The evolutionary and ecological significance of heterophylly in aquatic plants: a case study in the genus Proserpinaca (Haloragaceae).

Carolyn L. Wells

University of Tennessee

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To the Graduate Council:

I am submitting herewith a dissertation written by Carolyn L. Wells entitled "The evolutionary and ecological significance of heterophylly in aquatic plants: a case study in the genus Proserpinaca (Haloragaceae)." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Botany.

Massimo Pigliucci, Major Professor

We have read this dissertation and recommend its acceptance:

Christine R.B. Boake, Mitch B. Cruzin, Otto J. Schwarz

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
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Accepted for the Council:

Interim Vice Provost and Dean of the Graduate School
The evolutionary and ecological significance of heterophylly in aquatic plants:
a case study in the genus *Proserpinaca* (Haloragaceae).

A Dissertation Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville

Carolyn L. Wells
August 2001
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Chapter 1

General Introduction

Heterogeneous environments and phenotypic plasticity

Natural habitats are temporally and spatially variable, and organisms express a multitude of strategies in coping with this environmental heterogeneity (Levins 1968). At the level of the individual, genotypes faced with such conditions may either produce the same phenotype (homeostasis) or express different phenotypes in response to specific environmental cues (phenotypic plasticity). In the case of the former, one may speak of populations consisting of different, coexisting yet tightly canalized phenotypes, each of which is adapted to a subset of the conditions experienced by the population (genetic polymorphism, Waddington 1942; Lerner 1954). With respect to the latter, populations may be comprised of individuals capable of functionally appropriate responses mediated through the processes of organismal development and physiology (Schlichting and Pigliucci 1998). Both of these constitute fundamental modes of adaptation to life in heterogeneous environments.

A common misconception surrounding the study of phenotypic plasticity is that it represents a non-genetic source of phenotypic variation (Schlichting 1986) and is therefore irrelevant to classic neo-Darwinian models of evolution via natural selection (Sultan 1992). However, that environmentally-induced responses may have a genetic basis was recognized early by Bradshaw (1965) and later substantiated by the documentation of ample genetic variation for degrees of plasticity to specific environmental conditions in natural populations (e.g., Cook and Johnson 1968; Quinn 1987; Macdonald and Chinnappa 1989; Platenkamp 1991; Pigliucci 1992; Oyama 1994; Dudley and Schmitt 1995). These observations lend empirical support to the contention that phenotypic plasticity can be directly targeted by natural selection, a subject that
has received considerable theoretical attention from evolutionary ecologists (Haldane 1946; Schmalhausen 1949; Levis 1963; Bradshaw 1965; Jain 1979; Via and Lande 1985; Levin 1988; Thompson 1991; van Tienderen 1991; Sultan 1992; Scheiner 1993; de Jong 1995; Schlichting and Pigliucci 1998).

The suggestion that phenotypic plasticity confers an increased ability to tolerate environmental variation rests upon several testable hypotheses (Dudley and Schmitt 1996; Schmitt et al. 1999). For phenotypic plasticity to be adaptive natural selection should favor the expression of different phenotypes in different environments—and organisms (or their progeny) should have a high probability of encountering these contrasting conditions. If environments are variable, but there is no differential fecundity among phenotypes across these conditions, then evolution via natural selection will not occur (i.e., the phenotypic distribution of the population will not change). If different phenotypes are differentially fecund across a range of environments, but only a subset (or just one) of these conditions is routinely encountered, then evolution via natural selection will lead to a phenotypic distribution that reflects the selective regime that is most frequently experienced. Thus, the evolution of phenotypic plasticity largely depends upon the extent to which environments are variable, and the relationships between phenotypic attributes and organismal fitness.

The study of natural selection through the use of multiple regression techniques that quantify the relationship between phenotypic traits and components of fitness was pioneered in a seminal paper by Lande and Arnold (1983) and has since received considerable attention (Manly 1985; Endler 1986; Mitchell-Olds and Shaw 1987; Brodie et al. 1995). This dissertation uses these multivariate statistical analyses to study the strength of association between several plant traits and components of fitness in different environments, in an evaluation of the hypothesis that plasticity in these traits is adaptive.
Adaptative phenotypic plasticity: a case study in aquatic plant species

Because of their sessile habit, plants encounter a wide range of temporal and spatial environmental heterogeneity. The study of plant phenotypes in relation to their environments has a long history in botany, a tradition dating to the development of the 'ecotype' concept during early investigations of widely distributed species along broad ecological gradients (e.g., Turesson 1922; Clausen et al. 1940). Some of the classic examples of adaptation in comparative plant ecology come from this literature, such as the environmental modification of leaf morphology (Bailey and Sinnott 1916; Vaughan and Wiehe 1939; Brougham 1962), of whole-plant architecture (Blackman and Wilson 1951; Blackman and Wilson 1954; Kays and Harper 1974), of germination (Cumming 1963; Harper et al. 1965) and of seedling establishment (Harper 1955; Grime and Jeffrey 1965) to name but a few. It should come as no surprise that many of these pioneering works also provide the earliest documentation of the ecological and evolutionary significance of phenotypic plasticity in plants (reviewed in Bradshaw 1965).

Freshwater habitats are markedly variable in terms of water availability, and many species that inhabit these environments exhibit life cycles that are responsive to these fluctuations. Seasonal oscillations in water availability evoke plasticity in the life histories of plants that occupy these habitats, as many aquatic and semi-aquatic (amphibious) species capable of clonal reproduction when submerged will respond to emergence by flowering and/or increased seedling recruitment from the seed bank (Blom et al. 1990; Barrett et al. 1993; Grace 1993; Robe and Griffiths 1998). Aquatic plants also exhibit phenomenal plasticity in their vegetative morphology, quite often to the frustration of classical taxonomists (e.g., McCully and Dale 1961; reviewed in Sculthorpe 1967).
Questions addressed in this dissertation

This dissertation consists of three parts. First (Chapter 2), I review one of the most well-characterized patterns of phenotypic plasticity exhibited by semi-aquatic plant species—variation in leaf form, or heterophylly—and examine what is currently known about the environmental mediation of this trait in aquatic plant taxa. This paper, co-authored with Massimo Pigliucci, has been published and is re-printed with permission from the editors of that journal (Wells and Pigliucci 2000). Next (Chapter 3) I take an ecological genetics approach toward explicit tests of the hypothesis that heterophylly is adaptive in aquatic plants, by comparing the relative fitness of co-occurring, closely related species that naturally exhibit variation for this trait in nature. Finally (Chapter 4), I broaden the scope of my inquiry into other potentially adaptive responses to flooding exhibited by aquatic plant taxa, via an examination of variation in additional traits with a presumed functional relationship to submergence (stomate density, aerenchyma tissue production, and changes in plant architecture).

Few studies have examined the degree of plasticity exhibited within and among co-occurring, closely related species in relation to the amount of environmental heterogeneity that they experience in nature. Moreover, quantitative descriptions of the adaptive significance of plasticity are also uncommon in the literature. The considerable plasticity exhibited by aquatic and amphibious plant species suggests myriad ways in which these plants may have adapted to the aquatic environment, and provide ideal circumstances in which to further our understanding of phenotypic plasticity and its role in ecological niche breadth.
Chapter 2

Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants

Abstract

Phenotypic plasticity may play a key role in the adaptation of organisms to changing environmental conditions. A special case of plasticity is represented by heterophylly, the ability of semi-aquatic plants to produce different types of leaves below and above water. Submerged leaves are thin and lack both a cuticle and stomata, whereas aerial leaves are thicker, cutinized and bear stomata. The striking variability in the submerged, floating and aerial leaves of heterophyllous (‘heteros’=different, ‘phyllos’=leaves) aquatics has historically been considered a paradigmatic example of adaptive phenotypic plasticity. An extensive body of developmental and physiological research reveals that heterophylly is quite often mediated by similar environmental cues across diverse taxa, which may imply a common underlying mechanism. Patterns of plasticity in response to environmental cues in the laboratory are consistent with the hypothesis of individual adaptation to heterogeneous environments, and the distribution of this trait among phylogenetically related aquatic angiosperms suggests either convergent or parallel evolution in their descent from terrestrial ancestors. Yet, critical evaluations of the ecological and evolutionary significance of this trait are scarce. In this essay, I discuss the patterns of plasticity revealed by experimental manipulative studies of heterophylly in the general context of adaptive phenotypic plasticity, and suggest avenues for future research that are needed in assessing the ecological and evolutionary significance of this trait.
Introduction

Phenotypic plasticity and leaf morphology

Phenotypic plasticity is the property of a genotype to alter its development in response to changes in the environment (Schmalhausen 1949). Some types of phenotypic change, especially those elicited by resource limitations, may represent inevitable responses to adverse conditions (Smith-Gill 1983); but other types of plasticity better equip a single genotype to survive and maintain reproductive fitness when faced with fluctuating environments (Sultan 1995).

The realization that phenotypes are environmentally dependent is not new, nor is the speculation that phenotypic plasticity may be advantageous to organisms which regularly encounter heterogeneous environments (Schmalhausen 1949; Levins 1963; Bradshaw 1965). More recently, considerable interest in the genetic basis of plasticity has revealed that it is not only heritable and potentially governed by specific regulatory loci, but that it also may be targeted by and responsive to natural selection (Schlichting and Levin 1986; Scheiner 1993; Schlichting and Pigliucci 1993; de Jong 1995). Moreover, empirical tests of adaptive plasticity hypotheses are being pursued with vigor (Winn and Evans 1991; Sultan and Bazzaz 1993; Sultan and Bazzaz 1993; Sultan and Bazzaz 1993; Schmitt et al. 1995; Dudley and Schmitt 1996).

Numerous authors have emphasized the presence of spatial and temporal variability at increasingly finer scales in natural habitats (Bell et al. 1991; Bell and Lechowicz 1991; Lechowicz and Bell 1991; Epperson 1995; Stratton 1995). However, the manner in which such heterogeneity is experienced is not identical for all organisms: the capacity for mobility that enables animals to minimize heterogeneity in resource availability has no parallel in plants, which initiate their life cycle under one set of conditions and complete it in another due to their sessile habit. Thus plant survival and reproduction depends upon toleration of, rather than escape from,
environmental extremes (Bradshaw 1965). This tolerance may be made possible by a repertoire of plastic responses distributed across phenological, morphological, cellular and physiological traits (Dudley 1996; Stuefer et al. 1996; Humphrey and Pyke 1998).

Yet not all instances of plasticity are adaptive (Sultan 1995). Critical evaluations of putatively adaptive plastic responses must incorporate an understanding of the frequency with which contrasting environments are encountered with demonstrations of the consequences to organismal fitness brought about by the expression of alternate phenotypes in each environment (Schmitt et al. 1995; Dudley and Schmitt 1996). A well-characterized example of adaptive plasticity in plants is the “shade avoidance response”: changes in light quality (red:far red ratio), detected by phytochrome molecules, induce a suite of alterations in growth strategy which maximize competitive ability for harvesting sunlight (Schmitt 1997).

An important type of potentially adaptive plasticity involves differences in the morphological, anatomical and physiological characteristics of leaves along environmental gradients (such as light and or water availability, Table 2.1; all Tables and Figures are organized by chapter in the Appendix). Variation in leaf traits can be found across species (guilds, Givnish 1987), among populations of the same species (ecotypic differentiation and/or across-individual plasticity, Clausen et al. 1940), and even between leaves produced by a single plant (within-individual plasticity, Winn and Evans 1991; Winn 1996). Furthermore, similar modifications of leaf structure and form in response to the environment appear at each of these levels (across or within species, populations or individuals). The second column of Table 2.1 lists several characteristic differences between the sun and shade leaves of terrestrial plants. Probably because high light intensity is often correlated with drought, many of these responses parallel those observed in reaction to low water availability (third column in Table 2.1). These traits may reasonably be expected to influence many of the generalized aspects of leaf function (e.g. stomatal density and rates of water and/or gas exchange, chlorophyll concentration and
photosynthetic efficiency, Lewis 1972). In general, adaptive plasticity hypotheses predict that individuals capable of exhibiting such differences in leaf architecture in response to heterogeneous light environments should have higher fitness relative to less plastic individuals, however there are few tests of this prediction in the literature (but see Sultan and Bazzaz 1993).

In this essay my aim is to assess the foundation upon which this one preeminent example of adaptive phenotypic plasticity is built, to illustrate strengths and weaknesses of the adaptive plasticity hypothesis as it pertains to heterophylly in aquatic plants, and to discuss avenues for future research.

*Environmental heterogeneity and plasticity in aquatic plants*

In addition to experiencing heterogeneous light environments, plants occupy a continuum of habitats from mesic terrestrial soils to continuously flooded substrates, along which individuals experience inundation of their roots and photosynthetic organs with varying frequency and duration. Positioned at the extremes of this gradient of water availability are plants tolerant of saturated soils (which may experience occasional inundation of their roots but whose leaves and stems remain essentially aerial) and plants exposed to constantly submerged habitats (those that complete their entire life cycle underwater). Within the framework of discussions of adaptations to environmental heterogeneity, taxa in which individual plants regularly encounter the contrasting environments are of particular interest. The diverse ways in which this is possible for shallow-rooted aquatics are depicted in Figure 2.1, which illustrates schematically how individuals exhibiting contrasting growth forms may experience air and water with different frequencies despite living in close proximity to one another. Whether rooted in the substrate and ascending vertically through the water column, or free-floating at or just below the water surface, plants of aquatic habitats may inevitably encounter markedly contrasting environments during the completion of their life cycles.
Many plant species modify their stem and/or leaf morphology in a manner reflecting a correlation with water availability. Characteristic flooding responses include rapid stem elongation to re-elevate photosynthetic tissues above water and the production of aerenchyma tissue in an effort to decrease diffusive resistance to O₂ (reviewed recently by Blom et al. 1990; Blom and Voesenek 1996; Arteca 1997). Yet another response to fluctuations in water level is the apparent association between leaf morphology and submergence: leaves produced while the shoot apex is submerged are thin and highly filamentous whereas those produced by an emergent shoot apex are thicker, exhibiting reduced surface area relative to their volume (Sculthorpe 1967). This type of within-individual plasticity is depicted in Figure 2.2, where the sequence from left to right shows the transition in leaf morphology observed as stems of *Proserpinaca palustris* (Haloragaceae) ascend vertically through the water into the air environment above. Heterophylly ('heteros' = different, 'phyllos' = leaves) is an example of phenotypic plasticity that has long attracted investigators (for an early review, consult Arber 1919), initially mostly for the nuisance it can create taxonomically (Sculthorpe 1967), and subsequently for the environmental contingencies it places upon plant development (Goliber and Feldman 1990; Young et al. 1990). This pattern of within-individual plasticity in leaf morphology is not restricted to aquatic plants, as it has been documented in response to temperature (Fisher 1960; Gurevitch 1992; Winn 1996), light availability (Lee and Richards 1991) and herbivory (Givnish et al. 1994) in terrestrial species.

The striking variability in the submerged, floating and aerial leaves observed in heterophyllous aquatic plants is considered by many to represent an intuitive, even paradigmatic, example of adaptive phenotypic plasticity (treated in some detail in Bradshaw's seminal 1965 review). Experimental investigations with heterophyllous aquatics have a long history, dating back at least to Lamarck (reviewed and referenced in Ashby 1948). In many cases, these works include meticulous attempts to characterize the influence of environmental conditions upon plant
development – despite the fact that they were initiated decades before the publication of even the earliest papers on phenotypic plasticity per se. This diffuse yet extensive body of research has been cogently summarized in the treatments of Arber (1920), Sculthorpe (1967) and Hutchinson (1975).

**Within-individual variation in leaf form**

Etymologically speaking, the term *heterophylly* applies to any observation of different leaves upon a single plant. Before proceeding with a discussion of heterophylly in aquatic plants, we need to take a brief historical tour of how the various manifestations of within-individual plasticity in leaf form have been interpreted (reviewed in Ashby 1948). An historical perspective is warranted because different authors have introduced some degree of subjectivity in the discussion by using a terminology based on the typological classification of leaf types such as “juvenile” or “adult” (defined ontogenetically) vs. “submerged” or “aerial” (defined ecologically), often in absence of the sorts of empirical data that are required to distinguish among these hypotheses.

Obviously, not all expressions of different leaf forms during the course of plant growth represent plastic responses to environmental conditions. Rather, in many plant taxa alterations in morphology appear coincident with maturation, such that the switch from a vegetative to a reproductive state is marked by a transition in leaf morphology. Goebel (1908) first introduced the term *heteroblasty* to describe the mode of plant development in which juvenile and adult phases of the life cycle were markedly different in appearance; thus he considered heterophylly to be the foliar manifestation of heteroblastic development (reviewed and referenced in Ashby 1948). A familiar example is *Hedera helix* (English Ivy), in which the lobed juvenile leaves of vegetative shoots are markedly different from the entire leaves borne by flowering stems.
(Robbins 1960). Subsequent investigations of heteroblastic species have shown that differences in the leaves are apparent early, at or near the point of their inception as young primordia - a pattern clearly suggestive of canalized (non-plastic) developmental change manifested as changes in leaf morphology.

However, Goebel was also among the first to note that certain conditions (e.g., low light intensity) appear to prolong or cause a reversion to the production of juvenile leaves in heteroblastic plants, thus leading to the concept of plastic heterophylly which is the main object of this article. To explain such seemingly aberrant patterns he advanced the hypothesis of arrested development (Goebel 1908), suggesting that leaves that are superficially similar in form are produced via a reiteration, at the level of the whole-plant, of the juvenile developmental program (reviewed and referenced in Ashby 1948). But how might a plant, which had long ago emerged from seed, come to once again reiterate the juvenile condition? Jones questions such interpretation, offering the alternative that the differences represent the plastic responses of individual leaves to shade (Jones 1995). To distinguish between these hypotheses she reasons that if, as is typical of heteroblastic development, the differences between juvenile and adult leaves are detectable at early stages in their development, then the primordia of leaves produced by mature plants in shade should more closely resemble juvenile leaf primordia than those of comparably aged plants grown in full sun. Using morphometric analyses of mature leaves and SEM studies of developing primordia to compare individuals of *Cucurbita argyrosperma* subsp. *sororia* (Cucurbitaceae) grown in contrasting environments, she finds that unlike the apparent differences between primordia of juvenile and adult leaves produced in the same environment, differences in the sun and shade leaves are not apparent until their lamina reach lengths surpassing 1000µm – corresponding to a later time of divergence than is typical for early (juvenile) and late (adult) leaves in this heteroblastic species. Thus heterophyllous leaves may reflect either a non-plastic developmental transition or a plastic response to environmental
conditions (environmentally-mediated, or plastic, heterophylly), or an interaction between the two, a conclusion reached by other authors working with heterophyllous species (e.g. Winn 1996). Clearly the distinction between non-plastic and plastic traits is not dichotomous, but can be used as a conceptual tool for understanding the relationship between genotype and environment in producing a given phenotype. In the discussion that follows and unless otherwise noted, whenever I refer to heterophylly I am referring to plastic heterophylly, induced by changes in environmental conditions.

Without exception, the primordia of submerged and aerial leaves in mature heterophyllic aquatic plants are indistinguishable until relatively late stages in their development and are not correlated with changes in the organization or size of the shoot apex (McCully and Dale 1961; Schmidt and Millington 1968; Deschamp and Cooke 1985; Goliber and Feldman 1990; Bruni et al. 1996). When submerged plants are transferred to aerial conditions, examination of leaves still in bud at the time of the transition reveals anatomical changes at the base of these leaves (the portion of the leaf which is last to emerge from the bud) prior to observable differences at the morphological level. This production of transitional leaves in some species also corroborates the interpretation of heterophylly as a very localized plastic response to the environment, occurring at the cellular level and reflecting the basipetal nature of leaf development (Goliber and Feldman 1990; Bruni et al. 1996). All of these observations suggest that heterophylly is a form of marked phenotypic plasticity in these species.

Few studies of heterophylly in aquatics have fully appreciated the quantitative nature of this plastic response. Rather, the literature abounds with typological references to "submerged" and "aerial" leaves elicited by subjecting plants to extreme conditions, rather than a range of ecologically relevant environments. This not only presumes that the salient differences between the leaf forms have been identified, but ascribes a certain qualitative distinction to the pattern that would appear to set it apart from similar plastic responses of terrestrial species. That this may be
unwarranted is evident from a study of the effects of temperature in *Ranunculus flabellaris*, in which Johnson (1967) demonstrated a linear response in two leaf traits (mean lobe number and mean blade length) over a range of 5 temperatures from 8 to 28°C. This illustrates that, despite the discrete leaf forms observed in nature, the underlying mechanism might best be understood as a quantitative character underlying an apparent threshold response. This situation has been described for other kinds of plasticity or morphological dimorphism, and can be modeled by standard quantitative genetic theory (Roff 1994).

**Heterophylly in aquatic plants**

*Environmental heterogeneity and anticipatory plasticity*

In his fundamental review on the evolution of plastic responses in plants, Bradshaw noted the potential for anticipatory plasticity, a situation in which an environmental cue induces a developmental response *before* the actual environmental change to which that response is adaptive (Bradshaw 1965). In highly predictable environments, reliance upon seasonal cues may facilitate preemptive responses to forthcoming changes in some limiting resource – the shade avoidance response mentioned previously is another such example of anticipatory plasticity. Another example is provided by the deciduous habit in terrestrial species, in which seasonal cues signal impending changes in temperature at the conclusion of the active growth season. In the case of the deciduous habit, shorter photoperiods signal the end of the growth season and increasingly colder temperatures; in the case of shade avoidance, depletion of red wavelengths by neighboring plants signals encroaching canopy shade. In either case, an indirect cue (photoperiod, the ratio of red:far red light) elicits the response (leaf senescence, stem elongation and/or early
flowering) before changes in temperature (deciduous habit) or light availability (shade avoidance) actually occur.

