	0	0	0	3	0	2		1	0	0	0	0	0	?	1	0
E.setacea	1	[01]	1	2	1	0	0	0	0	0	0	0	?	0	1	0	0	0	0	?	?	?	0	0	0	0	0	3	0	2		1	0	0	0	0	[01]	1	1	0
E.triandra	1	0	1	1	1	0	1	0	1	0	1	0	?	1	0	1	0	1	1	0	1	0	0	0	0	0	1	1	2		0	1	0	0	1	1	1	0	0
E.thunbergii	1	0	1	1	1	1	0	1	0	2	1	0	?	1	0	2	1	0	1	0	0	0	0	0	0	1	0	3	1	2		0	0	0	0	0	1	0	1	0
E.villosa	1	0	1	2	1	1	0	1	0	2	1	0	?	1	0	2	1	0	1	0	0	0	0	0	0	1	0	3	1	2		0	0	0	0	0	1	0	1	0
M.avenacea	0	0	1	0	0	0	0	2	0	1	0	0	?	0	0	1	0	0	0	?	?	?	0	0	1	0	0	0	0	[01]	1	0	0	0	0	1	1	1	0
M.polynoda	1	0	1	0	1	0	0	2	0	0	0	0	?	0	0	1	0	0	0	?	?	?	0	0	1	0	0	2	0	0	?	0	0	0	0	1	1	1	0
M.tasmanica	0	0	1	0	0	0	0	2	0	1	0	0	?	0	0	1	0	0	0	?	?	?	0	0	1	0	[01]	0	0	[01]	1	0	0	0	0	1	1	1	0
M.stipoides	1	0	1	0	1	0	0	2	0	0	0	0	?	0	0	1	0	0	0	?	?	?	0	0	1	0	0	[02]	0	0	?	0	0	0	0	1	1	[01]	0
T.acuminata	1	1	0	0	0	0	0	0	0	0	0	0	?	0	0	2	0	0	0	?	?	?	0	0	1	0	0	2	0	0	?	0	0	0	0	1	1	0	0
T.distichophylla	1	1	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	?	?	?	0	0	1	0	0	2	0	0	?	0	0	0	0	0	?	1	0
T.juncea	1	1	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	?	?	?	0	0	1	0	0	2	0	0	?	0	0	0	0	0	?	1	0
T.laevis	1	[01]	1	1	[01]	0	0	0	0	0	0	0	?	0	0	0	0	0	0	?	?	?	0	0	1	0	0	2	0	0	?	0	0	0	0	1	1	1	0
T.turfosa	1	1	0	0	0	0	0	0	0	0	0	0	?	0	0	0	0	0	0	?	?	?	0	0	1	0	0	2	0	0	?	0	0	0	0	0	?	1	0
Z.colensoi	1	0	1	1	0	0	0	2	0	1	0	0	?	0	0	1	0	0	0	?	?	?	0	0	0	0	0	0	0	1	1	0	0	0	0	0	?	1	0
Z.thomsonii	1	0	1	1	0	0	0	2	0	1	0	0	?	0	0	1	0	0	0	?	?	?	0	0	0	0	0	0	0	1	0	0	0	0	0	0	?	0	0
Oryza	1	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	?	1	1	1	0	0	1	0	0	?	0	1	1	0	1	1	0	0
Leersia	1	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	?	1	1	1	0	0	1	0	0	?	0	1	1	0	1	1	1	0
Aegilops	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
ORYZOID	1	0	1	0	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	?	1	1	1	0	0	1	0	0	?	0	1	1	0	1	1	1	0

APPENDIX 4. Matrix used in parsimony based reconstruction of growth form attributes in Ehrharteae. Character states follow Table 3.1 and are repeated below[a]. Uncertainty is indicated by '?'.

Species	Growth form attribute					
	1.	2.	3.	4.	5.	6.
E. avenacea	1	1	0	1	0	?
E. barbinodis	1	0	1	1	1	1
E. brevifolia	0	1	0	1	0	1
E. bulbosa	1	1	0	0	1	1
E. calycina	1	0/1	0/1	1	0	1
E. capensis	1	1	0	0	1	1
E. delicatula	0	1	0	1	0	1
E. dura	1	1	0	1	1	?
E. eburnea	1	1	0	0	1	1
E. erecta	1	0/1	0/1	1	0	0/1
E. longiflora	0	1	0	1	0	1
E. longifolia	1	1	0	0	1	0
E. longigluma	1	1	0	1	0	?
E. melicoides	1	1	0	1	0	1
E. microlaena	1	1	0	1	1	?
E. ottonis	1	1	0	0	1	?
E. pusilla	0	1	0	1	0	1
E. ramosa	1	0	1	1	0	1
E. rehmannii	1	0/1	1	1	0	1
E. rupestris	1	0	0	1	0	0
E. setacea	1	0	0	1	0	0
E. thunbergii	1	0	1	1	1	1
E. triandra	0	1	0	1	0	1
E. villosa	1	0	1	1	1	1
M. avenacea	1	1	0	1	0	?
M. polynoda	1	0	?	1	0	?
M. stipoides	1	1	0	1	0	?
M. tasmanica	1	1	0	1	0	?
T. acuminata	1	0/1	?	1	0	?
T. distichophylla	1	0	0	1	0	?
T. juncea	1	0	?	1	0	?
T. laevis	1	0/1	?	1	0	?
T. turfosa	1	0	0	1	0	?
Z. colensoi	1	0	0	1	0	?
Z. thomsonii	1	0	0	1	0	?

