ORIGINAL ARTICLE

doi:10.1111/j.1558-5646.2008.00562.x

EVOLUTION OF LEAF FORM IN MARSILEACEOUS FERNS: EVIDENCE FOR HETEROCHRONY

Kathleen M. Pryer[1,2] and David J. Hearn[3,4]

[1]*Department of Biology, Duke University, Durham, North Carolina 27708*

[2]*E-mail: pryer@duke.edu*

[3]*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721*

[4]*E-mail: dhearn@email.arizona.edu*

Received September 27, 2007
Accepted October 13, 2008

Using an explicit phylogenetic framework, ontogenetic patterns of leaf form are compared among the three genera of marsileaceous ferns (*Marsilea*, *Regnellidium*, and *Pilularia*) with the outgroup *Asplenium* to address the hypothesis that heterochrony played a role in their evolution. We performed a Fourier analysis on a developmental sequence of leaves from individuals of these genera. Principal components analysis of the harmonic coefficients was used to characterize the ontogenetic trajectories of leaf form in a smaller dimensional space. Results of this study suggest that the "evolutionary juvenilization" observed in these leaf sequences is best described using a mixed model of heterochrony (accelerated growth rate and early termination at a simplified leaf form). The later stages of the ancestral, more complex, ontogenetic pattern were lost in Marsileaceae, giving rise to the simplified adult leaves of *Marsilea*, *Regnellidium*, and *Pilularia*. Life-history traits such as ephemeral and uncertain habitats, high reproductive rates, and accelerated maturation, which are typical for marsileaceous ferns, suggest that they may be "*r* strategists." The evidence for heterochrony presented here illustrates that it has resulted in profound ecological and morphological consequences for the entire life history of Marsileaceae.

KEY WORDS: **Elliptic Fourier analysis, heteroblasty, heterochrony, leaf shape, principal components analysis.**

During the course of development from embryo to adult, many land plants exhibit dramatic changes in organ form along the shoot. In his Organography of Plants, Goebel (1900) distinguished between heteroblastic development, in which the differences between juvenile and adult stages are well marked, and homoblastic development, in which the differences are slight. Leaf development offers perhaps the most conspicuous examples of heteroblastic series in vascular plants (Allsopp 1965). The idea that the heteroblastic sequence of leaf shapes produced along the shoot during ontogeny might recapitulate a group's evolutionary history of change in leaf shape has been of interest for at least 100 years (Goebel 1900; Sahni 1925; Ashby 1948). For example, Goebel (1900) proposed that the bipinnate, juvenile leaves in the heteroblastic, phyllode-forming species of *Acacia* resembled the adult leaves in presumably ancestral species (Kaplan 1980). During ontogeny, some organisms may indeed "recapitulate" the adult stages of their ancestors; however, there has been much criticism of Haeckel's biogenetic law that ontogeny preserves the historical stages in the evolution of a particular organ form (e.g., de Beer 1930; Gould 1977). Circumstances in which there is a change in the relative timing of events, or in the sequence of developmental events, are in conflict with Haeckel's biogenetic law (Alberch et al. 1979).

Gould (1977) defined heterochrony as an evolutionary change in the relative timing (e.g., acceleration or deceleration) of events during development compared to the ancestral ontogeny.