Heterophyllous aquatics exhibit many responses to the environment that are similarly consistent with our understanding of anticipatory plasticity. Despite the initial tendency to describe the leaves of these species typologically relative to their position in the water column (e.g., submerged, floating, aerial), the association between leaf form and submergence is less direct than one might suspect (Tables 2.2 and 2.3, and references therein). In addition to submergence, these species exhibit plasticity in response to daylength, light quality, temperature, relative humidity or some combination of these factors.

At a point in the history of biology when phenotypic plasticity was eschewed as Lamarckian and deemed little more than “environmental noise”, the incompleteness of the correspondence between leaf morphology and submergence quite often vexed some early students of heterophylly, who reacted by questioning the tendency to interpret it as an adaptation to the aquatic environment (Arber 1919). Yet, as later authors soon realized, the fact that these species should exhibit plasticity to each of these factors is less surprising when we consider the propensity for several environmental factors to vary synchronously in many habitats: in shallow wetlands of temperate latitudes, water levels often rise in winter in concert with increasingly shorter days and cooler temperatures, and fall again the following summer as days lengthen and temperatures increase.

Here I consider how plasticity in response to factors other than submergence may enable heterophyllous taxa to detect impending changes in the position of their shoot apices relative to the water column. I have chosen two species to illustrate this point, although several heterophyllous taxa are known to exhibit the patterns depicted here (Tables 2.2 and 2.3). These particular case studies have been selected for several reasons. First, a substantial body of literature characterizes the expression of heterophylly in each, ensuring that the responses I
describe are at least qualitatively consistent across many studies and authors. Second, the environments they inhabit and signals they use to 'anticipate' the environmental change and make the transitions from submerged to aerial conditions are different, thus the pair provides an opportunity to illustrate some of the alternative ways in which anticipatory plasticity may manifest itself in heterophyllous aquatics. Third, the mechanisms invoked in the regulation of plasticity in each case (signal transduction via hormones in the first, light transduction via phytochromes in the second) are the target of active research in physiology and molecular biology, leading to the possibility of not only understanding the ecology of a plastic response, but also its mechanistic basis.

Case study 1: *Proserpinaca palustris* (Haloragaceae)

Shallow, freshwater wetland habitats are apt to vary at many scales: the ‘hummock/hollow’ micro-topography generated from uneven biomass accumulation and patchy vegetation growth results in a mosaic of hills and depressions across the wetland floor, in turn creating heterogeneity in the amount of flooded and exposed substrate (Harper et al. 1965; Sheldon 1974). Seasonal fluctuations also occur when water accumulation is largely dependent upon rainfall, such that higher water levels predominate during cooler months, as evapotranspiration decreases. In these spatially and temporally variable habitats, it may be quite unlikely that a seed will be dispersed to and germinate under similar conditions as the maternal plant that produced it. The high incidence of both clonal growth and the perennial habit of aquatic species (Grace 1993) also increase the likelihood that an individual will experience environmental heterogeneity on a spatial (clonal growth) and/or temporal (perennial habit) scale.

A majority of heterophyllous species that have served as subjects of experimental investigation are inhabitants of these shallow and seasonally flooded depressions. *Proserpinaca*
*palustris*, a species commonly encountered in such habitats, exhibits marked plasticity to daylength, light intensity and temperature in addition to submergence (McCallum 1902; Wallenstein and Albert 1963; Davis 1967; Schmidt and Millington 1968; Kane and Albert 1987). The phenology and natural history of this species has been considered in detail by several authors (McCallum 1902; Wallenstein and Albert 1963). This perennial plant is rarely reported from depths greater than 1 m, and alternates between a submerged and aerial existence via the combination of plastic geotropic shoot responses and reactions to seasonal fluctuations in water level. During the winter shoots grow prostrate underwater, producing highly dissected leaves. In early spring, growth at the stem apices and lateral branches becomes vertical. As these shoots ascend through the water column, leaf morphology changes from a highly filamentous to an entire, lanceolate leaf with serrated margins (Figure 2.2). In addition to these changes in leaf morphology, internode elongation and the eventual induction of flowering (on erect shoots bearing lanceolate leaves) accompany the transition from the submerged to the aerial state.

In addition to exhibiting marked plasticity to submergence, heterophylly in *P. palustris* is also mediated by the effect of daylength on aerial shoots (Davis 1956; Wallenstein and Albert 1963; Davis 1967; Schmidt and Millington 1968). However, submergence overrides this effect: under long days (16h), aerial shoots produce lanceolate, entire leaves with serrated margins (typical aerial leaves in this species), whereas under short days (8h) leaf morphology becomes highly dissected (typical of submerged leaves) despite having developed in air (Schmidt and Millington 1968). When shoots are submerged, new leaves are dissected regardless of daylength; however when exposed to long days (ca. 16h) in conjunction with either high temperature (≥ 30°C) or high light intensity (9000 ft-c) laminar expansion (resulting in the production of entire, aerial leaves) occurs on submerged shoots. The fact that daylength and average summer temperatures often reach their annual maxima prior to drops in the water table, combined with the
observation that these same conditions are capable of eliciting aerial leaves on submerged shoots, is therefore consistent with a scenario of anticipatory plasticity.

Case study 2: *Hippuris vulgaris* (Hippuridaceae)

*Hippuris vulgaris* is another heterophyllous aquatic that, like *P. palustris*, has been well characterized for its responses to a variety of environmental conditions (McCully and Dale 1961; McCully and Dale 1961; Bodkin et al. 1980; Kane and Albert 1982; Goliber and Feldman 1989; Goliber and Feldman 1990). A number of studies in *H. vulgaris* take their impetus from the taxonomic confusion surrounding this species (formerly a member of the Haloragaceae, now assigned to its own monotypic family). The extensive morphological plasticity exhibited by *H. vulgaris* has led to its description under numerous species, varieties and forms which were, upon closer investigation, determined to represent the plastic responses of a single species (McCully and Dale 1961). Although similar to *P. palustris* in being rooted to the substrate and growing vertically through the water column, it differs in that it can be found growing at depths exceeding 3m (Bodkin et al. 1980). At these depths, far-red wavelengths are rapidly depleted (Spence 1976), altering the ratio of red:far-red light in a manner directly opposite to the pattern caused by vegetation shade (Smith 1982). While this wavelength-specific light attenuation is unlikely to be detected by plants inhabiting shallow water (such as *P. palustris*), *H. vulgaris* exhibits heterophyllly directly in response to the balance between red and far-red light (and does not exhibit notable plasticity to daylength: (Bodkin et al. 1980). In nature, aerial leaves are often observed on submerged stems at a nearly constant distance from the water surface (usually within the first 1.5m). In a series of elegant experiments, Bodkin *et al.* illustrate that heterophyllly in *H. vulgaris* is inducible by either supplementing the incident radiation with far-red, or providing post-photoperiodic bursts of far-red light. Based on these observations, they convincingly argue
that this photoreversible response to emergence may be mediated by phytochromes. The parallels to the "shade-avoidance" response are certainly suggestive of anticipatory plasticity in *H. vulgaris*: changes in R:FR (as opposed to increasing light intensity and/or temperature in the shallow, seasonally flooded habitats colonized by *Proserpinaca* spp.) signal an impending change from submerged to aerial conditions that evokes a shift in leaf morphology prior to the emergence of the growing shoot apex. That the shift occurs prior to emergence, resulting in aerial leaves beneath the surface leads to a variety of possible scenarios: (a) the cue is unreliable and the response is maladaptive; (b) the production of aerial leaves on submerged shoots is less detrimental than exposing submerged leaves, devoid of cuticle and stomata, to aerial conditions; or (c) despite overall similarities in morphology, "aerial" leaves produced underwater are functionally more similar to submerged leaves with regard to the thickness of their cuticle and density of stomata. These are testable hypotheses that require appropriate empirical investigation. Regardless, given the demonstrable adaptive significance associated with anticipatory shade-avoidance in terrestrial taxa (Schmitt et al. 1999), further investigation of phytochrome-mediated plasticity in this species is warranted. It is also of considerable interest that plasticity in response to light quality in *H. vulgaris* is unique among heterophyllous aquatic angiosperms, although it has been reported in *Marsilea vestita*, an aquatic fern (Gaudet 1963). This likely reflects the greater depths at which *H. vulgaris* grows relative to the other heterophyllous species that have been subjected to similar experimental investigation.

*The role of plant hormones*

Exogenous applications of abscisic acid (ABA; Table 2.4) and gibberellic acid (GA; Table 2.5) have revealed that these hormones directly influence leaf morphology (references in Tables). Application of either ABA or GA to developing shoot apices mimics the effects of growing plants under certain conditions, suggesting that changes in endogenous levels of these
hormones may catalyze the heterophyllous responses observed under natural conditions. The
effects of ABA have been investigated in numerous heterophyllous aquatics, and show consistent
results across all species investigated so far: plants exposed to ABA produce aerial leaf
morphology while submerged (Table 2.4). ABA is widely recognized as a drought-stress
hormone in terrestrial species, and endogenous levels of ABA have been shown to increase in the
leaves of water-stressed terrestrial plants (Walton and Li 1995), as well as aerial and osmotically
stressed submerged shoots of the heterophyllous aquatic *Hippuris vulgaris* (Goliber and Feldman
1989). Therefore, the hormone that regulates water-relations in terrestrial species appears to serve
a similar function in moderating the response of aquatics to changes in water availability,
bringing us one step closer to understanding how plasticity to submergence may be controlled
across a wide ecological range. Yet we still have far to go: research in *Marsilea quadrifolia*
(Marsiliaceae, Lin and Yang 1999) suggests that blue light is also capable of eliciting aerial leaf
morphology in this species - and that this response occurs independently of ABA biosynthesis.

In heterophyllous aquatics as well as terrestrial species, the effects of gibberellins are
more varied (Table 2.5). *Marsilea drummondii* (Marsiliaceae, a fern), *Potamogeton nodosus*
(Potamogetonaceae, a monocot) and *Callitriche heterophylla* (Callitrichaceae, a dicot) respond to
gibberellic acid with the production of leaves characteristic of submerged plants. This directly
opposes the response to ABA in these species, suggesting that the relative concentration of these
hormones may mediate the production of submerged and aerial leaves (see references in Table
2.5). In contrast, *Eichhornia crassipes* (water hyacinth, Pontedariaceae, a monocot) responds to
exogenous GA with the production of aerial (canopy) leaves (Watson et al. 1982). This concurs
with the responses in species of *Proserpinaca* (Haloragaceae, a dicot) in which exogenous
applications of GA elicit entire, lanceolate (i.e., aerial) leaves on short-day plants grown in 8h
photoperiods, conditions which would normally result in dissected (i.e., submerged) leaves in
these taxa (Wallenstein and Albert 1963; Davis 1967; Kane and Albert 1987). In this genus then,
plasticity to photoperiod may be mediated by changes in endogenous levels of gibberellic acid, whereas plasticity to water availability appears mediated by ABA. Additionally, although aerial leaves in both *E. crassipes* and *P. palustris* are typically associated with flowering, only *E. crassipes* is induced to flower early by exogenous applications of GA (Watson et al. 1982). In *P. palustris*, the onset of sexual reproduction is instead delayed when this hormone is applied (Davis 1967). Thus, it appears that GA is capable of regulating vegetative and reproductive traits somewhat independently, in agreement with the effects of this hormone in terrestrial species and some mutants of *Arabidopsis thaliana* and *Zea mays* (reviewed in Lawson and Poethig 1995).

*Plasticity in anatomy, ultrastructure and physiology*

In addition to the aforementioned patterns in leaf morphology, differences in the submerged and aerial leaves also extend to several aspects of their cellular anatomy, such as stomatal density, thickness of the cuticle, presence of epidermal chloroplasts, and the extent of lamina (especially mesophyll) development (Table 1, Schmidt and Millington 1968; Deschamp and Cooke 1984; Deschamp and Cooke 1985; Young et al. 1987). Some of the most extensive documentation of plasticity in these traits comes from a series of papers exploring the effects of ABA on leaf morphology (Young et al. 1995), anatomy (Young et al. 1987) and ultrastructure (Young et al. 1990) in *Ranunculus flabellaris* (Ranunculaceae). These studies clearly demonstrate consistent differences between the leaves of submerged plants and those produced either in air or in ABA solution (following the general trends listed in Table 2.1). In addition, many of these differences closely parallel those observed in sun and shade leaves (Table 2.1). However, the submerged leaves of *R. flabellaris* possess less total chlorophyll and fewer chloroplasts - contrary to predictions based upon a strict parallel with shade leaves of other species.

In a comparison of five *Potamogeton* species (Potamogetonaceae), floating leaves exhibit higher chlorophyll per unit surface area, but lower chlorophyll per unit volume, than submerged
leaves (no differences are revealed when total chlorophyll is considered on a fresh weight basis, Frost-Christensen and Sand-Jensen 1995). These differences are correlated with contrasting rates of photosynthesis: floating leaves achieve ten-fold higher rates of photosynthesis than submerged leaves in air - a discrepancy generated predominately by extremely high respiration rates in submerged leaves when transferred to this environment. When compared under water, rates of photosynthesis increased (two-fold) in submerged leaves and decreased (four-fold) in floating leaves relative to the performance of the same leaf type in air. Thus, each leaf form not only exhibits higher rates of photosynthesis in its respective environment, but also appears better suited to function in that environment than the alternative leaf - in direct accordance with the adaptive plasticity hypothesis. These findings are corroborated by another study in *Batrachium peltatum* (Ranunculaceae, Nielsen 1993), in which the floating and aerial leaves exhibit reduced rates of photosynthesis when submerged, relative both to the performance of the same leaf type in air and to the submerged leaves in the same environment (rates of submerged leaves in air were not determined). However, contrary to the *Potamogeton* species discussed above, the differences in photosynthetic rate exhibited across the leaf forms of *B. peltatum* are not attributable to consistent differences in total chlorophyll, RUBISCO, or nitrogen content of the leaves. Instead, the physiological differences appear to be directly related to the morphological ones.

The influence of traits like stomatal density, cuticle thickness, chlorophyll concentration and chloroplast density upon generalized leaf function (such as the exchange of gases and water vapor) suggests an underlying functional significance of the production of submerged and aerial leaves. Hypotheses that ascribe an adaptive basis to heterophylly on the basis of such traits thus presume a firm correlation across morphological, anatomical, ultrastructural and physiological levels of organization. While this is indeed supported (in most, but not all, traits examined) by studies such as those conducted in *Ranunculus flabellaris* (Young and Horton 1985; 1987; 1990), research on organogenesis in heterophyllous aquatics reveal that anatomical traits are more labile
than gross leaf morphology. When heterophyllous plants are transferred to new conditions, leaves developing at the time of the transfer show sectors of cells bearing the characteristic differences in architecture prior to changes in overall shape (Schmidt and Millington 1968; Young et al. 1987; Goliber and Feldman 1990; Bruni et al. 1996).

These putative dissociations between the morphological, anatomical and physiological traits (and plasticities) of submerged and aerial leaves confirms several points initially addressed in Bradshaw's (Bradshaw 1965) review, and later extended under the concept of "phenotypic plasticity integration" in several papers by Schlichting (1984; 1989; 1989). First, plasticity is a character-specific, not a genotype-specific attribute: there is little reason to expect a priori that different organismal traits will be equivalently plastic. Second, and partly because of this, plasticities (as with other traits) may be viewed somewhat hierarchically, with physiological and gross morphological plasticities forming end-points along a continuum. With regard to heterophyllous aquatics, we clearly need a more thorough understanding of the correlations among traits and their plasticities before ascribing adaptive significance to a pattern of morphological plasticity on the presumption that it is associated with plasticity in other (anatomical or physiological) attributes of leaf function. Given that few non-heterophyllous aquatic plant taxa have been scrutinized for plasticity in the morphological, anatomical, and ultrastructural characteristics of their leaves to the extent that heterophyllous species have, it is at least plausible that these taxa possess plasticity at other levels of organization, despite lacking plasticity in gross leaf morphology. Evidence of plasticity in these traits in non-heterophyllous aquatics would also be of use in evaluating the adaptive significance of plasticity in overall leaf morphology. An intriguing example of dissociations between morphological and physiological plasticity that is receiving increased attention from physiological ecologists is the differential affinity toward alternative carbon sources (such as CO2 and HCO3-), found to exist independent of leaf surface area (Maberly and Madsen 1998).
Ecological and evolutionary significance

Developmental biologists and experimental morphologists have paid far more attention to the expression of heterophylly than have evolutionary ecologists. In contrast to the wealth of information describing the patterns of leaf development exhibited as a response to specific experimental conditions and hormonal applications, documentation of the functional and adaptive significance of heterophylly in aquatic taxa remains uncommon (but see Cook and Johnson 1968; Clevering et al. 1996), and little is known about the evolution of the heterophyllous habit.

The usual exception is Bradshaw who, in considering the evolutionary significance of plasticity in plants, noted that numerous congeners of heterophyllous taxa fail to express plasticity in overall leaf form (Table 6, Bradshaw 1965). He also pointed out that in many cases non-heterophyllous species (or in some cases less heterophyllous, conspecific populations) appear to lack morphological plasticity despite the fact that they occur in similar habitats and express similar growth form(s) as their heterophyllous relatives. These observations lead to fundamental ecological and evolutionary questions: Does the pattern of variable expression of leaf production within and among populations of heterophyllous aquatics concur with expectations based on the adaptive plasticity hypothesis? Are populations regularly subjected to fluctuations in water availability more heterophyllous than those exposed to more stable terrestrial or submerged conditions? More in general, what are the patterns of evolution in heterophyllous taxa when their phylogenetic history is considered together with their ecology? These questions belong to the twin fields of ecological genetics and evolutionary ecology, conceptual frameworks which I use below to organize the available evidence and my thoughts on the matter.
Ecological genetics

In a study on heterophylly and its bearing upon ecological amplitude, Cook and Johnson (Cook and Johnson 1968) demonstrated that populations regularly experiencing more heterogeneous water levels do retain the greatest plasticity in leaf morphology, and that as the heterogeneity in water level decreases, plasticity in leaf morphology (between submerged and aerial leaves) likewise decreases. This finding is in direct accordance with the expectations of the adaptive plasticity hypothesis; plasticity should be selected for only under some patterns of heterogeneous conditions (Levene 1953) (van Tienderen 1991) (Schlichting and Pigliucci 1995). The lack of plasticity in populations from more homogeneous environments also suggests that there may be a cost (DeWitt et al. 1998) to retaining the ability to be heterophyllous, and that when not selected for plasticity may be rapidly lost. Furthermore, comparisons of variation for plasticity across individuals (presumed to represent different genotypes) revealed a tendency for plants of lower, moister areas to be individually more plastic yet as a group less variable relative to plants of more terrestrial sites. This is in agreement with the expectation that heterogeneous conditions select for phenotypic plasticity: the lack of variation in plasticity among individuals from the heterogeneous environment suggests little genetic variation in this trait among these individuals, possibly as a result of stabilizing selection on the reaction norm. In contrast, plants of more homogeneous habitats (in which plasticity would not be expected to be subject to selection) are more variable in their patterns of plasticity (if expressed at all).

Remarkably, this study remains unsurpassed in its attention to variation for heterophylly expressed both within and among populations. Most other investigations are based upon too few genotypes for even a cursory understanding of genetic variation in this trait (in many cases only a single genotype forms the basis of all observations reported, in others no mention of the genetic material is given). In contrast to the extensive documentation of genetic variation for plasticity for a variety of traits in both animals and plants (Scheiner 1993), I am aware of a single report of
genetic variation for plasticity in heterophylly [Geber, 1992 #830; for a comparable example in terrestrial species see (Winn 1996) and of no estimations of the action of natural selection on heterophylly in aquatic plants. In fact, the only quantitative investigation detailing the nature of selection of which I am aware found no indication of selection for increased plasticity in leaf morphology in a heterophyllous terrestrial species (Winn 1999). This dearth of empirical evidence is surprising given that heterophylly represents a form of phenotypic plasticity that is sufficiently common in aquatics as to have attained nearly universal recognition as an adaptive response to the aquatic environment (Cook and Johnson 1968; Lockhart 1996).

Evolutionary origins

The preponderance of heterophylly across distantly related taxa strongly suggests convergent or parallel evolution in response to the aquatic environment. With regard to the evolution of aquatic angiosperms, several patterns emerge that are germane to the discussion of the association between heterophylly and aquatic life. Whereas the monocots represent a monophyletic group characterized by numerous aquatic taxa, comparably few members of the dicots are aquatic (Sculthorpe 1967). Furthermore, the location of aquatic taxa in the least specialized super-order of the monocots (the Alismatidae) suggests that the aquatic habit arose quite early during the evolution of this group (Grace 1993). Such a unified return to aquatic life from terrestrial ancestors does not appear to have been the case in the dicots. Here aquatic lineages are scattered throughout otherwise terrestrial groups, indicating multiple evolutionary events giving rise to predominately aquatic taxa. (Grace 1993) has noted the overwhelming convergence upon the clonal habit in aquatic monocots and dicots alike. The same observation could be made regarding the prevalence of heterophylly in aquatics, with the added (and intriguing) possibility that plasticity in vegetative traits was selected for as a consequence of the increased duration of the life cycle brought about by the clonal (vegetative, perennial) habit.
Is there an evolutionary connection between environmentally-invariant and environmentally-mediated heterophylly? The observation that several aquatic plants that exhibit marked plasticity in their mature leaves also express heterophyllous sequences as seedlings makes it conceivable that the two patterns are related at some level (Arber 1920; Sculthorpe 1967). The fact that these two forms of foliar modification seem to grade into one another makes it easier to understand them both under the broader heading of leaf development, rather than ascribing to each its own independent regulatory control and evolutionary origin. Was a pattern of increasing plasticity in heterophylly superimposed upon an initially non-plastic developmental sequence as plants returned to aquatic habitats? Several authors have advanced the hypothesis that plastic heterophyllic aquatic plants arose from non-plastic heterophyllic ancestors (Hutchinson 1975; Goliber and Feldman 1990). From an evolutionary perspective, it is indeed suggestive that some of the most notorious cases of non-plastic heterophyllic development in aquatic plants are members of evolutionarily primitive lineages (Sculthorpe 1967; Hutchinson 1975; Les et al. 1991). For example, seedlings of *Sagittaria* (Alismataceae) and *Nuphar* (Nymphaceae) characteristically produce early leaves that most closely resemble the submerged form, regardless of whether growth is initiated under aquatic or terrestrial conditions. In the Nymphaceae, immature leaves are preformed and remain for years as young primordia along the apex of horizontal shoot apices (Cutter 1957; Cutter 1958). A subset of these leaves develops from the rhizome each year, and these exhibit a fairly non-plastic ontogeny - a pattern very similar to preformation in some alpine and arctic species (Aydelotte and Diggle 1997; Diggle 1997).