[a]1. Plant lifespan: **0**=annual, **1**=perennial
2. Culm branching: **0**=branching, **1**=not branching
3. Culm chlorenchyma: **0**=weakly developed, **1**=strongly developed
4. Culm base swelling: **0**=bulbous, **1**=not bulbous
5. Culm base burial depth: **0**=less than 5cm below ground, **1**=more than 5cm below ground
6. Foliage phenology: **0**=summer-green, **1**=summer-deciduous

APPENDIX 5. Matrix used in parsimony based reconstruction of habitat preferences in Ehrharteae. Character states follow Table 3.2 and are repeated below[a]. Uncertainty is indicated by '?'.

Species	Habitat variable					
	1.	2.	3.	4.	5.	6.
E. avenacea	5/6	?	?	?	?	0
E. barbinodis	0/1	0	7/8	0/1/2	1	3
E. brevifolia	0/1	0/1	7/8	0/1/2/3/4	1/3	2/3/5
E. bulbosa	2/3/4	3/4	2/3/4/5/6	5/6/7/8	0/1/2	1/2
E. calycina	0/1/2/3	0/1/2/3	6/7/8/9	0/1/2/3/4/5/6	1/2/3	2/3
E. capensis	2/3	0/1/2	5/6/7/8	2/3/4/5/6	0/1/2	1/2
E. delicatula	0/1	0	7/8	0/1/2/3	1/2	3
E. dura	5/6	3/4/5	0/1/2/3/4	7/8	0	1
E. eburnea	1	0	8	0/1/2	2	3
E. erecta	2/3/4/5/6	2/3/4	1/2/3/4/5/6/7	4/5/6/7/8	1/2	0
E. longiflora	0/1/2/3	0/1/2	6/7/8	0/1/2/3/4/5/6	1/2	2/3
E. longifolia	2/3/4	0/1/2	5/6/7/8	4/5	0	1
E. longigluma	4/5/6	5	0	4/5/6/7/8	4	4
E. melicoides	0/1/2/3	0/1/2	6/7/8/9	0/1/2/3/4/5	2	2
E. microlaena	5/6	3/4/5	0/1/2/3/4	7/8	0	1
E. ottonis	2/3/4	2/3/4	4/5/6	5/6/7/8	0	1
E. pusilla	0	0	8	0	1	3
E. ramosa	2/3/4/5/6	0/1/2/3	0/1/2/3/4/5/6/7/8	5/6/7/8	0	1
E. rehmannii	2/3/4/5/6	2/3/4	0/1/2/3/4/5	5/6/7/8	0	1
E. rupestris	5/6	3/4/5	0/1/2/3/4	7/8	0	1
E. setacea	5/6	3/4/5	0/1/2/3/4	7/8	0	1
E. thunbergii	1/2	0/1/2	7/8/9	2/3/4/5	0/1	2
E. triandra	0	0	8/9	0	1/2	3
E. villosa	2/3	1/2	5/6/7	4/5/6/7	3	5
M. avenacea	5/6	?	?	?	?	0
M. polynoda	3/4/5/6	?	?	?	?	0
M. stipoides	3/4/5/6	?	?	?	?	0
M. tasmanica	3/4/5/6	?	?	?	?	?
T. acuminata	2/3/4/5/6	?	?	?	?	0
T. distichophylla	2/3/4/5/6	?	?	?	?	0
T. juncea	2/3/4/5/6	?	?	?	?	0
T. laevis	2/3/4/5/6	?	?	?	?	0
T. turfosa	2/3/4/5/6	?	?	?	?	0
Z. colensoi	5/6	?	?	?	?	4
Z. thomsonii	5/6	?	?	?	?	4

[a]1. Mean annual rainfall: **0**=0-200mm, **1**=200-400mm, **2**=400-600mm, **3**=600-800mm, **4**=800-1000mm, **5**=1000-1200mm, **6**=more than 1200mm
2. Median January rainfall: **0**=0-5mm, **1**=5-10mm, **2**=10-20mm, **3**=20-40mm, **4**=40-60mm, **5**=more than 60mm
3. January potential evaporation: **0**=less than 180mm, **1**=180-200mm, **2**=200-220mm, **3**=220-240mm, **4**=240-260mm, **5**=260-280mm, **6**=280-300mm, **7**=300-320mm, **8**=320-340mm, **9**=more than 340mm
4. Duration of moisture growing season: **0**=0-25d.yr^{-1}, **1**=25-50d.yr^{-1}, **2**=50-100d.yr^{-1}, **3**=100-125d.yr^{-1}, **4**=125-150d.yr^{-1}, **5**=150-175d.yr^{-1}, **6**=175-200d.yr^{-1}, **7**=200-225d.yr^{-1}, **8**=more than 225d.yr^{-1}
5. Substrate parent material: **0**=sandstone, **1**=granite, **2**=shale or dolerite, **3**=basic, quarternary sands
6. Vegetation type: **0**=forest, **1**=fynbos shrubland (sclerophyll heathland), **2**=karroid and renoster shrubland, **3**=succulent shrubland, **4**=grassland, **5**=dune thicket