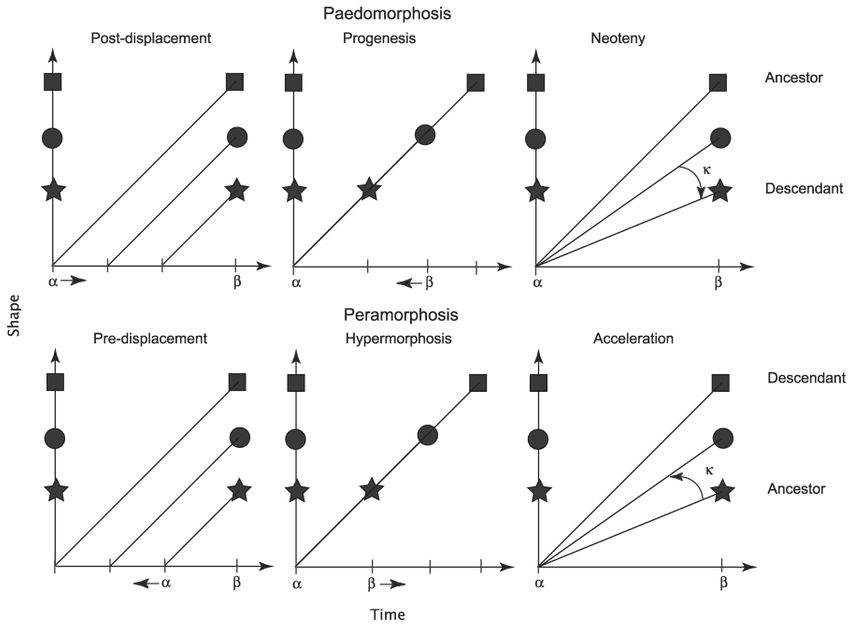

Figure 1. Diagrammatic representation of the effect of heterochrony resulting in pedomorphic and peramorphic morphological evolution, according to the model proposed by Alberch et al. (1979). *x*-axis = time, *y*-axis = shape measure. α = age of the onset of developmental process, β = age of the offset or culmination of process. Pedomorphic descendants can be produced by starting the onset of development late relative to an ancestor (α→, postdisplacement), decreasing the age of maturation (←β, progenesis), or reducing *k*, the rate of development (neoteny). Peramorphic descendants can be produced by starting the onset of development early relative to an ancestor (←α, predisplacement), increasing the age of maturation (β→, hypermorphosis), or increasing *k*, the rate of development (acceleration). Symbols depict stylized shapes of ancestor and descendant taxa.

Interest in the role of heterochrony in morphological evolution was revived by his 1977 publication of Ontogeny and Phylogeny (e.g., Alberch et al. 1979; Alberch 1980, 1982; Guerrant 1982; Raff and Kaufman 1983; Alberch 1985; McKinney 1988). Alberch et al. (1979) developed a model using size, shape, and age as independent variables to show how heterochrony can account for morphological differences among related taxa. Under their scheme (Fig. 1), paedomorphosis results from development that, when compared to an ancestor, was delayed in its initiation (postdisplacement), abbreviated in time (progenesis), or decreased in rate (neoteny). In contrast, peramorphosis results from a developmental process that was initiated sooner (predisplacement), extended in time (hypermorphosis), or increased in rate (acceleration) compared to the ancestral ontogeny (Fig. 1). Prior to Alberch et al. (1979), the meanings and usage of categories of heterochrony varied among researchers (Gould 1977; Smith 2001), but we follow their usage here.

The earliest investigations of heterochrony in plants using the Alberch et al. (1979) methodology were those of Guerrant (1982) and Lord (1982) on flowers. These studies inspired other research that supports the hypothesis that heterochrony is important in the evolution of land plants (Mishler 1986, 1987; Lord and Hill 1987; Guerrant 1988; Mishler 1988; Kellogg 1990; Lammers 1990; McLellan 1990; Kato 1991; Mishler and De Luna 1991; Jones 1992, 1993; Gallardo et al. 1993; McLellan 1993; Robson et al. 1993; Hufford 1995; Friedman and Carmichael 1998; Olson and Rosell 2006; Olson 2007).

With regard to ferns, Bower (1923) was of the opinion that the ontogeny of leaves recapitulates phylogeny and that there is a direct relationship between the sequence of leaves produced during the ontogeny of an individual and the evolution of species, with the ancestral condition being less complex relative to the derived state. Bower (1923, 1935) suggested that successive juvenile leaves of young fern sporophytes illustrate the probable course of shape transition during evolution and, in particular, demonstrate that the pinnate fern leaf was derived by the overtopping of a dichotomous, ancestral leaf type. As typified by leaves of *Asplenium* (Fig. 2—adapted from Wagner 1952c: p. 582), the first fern leaf is simple, with a single vein, although usually the young leaves first show dichotomous veins, whereas the later leaves that eventually assume the adult pinnate leaf form show inequality of the forking of lobes and veins. Bower (1923) concluded that "... equal dichotomy was the prior, and probably the original state in the construction of the fern leaf, and that some form or another of dichopodium of the main veins is a state derivative from it." In contrast, Wagner's (1952c) extensive study of the