A fundamental difference between environment-independent heterophylly and the more labile strategy observed in so many plastic heterophyllous aquatics lies in the concept of reversibility. Decades of research with heterophyllous aquatics has revealed their adult foliage to be utterly contingent upon the prevailing conditions, and the production of an aerial leaf at one node does not preclude a switch back to submerged leaf morphology, should the conditions
change (Davis 1956; Cook 1968; Bodkin et al. 1980; Goliber and Feldman 1990; Bruni et al. 1996). Although some of these species indeed do exhibit a non-plastic heterophyllous sequence as seedlings develop, in mature plants the determination of final leaf form appears to be a gradual process that occurs as a direct response to prevailing external conditions. The repeated demonstration of transitional leaves when plants are experimentally transferred from one environment to another further illustrates how the open-ended architecture at the whole-plant level makes reversible responses possible (Goliber and Feldman 1990; Bruni et al. 1996).

If plastic heterophyllous aquatics are indeed descendants of non-plastic ancestors, the relevant question to ask is: why would not a non-plastic sequence, in which juvenile leaves are produced underwater while the adult leaves are produced above, suffice? When considering the evolution of plastic heterophyllly from a more invariant sequence of developmental events, an issue of particular relevance is the degree to which environmental heterogeneity is experienced: this is determined by the life cycle of the plant, and the growth habit adopted. Returning to Figure 1, I reiterate that either as a consequence of their own vertical ascent through the water, or of the seasonal drop in the water table during summer, an emergent aquatic plant will inevitably encounter first a submerged and then an aerial environment as it matures. In a seasonally fluctuating habitat, I note that only through extension of the life cycle (as may be expected with an increasingly clonal, perennial habit) would an individual be likely to re-encounter submergence, as water levels rise again toward the conclusion of the growth season. Thus, we might predict an increased preponderance of highly reversible, plastic responses among those taxa that routinely encounter not only the drops in water level associated with summer, but the return of higher water levels toward the end of the growth season. In contrast, those taxa that conclude their life cycle (either by senescence of the entire plant, or by returning to a dormant, rhizomatous state) shortly after emergence would be unlikely to re-encounter a submerged existence, and would likewise be less apt to express highly reversible modifications of leaf form.
Whether or not close relatives of the more plastic heterophyllic aquatics exhibit an increased propensity toward non-plastic heterophylly (suggesting that plasticity was selected for by the aquatic environment directly) is a question for phylogenetically informed comparative studies. An alternative, yet not necessarily mutually exclusive, hypothesis may be that the terrestrial ancestors of plastic heterophyllous aquatics were predisposed toward an aquatic existence by possessing extraordinary plasticity of leaf morphology in response to some of the other multifarious environmental factors known to covary with water levels in aquatic habitats (discussed in the section on anticipatory plasticity, above). In particular, it would be of interest to understand the relative contribution of each scenario (or combinations of the two in which a non-plastic response led first to terrestrial plasticity and then to aquatic plastic heterophylly), not only in determining which lineages of angiosperms successfully returned to an aquatic environment, but in influencing the colonization of and adaptation to novel (stressful) habitats in general.

Additionally, the observation that several congeners of plastic heterophyllous aquatics lack discernible plasticity in leaf morphology likewise merits further consideration with regard to the relative rates of evolution in morphological and physiological traits. Are these non-plastic taxa simply inhabitants of more homogeneous environments, or have they evolved alternative strategies of coping with aerial and submerged existence? Is there an evolutionary trend toward or away from morphological plasticity with increasing specialization to an aquatic milieu? Are there taxa in which morphological plasticity has been supplanted by more rapidly reversible, possibly less costly, physiological plasticity? Clearly, the study of heterophylly, which began before the publication of Darwin's *Origin*, still offers a panoply of questions addressing fundamental aspects of the evolution and ecology of plant responses to environmental conditions.
Plants of freshwater wetland habitats display a wide variety of putatively adaptive physiological, morphological, and phenological responses to seasonal and local fluctuations in their environment. One of the most notorious patterns of plasticity exhibited by these taxa is the production of distinct leaf forms in association with seasonal and/or spatial fluctuations in water depth (heterophylly). I compared patterns of seasonal variation in leaf morphology among species of the amphibious (semi-emergent) plant genus *Proserpinaca* (Haloragaceae) under field conditions, in an explicit test of the hypothesis that plasticity in leaf morphology is adaptive. Plants with greater plasticity in leaf morphology (i.e., more heterophyllous) exhibited higher relative biomass and greater relative flower and fruit production across two consecutive field seasons, indicating that this pattern of plasticity is adaptive and is currently being maintained by natural selection. This is the first study to report a quantitative estimate of natural selection operating upon patterns of plasticity in leaf morphology in heterophyllous aquatic plant taxa, and one of few comparative studies of adaptive phenotypic plasticity in multiple, co-occurring plant taxa in nature.
Introduction

The pervasiveness of phenotypic plasticity in natural populations (Chapter 1, General Introduction) provides compelling evidence that responsiveness to environmental change may be targeted by natural selection, however explicit tests of this hypothesis remain uncommon in the literature. For plasticity to be adaptive, in the sense of currently advantageous, the expression of an environmentally-induced phenotype should confer higher fitness relative to non-plastic phenotypes when these are compared in the environment in which the pattern of plasticity is presumed adaptive (Schmitt et al. 1999). This enables an operational distinction to be made between plastic responses that are active, functional responses to a known environmental cue from those that are passive, inevitable outcomes of growth under resource-poor conditions (Sultan 1995). For phenotypic plasticity to evolve, genetic variation for plasticity must also be present and natural selection must favor different phenotypes in different environments. Although a number of studies have demonstrated the presence of genetic variation for plasticity in natural populations (e.g., Schlichting and Levin 1990; Pigliucci et al. 1997; Skalova et al. 1997; Smith 1998; St Clair and Sniezko 1999; Donohue et al. 2000), fewer have addressed whether selection is currently acting to maintain phenotypic plasticity in nature (Weis and Gorman 1990; Weis et al. 1992; Miller et al. 1994; Dudley 1996).

The point has been repeatedly made that, due to their sessile habit, plants must tolerate and respond to a wide array of environmental conditions—and may do so via a repertoire of adaptively plastic responses in physiological, morphological, and life history traits (Bradshaw 1965; Grime et al. 1986; Schlichting 1986; Sultan 1995). Strong support for this contention comes from the shade-avoidance responses of plants, in which there is ample evidence of adaptive plasticity, population divergence, and natural selection in favor of earlier flowering (Arabidopsis thaliana, Callahan and Pigliucci in press) and/or stem elongation (Impatiens
capensis, Schmitt et al. 1995; Schmitt et al. 1999; Donohue et al. 2000; Abutilon theophrasti, Weinig 2000) in response to changes in light quality and quantity caused by the growth of neighboring vegetation and resultant increases in competition for sunlight (Schmitt 1997; Aphalo et al. 1999). We should therefore expect phenotypic plasticity to be especially favored in heterogeneous environments, in those organisms whose life history strategies are such that they (or their progeny) have a relatively high likelihood of experiencing that environmental heterogeneity.

Freshwater habitats exhibit marked seasonal and spatial variation in water availability, and many species that inhabit these environments exhibit life cycles that are responsive to these fluctuations. For instance, amphibians with an aquatic larval stage may accelerate the timing and/or rate of metamorphosis in response to pond desiccation (Griffiths 1997; Denver et al. 1998; but see also Leips et al. 2000; Merila et al. 2000). These seasonal fluctuations in water availability also evoke plasticity in the life histories of plants that occupy these habitats, as many aquatic and semi-aquatic (amphibious) species capable of clonal reproduction under flooded conditions will respond to draw-down conditions by flowering and/or increased seedling recruitment from the seed bank (Blom et al. 1990; Barrett et al. 1993; Grace 1993; Robe and Griffiths 1998).

Heterophylly, the striking divergence in the morphology of submerged and aerial leaves borne along the stems of amphibious aquatic plants, has held the attention of researchers since before the turn of the last century (Wells and Pigliucci 2000). Throughout this time, the pattern has been presumed to reflect great adaptive significance (Arber 1920; Sculthorpe 1967; Cook 1968; Hutchinson 1975). The observation that numerous species of amphibious aquatics—scattered across divergent evolutionary lineages—express similar patterns of plasticity in leaf morphology has been hypothesized to be the result of convergent evolution during the return to the aquatic environment by descendants of terrestrial ancestors. Early students of wetland plant
zonation also commented on the preponderance of this trait among those plants occupying a semi-emergent niche in transitional areas between intermittently flooded soils and deep-water habitats (Figure 1.1, see also Spence et al. 1973; Hutchinson 1975). This observation is further supported by a recent study of wetland plant community establishment across a range of flooding depths, durations and frequencies which finds that heterophyllous species tend to be particularly common in habitats characterized by short but recurrent floods (Casanova and Brock 2000). In an early investigation of phenotypic plasticity and its bearing upon ecological amplitude, Cook and Johnson (1968) found that populations of *Ranunculus flammula* (Ranunculaceae) that routinely encountered fluctuating water levels were more heterophyllous than those in more stable environments, whether more consistently submerged or emergent. Heterophylly has also been implicated as a contributing factor in the establishment and spread of the invasive melaleuca tree (*Melaleuca quinquenervia*, Myrtaceae, Lockhart 1996). All of these observations lend further empirical support for the hypothesis that heterophylly may be a particularly favorable morphological response to the variable conditions encountered by emergent aquatic vegetation.

Although a great deal of attention has been paid to the patterns of heterophylly in aquatic plant taxa, I am aware of but a single published account of genetic variation in this trait (among populations of water hyacinth, *Eichhornia crassipes*, Geber et al. 1992), and no attempts to measure natural selection on heterophylly in aquatic plant species. To my knowledge, only one other study has examined the force of natural selection acting upon heterophylly under field conditions (Winn 1999). In that investigation, Winn found no evidence of selection for heterophylly in the terrestrial annual *Dicerandra linearifolia* (Lamiaceae), a finding that contradicts the predictions of the adaptive plasticity hypothesis while challenging our understandings of the ecological significance of plasticity in leaf morphology.

As an explicit test of the hypothesis that heterophylly is an adaptive response to the aquatic plant environment, I evaluated the pattern and strength of natural selection for this trait in
co-occurring species of *Proserpinaca* (Haloragaceae) under field conditions. I examined the relationship between heterophylly and two components of relative fitness (total plant biomass and total flower and fruit production), and report an estimate of contemporary levels of selection acting upon this trait in nature. I address the following questions: (1) Do comparisons among species that exhibit variation in heterophylly provide empirical support for the adaptive phenotypic plasticity hypothesis, in which more plants with greater plasticity in leaf shape also exhibit higher fitness? (2) Do the patterns of selection detected for heterophylly apply equally to vegetative and sexual reproduction? (3) How consistent is the pattern and intensity of selection for heterophylly among consecutive field seasons? I report estimates of genetic variation for plasticity elsewhere (Chapter 4).

**Materials and Methods**

**Study system**

The genus *Proserpinaca* is a member of the Haloragaceae, a predominately aquatic plant family with some members (esp. *Myriophyllum* spp., the water-milfoils) exhibiting a cosmopolitan distribution and at times becoming invasive aquatic weeds. *Proserpinaca* is the only member of this family whose contemporary species’ ranges are restricted to the Americas, spanning latitudes from Nova Scotia southwards to Brazil. The genus consists of two distinct species, *P. palustris* and *P. pectinata*, and a putative hybrid taxon, *P. intermedia*. *P. palustris* and *P. pectinata* are relatively common in the eastern half of North America, particularly along the Atlantic and Gulf coastal plains.

*Proserpinaca* spp. occur in a variety of wetland habitats, ranging from small ephemeral ponds to freshwater marshes, that are typically dry in the summer and flooded in the winter. The
species are perennial and have both vegetative (e.g., stem fragmentation) and sexual modes of reproduction, the latter occurring over several weeks during periods of emergence in late summer. The prominent feature that differentiates the three species of *Proserpinaca* is the morphology of their aerial leaves (in effect, the degree of heterophyll expressed). All of the species produce highly dissected and filamentous submerged leaves (Figure 3.1, center), and the aerial leaves of both *P. pectinata* and *P. intermedia* differ little from the submerged ones in overall outline (with the exception of some subtle increases in the thickness of the midrib; Figure 3.1, bottom right and top, respectively). In contrast, the transition from submerged to aerial leaves in the heterophyllous *P. palustris* is quite pronounced: aerial leaves are lanceolate with serrate margins, and bear little resemblance to the submerged leaves produced by these plants earlier in the season (Figure 3.1, bottom left). Thus, the genus *Proserpinaca* consists of three species that express variable levels of within-plant plasticity in leaf morphology: the markedly plastic *P. palustris*, the marginally plastic *P. intermedia* and the least plastic member of the group, *P. pectinata*.

**Study location**

I conducted my investigations in Goose Pond, one of several karst pan wetlands concentrated in an area on the Eastern Highland Rim of Tennessee known locally as “The Barrens” that is noted for its diverse collections of rare plant and animal species, including several disjunct taxa from both the Atlantic and Gulf coastal plains (DeSelm 1989; DeSelm 1990; DeSelm 1994; DeSelm 1995). All 3 species of *Proserpinaca* co-occur in this protected wetland, making it ideal for field studies. Additionally, paleoecological investigations indicate that these *Proserpinaca* species have occurred in the eastern Highland Rim of Middle Tennessee for some time. Macrofossils of *P. pectinata* have been reported in sediments dating 12,750 to 12,500 years BP at Anderson Pond, ca. 50 miles northeast of Goose Pond in White County. Macrofossils of *P.
*palustris* have also been located in more recent (12,500 yr BP to present) sediments at Mingo Pond, ca. 20 miles south of Goose Pond in Franklin County (Delcourt 1979).

Goose Pond consists of approximately 20 acres of open marsh that is bordered by a mixed deciduous forest of red maple (*Acer rubrum*) and sweet gum (*Liquidambar styracifolia*) with white oaks (*Quercus alba*) becoming increasingly dominant toward the periphery (Figure 3.2). Toward the interior of this fringing forest, the marsh is predominately characterized by a tall (2-3m) dense canopy of the perennial grass *Panicum hemitomon* (Poaceae), that is occasionally interrupted by several oblong (ca. 20m) open areas in which various herbaceous emergent aquatic species (e.g., *Dulichium arundinaceum*, *Eleocharis quadrangulata*, *Ludwigia linearis*, *Pontedaria cordata*, *Sagittaria graminea*, *S. latifolia* and *Xyris* spp.) occur. The decumbent stems of *Proserpinaca* spp. grow interdigitated throughout the marsh, both beneath and outside of the *P. hemitomon* canopy. Water depths throughout the marsh fluctuate across the growth season: during the years of this study (1998 and 1999) the depth of standing water fluctuated from 50-60cm (late winter to early spring) to saturated soils with no standing water (late summer to early fall). In addition to these temporal fluctuations, water depths also vary spatially: when measured at the center of 1m$^2$ quadrats placed along transects (see below), differences in the depth of standing water between adjacent quadrats were as great or greater than differences across transects located meters apart (Figure 3.3).

1998 field season

Cursory observations of the distribution of *Proserpinaca* species at Goose Pond suggested that heterophyllous individuals were more common outside the *Panicum* canopy than beneath it. *Proserpinaca* spp. growing underneath and outside the grass canopy may encounter differences in temperature, water availability and light intensity and /or quality, all factors that affect the expression of heterophylly in this genus (McCallum 1902; Wallenstein 1963; Kane and
Albert 1982). In order to determine if heterophyllous and non-heterophyllous plants were indeed unequally represented in these two habitats, and to compare general patterns of growth and reproduction across *Proserpinaca* spp. at the Goose Pond study site, in May 1998 I established six paired (3 in the grass canopy, 3 in the open) 17m transects throughout the marsh. Transect length was determined by the natural length of the open areas in which transects were placed.

In June 1998 I sampled along four (2 closed, 2 open) of these six transects, and repeated this sampling regime (2 closed, 2 open transects) in July 1998, with the exception that I selected one new pair of transects to maximize the spatial scale covered by the 1998 census of *Proserpinaca*. Thus, although two pairs of transects were sampled each time, only one pair of transects is common to both the June and July 1998 census. I limited each census to four of the six transects because of the labor-intensive nature of processing fresh plants, coupled with the observation that plants stored in the cold room (at 4°C) for longer than two weeks began to resume growth (production of new branches and leaves).

During each census, I sampled along each transect in 17 contiguous 1m² quadrats, haphazardly removing four stems of *Proserpinaca* from each. Because *Proserpinaca* species propagate vegetatively as well as from seed, stems collected in this manner are more appropriately viewed as ramets that may have become detached from the genet rather than whole plants that originated from seed. These ramets were placed in plastic bags, brought to the laboratory and stored in a cold room (4°C) until they could be processed for the measurements of traits that are described below.

To obtain biomass measurements, plants were first washed in tap water to remove mud and epiphytic algae, separated into roots, shoots, and leaves and dried to constant weight at 50°C.
1999 field season

During the 1998 field season several quadrats along closed canopy transects were altogether devoid of *Proserpinaca* stems, contributing to unequal sample sizes in each habitat and an inability to approximate quasi-random sampling in those quadrats with particularly low densities of *Proserpinaca* stems. These results, coupled with the consideration that the open canopy habitat at Goose Pond is generally more typical of the habitats frequented by *Proserpinaca* species throughout their range in the southeastern U.S, prompted me to sample along only open transects in a third census the following year. Therefore, in August 1999 I added one additional open canopy transect to the sampling regime (for a total of four transects), and repeated the sampling strategy described above for June and July 1998 census dates (see above).

Measurements of leaf morphology

At the Goose Pond study site, the presence of *P. intermedia* creates a gradient of leaf morphology between the dissected aerial leaves of *P. pectinata* and the lanceolate aerial leaves of *P. palustris* (e.g., Figure 3.1). Consequently, the expression of heterophyll in these species cannot easily be scored as a binary character. Furthermore, although methods for binary trait data do exist, techniques for the estimation of phenotypic selection (sensu Endler 1986 and Lande and Arnold 1983) are commonly tailored toward the use of quantitative traits, and I considered such measurements of phenotypic selection to be a primary objective of this study.

To obtain a quantitative description of differences in leaf morphology for plants collected during the 1998 and 1999 censuses, I removed and obtained a digital image of three leaves per plant (these leaves were taken from the 1st, 5th and 10th node along the main stem, with the 1st node defined as that nearest the apex bearing the first fully expanded leaf). I used ImagePro (Media Cybernetics, Inc.) to trace the perimeter and determine the area of these leaves from their
digitized images. I calculated the ratio of perimeter to area (P/A ratio) for each leaf, which
provided a measurement of the degree of leaf dissection, a trait that differentiates the aerial leaves
of Proserpinaca species. High P/A ratios typify the highly dissected submerged leaves produced
by all Proserpinaca species as well as the aerial leaves of P. pectinata; slightly lower P/A ratios
characterize the aerial leaves of P. intermedia; and the lowest P/A ratios describe the lanceolate,
serrated aerial leaves of Proserpinaca palustris. This relationship between P/A and leaf
morphology is illustrated by the transition from submerged to aerial leaves shown in Figure 3.4.
Because the species of Proserpinaca all produce highly dissected submerged leaves (and there is
little phenotypic or genotypic variation in this trait, Chapter 4), differences in heterophylly across
these taxa result from differences in their aerial leaf morphology: the most heterophyllous plants
are those with the least dissected aerial leaves. Because submerged leaves rapidly senesce upon
emergence, the majority of plants collected during each census did not possess submerged leaves.
I therefore selected the lowest P/A ratio from the three P/A ratios obtained per plant as a
measurement of the most lanceolate leaf produced by that plant during the growth season in the
field, and a proxy for the extent of heterophylly expressed.

Statistical analyses

I used a general linear model to examine patterns of spatial variation in leaf morphology,
total stem length, total plant biomass and flower/fruit production among habitats (open or closed,
present in 1998 analyses only), transects (nested within habitats in 1998), and quadrats (nested
within transects in both years) across the 1998 and 1999 censuses. Throughout these analyses the
effect of habitat was considered fixed, whereas transects and quadrats were treated as random
effects (Sokal and Rohlf 1995). In accordance with the hierarchical nature of these models, the
effects of quadrat (sub-subgroups) were tested over the mean square error term, the effects of
transect (subgroup) over quadrats nested within transects (sub-subgroups within subgroups), and
finally that of habitat (groups) over transect nested within habitat (subgroups nested within groups). Variances for error terms, interaction terms and main effects were estimated using the General Linear Model (GLM) procedure in SYSTAT version 10.0 (SYSTAT 2000). Type III mean squares are reported for a series of univariate ANOVAs in which each trait (leaf morphology, total stem length, biomass and flower/fruit production) was included as a dependent variable. Because I measured multiple traits per individual plant, all analyses using the same linear model were corrected for multiple comparisons using a sequential Bonferroni procedure (Rice 1989). To better meet assumptions of normality and homogeneous variances (homoscedasticity), total length and total plant biomass were log-transformed and P/A ratios were standardized to the highest value.