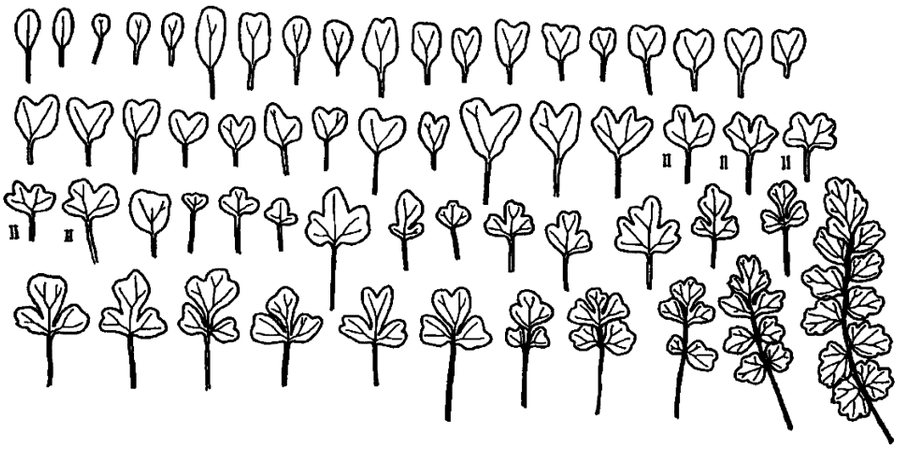

Figure 2. Representative series of juvenile leaves in *Asplenium*; figure modified from Wagner 1952c, p. 582. The juvenile leaves are unlike the adult leaves. The first leaves are simple with a single vein, later leaves show dichotomous venation, and the more complex, pinnate leaves are produced only once the plant has reached a sufficient size.

juvenile leaves of various genera of ferns concluded that adult leaves exhibiting juvenile-appearing, foliar dichotomy, including vascular dichotomy, were derived from ancestors with pinnately organized leaves.

Although juvenile stages of fern leaves have been investigated in detailed anatomical studies (Wagner 1952a,b,c, 1957; Tryon 1960; White 1971; Kato and Iwatsuki 1985; Imachi and Kato 1993; Tuomisto and Groot 1995), taxonomic treatments with descriptions of young leaves (Hennipman 1977), identification keys with descriptions (Tuomisto and Groot 1995), and sporadic comments on juvenile leaves in major reference works (Tryon and Tryon 1982), few studies present detailed documentation of the sequence of leaves produced during early sporophyte development. In contrast, young sporophyte development of the semiaquatic fern, *Marsilea*, is well documented. *Marsilea* is a member of Marsileaceae, a family comprising three extant genera (*Marsilea*, *Regnellidium*, and *Pilularia*) each with unique vegetative and reproductive characteristics. Given the complexity of most fern leaves, the marsileaceous leaf is remarkable for its simplicity. It consists of a petiole and four leaflets in *Marsilea*, a petiole and two leaflets in *Regnellidium*, and an undifferentiated, filiform leaf in *Pilularia* (Fig. 3). *Marsilea* is remarkably easy to grow in culture and has a comparatively rapid life history; consequently, it has received much attention from a physiological and morphological–anatomical point of view with studies focusing on leaf form and development (Allsopp 1951, 1959, 1963; White 1966, 1968; Schmidt 1973, 1978).

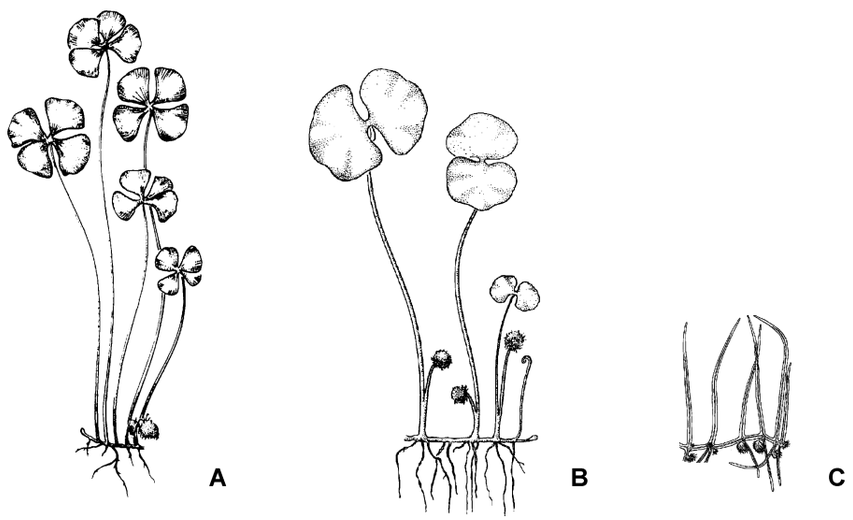

Figure 3. (A) *Marsilea.* (B) *Regnellidium.* (C) *Pilularia.* Figure modified from Pryer 1999, p. 932.