I conducted phenotypic selection analyses to evaluate the adaptive significance of the variance in phenotypic traits (the lowest P/A ratio and total stem length per plant) observed during this study (Lande and Arnold 1983). I evaluated a separate multivariate regression model for each component of fitness (total biomass and total fruit production) measured during each census (June 1998, July 1998, and August 1999). For Proserpinaca, as well as numerous other aquatic and semi-aquatic plant taxa, propagation via vegetative means (e.g., stem fragmentation) represents a well-documented mode of population growth, and thus provides more than an index of resource allocation. However, because the species of Proserpinaca also reproduce via seeds, I include this estimate of plant fitness here. For each component of plant fitness, relative fitness was calculated as total fitness (untransformed) divided by the mean fitness within each sampling date (Lande and Arnold 1983). I report selection differentials and selection gradients, which provide estimates of total selection and direct selection, respectively. Selection differentials (s) were calculated as the regression coefficient from separate regressions of relative fitness upon each trait (standardized within each census). Standardized selection gradients were computed as the partial regression coefficients resulting from a multiple regression of relative fitness upon all
traits (standardized as above). Linear (β) and quadratic (γ) selection gradients were estimated separately (Lande and Arnold 1983).

Results

Variation in P/A ratios

Measurements of P/A ratios were generally comparable across the two field seasons (Table 3.1), and revealed a bimodal distribution in the aerial leaf morphology expressed across all individuals (ramets) within each census (Figure 3.5). This reflects the presence of lanceolate-leaved representatives of P. palustris (left peak) and dissected-leaved representatives of P. pectinata (right peak), as well as their presumed hybrid, P. intermedia, at the Goose Pond study site.

The hypothesis that heterophyllous individuals are more concentrated in open areas (with decreased Panicum hemitomon cover) predicts that P/A ratios in this habitat would be consistently lower. During the 1998 census dates, the effects of habitat were marginally significant (but not after Bonferroni comparisons) with respect to this trait (Table 3.2a). Due to their hierarchical structure, nested experimental designs (and sampling regimes) carry less power to detect variation among groups (e.g., habitats) than subgroups (transects and quadrats), so my inability to detect statistically significant differences between the two habitats is not surprising. I observed strong differences among transects during each census in 1998 (again marginal for June, but highly significant for July, Table 3.2a), and comparisons among transects revealed that plants from open transects did in fact tend to have less dissected aerial leaves (Figure 3.6 and 3.7). Differences among transects were not expected in August 1999, when all transects were located in open habitats. However, the anomalous transect (number 6, Figure 3.8) was smaller than the
other three and considerably more dense with *Panicum hemitomon* and associated vegetation, conditions which (based on the 1998 trends) appear to favor the presence of *P. pectinata* over *P. palustris*. Finally, I also detected variation among quadrats, the smallest spatial scale represented in my sampling, during all census dates (Table 3.2a).

In addition to leaf morphology, I measured the total stem length, biomass and number of flowers and fruits produced on all plants collected at each census. Plants collected from the closed and open habitats (1998 only) did not differ with respect to any of these traits (Tables 3.3 through 3.5). Total stem length differed among transects in July 1998 and August 1999, and differed among quadrats in June 1998 and August 1999 (Table 3.3). Total plant biomass, measured in June 1998 and August 1999, showed marginal variation among transects in 1998 (not significant after Bonferroni corrections), and among quadrats in both years (Table 3.4). Total flower and fruit production, measured in July 1998 and August 1999, exhibited less variation than total biomass—significant variation was detected only at the transect level in 1999 (Table 3.5).

*Selection for heterophylly*

Plants with less dissected aerial leaves exhibited significantly higher relative biomass in both years and more flowers and fruits in August 1999 (Table 3.6, Figures 3.9 and 3.10, respectively). Not surprisingly, plants with longer stems also exhibited greater relative biomass and flower/fruit production (with the exception of the August 1999 census, Table 3.6). Estimates of total selection and direct selection did not differ for aerial P/A ratios, indicating that less dissected aerial leaves are still associated with higher relative fitness once differences in the total length of these plants are taken into account. Therefore, the relationship between aerial leaf morphology and relative fitness is not simply an artifact of longer stems bearing leaves that are more lanceolate. Although I included quadratic and cross-product terms in the model, partial
regression coefficients for these analyses never approached statistical significance ($P>0.1$ in all cases) and are therefore not reported.

**Discussion**

Semi-emergent aquatic plants experience markedly contrasting environments during their life cycles. While these plants are submerged, changes in light quality and quantity coupled with the slower diffusion of CO$_2$ may impose limits upon photosynthesis, and anaerobic soils may hinder respiration and nutrient uptake (Sculthorpe 1967; Spence 1976; Bodkin et al. 1980). Although these conditions change upon emergence, water loss quickly becomes a potential limitation to metabolism in this environment. Over the course of the growth season, the majority of plants living in shallow-water habitats will experience both of these environments either as a result of seasonal water table fluctuations or their own vertical growth. Furthermore, no matter how rapidly the transition from a submerged to an aerial existence occurs, the majority of amphibious plants encounter both of these conditions *simultaneously* for a period of time that may range from days to months. At the Goose Pond study site, the species of *Proserpinaca* persist in a partially-emerged state for several months at the beginning and end of the growth season. They may also be partially emerged throughout the growth season during a particularly wet summer.

The primary aim of my investigation was to test the hypothesis that the expression of heterophylly in *Proserpinaca* is an adaptive morphological response to seasonal water level fluctuations. The species of *Proserpinaca* are not divergent with respect to the morphology of their submerged leaves, but differ with respect to their aerial leaf morphology. If plants with less dissected aerial leaf morphology (lower P/A ratios) have higher relative fitness than individuals with more highly dissected aerial leaves (higher P/A ratios), then it can be said that natural
selection favors the pattern of heterophylly expressed in *P. palustris* relative to the more highly dissected aerial leaves of *P. pectinata*. I found this to be the case across two consecutive growth seasons, in which more heterophyllous plants exhibited greater relative biomass in June 1998 and both greater biomass and increased flower and fruit production in August 1999.

At the same time, my results contradict the lack of selection for heterophylly in *Dicerandra linearifolia*, a terrestrial annual plant of North American coastal plain environments (Winn 1999), the only other published selection analysis of heterophylly. This species exhibits within-individual plasticity in leaf anatomy and morphology that coincides with changes in temperature across seasons: the first true leaves produced in winter have lower stomate densities than leaves produced later in the summer, and are also shorter (approximately one-third the length of summer leaves, Winn 1999). Although *D. linearifolia* exhibits these patterns of plasticity, they do not appear to be adaptive. When measured across two consecutive field seasons, selection favored longer leaves in both the winter and summer months, and although reduced stomate density was favored during the winter, this trait was not significantly related to fitness in the summer of either year.

Differences in the life history strategies of *D. linearifolia* and *Proserpinaca* spp. influence the extent of environmental heterogeneity that they experience, and may partially explain why heterophylly is adaptive in the latter and not in the former. *D. linearifolia* is an annual species whose individuals germinate from seed in December and flower the following October (Winn 1999). Thus, Winn notes that these seedlings may be constrained to produce shorter leaves – so the fact that individuals of this species possess smaller leaves in winter is confounded with the developmental stage of these plants at this time of year. In contrast, the species of *Proserpinaca* are perennial and occur in shallow water environments that are usually dry during the late summer months, flooded during the winter and also variable locally in response to heavy rains. In addition to their seasonal trends in leaf morphology, *Proserpinaca*
spp. show seasonally-mediated plasticity in their mode of stem growth: after flowering in late summer, erect stems shift to a prostrate (plagiotropic) habit and return beneath the water where they persist, submerged, throughout the winter months (Burns 1904; Wallenstein 1963). In spring, vertical growth resumes from the apical and/or lateral meristems of these prostrate stems. Thus, a vegetative stem may live for several years in which it alternates between a submerged and an emergent existence. Results from greenhouse studies indicate that photoperiod and temperature evoke these patterns of plasticity in stem orientation, just as they mediate the effects of water level on plasticity in leaf morphology. Transfer experiments also show that patterns of stem orientation and leaf morphology exhibited by *P. palustris* (and to a lesser extent, *P. intermedia*) are readily reversible when plants are exposed to new conditions (McCallum 1902; Wallenstein 1963; Schmidt and Millington 1968; Kane and Albert 1982; Kane and Albert 1987). As a consequence of their longevity, plants of *Proserpinaca* spp. encounter temporal (perennial habit) and/or spatial (clonal growth) resource heterogeneity. Ample evidence suggests that they respond to these changes via a variety of plastic responses to seasonal and local environmental cues.

If heterophylly is an adaptive strategy for amphibious plants, then how do non-heterophyllous taxa persist in these environments? Heterophylly, while a common characteristic of semi-emergent plants, is not ubiquitous across these taxa (Arber 1920; Bradshaw 1965; Sculthorpe 1967; Hutchinson 1975). For instance, *Proserpinaca pectinata* appears to inhabit qualitatively similar environments as does the markedly heterophyllous *P. palustris*—conditions that favor morphological plasticity in leaf form at the Goose Pond study site. Yet, *P. pectinata* exhibits only marginal plasticity in leaf morphology (Figure 3.1). Quantitative comparisons of flooding depth, duration and frequency across these species’ habitats may reveal less heterogeneity in the habitats preferred by *P. pectinata*, but it is also possible that this species responds to fluctuations in water level via plasticity in other traits than those measured here. The idea that plasticity in some traits may confer homeostasis in others was suggested by Bradshaw.
and is related to the concept of plasticity integration developed by Schlichting (1986). This hypothesis is especially appealing with respect to amphibious plants, for which there exists ample evidence of physiological plasticity in the photosynthetic phenotype that may occur either in conjunction with or independent of plasticity in leaf morphology (Nielsen 1993; Spencer and Terri 1994). The submerged leaves of aquatic and amphibious plants have been found to employ a variety of CO₂-concentrating mechanisms, including facultative uptake of alternative carbon sources such as HCO₃⁻ (Allen and Spence 1981; Spence and Maberly 1985), or even the use of CAM or C₄ photosynthetic pathways (Van et al. 1976; Salvucci and Bowes 1981; Keeley 1998). Investigations of the adaptive significance of these forms of physiological plasticity would be especially noteworthy given that physiological traits are often omitted from studies of selection in natural populations (Kingsolver et al. 2001). In Chapter 4 of this dissertation, I examine levels of plasticity in other traits functionally related to submergence in Proserpinaca species.
Chapter 4

Heterophylly in Proserpinaca. II.
Comparisons of morphological and anatomical plasticity in response to water depth under greenhouse conditions.

Abstract

I compared patterns of plasticity in traits commonly associated with flooding tolerance among three species of the semi-aquatic plant genus Proserpinaca (Haloragaceae) in a greenhouse experiment. I investigated if patterns of plasticity in response to contrasting flooding regimes differed among these species, whether these patterns of plasticity were adaptive, and if genetic variation for plasticity was detectable for any of these traits. I found that selection in favor of heterophylly was stronger under consistently flooded conditions. Species that exhibited greater morphological plasticity in leaf shape also exhibited the greatest plasticity in stomate density across submerged, transitional and aerial leaves. The production of aerenchyma tissue significantly reduced flower and fruit production under consistently flooded conditions, suggesting that this trait may result in a trade-off with sexual reproduction. I also observed weak patterns of plasticity in plant architecture, which I discuss in the context of the life history strategy of these and other wetland plant species.
Introduction

Phenotypic plasticity is a consequence of a genotype's interaction with its environment, and represents an integral component of the process by which individuals, populations and species tolerate and adapt to environmental change (Levins 1963; Jain 1979; Bradshaw and Hardwick 1989; Pigliucci in press). Although it may be tempting to think otherwise, phenotypic plasticity is also a trait-specific, rather than a genotype-specific, attribute: an organism's phenotype can be described in terms of a seemingly endless number of traits, each of which may show a range of responsiveness (including no response) to the same environmental cue (Bradshaw 1965). In recent decades, growing interest in the genetic basis of developmental responses to environmental stimuli has revealed that plasticity often has a genetic basis (Pigliucci 1996), and as such, can be targeted by and responsive to natural selection (Chapter 3, see also Schlichting and Pigliucci 1993; Schlichting and Pigliucci 1998; Scheiner and Callahan 1999).

Populations vary considerably in their patterns of response to similar environmental cues (Schlichting 1986; Donohue et al. 2000). These patterns of divergence are generally consistent with a hypothesis of local adaptation to differentially heterogeneous environments (selective regimes) throughout a species' range (Cook and Johnson 1968; Quinn 1987; Macdonald and Chinnappa 1989; Platenkamp 1991; Pigliucci 1992; Oyama 1994; Dudley and Schmitt 1995). Moreover, interpopulation or even interspecific divergence in the plasticity of any one trait is not necessarily correlated with equal divergence in the plasticity expressed in other traits (Clausen et al. 1940; Schlichting and Levin 1986; Schlichting 1989; Schlichting and Pigliucci 1998). All of these observations are consistent with the early proposition that "plasticity of certain characters [traits] may lead to homeostasis of others" (Bradshaw 1965).
The marked plasticity in leaf morphology exhibited by semi-aquatic plants has long held the fascination of developmental biologists as well as taxonomists (Arber 1920; Sculthorpe 1967; Hutchinson 1975). Although the term ‘heterophylly’ is conventionally applied to the morphological differences between submerged, floating and/or aerial leaves in these species, it has long been recognized that changes in leaf shape or form are often accompanied by plasticity in anatomical, ultrastructural and even physiological traits (Schmidt and Millington 1968; Salvucci and Bowes 1981; Anderson 1982; Deschamp and Cooke 1983; Deschamp and Cooke 1984; Young et al. 1987; Goliber and Feldman 1990; Les and Sheridan 1990; Young et al. 1990). At the same time, far less is known about the responses (which may not be morphological in nature) exhibited by non-heterophyllous aquatic plant species that inhabit similar environments and are close relatives of heterophyllous taxa (Bradshaw 1965). Because trait plasticities may be independent, it is an empirical question as to whether or not species with canalized leaf morphology nonetheless exhibit responses to water at other levels of organization (e.g., anatomical, ultrastructural, physiological or biochemical plasticity). As I have articulated elsewhere (Wells and Pigliucci 2000), the fact that heterophyllous aquatic species often exhibit anatomical responses independently of changes in leaf shape when transferred to new environments suggests a certain degree of dissociation between morphological and anatomical traits. By extension, these observations also render it plausible that species that lack morphological plasticity nonetheless exhibit functionally appropriate (i.e., adaptive) responses at other scales of organization.

To examine the relationship between morphological and anatomical plasticity among related species, I examined flooding responses in members of the semi-aquatic plant genus *Proserpinaca* (Haloragaceae). I chose these taxa because I wished to compare the patterns and amounts of response exhibited in co-occurring species that may routinely experience similar temporal and spatial selection pressures because of their coexistence in nature. The species of
Proserpinaca exhibit marked differences in their patterns of plasticity in leaf shape, and the morphologically plastic *P. palustris* has also been shown to exhibit plasticity in other anatomical and physiological traits (McCallum 1902; Wallenstein 1963; Schmidt and Millington 1968). The responses of *P. pectinata* to water depth have not previously been characterized, perhaps because the species is generally regarded as non-heterophyllous by taxonomists (but for brief mention of responses to growth substances, see Davis 1956). In this study, I compare patterns of plasticity in leaf shape, stomate density, aerenchyma tissue production and plant architecture in response to water availability. These traits have all been implicated as adaptive responses to flooding in semi-aquatic plants (Blom et al. 1994; Blom 1999; Casanova and Brock 2000). As a direct test of this hypothesis, I examined the relationship between plasticity in these traits and components of plant fitness under contrasting flooding regimes.

I address the following questions: (1) Although *P. pectinata* does not express notable plasticity in leaf shape, does this species show plasticity in other traits functionally related to submergence? If this non-morphologically plastic species exhibits patterns of plasticity in these traits, this would caution against undue emphasis upon the functional significance of plasticity in leaf morphology *per se* in heterophyllous aquatic taxa. (2) How do the flooding responses of *P. pectinata* compare to those of its congeners (the markedly heterophyllous *P. palustris* and the moderately heterophyllous *P. intermedia*)? (3) When considered independently of leaf morphology, is plasticity in stomate density, aerenchyma tissue production and/or plant architecture adaptive? Finally, (4) do the species of *Proserpinaca* exhibit genetic variation for patterns of plasticity? Although these questions are of particular interest with respect to evolution of the semi-aquatic plant habit, they also relate more broadly to the role of phenotypic plasticity in the tolerance of environmental change, the evolution of ecological niche breadth and the process of adaptation to heterogeneous habitats.
**The experimental population**

To form a stock population of vegetative material, on 10 May 1999 I collected 60 stems from *Proserpinaca* species from a location in middle Tennessee where these species co-occur (Goose Pond, Chapter 3). Because stems of *Proserpinaca* species may routinely reach 40-80 cm in length, stems were collected at a minimum of 5 m apart in order to reduce the likelihood of sampling repeatedly from the same genet. Therefore, I note that I am employing an operational definition of genotype in this chapter, with the underlying assumption that each stem collected in this manner represents a ramet from a different genet, and hence a distinct genotype. To the extent that this assumption does not hold true, this merely renders my ability to estimate genetically-based variation more conservative. Therefore, the initial 60 stems so obtained from Goose Pond are hereafter referred to as 60 different genets, a sub-set of which I later propagated into multiple replicates, or ramets, for use in my experimental population (described below).

On 10 May 1999, the standing water in Goose Pond was greater than 20 cm deep, and *Proserpinaca* stems had only just begun to orient vertically. Consequently, the majority of stems were not yet producing aerial leaves. Because the species of *Proserpinaca* are distinguished on the basis of their aerial leaf morphology (Chapter 3), the 60 genets were taken to the UTK Botany Departmental greenhouses and maintained under uniform growth conditions (described below) until all had produced aerial leaves and could be identified to species (October 1999).

**Growth conditions**

In the greenhouse genets were placed individually in pots filled with 4 cm of peat moss topped with a 1 cm layer of sand (to weigh down the pots). The pots were submerged in one of
four square tanks each consisting of a wooden frame lined with 20 mil plastic and filled to a depth of 10 cm with tap water that was filtered to remove chlorine. Initial growth conditions consisted of ambient greenhouse temperatures and a 16 hr photoperiod (16 hours of light, 8 hours of dark). Water was constantly circulated through these tanks to avoid algal and cyanobacterial blooms, which were frequently associated with high mortality of *Proserpinaca* plants in my pilot studies. Nonetheless, I acknowledge that the introduction of a slight current added to the novelty of the greenhouse environment when compared to the stagnant conditions that are more characteristic of the habitats in which *Proserpinaca* normally occurs.

After five months of growth under these conditions, all genets were producing aerial leaves and could be distinguished taxonomically. To increase the amount of plant material available for the experimental population, between 10-15 October 1999 I propagated all genets by cutting their stems into 5 cm fragments (ramets). These were labeled according to their genet of origin, potted individually, submerged to a depth of 10 cm and allowed to grow for one month prior to the start of the experiment.

**Experimental design**

During 4-10 November 1999 I selected seven genets from each of the three species to form the experimental population, on the basis of the amount of vegetative material available per genet. I took 16 stem cuttings from each of these 21 genotypes (via the same propagation techniques described above), for a total experimental population of 336 plants (3 species x 7 genotypes/species x 16 replicates/genotype). At the time of this last propagation, I collected the following data: (1) the fresh weight of each 5 cm stem segment (initial weight), (2) whether or not the section contained an apical meristem (apical/not apical, a binary trait), (3) the total number of leaves present, and (4) the number of leaves above the soil after planting. The new cuttings were
allowed to establish for two weeks, and on 24 November 1999 they were assigned a random location across the four tanks. The entire experiment lasted approximately 20 weeks (24 November 1999 to 15 April 2000). Plants were re-randomized across tanks once more three weeks later (15 December 1999) to minimize the effects of a light failure above one of the four tanks (which was fixed prior to the start of the treatments).

The experiment was conducted within four adjacent tanks, each of which had a center stand-pipe that allowed water depth to be manipulated independently. A pair of adjacent tanks represented a block, for a total of two replicates of the entire experiment. Each block contained two treatments, one per tank. In one tank, plants were subjected to constant flooding (water was held at 15 cm throughout the experiment. In the other tank, plants experienced a draw-down (water availability was reduced from 15 cm to saturated soils roughly midway through the experiment on 26 January 2000).

Each of the four tanks contained 84 plants (4 replicates/genotype x 7 genotypes/species x 3 species) positioned in a randomized array with a border of pots (filled with peat moss covered by a layer of sand) surrounding the experimental plants to reduce edge effects. Because plant sections that contained an apical meristem tended to exhibit strong apical dominance during pilot studies, replicates were randomly assigned to tanks with the provision that at least one apical section per genet be represented in each of the four tanks.

Plants were monitored for establishment and growth from 24 November 1999 until 26 January 2000, at which time I reduced water levels from 15 cm to saturated soils in two tanks (one tank per block), while maintaining a depth of 15 cm in the two remaining tanks. Water was drained from the tanks over the course of 6 days (26-31 January), at approximately 2 cm per day, until less than 1 cm standing water remained in the tank. These conditions were maintained until the conclusion of the experiment on 15 April 2000.
**Traits measured**

Between the initiation of the experiment (24 November 1999) and the beginning of treatments (26 January 2000), I tracked the number of days it took for the submerged stem apex to reach the water surface (‘days to emergence’). This provided an indication of the responses to stem fragmentation exhibited by genotypes and species, as well as an initial estimate of their respective rates of growth.

To measure plasticity in leaf traits within and among individual plants, genotypes, species and treatments, I collected a submerged, transitional and aerial leaf from each plant over the course of the experiment (Figure 4.1). The basal-most intact leaf (a submerged leaf) and the apical-most intact, fully expanded leaf (a transitional leaf) was removed from the main stem of each plant on 24 or 25 January 2000, prior to the start of the treatments. At the end of the experiment I removed the apical-most intact, fully expanded leaf from the main stem of each plant (because the apices of most plants were positioned above water by this time, these leaves are hereafter referred to as ‘aerial’ leaves). All leaves were carefully removed with forceps and immediately transferred to individual 5 ml vials containing a tissue fixative (FAA, formalin-acetic acid). I measured the perimeter-area ratio (P/A, an index of leaf dissection; see Chapter 3, Materials and Methods) of each leaf from a digital photograph and counted stomates on both lower (lower) and upper (upper) leaf surfaces. I performed these stomate counts directly on the surfaces of preserved leaves using epi-fluorescence microscopy (Olympus System microscope model BX60), in which tissue specimens are surface-illuminated by ultra-violet light (420-480nm). For each leaf surface, stomate counts were summed across three separate fields of view (delimited by a 5mm$^2$ ocular grid micrometer) at the base, middle, and apex of the leaf. These counts are reported as stomata/mm$^2$. 

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Because the production of aerenchyma tissue (in both the stems and roots of aquatic, semi-aquatic and terrestrial plant species) is associated with flooding tolerance, I measured this trait by obtaining a digital photograph of a stem cross-section taken from the base of the main stem of each plant (Figure 4.2). Using the morphometric software program ImagePro (Media Cybernetics, Inc.), I measured the total stem diameter (labeled ‘A’ in Figure 4.2), the inner stem diameter (labeled ‘B’) and the diameter of the central vascular cylinder, or the stele (labeled ‘C’) from digital images of these stem cross-sections. I calculated the extent of aerenchyma tissue as the stele diameter subtracted from the inner stem diameter, corrected by the total stem diameter of each cross section.

In addition to these traits, I measured several aspects of plant growth and architecture at the conclusion of the experiment: number of basal stems (defined as those arising from either the apical or axillary meristems of the original cutting), number of non-basal stems (those arising from axillary meristems on the basal stem), total basal stem length (the cumulative length of all basal stems), and total non-basal stem length (the cumulative length of all non-basal stems). I also measured plant biomass and fruit and flower production at the end of the experiment. All roots, stems and leaves were washed in tap water to remove mud, sand and epiphytic algae, blotted dry and weighed separately (fresh weight to the nearest 0.001 g) before being placed in a drying oven at 60°C. Expected dry weight was obtained from a regression of dry weight on fresh weight for a sub-sample of the experimental plants (n=105, Figure 4.3).

Statistical analyses

A series of univariate ANOVAs revealed that all dependent variables (with the exception of aerenchyma tissue production) measured during the experiment were significantly influenced (p < 0.05) by the initial weight of the vegetative fragment and/or by whether or not that fragment
contained an apical meristem from the parent genet (apical/not apical, results not shown). In contrast, dependent variables were not influenced by either the total number of leaves initially present or by the number of leaves above the soil after planting. Specifically, stomate densities on transitional and aerial leaves, the length of basal and non-basal stems, the number of non-basal stems, all biomass estimates and flower and fruit production were influenced by initial weight. The date of emergence as well as transitional and aerial leaf dissection (P/A ratios) were influenced by both initial weight and the presence of an apical meristem. To account for these influences, I used the residuals from a regression of initial trait values upon initial weight and/or the presence of an apical meristem (as appropriate, based upon the results of these univariate tests) in all subsequent analyses, unless otherwise noted. When transformations were required to meet assumptions of normality and homoscedasticity in analyses of variance (see below), these were conducted on the original trait values prior to obtaining the residuals from a regression on weight and/or the presence of an apical meristem.

To examine patterns of plasticity in submerged, transitional and aerial leaf traits among species and genotypes, I conducted repeated measures ANOVAs among the set of leaves collected from each plant, using leaf dissection (P/A ratios) and stomatal densities (lower and upper leaf surfaces) as dependent variables. Provided that the covariance structure of the repeated measures meets certain criteria (the so-called H-F condition, Huynh and Feldt 1970) repeated measures analysis can be thought of in terms of two univariate analyses: an analysis *among* subjects, and an analysis *within* subjects (for further discussion of repeated measures analysis, and an explanation of these terms, see Gurevitch and Chester Jr. 1986; Littell 1989). The among subjects analysis (among all plants in this study) is based upon the mean of the dependent variable that has been repeatedly measured over time, and examines the influence of main effects upon that trait. The within subjects analysis reports trends in the traits (e.g., leaf shape, stomate
counts) that have been repeatedly measured over the course of the experiment, via a set of interactions among the main effects (species, genotypes) with time (submerged, transitional and aerial leaves) with respect to each dependent variable. Because failure to meet the H-F condition contributes to inflated Type I error rates in the analysis of within subjects effects, I followed the recommendation of Littell (1989) in reporting adjusted p-values (the so-called G-G adjustment, Greenhouse and Geisser 1959) in instances where the H-F condition failed to hold for my data. Variances for error terms, interaction terms and main effects were estimated using the General Linear Model (GLM) procedure for Repeated Measures Analysis in SYSTAT version 10.0 (SYSTAT 2000). Leaf dissection measurements (P/A ratios) were square-root transformed to better meet assumptions of normality of error and homoscedasticity. Despite numerous attempts at transformation (including ranking the data), stomate counts from the submerged leaves were omitted from the repeated measures analysis due to extreme departures from normality (most submerged leaves did not possess stomata, Table 4.1). Stomate counts on both the lower and upper surfaces of transitional and aerial leaves were normally distributed with homogeneous variances and did not require transformation.

I used a general linear model to examine the effects of blocks (indicating heterogeneity among the two replicates of the experiment), treatments (indicating phenotypic plasticity in response to constant vs. reduced water depth), species (differences among the three species of Proserpinaca) and genotypes (differences among genotypes within each species) for several dependent variables. These dependent variables were: aerial leaf dissection and (lower and upper) stomate densities, aerenchyma tissue production, plant architecture (the number and length of basal and non-basal stems), biomass (of stems, leaves and roots) and flower and fruit production. My model also included two sources of genetically based variation in patterns of phenotypic plasticity: treatment by species (differences in patterns of plasticity exhibited among species) and
treatment by genotype (differences in patterns of plasticity exhibited among genotypes within species). Because time to emergence was measured before the start of the treatments, this trait was analyzed using a reduced model in which the treatment main effect and interaction terms were omitted. Throughout these analyses the effects of treatment and species were considered fixed, whereas block and genotypes (nested within species) were treated as random effects. Because genotypes were nested within species, the main effect of species was tested over the genotype(species) mean square term. The main effects of treatment and genotype(species) were tested over the treatment x genotype interaction mean square error term (Sokal and Rohlf 1995). Type III mean squares are reported. As in the repeated measures analyses, measurements of leaf dissection (P/A ratios) were square root transformed to better meet assumptions of normality and homogeneity of variances. Because I measured multiple traits per individual plant, all analyses using the same linear model were corrected for multiple comparisons using a sequential Bonferroni procedure (Rice 1989).

I conducted multivariate matrix comparison tests to examine trait correlations within each environment (constant flooding and draw-down conditions) using parametric Mantel tests (Manly 1986) carried out with the NTSYS-pc2 software package (Rohlf 1998). Mantel tests assess the relationship between two independently derived matrices via the computation of a statistic of association between them (the Mantel test statistic, Z). The observed Z value is then contrasted with an empirical distribution of Z values obtained by 1000 random permutations of one of the two matrices being compared, and evaluated against the null hypothesis (H₀) of no matrix similarity (i.e., that the matrices being compared share no elements in common). While the relative utility of matrix permutation tests (such as the Mantel test) versus other methods of matrix comparison (e.g., restricted maximum likelihood tests) is controversial (Cowely and Atchley 1992; Shaw 1992), I used the Mantel test here because it allowed a quantitative
Description of the similarity in trait correlations among environments. I report the value of the calculated matrix correlation coefficient ($r$) between the pair of matrices along with the corresponding p-value obtained by randomization ($p_{1000}$). Pearson product-moment correlation coefficient matrices are reported separately for each environment in Appendix I. To visualize the extent of similarities in trait associations across environments, I generated dendograms based upon a UPGMA clustering algorithm using the NTSYS-pc2 software package (Rohlf 1998).

I conducted phenotypic selection analyses to evaluate the adaptive significance of the phenotypic traits measured during this study (Lande and Arnold 1983) and to compare these estimates to the nature and intensity of selection upon leaf morphology that I observed in *Proserpinaca* species in the field (Chapter 3). These analyses included the following independent variables: leaf morphology (P/A ratios for submerged, transitional and aerial leaves), transitional stomate density (averaged across lower and upper surfaces), aerial leaf stomate density (lower and upper surfaces treated separately), aerenchyma tissue production, and components of plant architecture (the number and length of basal stems and the number and length of non-basal stems).

Within each environment, I conducted a separate set of regressions for each of two components of plant fitness, one vegetative (plant biomass) and the other sexual (flower and fruit production). For *Proserpinaca*, as well as numerous other aquatic and semi-aquatic plant taxa, propagation via vegetative means (e.g., stem fragmentation) is a well-documented mode of population increase. However, because the species of *Proserpinaca* also reproduce via seeds, I also include this estimate of plant fitness. For each component of plant fitness, relative fitness was calculated as total fitness (untransformed) divided by the mean fitness within each environment (Lande and Arnold 1983). I report *selection differentials* and *selection gradients*, which provide estimates of total selection and direct selection, respectively (Chapter 1). Selection
differentials (s) were calculated as the coefficients from separate regressions of relative fitness upon each trait (corrected for the effects of block and initial weight and/or the presence of an apical meristem where appropriate, and standardized prior to analysis). Standardized linear selection gradients (β) were measured as the partial regression coefficients resulting from a multiple linear regression of relative fitness upon all traits collectively (corrected and standardized as above). Preliminary multiple regression analyses (on abbreviated models of selection including fewer traits) indicated few significant quadratic partial regression coefficients, and because these were not strictly interpretable as either stabilizing or disruptive selection (visual inspection of the data ranges for these traits revealed no local maxima or minima within the range of the data, Mitchell-Olds and Shaw 1987), quadratic selection gradients were not estimated in the analyses presented here. Because of the large number of dependent variables examined, I do not report estimates of correlational selection since they would suffer from a lack of statistical power.

To determine if the patterns of selection varied across species, I conducted an analysis of covariance with species included as a main effect, standardized trait values (corrected for block and initial weight and/or apical meristems as above) as covariates, and relative fitness (either biomass or flowers and fruits) as the dependent variable. In these analyses, significant interactions between species and standardized trait values indicate that patterns of selection differed among the species of Proserpinaca. Because I lacked sufficient statistical power to include all 11 traits and their interaction with the main effect of ‘species’ simultaneously, I conducted these ANCOVAs separately for each trait in which I observed significant selection differentials. The results of these separate analyses were then Bonferroni corrected for multiple comparisons. When significant species by trait interactions for relative fitness were observed, I conducted post-hoc comparisons (using the ‘SPECIFY’ command, Systat, version 10.0) to identify significant comparisons.
Results

Species and genotypes differed in their initial growth rates following propagation (p=0.0214 and <0.0001, respectively, results not shown). A more detailed inspection of this trend revealed that newly propagated cuttings of \textit{P. intermedia} reached the water surface faster than those of the other species (Figure 4.4).

\textit{Within plant plasticity}

The extent of leaf dissection (P/A ratios) decreased across submerged, transitional and aerial leaves, reflecting a shift toward less-dissected leaves over the course of the experiment (Table 4.1) and mimicking the pattern of plasticity expressed by \textit{Proserpinaca} species (to varying degrees) in natural populations across the growth season. I observed little variation among plants in their submerged leaf morphology, in contrast to a nearly bimodal distribution in the distribution of aerial leaf dissection (Figure 4.5). Plants in this experimental population also expressed plasticity in stomate density: the majority of submerged leaves lacked stomata altogether, and average stomate density was highest in the lower surfaces of aerial leaves (Table 4.1). In contrast to aerial leaves, transitional leaves expressed similar stomatal densities on their upper and lower surfaces.

Repeated measures ANOVAs revealed highly significant effects of species and genotype when P/A ratios and stomate densities were averaged across submerged, transitional and aerial leaves (among subjects analyses, Table 4.2). The significant \textit{emergence by species} and \textit{emergence by genotype} interactions indicated that the patterns of leaf dissection and stomate density
expressed across submerged, transitional and aerial leaves differed among species and genotypes (within subjects analyses, Table 4.2). These patterns of within-individual plasticity in leaf traits are depicted in Figure 4.6 (across species) and 4.7 (across genotypes). F-tests of the species main effect upon leaf morphology revealed that species did not differ with respect to the amount of dissection in their submerged leaves (Type III MS = 0.6007; P=0.4747), and that the genotypes representing these species were also homogeneous for this trait (Type III MS = 0.7734; P=0.0827). In contrast, species were divergent in the morphology of their transitional (Type III MS = 28.0643; P=0.0154) and aerial (Type III MS = 82.3754; P<0.001) leaves (as were their genotypes, P<0.0001 for the effect of genotype upon both transitional and aerial leaf morphology). Pairwise post-hoc comparisons of these trends (Figure 4.6, top) revealed that *P. pectinata* exhibited more dissected transitional leaves than either *P. intermedia* (Type III MS = 51.8790; P= 0.0058) or *P. palustris* (Type III MS 29.1697; P= 0.0305), which did not differ from one another with respect to transitional leaf morphology (Type III MS = 3.2620; P=0.4425). However, at the conclusion of the experiment, the aerial leaves of all species were significantly different from one another (p < 0.01 for all pairwise post-hoc comparisons; Figure 4.6, top).

At the species level, divergence in leaf morphology was not always accompanied by differences in anatomy. Although the transitional leaves of *P. pectinata* were significantly different from those of either congener with respect to leaf morphology (Figure 4.6, top), F-tests revealed no differences among species with respect to the stomate densities on either surface of their transitional leaves (*lower*: Type III MS = 612.7495, P=0.2711; *upper*: Type III MS = 405.9123, P=0.3175; Figure 4.6, bottom). However, some degree of interspecific divergence was apparent by the conclusion of the experiment, as the species differed with respect to the stomate densities expressed on the lower surfaces of their aerial leaves (Type III MS = 2946.9107, P=0.0002). Pairwise post-hoc comparisons of this result (Figure 4.6, bottom) revealed that
*P. pectinata* had significantly fewer stomates on its lower leaf surfaces than either *P. palustris* (Type III MS = 5347.3499, P=0.0001) or *P. intermedia* (Type III MS = 3243.2202, P=0.0011). Differences among species with respect to the stomate densities on the upper surfaces of aerial leaves were not significant after correction for multiple comparisons (Type III MS = 824.8872, P=0.0419).

**Plasticity in response to contrasting flooding regimes**

A multivariate analysis of variance (MANOVA) including all traits measured after the initiation of treatments (aerial leaf and plant architecture traits, aerenchyma tissue production, biomass and flower and fruit production) as dependent variables revealed highly significant differences among treatments, species and genotypes (Table 4.3). Although the treatment by species interaction term was significant, the treatment by genotype was not. Individual univariate ANOVA results are presented in Table 4.4a-c.

Despite a highly significant treatment effect in the univariate ANOVA for aerial leaf dissection (Table 4.4a), comparisons of this trait among species (Figure 4.8a) and genotypes (Figure 4.9a) revealed only marginal plasticity across environments. Although the trend was weak, the species tended to produce slightly more dissected aerial leaves under draw-down conditions (Figure 4.8a). Differences between species within environments were more striking than their patterns of plasticity among environments, and were consistent with the patterns of aerial leaf shape that form the basis of species descriptions in this genus: in both environments, *P. pectinata* exhibited more dissected aerial leaves than either *P. intermedia* or *P. palustris*.

Treatments did not exhibit significant effects upon stomatal densities in aerial leaves (Table 4.4a and Figure 4.8b-c), which were higher on the lower than the upper surfaces. Furthermore, *P. pectinata* exhibited less divergence in stomate densities across the lower and upper surfaces of its
aerial leaves, in contrast with both *P. intermedia* and *P. palustris*. I also detected significant variation among the genotypes within species for all aerial leaf traits (Table 4.4a), with the genotypes of *P. pectinata* exhibiting the greatest amount of variation (Figure 4.9b-c).

The treatment effect was highly statistically significant for aerenchyma tissue (Table 4.4b). However, overall patterns of plasticity in aerenchyma tissue production were not particularly striking (Figure 4.10). Species and genotypes also differed in this trait (Table 4.4b). Although both *P. palustris* and *P. pectinata* showed weak responses consistent with adaptive hypotheses (increased aerenchyma tissue under flooding) the responses of these species bracketed those of *P. intermedia*, which was characterized by high but non-plastic values for this trait. However, *P. pectinata* differed from both *P. palustris* (Type III MS = 0.1934, P=0.0123) and *P. intermedia* (Type III MS = 0.1448, P=0.0270) in its production of less aerenchyma tissue under draw-down conditions (Figure 4.10).

I observed highly significant treatment effects on basal, but not non-basal, stem traits (Table 4.4b). The length of basal stems differed among treatments, species and genotypes (Table 4.4b), an effect largely attributable to the longer basal stems of *P. intermedia* relative to either *P. palustris* or *P. pectinata* under continually flooded conditions (Figure 4.11, left). Although differences among species were not significant with respect to the length of non-basal stems (Table 4.4b), these were generally greater in *P. intermedia* (Figure 4.11, left). For most aspects of whole-plant architecture, the reaction norms of *P. intermedia* and *P. palustris* were more similar to each other than either was to *P. pectinata* (Figure 4.11a-b). The responses of *P. pectinata* tended to be somewhat distinct from those of its congeners, although these trends were usually not significant.

I detected significant differences among the treatments, species and genotypes for all components of plant biomass (with the exception of root biomass, in which the main effect of
species was not significant after Bonferroni corrections, Table 4.4c). In general, biomass decreased under draw-down conditions (Figure 4.12) and resulted in moderate decreases in total plant biomass—a vegetative component of plant fitness in this perennial species—in this environment (Figure 4.13, left). Flower and fruit production—another component of fitness—also exhibited differences among treatments, species and genotypes (Table 4.4c), and likewise tended to decrease under draw-down conditions (Figure 4.13, right).

**Phenotypic integration**

The term ‘phenotypic integration’ describes the tendency for organismal responses to environmental stimuli to be comprised of suites of functionally related traits. I examined the relationships among the phenotypic traits measured during this study using a Mantel test of correlation matrices calculated separately within each treatment. This test indicated similarity among trait correlations in the flooded and draw-down environments (calculated correlation coefficient for the two matrices, $r = 0.89798$, $p_{1000}=0.0020$), suggesting that trait relationships were largely conserved between the treatments. At the same time, visual inspection of UPGMA dendograms revealed subtle differences in the relative magnitude, but not the sign, of trait correlations between environments (Figure 4.14a-b). Complete Pearson product-moment correlation matrices, along with Bonferroni corrected tests of their significance, are presented for each environment in Appendix I.

Components of plant architecture (basal and non-basal stem traits) and transitional leaf stomate densities (lower and upper surfaces) were positively correlated with total plant biomass and flower/fruit production in each environment. In contrast, the date of emergence, transitional and aerial leaf dissection, and the density of upper (upper) aerial leaf stomata showed inverse relationships with all other traits. Not surprisingly, the number and length of basal stems were
positively correlated, as were the number and length of non-basal stems—although the strength of
the correlation between number and length of basal stems was stronger under flooding than draw-
down conditions (Figure 4.14, Table 4.7a-b).

Stomate densities on the upper and lower surfaces of transitional leaves were positively
correlated with each other, whereas stomatal densities on aerial leaf surfaces were not (Figure
4.14, Table 4.7). The negative correlation between transitional leaf dissection and transitional leaf
stomate densities (both lower and upper surfaces) corroborates the observation that the more
highly dissected transitional leaves of *P. pectinata* possessed slightly fewer stomata per unit area
than those of *P. palustris* or *P. intermedia* (Figure 4.6b-c). The number of stomates on the lower
surfaces of aerial leaves were negatively correlated with the amount of dissection in these leaves,
whereas the number of stomates on the upper surface was positively correlated with aerial leaf
dissection. These trends can be partially explained by the patterns of plasticity exhibited by *P.
pectinata*, whose highly dissected aerial leaves tended to possess fewer lower, and more upper,
stomata than those of the other two species (Figure 4.6b-c). Finally, increased stomata/mm² on
transitional leaves contributed to higher biomass (upper and lower surfaces) and greater flower
and fruit production (upper surfaces only), in contrast with a negative correlation between
stomate density and biomass with respect to the upper surfaces of aerial leaves (Figure 4.14,
Table 4.7).

Because transitional leaves are generally produced just below, at or above the water
surface, I did not expect divergence in the lower and upper stomate densities of transitional
leaves—and found these values to be correlated (Figure 4.14, Table 4.7). As a result, I calculated
the average stomate density across these surfaces for use in the selection model. In contrast, the
potential for an increased risk of water loss due to evapotranspiration on upper leaf surfaces
suggests that increased stomatal density should be favored on lower (but not upper) surfaces of
aerial leaves. Correlations between the lower and upper stomate densities of aerial leaves were generally weak and non-significant, and I therefore retained lower and upper stomate densities as separate traits in the selection analyses in order to examine their relative contributions to plant fitness (see below).

Aerenchyma tissue production was negatively correlated with aerial leaf dissection under draw-down conditions (Table 4.7), reflecting greater aerenchyma tissue production in *P. palustris* and *P. intermedia* relative to *P. pectinata* (Figure 4.10). Unlike the majority of plant architecture and leaf traits, this trait showed weak and non-significant correlations with biomass and flower and fruit production (Table 4.7).