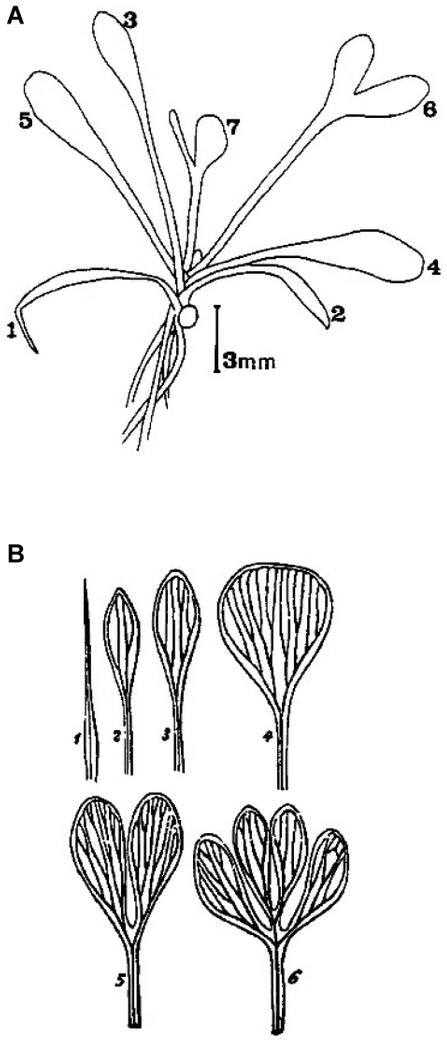

Figure 4. Heteroblastic ontogenetic leaf sequence of *Marsilea*. (A) Young sporophyte of *M. polycarpa* with seven juvenile leaves. The sixth and seventh leaves have two leaflets. (B) Leaf sequence of *M. drummondii* demonstrating parallels with the mature morphology of related genera: the first leaf is filiform as in *Pilularia*, the bifoliate juvenile leaves resemble those of *Regnellidium*. Figure modified from Bower (1926) and Schmidt (1978).

The ontogenetic leaf sequence of *Marsilea* is markedly heteroblastic. During its development, parallels are seen with the mature morphology of related genera: there is a transition from the first filiform leaf (as in *Pilularia*), through spatulate, then bifoliate juvenile leaves (as in *Regnellidium*), to the quadrifoliate adult leaf form of *Marsilea* (Fig. 4; Bower 1926; Gupta 1962; Schmidt 1978). Plants of *Marsilea* have always been observed to begin development with *Pilularia*-like leaves, followed by *Regnellidium*-like leaves, before attaining the mature leaf form. This pattern is suggestive of heterochrony, specifically some form of paedomorphosis, as being responsible for the reduction of leaf complexity in *Pilularia* and *Regnellidium* in particular (Takhtajan 1953; Allsopp 1967). However, the phylogenetic and systematic implications of these observations have never been investigated. Recent, well-resolved phylogenetic relationships within Marsileaceae (Pryer 1999; Nagalingum et al. 2007, 2008) provide an explicit framework to analyze the direction of morphological transformation (Fink 1988) and interpret character state transformations (Fink 1982; Kluge 1988; Brooks and McLennan 1991) relative to hypotheses of heterochrony.

In this article, ontogenetic sequences of leaf forms are qualitatively and quantitatively compared for *Marsilea*, *Pilularia*, and *Regnellidium*, relative to the outgroup taxon *Asplenium*. Quantitative analyses of ontogenetic sequences provide a formalization of traditional narrative descriptions of development, emphasizing the sequences of transitions in form and evolutionary events that alter forms during development (Hufford 2001). Sequences in leaf-shape transitions from node to node are characterized here in the form of ontogenetic trajectories based on a quantitative interpretation of leaf-shape data that use Fourier coefficients as shape descriptors. The ontogenetic sequences are then interpreted in terms of a phylogenetic hypothesis of ancestral ontogeny to characterize homology and the mode of evolutionary transformation (paedomorphosis vs. peramorphosis). In addition, we contrast the typical and original use of the term heterochrony for the development of a single structure (such as the change of size and shape of a bone) to the application of heterochrony to describe alterations in shape and size of mature structures (such as leaves) in series. Finally, we discuss ecological causes and consequences of the observed patterns of developmental change.