**Selection analyses**

Decreased aerial leaf dissection was associated with higher relative fitness in both environments, but the intensity of total selection was only significant (after correction for multiple comparisons) under consistently flooded conditions (Table 4.5a). I detected direct selection on aerial leaf morphology with respect to flowers and fruits in both environments, and the intensity of selection approached significance with respect to biomass under flooding but not under draw-down conditions (Figure 4.15). Tests of the heterogeneity of these slopes revealed that the relationship between aerial leaf morphology and biomass differed among species in both environments (Table 4.6). Pairwise comparisons among species revealed that no species pair exhibited the same relationship between aerial leaf morphology and fitness under flooding (*P. intermedia* versus *P. palustris*: Type III MS = 0.9210, *P* = 0.0367; *P. intermedia* versus *P. pectinata*: Type III MS = 4.9787, *P* < 0.0001; *P. palustris* versus *P. pectinata*: Type III MS = 1.1451, *P* = 0.0200) or draw-down conditions (*P. intermedia* versus *P. palustris*: Type III MS = 1.8019, *P* < 0.0001; *P. intermedia* versus *P. pectinata*: Type III MS = 8.0058, *P* < 0.0001;
*P. palustris* versus *P. pectinata*: Type III MS = 1.8346; \( P = 0.0077 \). Visual inspection of these trends revealed a strong pattern of selection favoring less dissected aerial leaves in both environments for *P. intermedia*, a weak trend in this direction for *P. palustris* under draw-down but not flooded conditions, and nearly neutral selection on leaf morphology in *P. pectinata* in both environments (Figure 4.16).

Total selection also favored less dissected transitional leaves in both environments, and the intensity of selection on this trait was stronger in draw-down conditions than flooding (Table 4.5a). Within environments, transitional leaf morphology influenced flower and fruit production more strongly than biomass (a pattern that I also observed with respect to aerial leaf morphology). However, directional selection gradients for transitional leaf morphology were small and non-significant, indicating that total selection on transitional leaf morphology occurred because it is correlated with another trait that is directly related to fitness. An analysis of covariance indicated that the relationship between transitional leaf morphology and fitness did not differ among the species of *Proserpinaca* in either environment (Table 4.6, Figure 4.17). In contrast to transitional and aerial leaves, the morphology of submerged leaves was not associated with either component of plant fitness in either environment (Table 4.5a).

Total selection also favored decreased stomata on the upper surfaces of aerial leaves under flooding with respect to plant biomass, and under draw-down with respect to flower/fruit production (Table 4.5a, Figure 4.18). Heterogeneity of slopes tests indicated that the relationship between upper aerial leaf stomata and relative biomass differed among species under flooded, but not draw-down, conditions (Table 4.6, Figure 4.18). Contrasts under flooding revealed differences among all species in the relationship between stomate density and biomass in this environment (*P. intermedia* versus *P. palustris*: Type III MS = 3.0448, \( P = 0.0002 \); *P. intermedia* versus *P. pectinata*: Type III MS = 9.9535, \( P < 0.0001 \); *P. palustris* versus *P. pectinata*: Type III
However, directional selection gradients were not significant for this trait, indicating stomate density per se is not the target of selection, but is correlated with a trait that is. In contrast to upper stomata on aerial leaves, total selection was neutral with respect to the density of lower stomata, which was not strongly associated with either component of fitness in either environment (Table 4.5a). Finally, total selection favored increased average stomatal density in transitional leaves in both environments, again with respect to both components of fitness, although the intensity of selection was not significant with respect to flower/fruit production under flooding after correction for multiple comparisons (Table 4.5a). Directional selection gradients were significant only with respect to biomass under draw-down conditions.

Total selection was neutral with respect to aerenchyma tissue production (Table 4.5b). However, once differences in other traits were taken into account, I observed significant direct selection against the production of aerenchyma tissue with respect to flower and fruit production under flooded conditions. Although not significant, the sign of the selection gradient under draw-down suggested that aerenchyma tissue decreased flower and fruit production in this environment.

Not surprisingly, longer basal stems consistently resulted in higher relative biomass and flower/fruit production under flooded and draw-down conditions (Table 4.5b). Longer non-basal stems contributed to greater relative biomass and more flowers and fruits in the consistently flooded environment, and greater relative biomass under draw-down conditions. However, the intensity of direct selection on non-basal stem length was only significant with respect to total plant biomass under constantly flooded conditions.

Total selection (with respect to biomass and flowers and fruits) also favored a higher number of basal stems in both environments. However, the positive association between the number of basal stems and the number of flowers and fruits appeared to be due to correlated
selection on length: once the length of basal stems was taken into account, plants with a higher number of basal stems actually showed significantly lower relative flower and fruit production under flooded conditions (Table 4.5b). Although the same pattern of selection (against increased basal stem number with respect to flowers and fruits) was also present under draw-down conditions, the intensity was weaker and non-significant.

Analyses of covariance indicated that the relationship between basal stem length and relative biomass differed among species under flooding (Table 4.6). Visual inspection of this trend (Figure 4.20) revealed a tendency toward smaller increases in biomass per unit increase in stem length in *P. pectinata* relative to either *P. palustris* (Type III MS = 2.5714, *P* < 0.0001) or *P. intermedia* (Type III MS = 4.5145, *P* < 0.0001), which did not differ in the relationship between these traits (Type III MS = 0.2181, *P* = 0.1428). The relationship between the number of basal stems and relative biomass also differed among species under flooding (Table 4.6). This pattern of divergence was due to differences between all of the species (*P. intermedia* versus *P. palustris*: Type III MS = 2.9271, *P* < 0.0001; *P. intermedia* versus *P. pectinata*: Type III MS = 12.5201, *P* < 0.0001; *P. palustris* versus *P. pectinata*: Type III MS = 2.9727, *P* < 0.0001), with the largest increases in biomass per unit increase in basal stem number occurring in *P. intermedia*, followed by *P. palustris* and then *P. pectinata* (Figure 4.21).

**Discussion**

The flooding responses exhibited by aquatic plant species have often been implicated as adaptive strategies in coping with environmental heterogeneity in freshwater wetland environments. Although countless works have demonstrated the physiological basis (e.g., hormonal regulation) of these responses (Voosenek et al. 1992; Voosenek and Blom 1996; Arteca
1997; Vartapetian and Jackson 1997), and others have associated the expression of these traits with increased survival under flooded conditions (Blom and Voesenek 1996; He et al. 1999), this study is among the first to directly quantify the relationship between these traits and two components of plant fitness—biomass and flower and fruit production—across contrasting flooding regimes in comparisons of co-occurring, closely related species. In my multivariate selection analyses, I found evidence of adaptive plasticity in leaf morphology (shape) and anatomy (stomate density) in response to flooding, but no evidence of adaptive plasticity in aerenchyma tissue production. Rather, the expression of aerenchyma in response to flooding was weakly associated with deceased flower and fruit production—suggesting that this trait results in a trade-off between sexual reproduction and plant survival under these conditions. While only further investigation can substantiate (or refute) this hypothesis, it is interesting to consider in light of the fact that aquatic species as a whole are characterized by their propensity for vegetative modes of reproduction (Sculthorpe 1967; Hutchinson 1975; Grace 1993).

*Is plasticity in leaf morphology adaptive?*

These results provide strong empirical support for the hypothesis that the pattern of heterophylly exhibited by semi-aquatic plant taxa is an adaptive response to flooding. The magnitudes and distribution of P/A ratios for aerial leaves produced in the greenhouse were comparable to those measured on aerial leaves from field-collected plants (Chapter 3, Table 3.1), indicating that the patterns of leaf morphology produced under controlled conditions were similar to those found in nature. Consistent with my observations of natural selection in the field, *Proserpinaca* plants with less dissected transitional and aerial leaves exhibited higher relative biomass and greater relative flower and fruit production. More lanceolate aerial leaves were also more favorable under flooded conditions, in direct accordance with the hypothesis that
divergence between submerged and aerial leaf shape is an adaptively plastic response to the semi-aquatic habitats colonized by these plants (reviewed in Casanova and Brock 2000; Wells and Pigliucci 2000).

The expression of heterophylly influenced flower and fruit production more strongly than plant biomass in the greenhouse, a finding that also corroborates prior observations of *Proserpinaca* species in the field (Chapter 3). This suggests that the adaptive significance of heterophylly is not simply a consequence of increased photosynthetic surface area in emergent leaves, which would be expected to increase total plant biomass. I observed significant selection gradients favoring less dissected aerial leaves with respect to flower and fruit production in both environments, consistent with the hypothesis that leaf morphology directly influences this component of plant fitness under flooded and draw-down conditions. However, discrepancies between estimates of total and direct selection indicate that the positive correlation between less dissected aerial leaves and higher plant biomass is attributable to correlations between leaf morphology and other traits.

At the same time, the divergence in aerial leaf morphology exhibited by these species appears to be due to different amounts, rather than patterns, of plasticity: even though the pattern is not particularly striking in *P. pectinata*, all of the species exhibit a reduction in the amount of leaf dissection during their transition from a submerged to an emergent habit. This observation suggests that different selective regimes may have favored different degrees of plasticity in these species (Day et al. 1994). Although far more extensive comparisons of environmental heterogeneity, plasticity and fitness across the ranges of *Proserpinaca* species would be required to test this hypothesis, circumstantial evidence lends it some credibility. For instance, although the trend was non-significant, I observed a tendency toward increased aerial leaf dissection under draw-down conditions during this experiment: this suggests that extremely dry conditions may
select for intermediate leaf size and higher water use efficiency in aerial leaves, as has been associated with drought tolerance in terrestrial species (Dudley 1996). According to this interpretation, the more dissected aerial leaves of *P. pectinata* may be more efficient under periods of sustained draw-down—which is consistent with the observation that increasingly lobed or dissected leaves are more efficient at convective heat loss at higher temperatures (Givnish 1987; Winn 1999).

Although *Proserpinaca* species are divergent in their aerial leaf morphology, they were notably uniform in their production of highly dissected submerged leaves (Figure 4.5, left). The lack of phenotypic and genotypic variation in submerged leaf morphology probably explains my inability to detect selection on this trait, while simultaneously suggesting the operation of strong selection in the past. This result is expected on the basis of Fisher’s fundamental theorem of natural selection, which posits that the sustained operation of directional or stabilizing selection over time should deplete additive genetic variation on the trait that is targeted by selection (Fisher 1930). If selection has been particularly effective with respect to submerged leaf morphology in the past, such that the highly dissected leaves that are currently expressed represent the optimal leaf phenotype in this environment, then we would expect little genetic variation in the trait to remain in natural populations.

Observations from this study, accompanied by the fact that multiple semi-aquatic plant species have converged upon similarly dissected submerged leaf morphology (Arber 1920; Fassett 1957; Sculthorpe 1967; Hutchinson 1975), provide good circumstantial evidence that the performance of submerged leaves is associated with their highly dissected form (probably because of this translates to increased surface area, Whitwer 1995). However, the relationship between surface area and acclimation to the aquatic environment is not perfect, as other species
have been found to meet the demands of an aquatic existence via other mechanisms, including the faculative uptake of $\text{HCO}_3^-$ (which may occur independently of surface area, Maberly and Madsen 1998).

Is plasticity in leaf shape accompanied by adaptive plasticity in other leaf traits?

In addition to leaf shape, the species of *Proserpinaca* exhibited striking within-plant plasticity in both the density and distribution of stomata, in accordance with previous observations of *P. palustris* (Schmidt and Millington 1968) and other heterophyllous aquatic plants (Anderson 1982; Deschamp and Cooke 1985; Young et al. 1987). Submerged leaves were more or less devoid of stomata, transitional leaves exhibited considerably more stomata than submerged leaves at approximately equal frequencies on their lower and upper surfaces, and aerial leaves exhibited the highest density of lower stomates and (with the exception of *P. pectinata*) fewer upper stomates than transitional leaves.

Although some authors have attempted to infer stomatal function from the presence of starch in the guard cells on submerged leaves (Wallenstein 1963), the traditional roles ascribed to stomata are of dubious importance in an aquatic environment. The extremely low density of stomata on the submerged leaves of *Proserpinaca* species argues against a functional role for stomata under water, and suggests that stomata may even be disadvantageous under such circumstances. Comparisons of photosynthetic efficiency or other indices of metabolic performance between submerged leaves with and without stomata would provide a direct test of this hypothesis. However, probably because submerged leaves do not naturally produce stomata, these comparisons do not exist in the literature. Nevertheless, comparisons of floating and submerged leaves in five *Potamogeton* species reveal that the photosynthetic performance of
floating leaves (which possess stomata and a thick cuticle) declines roughly four-fold when submerged—which suggests that these traits may be a liability in these conditions (Frost-Christensen and Sand-Jensen 1995).

The floating leaves of many aquatics such as the water lilies (*Nymphaea* spp., Nymphaeaceae) often possess stomata on the upper surfaces of their leaves (Hopkins 1999). *Proserpinaca* species do not possess floating leaves, but their transitional leaves are generally produced just below, at or above the water surface. It is possible that these leaves routinely encounter both environments, and may also rest upon the water in a manner analogous to the floating leaves of other species. The transitional leaves produced by plants in my experimental population had more stomata than submerged leaves, and stomate counts were similar across the lower and upper surfaces of these transitional leaves. Furthermore, increased stomate density in transitional leaves was associated with both measures of fitness: higher relative biomass in both environments, and higher flower and fruit production under draw-down conditions.

Increased stomatal density on transitional leaves was also favored by selection under draw-down than flooded conditions, but discrepancies between selection differentials and gradients indicate that stomate density was often selected indirectly through correlations with other traits. This may reflect the fact that transitional leaves were exposed to air in this environment, whereas in the consistently flooded treatment these leaves remained more or less submerged—an environment in which the function of stomata is questionable.

Although it is tempting to infer that putative costs associated with occasionally submerged (and presumably non-functional) stomata are outweighed by the benefits of possessing functional stomata should transitional leaves become emergent, an explicit test of this hypothesis would involve comparisons of the relative performance of transitional leaves in submerged and emergent environments. I did not conduct such comparisons in this study, but
note that while submerged leaves undergo rapid senescence upon exposure to air during seasonal draw-down cycles, transitional leaves often remain attached to vertically-oriented stems throughout the growth season in the field (personal observation). This observation provides further circumstantial evidence that submerged leaves are strictly adapted to aquatic environments, whereas the performance of transitional leaves may be more facultative. This performance may be partially attributable to the presence of functional stomata on these leaf surfaces.

The leaves of many terrestrial dicots typically have more stomata on their lower leaf surfaces relative to the upper surfaces of these same leaves (Hopkins 1999), presumably because reduced rates of evapotranspiration allow the intake of CO₂ to be maximized while minimizing water loss. The species of *Proserpinaca* also expressed this pattern of asymmetrical stomate distribution in their aerial leaves. Furthermore, the more morphologically plastic species (*P. palustris* and *P. intermedia*) also exhibited greater increases in stomate density on their lower leaf surfaces in response to emergence.

The species were characterized by different patterns of stomate distribution across the upper surfaces of their aerial leaves. Although species means were not significantly different after multiple comparisons, *P. pectinata* exhibited a tendency toward higher upper surface stomate density in its aerial leaves than either *P. palustris* or *P. intermedia*, which both reduced the density of upper surface stomata across their transitional and aerial leaves. Observations from terrestrial species (see above) suggest that upper stomata should be mal-adaptive, and these results confirm this hypothesis: I found that increased upper stomate density was associated with decreased relative biomass under flooding as well as lower flower and fruit production under draw-down conditions. However, selection against stomata on the upper surfaces of aerial leaves was only apparent for *P. intermedia*, and neutral with respect to this trait in both *P. pectinata* and
P. palustris. Therefore, even small increases in stomata on upper leaf surfaces were mal-adaptive for P. intermedia, whereas increased stomate density on upper leaf surfaces did not have an adverse effect on plant biomass in either P. pectinata or P. palustris. This result may be explained by other characteristics of stomate density and/or distribution not measured here (such as the degree to which stomates are sunken within the epidermis), or other aspects of leaf anatomy (e.g., species differences in cuticle thickness).

Is the production of aerenchyma adaptive?

The production of aerenchyma tissue is one of the more characteristic flooding responses exhibited by wetland and facultative wetland species (Smirnoff and Crawford 1983; Blom 1999; Jackson and Armstrong 1999). This response, mediated in part by the synthesis of and changes in receptivity to the hormone ethylene, reduces the diffusive resistance to oxygen under the O₂ limiting conditions encountered by plants living in anaerobic soils and may allow photosynthetically-derived O₂ to be recycled for use in respiration (Jackson and Armstrong 1999). Plants in the study population were all characterized by the presence of aerenchyma tissue in their lower stems and roots, however I observed little plasticity in aerenchyma tissue production among the flooded and draw-down treatments.

Plants that exhibited more aerenchyma tissue also produced fewer flowers and fruits under consistently flooded conditions—a finding that suggests that this anatomical response to flooding may be accompanied by a metabolic cost that contributes to lower reproductive output. However, a straightforward interpretation of this result is complicated by the fact that Proserpinaca spp. (like the majority of aquatics) are perennial: a short-term reduction in flower and fruit production may be offset by the ability to flower in subsequent years. Because all of the plants in this study (except one) survived the course of the experiment, I could not measure
viability selection. However, the fact that aerenchyma tissue production has been directly correlated with survival in several aquatic species is consistent with this explanation (Loreti and Oesterheld 1996; He et al. 1999). The short time scale encompassed by my experiment cannot address this possibility, but if this is the case, then it suggests a life history trade-off (Sinervo and Svensson 1998): aerenchyma may be positively correlated with viability and yet exert negative influences upon fecundity.

My characterization of aerenchyma tissue in these species is limited for two reasons. First, although pilot studies indicated the presence of aerenchyma in the stems of *Proserpinaca* plants, this response may be more common in the roots of wetland species (Blom et al. 1994; Jackson and Armstrong 1999). Like many wetland species, *Proserpinaca* spp. are characterized by adventitious roots that form along prostrate stems in the water column. Because I did not measure aerenchyma in these structures, and because I do not know whether the production of aerenchyma in lower stem tissues corresponds to the extent of aerenchyma found in adventitious roots, it is possible that these organs will express different patterns of plasticity in this flooding response. Second, and more importantly, the plants in the draw-down treatment were first grown submerged and then emergent in the experiment. Because the cells of aerenchyma tissue are lignified, plants are not able to reverse the production of aerenchyma tissue once produced. As a result, my ability to detect plasticity in aerenchyma tissue production was probably compromised.

*Is plasticity in plant architecture a component of the flooding response?*

In addition to the expression of heterophylly and the production of aerenchyma tissue, yet another characteristic flooding response of many wetland plants is the elongation of stems, petioles and/or leaves such that these photosynthetic organs can be reoriented above the water surface (Blom 1999). Comparisons of several *Ranunculus* (Ranunculaceae) species from habitats
differing in flooding frequency and duration reveal that *R. seleratus*, a species of shallow mudflats, responds to flooding via petiole elongation; failure to elongate above the water results in mortality rates comparable to those in the flooding-intolerant *R. bulbosus*, a species of seldom flooded river levees (He et al. 1999). The species of *Proserpinaca* all exhibited longer basal stems under flooding, indicating that they too employ this strategy in response to inundation.

The elongation response is also associated with flowering in many wetland species, which despite their semi-aquatic habit generally express ancestral modes of pollination in which flowers must be elevated above the water (Barrett et al. 1993). Although the reproductive biology of *Proserpinaca* species is poorly understood, *P. palustris* generally does not flower while submerged (and flowering has been tightly linked to the production of aerial leaves in this species, Davis 1967). In this experiment, the number of basal and non-basal stems was positively correlated with biomass but negatively correlated with flower and fruit production under flooding (after differences in stem length were taken into account). While I did not measure the distribution of flowers and fruits across basal and non-basal stems, it is possible that allocation to more stems slowed the net rate of growth for each stem, thereby delaying emergence above water and the ability for each stem to flower and set fruit.

Increased allocation to vegetative growth may come at the expense of sexual reproduction in the species of *Proserpinaca*, similar to the trade-off suggested with respect to aerenchyma production in these species. Although the short temporal scale encompassed by my greenhouse study cannot address the implications of these patterns to lifetime fitness in these perennial species, the preponderance of clonal growth and low or sporadic seed set in aquatic angiosperms has been appreciated by numerous authors (Sculthorpe 1967; Hutchinson 1975; Grace 1993). The seasonal and spatial variability in water depth that characterizes shallow freshwater habitats may promote clonal growth during periods of submergence, and seedling
recruitment during draw-down cycles (Blom 1999; Casanova and Brock 2000). The observation that many aquatic species exhibit facultative shifts between vegetative and sexual modes of reproduction provides anecdotal evidence consistent with the hypothesis that heterogeneous environments may maintain plasticity in life histories (Bowers 1996; Prati and Schmid 2000; Mandujano et al. 2001).

Conclusions

These observations of Proserpinaca species are consistent with the hypothesis that less dissected aerial leaves are directly favored by natural selection. This finding also corroborates a general pattern of convergence toward heterophyllous leaf expression in amphibious plants, which is commonly cited as one of the strongest examples of adaptive phenotypic plasticity (Bradshaw 1965; Cook 1968; Schlichting and Pigliucci 1998). At the same time, this study also documents the existence of adaptive patterns of plasticity in stomate density in a species (P. pectinata) with canalized leaf morphology, illustrating that patterns of divergence in aerial leaf morphology may not hold for other traits. While it has long been recognized that heterophyllous aquatics exhibit plasticity in numerous other morphological, anatomical and physiological traits (reviewed in Wells and Pigliucci 2000), far less attention has been paid to patterns of plasticity in the non-heterophyllous relatives of these species. This dearth of phylogenetically informed comparisons is unfortunate, because such studies may provide crucial clues on adaptive divergence in flooding responses in particular and on adaptive phenotypic plasticity in general (Ackerly 2000).

Numerous submerged aquatic plant species exhibit plasticity in their modes of inorganic carbon acquisition and fixation with little or no concomitant change in leaf morphology (Allen
and Spence 1981; Spence and Maberly 1985; Bowes 1989). It has been suggested repeatedly that these patterns of plasticity reflect a repertoire of strategies for coping with fluctuations in water pH and the availability of dissolved inorganic carbon (Madsen and Sand-Jensen 1991; Bodner 1994; Maberly and Madsen 1998). Furthermore, a diverse array of strategies exist across species: despite morphological and anatomical similarities, *Hydrilla verticillata* and *Elodea canadensis* (both submersed aquatic plants of the Hydrocharitaceae) differ in that the former exhibits facultative shifts to C4-like metabolism when faced with carbon-limited conditions (Salvucci and Bowes 1981), whereas *E. canadensis* turns to HCO$_3^-$ as an alternative source of inorganic carbon (Madsen et al. 1996). Characterizations of physiological and morphological responses to CO$_2$ and HCO$_3^-$ in *Elodea canadensis*, *Ranunculus peltatus* and *Callitriche cophocarpa* reveal a great degree of physiological plasticity in *E. canadensis* with little change in specific leaf area, compared with physiological and morphological responses in *R. peltatus*, and a seeming emphasis upon morphological responses in *C. cophocarpa*.

It is informative to consider the distribution of these morphological and physiological strategies in relation to the scale of environmental heterogeneity experienced by these species: Are non-morphological responses more prevalent in fine-scale environments that change often and less predictably, because of the shorter response time and inherent reversibility of these processes? If so, one might observe physiological responses to be more common among plants, populations and species that experience less predictable environmental heterogeneity, whereas morphological responses may be more common among plants that encounter heterogeneity on a more predictable scale. With respect to the species mentioned above, *Hydrilla verticillata* and *Elodea canadensis* are submersed aquatic macrophytes, whereas *Ranunculus peltatus* and *Callitriche cophocarpa* are both amphibious species. To the extent that submersed species
encounter fine-scale fluctuations in water chemistry (e.g., pH and/or dissolved inorganic carbon availability) more often than seasonal variation in temperature, photoperiod and water availability, one might expect a preponderance of physiological plasticity in these species.

Patterns of plasticity in morphological, structural, physiological and life history attributes have been extensively documented in diverse lineages of semi-aquatic plant groups. In many cases the physiological basis of these responses (e.g., hormonal regulation) is well understood and consistent with adaptive hypotheses; however several fundamental questions need to be examined further. There are few comparisons of the degree of plasticity exhibited within and among taxa in relation to the amount of environmental heterogeneity that they experience, and quantitative descriptions of the adaptive significance of plasticity in these traits is lacking. These tactics of flood avoidance illustrate the myriad ways in which wetland plants may have adapted to the aquatic environment. They also present intriguing systems in which to study some of the more tantalizing questions with respect to the origin, maintenance, and evolution of phenotypic plasticity in heterogeneous habitats.
Chapter 5
General Discussion

The degree of plasticity expressed in aquatic and amphibious plants and the conspicuous heterogeneity of their habitats provided ideal opportunities for me to test the deceptively simple hypothesis that adaptive phenotypic plasticity confers increased tolerance of environmental variation (see reviews by Arber 1920; Sculthorpe 1967; Hutchinson 1975). My research is the first documented account of natural selection acting in favor of heterophylly. Comparisons of Proserpinaca species revealed that more heterophyllous plants exhibited higher relative biomass and/or greater flower and fruit production—and in the greenhouse, the relationship between heterophylly and these components of fitness was strongest under flooded conditions. My findings are in strong agreement with the adaptive plasticity hypothesis, while they are in contrast with the only other published account of selection analyses with respect to this trait, which revealed an absence of selection for heterophylly in a terrestrial plant (Dicerandra linearifolia, Lamiaceae) of coastal plain environments in the southeastern U.S. (Winn 1999). It is likely that these different results are due to differences in developmental patterns and life history strategies in these systems. Proserpinaca species are long lived perennials with indeterminate clonal growth that routinely encounter seasonal and spatial fluctuations in water availability. In comparison, Dicerandra linearifolia may be developmentally constrained from the expression of marked phenotypic plasticity due to its annual habit. Although the natural habitats of both Proserpinaca and Dicerandra are variable, the life cycle of Proserpinaca (like many aquatics) maximizes both the frequency at which this heterogeneity is encountered and the ability to exhibit reversible plastic responses to these fluctuations.

My work is also among the first to document plasticity in multiple, functionally related traits—and represents one of very few comparative studies of closely related heterophyllous and
non-heterophyllous species. The results illustrate that patterns and amounts of plasticity do not always correspond across multiple traits—even if they share a common functional relationship with the same environmental cue. Despite its canalized leaf morphology, the non-heterophyllous *Proserpinaca pectinata* exhibited functionally appropriate plasticity in stomate density. This suggests that different selective regimes may favor different degrees of plasticity among closely related species during the process of their ecological and evolutionary divergence (Day et al. 1994). In *Proserpinaca*, divergence in aerial leaf morphology could be associated with drought tolerance: I observed a weak trend toward increased aerial leaf dissection under draw-down conditions in this greenhouse experiment, consistent with the fact that dissected or lobed leaves are expected to be more effective at dissipating heat and restricting water loss (Lewis 1972; Givnish 1987; Winn 1999). It is possible—although speculative at this time—that throughout their ranges in the southeastern U.S. the habitats of *P. pectinata* are generally characterized by longer, more extensive periods of draw-down than those of *P. palustris*.

These results also suggest a trade-off between flooding tolerance and flowering in *Proserpinaca* species—at least over the short term. The life history strategies of amphibious plants are also notoriously plastic, as many of these species express facultative shifts between asexual (vegetative) and sexual (flowering, seed set) modes of reproduction contingent upon the frequency, duration and extent of seasonal draw-down cycles (Sculthorpe 1967; Hutchinson 1975; Barrett et al. 1993; Grace 1993; vanGroenendael et al. 1996). At the same time, these authors have also noted a trend away from sexual reproduction in aquatics—but the extent to which this is the result of unsuitable environmental conditions for flowering and/or seedling establishment as opposed to a genetically-fixed loss of sexual reproduction in these taxa is still poorly understood (reviewed in Barrett et al. 1993).
Although my dissertation focuses on the possible adaptive significance of heterophyly in aquatic species, terrestrial plants also exhibit notable modifications of leaf form in response to seasonal and local variations in their environment. Because aquatic and amphibious angiosperms are descended from the land plants, and because the cues that regulate leaf morphology are similar in aquatic and terrestrial species (e.g., photoperiod, temperature and moisture), it is intriguing to consider that the expression of phenotypic plasticity may have predisposed certain lineages toward the colonization of aquatic habitats. This possibility has been recognized by others, and summarized lucidly by Arber (1920) when she hypothesized that “... the aquatic Angiosperms thus include, by a process of sifting, those plants whose terrestrial ancestors were endowed with a strong tendency towards heterophyly.”

The hypothesis that the ancestors of heterophyllous aquatics may also have exhibited strong plasticity in leaf morphology suggests that phenotypic plasticity may play a role in adaptive radiations (Schmalhausen 1949; Waddington 1953). Despite the fascinating implications of this hypothesis, the macroevolutionary significance of adaptive phenotypic plasticity has rarely been addressed (but see West-Eberhard 1989; Day et al. 1994; Losos et al. 2000). The possibility that plasticity may facilitate the invasion of new habitats (perhaps by peripheral populations) clearly has far-reaching implications for our understanding of patterns of ecological niche breadth, speciation and the maintenance of biological diversity.
References


*Evolution* 53: 1704-1713.


Appendix
Table 2.1. Characteristic responses to light (sun vs. shade leaves) and water availability (submerged vs. emergent leaves) in terrestrial and heterophyllous aquatic species, respectively.

<table>
<thead>
<tr>
<th>trait</th>
<th>response to decreased light intensity in terrestrial species</th>
<th>response to submergence in heterophyllous aquatic species</th>
</tr>
</thead>
<tbody>
<tr>
<td>specific leaf area</td>
<td>larger</td>
<td>larger</td>
</tr>
<tr>
<td>leaf thickness</td>
<td>thinner</td>
<td>thinner</td>
</tr>
<tr>
<td>leaf margins</td>
<td>less lobed and/or toothed</td>
<td>variable *: more linear/lobed</td>
</tr>
<tr>
<td>stomatal frequency</td>
<td>lower</td>
<td>lower</td>
</tr>
<tr>
<td>mesophyll</td>
<td>reduced palisade layer (shorter cells and fewer layers)</td>
<td>reduction to complete absence of palisade layer</td>
</tr>
<tr>
<td>venation</td>
<td>reduced vein density</td>
<td>reduced vein density</td>
</tr>
<tr>
<td>cuticle</td>
<td>reduced</td>
<td>reduced or absent</td>
</tr>
<tr>
<td>epidermal cells</td>
<td>larger, more undulate margins</td>
<td>larger, long and narrow</td>
</tr>
<tr>
<td>location of chloroplasts</td>
<td>more epidermal</td>
<td>found in epidermis</td>
</tr>
</tbody>
</table>

*Depends upon generalized leaf form: monocot leaves typically become more linear (sometimes wavy at margins), and dicots more lobed/divided, underwater (e.g., Figure 2.2).

1 Lewis 1972 and references therein
2 Givnish 1987 and references therein
3 Sculthorpe 1967
Table 2.2. Experimental conditions capable of eliciting aerial-type foliage on submerged shoots. Information presented here is expanded from the summary given in Table 5 of Deschamp and Cooke, 1984.

<table>
<thead>
<tr>
<th></th>
<th>exogenous ABA</th>
<th>high temperature *</th>
<th>long photoperiods</th>
<th>high light intensity *</th>
<th>low R:FR ratio</th>
<th>osmotic stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>FERNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsilea quadrifolia</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M vestita</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MONOCOTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potamogeton nodosus</td>
<td>+3,4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DICOTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callitriche heterophylla</td>
<td>+5</td>
<td>+5</td>
<td></td>
<td></td>
<td>+5</td>
<td></td>
</tr>
<tr>
<td>C. intermedia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eryngium vesiculosum</td>
<td></td>
<td>+7</td>
<td></td>
<td></td>
<td>+6</td>
<td></td>
</tr>
<tr>
<td>Hippuris vulgaris</td>
<td>+8,9</td>
<td>+9</td>
<td></td>
<td></td>
<td>+11,8</td>
<td></td>
</tr>
<tr>
<td>Limnophila indica</td>
<td>+12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proserpinaca intermedia</td>
<td>+13</td>
<td></td>
<td></td>
<td>+16</td>
<td>+16</td>
<td></td>
</tr>
<tr>
<td>Proserpinaca palustris</td>
<td>+13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranunculus flabellaris</td>
<td>+17</td>
<td>+18,19</td>
<td></td>
<td>+18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. aquatilis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+20</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: submerged leaves are not defined by consistent criteria in all studies, thus some of the studies summarized above examined only anatomical characters (e.g., stomatal density) whereas others looked at gross leaf morphology. See references for further information. * As noted by Deschamp and Cooke, the effects of these factors are often contingent upon photoperiods of a certain length. References: 1 Liu 1984; 2 Gaudet 1963; 3 Anderson 1982; 4 Gass and Anderson 1998; 5 Deschamp and Cooke 1984; 6 Jones 1955; 7 Webb 1984; 8 Kane and Albert 1982b; 9 Goliber and Feldman 1990; 10 Bodkin, et al. (*only in conjunction with high light intensity); 11 McCully and Dale 1961a; 12 Ram and Rao 1982; 13 Kane and Albert 1982a; 14 Kane and Albert 1987; 15 Schmidt and Millington 1968; 16 McCallum 1902; 17 Young and Horton 1985; 18 Bostrack and Millington 1962; 19 Johnson 1967; 20 Cooke, 1969.
Table 2.3. Experimental conditions capable of eliciting submerged-type leaves on shoots grown aerially. Information presented here is expanded from the summary given in Table 5 of Deschamp and Cooke, 1984.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>exogenous GA</th>
<th>low temperature</th>
<th>short photoperiods</th>
<th>low light intensity</th>
<th>high relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FERNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Marsilea drummondii</em></td>
<td>+21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DICOTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Callitriche heterophylla</em></td>
<td>+5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hippuris vulgaris</em></td>
<td></td>
<td></td>
<td></td>
<td>+11</td>
<td>+11*</td>
</tr>
<tr>
<td><em>Proserpinaca intermedia</em></td>
<td>+14</td>
<td>+20,13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. palustris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+16</td>
</tr>
<tr>
<td><em>Ranunculus flabellaris</em></td>
<td>+19</td>
<td>+23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. aquatilis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+17</td>
</tr>
<tr>
<td><em>Synnema triflorum</em></td>
<td>+23</td>
<td>+20</td>
<td></td>
<td>+23</td>
<td>+23</td>
</tr>
</tbody>
</table>

References: numbers 1-20 are same as Table 1-2. 21Allsopp 1962; 22Wallenstein and Albert 1963; 23Cook 1968.
Table 2.4. Characteristics of leaves produced by heterophyllous aquatics when grown submerged in solutions of abscisic acid (ABA)

<table>
<thead>
<tr>
<th>leaf trait</th>
<th>affect of ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>general leaf form</td>
<td>aerial</td>
</tr>
<tr>
<td>leaf venation</td>
<td>decreased</td>
</tr>
<tr>
<td>stomatal density</td>
<td>increased</td>
</tr>
<tr>
<td>epidermal cells</td>
<td>less elongate</td>
</tr>
<tr>
<td>cuticle</td>
<td>thicker</td>
</tr>
<tr>
<td>flowering</td>
<td>accelerated</td>
</tr>
</tbody>
</table>

### Table 2.5. A comparison of the effects of gibberellic acid on select heterophyllous aquatics with some heteroblastic terrestrial species.

<table>
<thead>
<tr>
<th></th>
<th>general leaf form</th>
<th>leaf morphology</th>
<th>leaf trait</th>
<th>internode/stem elongation</th>
<th>flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HETEROPHYLLOUS AQUATICS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callitriche heterophylla$^1$</td>
<td>submerged</td>
<td>more linear</td>
<td>decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callitriche platycarpa$^2$</td>
<td>submerged</td>
<td>more linear</td>
<td>increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marsilea drummondii$^3$</td>
<td>submerged</td>
<td>more dissected</td>
<td>decreased</td>
<td></td>
<td>increased</td>
</tr>
<tr>
<td>Potamogeton nodosus$^4$</td>
<td>submerged</td>
<td>less dissected</td>
<td>decreased</td>
<td></td>
<td>increased</td>
</tr>
<tr>
<td>Proserpinaca palustris$^5,6,7$</td>
<td>aerial$^5$</td>
<td>less dissected</td>
<td>increased</td>
<td>delayed/inhibited</td>
<td></td>
</tr>
<tr>
<td><strong>TERRESTRIAL SPP.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eucalyptus$^8$</td>
<td>adult</td>
<td></td>
<td>accelerated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hedera$^9,10$</td>
<td>juvenile</td>
<td>more lobed</td>
<td>delayed/inhibited</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$As defined by the author(s).

$^2$In this study GA$_1$ was examined only in conjunction with ABA; thus results are relative to ABA treatments.

$^3$This response is contingent upon short photoperiods; in Proserpinaca, GA$_1$ supplants LD and/or high temperatures w/ regard to leaf form but not flowering (see Davis 1967).

References: 1 Descharmp and Cook 1984; 2 Musgrave et al 1972; 3 Allsopp 1962; 4 Anderson 1982; 5 Davis 1967; 6 Kane and Albert 1982a; 7 Kane and Albert 1987; 8 Scurfield and Moore 1958;

Table 2.6. Examples of non-heterophyllous taxa exhibiting similar growth form as their congeneric heterophyllous relatives. Expanded from Bradshaw, 1965 to include non-heterophyllous representatives of the genera noted in the text and tables of this review.

<table>
<thead>
<tr>
<th>MONOCOTS</th>
<th>heterophyllous spp.</th>
<th>non-heterophyllous spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potamogeton</td>
<td>P. nodous</td>
<td>P. lucens</td>
</tr>
<tr>
<td></td>
<td>P. natans</td>
<td>P. perfoliatu s</td>
</tr>
<tr>
<td></td>
<td>P. polygonifolius</td>
<td></td>
</tr>
<tr>
<td>Juncus</td>
<td>J. heterophylla</td>
<td>J. obtusifolius</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DICOTS</th>
<th>heterophyllous spp.</th>
<th>non-heterophyllous spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callitriche</td>
<td>C. intermedia</td>
<td>C. stagnalis</td>
</tr>
<tr>
<td></td>
<td>C. heterophylla</td>
<td></td>
</tr>
<tr>
<td>Proserpinaca</td>
<td>P. intermedia</td>
<td>P. pectinata</td>
</tr>
<tr>
<td></td>
<td>P. palustris</td>
<td></td>
</tr>
<tr>
<td>Ranunculus</td>
<td>R. aquatilis</td>
<td>R. hederaceus</td>
</tr>
<tr>
<td></td>
<td>R. flabellaris</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. flammula</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.1. A schematic representation of the multifarious growth forms found in aquatic plants, with particular emphasis upon the various environments experienced by their leaves. A: floating rosettes, leaves which rest at the air-water interface are often morphologically homogeneous within individual plants but bear stomates on their upper surfaces only. B and C: free-floating, non-rooted plants and/or consistently submerged species are often characterized by fairly uniform leaf morphology; D, E and F: rooted plants may produce floating and/or aerial leaves, depending largely upon whether or not growth occurs from a rosette or a vertical stem.
Figure 2.2. Three leaves removed from an individual of Proserpinaca palustris (Haloragaceae). From left to right, the leaves represent the transition from submerged to aerial leaf morphology observed in the vertically ascending stems of this species. Submerged and aerial leaves also exhibit notable differences in anatomical characteristics (Schmidt & Millington 1968).
Table 3.1. Means, ranges and coefficients of variation (CV) of phenotypic traits measured during each field census. The number of ramets (individuals) collected per census are indicated in parentheses.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 230)</td>
<td>(n = 252)</td>
<td>(n = 263)</td>
</tr>
<tr>
<td>Total stem length</td>
<td>44.781 ± 15.133</td>
<td>44.841 ± 10.893</td>
<td>34.042 ± 12.868</td>
</tr>
<tr>
<td>Total plant biomass</td>
<td>0.2594 ± 0.1353</td>
<td>--</td>
<td>0.2971 ± 0.1388</td>
</tr>
<tr>
<td>Flower and fruit production</td>
<td>--</td>
<td>9.5317 ± 7.7754</td>
<td>5.1407 ± 6.2449</td>
</tr>
</tbody>
</table>

-- Trait not measured.
Table 3.2. Minimum perimeter-to-area ratios (a) and total stem length (b) across the three census dates. Type III Mean Squares and p-values are shown. Boldface type indicates effects significant at the p < 0.05 level after a sequential Bonferroni procedure corrected for multiple tests within each census date.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f</td>
<td>MS</td>
<td>p</td>
<td>d.f</td>
<td>MS</td>
<td>p</td>
<td>d.f</td>
</tr>
<tr>
<td>a. P/A ratios</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>1</td>
<td>6.968</td>
<td>0.0305</td>
<td>1</td>
<td>7.890</td>
<td>0.0488</td>
<td>--</td>
</tr>
<tr>
<td>Transect</td>
<td>2</td>
<td>0.222</td>
<td>0.0133</td>
<td>2</td>
<td>0.416</td>
<td>0.0029</td>
<td>3</td>
</tr>
<tr>
<td>Quadrat</td>
<td>61</td>
<td>0.048</td>
<td><strong>0.0020</strong></td>
<td>62</td>
<td>0.065</td>
<td><strong>0.0006</strong></td>
<td>63</td>
</tr>
<tr>
<td>Error</td>
<td>165</td>
<td>0.0268</td>
<td></td>
<td>186</td>
<td>0.0343</td>
<td></td>
<td>196</td>
</tr>
<tr>
<td>b. total stem length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>1</td>
<td>0.656</td>
<td>0.1973</td>
<td>1</td>
<td>0.099</td>
<td>0.6787</td>
<td>--</td>
</tr>
<tr>
<td>Transect</td>
<td>2</td>
<td>0.181</td>
<td>0.1747</td>
<td>2</td>
<td>0.432</td>
<td><strong>0.0007</strong></td>
<td>3</td>
</tr>
<tr>
<td>Quadrat</td>
<td>61</td>
<td>0.101</td>
<td><strong>0.0003</strong></td>
<td>62</td>
<td>0.052</td>
<td>0.3324</td>
<td>63</td>
</tr>
<tr>
<td>Error</td>
<td>165</td>
<td>0.0502</td>
<td></td>
<td>186</td>
<td>0.0482</td>
<td></td>
<td>196</td>
</tr>
</tbody>
</table>
Table 3.3. Total plant biomass and flower and fruit production across census dates. Type III Mean Squares and p-values are shown. Boldface type indicates effects significant at the p < 0.05 level after a sequential Bonferroni procedure corrected for multiple tests within each census date.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.f</td>
<td>MS</td>
<td>P</td>
<td>d.f</td>
<td>MS</td>
<td>P</td>
<td>d.f</td>
<td>MS</td>
<td>P</td>
</tr>
<tr>
<td>a. plant biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>1</td>
<td>12.506</td>
<td>0.0974</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transect</td>
<td>2</td>
<td>1.423</td>
<td>0.0329</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadrat</td>
<td>61</td>
<td>0.394</td>
<td>&lt;0.0001</td>
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<td>--</td>
<td>196</td>
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</tr>
<tr>
<td>b. flowers/fruits</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1</td>
<td>0.081</td>
<td>0.9742</td>
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<td>Transect</td>
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<td>60.475</td>
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<td>302.694</td>
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<td>0.1031</td>
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<td>Error</td>
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<td>--</td>
<td>--</td>
<td>186</td>
<td>56.848</td>
<td></td>
<td>196</td>
<td>32.935</td>
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</tr>
</tbody>
</table>

-- Trait not measured.
Table 3.4. The relationship between plant traits and two components of relative fitness across three census dates. Boldfaced type indicates significance after adjusting for multiple tests. Significant quadratic selection gradients (γ) are reported in the text.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Biomass</th>
<th>Flowers &amp; Fruits</th>
<th>Flowers &amp; Fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total selection</td>
<td>Direct selection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(s)</td>
<td>(β)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total selection</td>
<td>Direct selection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(s)</td>
<td>(β)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total selection</td>
<td>Direct selection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(s)</td>
<td>(β)</td>
<td></td>
</tr>
<tr>
<td>June 1998 (n=230)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf shape</td>
<td>-0.2508</td>
<td>-0.1695</td>
<td>---</td>
</tr>
<tr>
<td>(P&lt;0.0001)</td>
<td>(P&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total stem length</td>
<td>0.3640</td>
<td>0.3211</td>
<td>---</td>
</tr>
<tr>
<td>(P&lt;0.0001)</td>
<td>(P&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 1998 (n=252)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf shape</td>
<td>---</td>
<td>---</td>
<td>-0.0803</td>
</tr>
<tr>
<td>(P=0.1279)</td>
<td>(P=0.7019)</td>
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<td>-0.0190</td>
</tr>
<tr>
<td>Total stem length</td>
<td>---</td>
<td>---</td>
<td>0.3306</td>
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<tr>
<td>(P&lt;0.0001)</td>
<td>(P&lt;0.0001)</td>
<td></td>
<td>0.3271</td>
</tr>
<tr>
<td>August 1999 (n=263)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Leaf shape</td>
<td>-0.0832</td>
<td>-0.0647</td>
<td>-0.2790</td>
</tr>
<tr>
<td>(P=0.0038)</td>
<td>(P=0.0063)</td>
<td></td>
<td>-0.2712</td>
</tr>
<tr>
<td>Total stem length</td>
<td>0.2669</td>
<td>0.2624</td>
<td>0.1298</td>
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<tr>
<td>(P&lt;0.0001)</td>
<td>(P&lt;0.0001)</td>
<td></td>
<td>0.1107</td>
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</tbody>
</table>

--Trait not measured.
Figure 3.1. Variation for plasticity in leaf morphology across the species of *Proserpinaca*. The leaf at center is representative of the submerged leaf morphology exhibited by all of these taxa during winter months, while stem apices are submerged. The three leaves shown for each taxon represent the leaf morphology exhibited by that taxon during summer months, while stem apices are above water.
Figure 3.4. Goose Pond.
Figure 3-3. Standing water depth along four transects sampled in June 1998.
Figure 3.4. Leaves removed from a single individual of *Proserpinaca palustris*. In this species, the transition from a submerged to aerial habit is marked by decreased dissection in aerial leaves. I measured differences in the degree of leaf dissection by the ratio of perimeter to area for each leaf removed from an individual plant. Above, this ratio is 47.32 for the highly dissected leaf at far left and 16.19 for the leaf at far right. Measurement error: ± 0.004.
Figure 3-5. Perimeter:area as a measurement of aerial leaf morphology. Bimodal distribution emphasizes the divergent leaf morphology exhibited in the aerial leaves of *P. palustris* (lanceolate leaves, at left) and *P. pectinata* (dissected leaves, at right).
Figure 3-6. Distribution of leaf morphology across 4 transects sampled in June 1998.
Figure 3-7. Distribution of leaf morphology across 4 transects sampled in July 1998.
Figure 3-8. Distribution of leaf morphology across 4 transects (all open) sampled in August 1999.
Figure 3-9. Plant biomass as a function of perimeter-area ratio. See Table 3.6 for statistical details.
Figure 3-10. Flower and fruit production as a function of perimeter-area ratio. See Table 3.6 for statistical details.
Table 4.1. Means and coefficients of variation (CV) of phenotypic traits measured during the experiment.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Submerged leaves</th>
<th></th>
<th>Transitional leaves</th>
<th></th>
<th>Aerial leaves</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± 1 SD</td>
<td>C.V.</td>
<td>Mean ± 1 SD</td>
<td>C.V.</td>
<td>Mean ± 1 SD</td>
<td>C.V.</td>
</tr>
<tr>
<td>Leaf shape: P/A</td>
<td>51.2326 ± 11.6990</td>
<td>0.2284</td>
<td>38.5376 ± 16.8292</td>
<td>0.2271</td>
<td>19.4074 ± 8.7210</td>
<td>0.4494</td>
</tr>
<tr>
<td>Stomate density: adaxial</td>
<td>1.0687 ± 1.0770</td>
<td>1.0078</td>
<td>8.8776 ± 6.5606</td>
<td>0.7390</td>
<td>6.3731 ± 5.8336</td>
<td>0.9153</td>
</tr>
<tr>
<td>Stomate density: abaxial</td>
<td>1.0090 ± 0.8634</td>
<td>0.8557</td>
<td>8.8866 ± 7.1852</td>
<td>0.8086</td>
<td>23.0985 ± 8.6342</td>
<td>0.3738</td>
</tr>
</tbody>
</table>
Table 4.2. Within-individual plasticity in leaf traits, analyzed via repeated measures ANOVAs. Type III MS and p-values are shown.

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>leaf shape: P/A</th>
<th>d.f.</th>
<th>stomate density: abaxial</th>
<th>d.f.</th>
<th>stomate density: adaxial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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<td>MS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>P</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td><strong>among subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>2</td>
<td>254.0911</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
<td>&lt;0.0001</td>
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<tr>
<td>Genotype</td>
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<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
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<tr>
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<td>63.7940</td>
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<td>48.3181</td>
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<td>0.2780</td>
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<td>0.1897</td>
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<td>0.1367</td>
</tr>
<tr>
<td>Error</td>
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<td>0.8368</td>
<td>313</td>
<td>36.9335</td>
<td>313</td>
<td>21.6994</td>
</tr>
<tr>
<td><strong>within subjects</strong></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Emergence * Species</td>
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<td>26.9427</td>
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<td>937.0338</td>
<td>2</td>
<td>976.7084</td>
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<tr>
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<td>&lt;0.0001</td>
<td></td>
<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
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<tr>
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<td>2.3862</td>
<td>18</td>
<td>324.7292</td>
<td>18</td>
<td>295.3998</td>
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<tr>
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<td>&lt;0.0001</td>
<td></td>
<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
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<td>241.7690</td>
<td>1</td>
<td>21.6935</td>
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<tr>
<td></td>
<td></td>
<td>0.1614</td>
<td></td>
<td>0.0066</td>
<td></td>
<td>0.2954</td>
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<tr>
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<td>32.3671</td>
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</table>
Table 4.3. MANOVA examining experiment-wide effects on aerial leaf traits (morphology and stomate density), plant architecture (the number and length of basal and non-basal stems), aerenchyma, biomass (stems, roots and leaves included as separate traits) and flower/fruit production. Type III Mean Squares, F-ratio and p-values are shown. Univariate ANOVAs for these traits are presented in Table 4.4a-c.

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>Wilks' Lambda</th>
<th>MS</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>0.0073</td>
<td>79.4011</td>
<td>&lt;0.0001</td>
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</tr>
<tr>
<td>Species</td>
<td>24, 14</td>
<td>0.0052</td>
<td>7.4924</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td>216, 98</td>
<td>0.0000</td>
<td>2.9523</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
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<td>24, 14</td>
<td>0.0182</td>
<td>3.7423</td>
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</tr>
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<td>0.8129</td>
<td>5.3904</td>
<td>&lt;0.0001</td>
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Table 4.4. Univariate ANOVAs examining (a) aerial leaf traits, (b) aerenchyma and plant architecture and (c) biomass and flower/fruit production. Type III Mean Squares and p-values are reported. Boldface type indicates significance after sequential Bonferroni correction for multiple comparisons.

(a) aerial leaf traits

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>MS</th>
<th>P</th>
<th>MS</th>
<th>P</th>
<th>MS</th>
<th>P</th>
</tr>
</thead>
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<td>1.9051</td>
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<td>0.0001</td>
<td>216.4367</td>
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</tr>
<tr>
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<td>0.4761</td>
<td>0.2492</td>
<td>40.3608</td>
<td>0.3024</td>
<td>3.4478</td>
<td>0.7300</td>
</tr>
<tr>
<td>Treatment * Genotype</td>
<td>18</td>
<td>0.3169</td>
<td>0.2431</td>
<td>31.5571</td>
<td>0.8299</td>
<td>10.7656</td>
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</tr>
<tr>
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<td>275.9144</td>
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<td>67.0215</td>
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<tr>
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<td>46.3641</td>
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</table>
Table 4.4. continued.

(b) aerenchyma tissue and plant architecture

<table>
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<tr>
<th>Effect</th>
<th>d.f.</th>
<th>aerenchyma tissue</th>
<th>number of basal stems</th>
<th>length of basal stems</th>
<th>number of non-basal stems</th>
<th>length of non-basal stems</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>MS</td>
<td>P</td>
<td>MS</td>
<td>P</td>
<td>MS</td>
</tr>
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<td>Treatment</td>
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<tr>
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<td>Genotype</td>
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<td>2296.7370</td>
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<tr>
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<td>0.0190</td>
<td>0.0968</td>
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<tr>
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</table>
Table 4.4. continued.

(c) biomass and flower and fruit production

<table>
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<tr>
<th>Effect</th>
<th>d.f.</th>
<th>stem biomass MS</th>
<th>P</th>
<th>leaf biomass MS</th>
<th>P</th>
<th>root biomass MS</th>
<th>P</th>
<th>flowers/fruits produced MS</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<td>0.0065</td>
<td>0.8212</td>
<td>0.0276</td>
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</tr>
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<td>0.0002</td>
<td>0.0191</td>
<td>0.0091</td>
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<td>$&lt;0.0001$</td>
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<tr>
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<td>0.0512</td>
<td>0.0203</td>
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</table>
Table 4.5. Total selection and standardized direct linear selection gradients illustrating the relationship between two components of plant fitness and (a) leaf traits and (b) aerenchyma and plant architecture in two environments. Selection differentials ($s$) were Bonferroni corrected; boldfaced type indicates significance after these adjustments for multiple comparisons.

**(a) leaf traits**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Constant Flooding (n=168)</th>
<th></th>
<th>Draw-down (n=167)</th>
<th></th>
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<tr>
<td></td>
<td>Biomass</td>
<td>Flowers &amp; Fruits</td>
<td>Biomass</td>
<td>Flowers &amp; Fruits</td>
</tr>
<tr>
<td></td>
<td>Total selection ($s$)</td>
<td>Direct selection ($\beta$)</td>
<td>Total selection ($s$)</td>
<td>Direct selection ($\beta$)</td>
</tr>
<tr>
<td>P/A: aerial leaf</td>
<td>-0.1637</td>
<td>-0.0593</td>
<td>-0.2979</td>
<td>-0.2220</td>
</tr>
<tr>
<td></td>
<td>(P=0.0001)</td>
<td>(P=0.0621)</td>
<td>(P=0.0001)</td>
<td>(P=0.0020)</td>
</tr>
<tr>
<td>P/A: transitional leaf</td>
<td>-0.1279</td>
<td>-0.0392</td>
<td>-0.2067</td>
<td>-0.0586</td>
</tr>
<tr>
<td></td>
<td>(P=0.0026)</td>
<td>(P=0.1704)</td>
<td>(P=0.0027)</td>
<td>(P=0.3523)</td>
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<tr>
<td>P/A: submerged leaf</td>
<td>-0.0229</td>
<td>0.0003</td>
<td>-0.1034</td>
<td>-0.0859</td>
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<tr>
<td></td>
<td>(P=0.5703)</td>
<td>(P=0.9890)</td>
<td>(P=0.1126)</td>
<td>(P=0.0894)</td>
</tr>
<tr>
<td>adaxial stomate density:</td>
<td>-0.1154</td>
<td>0.0300</td>
<td>-0.0927</td>
<td>0.0958</td>
</tr>
<tr>
<td>aerial leaf</td>
<td>(P=0.0067)</td>
<td>(P=0.2706)</td>
<td>(P=0.1833)</td>
<td>(P=0.1127)</td>
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<td>0.0885</td>
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<td>aerial leaf</td>
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<td>(P=0.1554)</td>
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<td>0.1778</td>
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<tr>
<td>transitional leaf</td>
<td>(P=0.0002)</td>
<td>(P=0.3601)</td>
<td>(P=0.0102)</td>
<td>(P=0.4601)</td>
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</table>

$\dagger$ Density calculated as the average between abaxial and adaxial surfaces.
Table 4.5. continued.

(b) aerenchyma tissue and plant architecture

<table>
<thead>
<tr>
<th>Trait</th>
<th>Constant Flooding</th>
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<th></th>
<th>Draw-down</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>(n=167)</td>
<td></td>
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<td>0.0163</td>
<td>-0.0912</td>
<td>-0.1314</td>
<td>0.0747</td>
<td>0.0238</td>
<td>0.0157</td>
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<td>0.4909</td>
<td>0.7117</td>
<td>0.4281</td>
<td>0.3044</td>
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<td>-0.2938</td>
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<td>0.1061</td>
<td>0.1923</td>
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<td>0.3934</td>
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Table 4.6. Analyses of covariance examining whether the relationship between phenotypic traits and fitness differed among species in each environment. F-ratios and p-values are shown for analyses conducted separately for each trait (see text). Boldface type indicates significance after Bonferroni corrections for multiple comparisons.

<table>
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<th>Draw-down (n=167)</th>
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<td>Flowers &amp; Fruits</td>
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<td>F-ratio</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>F-ratio</td>
<td>P</td>
</tr>
<tr>
<td>P/A: aerial leaf</td>
<td>6.2861</td>
<td>0.0023</td>
</tr>
<tr>
<td>P/A: transitional leaf</td>
<td>0.5290</td>
<td>0.5902</td>
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<tr>
<td>P/A: submerged leaf</td>
<td>2.0926</td>
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<tr>
<td>adaxial stomate density: aerial leaf</td>
<td>6.5684</td>
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</tr>
<tr>
<td>adaxial stomate density: submersed leaf</td>
<td>0.9670</td>
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<tr>
<td>average stomate density: transitional leaf</td>
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<td>aerenchyma tissue production</td>
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<td>number of non-basal stems</td>
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1Density calculated as the average between abaxial and adaxial surfaces.
Table 4.7. Pearson product-moment correlation coefficients for (a) constant flooding and (b) draw down conditions. Boldface type indicates significance after sequential Bonferroni correction for multiple comparisons.

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<th>NUMBST</th>
<th>BSTLEN</th>
<th>NUMNBST</th>
<th>NBSTLEN</th>
<th>L2BTM</th>
<th>L2TOP</th>
<th>L3BTM</th>
<th>L3TOP</th>
<th>PAB</th>
<th>PAC</th>
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<tr>
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<tr>
<td>BSTLEN</td>
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<td>NBSTLEN</td>
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<tr>
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<tr>
<td>L2TOP</td>
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<td>0.271361</td>
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<td>L3BTM</td>
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<td>0.173547</td>
<td>-0.26247</td>
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</tbody>
</table>

Trait abbreviations: FLWFRT = number of flower and fruits produced; TOTWT = total plant biomass; NUMBST = number of basal stems; BSTLEN = basal stem length; NUMNBST = number of non-basal stems; NBSTLEN = non-basal stem length; L2BTM = stomates/mm$^2$, abaxial surface of transitional leaf; L2TOP = stomates/mm$^2$, adaxial surface of transitional leaf; L3BTM = stomates/mm$^2$, abaxial surface of aerial leaf; L3TOP = stomates/mm$^2$, adaxial surface of aerial leaf; PAB = perimeter-area ratio, transitional leaf; PAC = perimeter-area ratio, aerial leaf.
<table>
<thead>
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<th></th>
<th>FLWFRT</th>
<th>TOTWT</th>
<th>NUMBST</th>
<th>BSTLEN</th>
<th>NUMNBST</th>
<th>NBSTLEN</th>
<th>L2BTM</th>
<th>L2TOP</th>
<th>L3BTM</th>
<th>L3TOP</th>
<th>PAB</th>
<th>PAC</th>
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</table>

Trait abbreviations: FLWFRT = number of flower and fruits produced; TOTWT = total plant biomass; NUMBST = number of basal stems; BSTLEN = basal stem length; NUMNBST = number of non-basal stems; NBSTLEN = non-basal stem length; L2BTM = stomates/mm², abaxial surface of transitional leaf; L2TOP = stomates/mm², adaxial surface of transitional leaf; L3BTM = stomates/mm², abaxial surface of aerial leaf; L3TOP = stomates/mm², adaxial surface of aerial leaf; PAB = perimeter-area ratio, transitional leaf; PAC = perimeter-area ratio, aerial leaf.
Figure 4.1. A stem of *P. palustris*, the most heterophyllous member of the genus *Proserpinaca*, illustrating plasticity in leaf shape expressed by an individual plant in response to changing environmental conditions.
Figure 4.2. Aerenchyma tissue production in stems of *Proserpinaca*. The stem section at left was taken from a plant that had been growing submerged, the section at right was taken from the stem of a plant growing above water. A=total stem diameter; B=inner stem diameter; C=stele diameter.
Figure 4.3. Dry weight in relation to fresh weight. Regression line plus 95% confidence intervals for 105 of 335 plants.
Figure 4.4. A box-plot depicting early patterns of growth across species. Post hoc comparisons of this trend revealed that *P. intermedia* cuttings reached the surface faster than either *P. palustris* ($p=0.0083$) or *P. pectinata* ($p=0.0367$). *P. palustris* and *P. pectinata* did not differ in their patterns of initial growth with respect to this trait ($p=0.4923$).
Figure 4.5. Histograms illustrating the distribution of perimeter:area (P/A) ratios across submerged, transitional and aerial leaves collected over the course of the experiment. The approximately bi-modal distribution for aerial leaf morphology (far right) reflects the presence of the markedly heterophyllous *P. palustris* (left peak), the non-heterophyllous *P. pectinata* (right peak) and their presumed hybrid, *P. intermedia*. 
**Figure 4.6.** Within-individual plasticity exhibited by *Proserpinaca* species during their growth through the water column from a submerged to an emergent state. Groups encircled by a common ellipse were not statistically different after post-hoc comparisons. Genotype responses are depicted in Figure 4.7.
Figure 4.7. Within-individual plasticity exhibited by the genotypes of each *Proserpinaca* species during their growth through the water column from a submerged to an emergent state.
Figure 4.8. Plasticity in aerial leaf traits exhibited by Proserpinaca species grown in contrasting environments. Groups encircled by a common ellipse were not statistically different from one another after post-hoc comparisons. For additional comparisons, treatment by genotype interactions are presented in Figure 4.9.
Figure 4.9. Within-individual plasticity exhibited by the genotypes of each Proserpinaca species grown in contrasting environments.
Figure 4.10. Plasticity in aerenchyma tissue production exhibited by *Proserpinaca* species grown in contrasting environments. Groups encircled by a common ellipse were not statistically different from one another after post-hoc comparisons.
Figure 4.11. Plasticity in plant architecture exhibited by Proserpinaca species grown in contrasting environments. Groups encircled by a common ellipse were not statistically different from one another after post-hoc comparisons.
b. non-basal stem traits

Figure 4.11. continued.
Figure 4.12. Plasticity in biomass exhibited by *Proserpinaca* species grown in contrasting environments. Groups occurring in a common ellipse are not statistically different from one another after post-hoc comparisons.
Figure 4.13. Plasticity in vegetative (total plant biomass, left) and sexual (flowers and fruits, right) components of fitness exhibited by Proserpinaca species grown in contrasting environments. Groups occurring in a common ellipse are not statistically different from one another after post-hoc comparisons.
a. constant flooding

Figure 4.14. Trait associations in (a) draw-down and (b) constant flooding conditions. Coefficient indicates degree of similarity among traits.
b. draw-down

Figure 4.14. continued.
Figure 4.15. Scatter plots of the relationship between aerial leaf dissection (P/A ratio) and two components of plant fitness (a) constant flooding and (b) draw-down in two environments. Total selection was significant (or marginally so) in for each component of fitness in each environment; directional selection gradients were significant with respect to flower and fruit production, but not biomass, in both environments. See text and/or Table 4.5 for further details.
Figure 4.16. Scatter plots of the relationship between aerial leaf dissection (P/A ratio) and relative plant biomass for the three species of Proserpinaca grown in two environments. For comparison, the same relationship is depicted for transitional leaves in Figure 17.
Figure 4.17. Scatter plots of the relationship between transitional leaf dissection (P/A ratio) and relative plant biomass for the three species of *Proserpinaca* grown in two environments. In contrast with aerial leaf morphology (Figure 4.16), the relationship between transitional leaf morphology and relative plant biomass did not significantly differ among species.
Figure 4.18. Scatter plots of the relationship between aerial leaf stomate density (upper surface) and components of fitness. Only significant relationships are shown (see text). The nature of the relationship between relative plant biomass and this trait differed among species, and is depicted graphically in Figure 4.19.
Species:

P. intermedia
P. palustris
P. pectinata

constant flooding

Figure 4.19. Scatter plots of the relationship between aerial adaxial stomate density and relative plant biomass for the three species of *Proserpinaca* under constantly flooded conditions (differences among species were not significant under draw-down conditions, see text).
Figure 4.20. Scatter plots of the relationship between basal stem length and relative plant biomass for the three species of *Proserpinaca* grown in two environments.
**Figure 4.21.** Scatter plots of the relationship between the number of basal stems and relative plant biomass for the three species of *Proserpinaca* grown in two environments.
Vita

Carolyn Leigh Wells was born in Atlanta, Georgia on February 12, 1972. She was raised in Greenville, South Carolina where she was a student in the Greenville County school system. After one year at Winthrop College in Rock Hill, South Carolina she transferred to the University of North Carolina at Asheville in the Fall of 1991. She received her Bachelors of Arts in Biology from UNCA in May of 1995. Her interests in wetland habitats and aquatic plant ecology began during her undergraduate studies at UNCA, when she participated in research at the Tulula wetlands in Graham County, North Carolina under the direction of Irene Rossell. Carolyn was accepted into the graduate program of the Botany Department at the University of Tennessee, Knoxville in August of 1996. She was awarded her Ph.D. in Botany in August of 2001.