Materials and Methods

TAXA, PLANT CULTURE, AND HANDLING

Four marsileaceous taxa were selected for this study: *Marsilea farinosa* Launert, *M. villosa* Kaulf., *Regnellidium diphyllum* Lindm., and *Pilularia americana* A. Br. Although there are minor interspecific differences in the shapes of *Marsilea* leaflets (Gupta 1962; Johnson 1986), all species of *Marsilea* produce adult leaves with four leaflets; therefore, only two divergent taxa (based on geography and sporocarp morphology) were selected to represent the genus. One to two sporocarps were removed from herbarium specimens or greenhouse material of each taxon (Table 1). Sporocarps were surface-sterilized for 20–30 min in half-strength commercial Clorox, and then rinsed in several changes of sterile, distilled water. In a sterile hood, using aseptic technique, each sporocarp was sliced at the tip with a sharp razor blade and placed in individual sterile petri dishes half-filled with sterilized Bold's mineral liquid medium (Deason and Bold 1960). The petri dishes were sealed with strips of parafilm to prevent water evaporation. The dishes were incubated at a temperature of 18–20°C with a

Table 1. Spore source for plants grown in ontogenetic leaf shape study of marsileaceous ferns.

Taxon	Spore Source
Marsilea farinosa	Kenya. Machakos District. R.F. Faden (US)
Marsilea villosa	Hawaii. Koko's Head. R.A. White (DUKE)
Pilularia americana	Georgia. Greene Co. K.M. Pryer 954, J. Klein, and J. Allison (DUKE)
Regnellidium diphyllum	Duke University Greenhouses. (Originally acquired from University of Wisconsin Greenhouses). K.M. Pryer 977 (DUKE)

16L:8D photoperiod using 20 watt florescent lights 30 cm above the cultures. Each culture produced, on average, 50–100 young sporophytes from a single sporocarp. Cultures were examined once a week over a two-month period, and at each observation period three representative young sporophytes from each dish were preserved in formalin: acetic acid: alcohol (FAA, 1:1:18).

DATA ACQUISITION

At the end of the study period, about 25–35 young sporophytes (all at different stages of development) had been preserved and examined for each taxon. Sporophytes were removed from the FAA preservative and representative sequential leaves were identified at successive nodes along the rhizomes and removed for outline tracing. Only developmentally mature, fully expanded leaves were used. Leaves had been softened by FAA and could be laid flat for tracing two-dimensional shapes. Leaf outlines were traced using a camera lucida device attached to a Wild M5A dissecting microscope. A series of 10 leaves was drawn for both *M. farinosa* and *M. villosa* to capture the heteroblastic sequence from the first leaf to the typical quadrifoliate leaf produced by mature plants (Fig. 5A,B). Only six leaves had to be drawn for *R. diphyllum* to depict the heteroblastic sequence from the first leaf to the bifoliate leaf characteristic of mature plants (Fig. 5C). For *P. americana*, the first seven leaves were drawn to illustrate the homoblastic sequence in leaf-shape ontogeny (Fig. 5D). In total, 33 two-dimensional marsileaceous leaf shape outlines were traced (Fig. 5).

When few or no landmarks are available on a structure (as is the case with fern leaves) the shape may be best captured by the coordinates of a sequence of points along its outline (Rohlf 1990). The leaf tracings shown in Figure 5 were individually placed on an electromagnetic digitizing tablet and, using Sigma Scan (Jandel Scientific 1988), a series of *x*,*y* coordinate measurements were recorded for blade regions of marsileaceous leaves. The blade portions of the 59 leaves shown in the *Asplenium* series in Figure 2 were also each digitized. Petioles were removed from analysis when leaves were differentiated into blade and petiole. It was assumed that each leaf contour begins with a homologous landmark at the blade–petiole junction (cf. Ray 1992), which was taken as the first point along the outline and treated as homologous from outline to outline. Using the digitizing tablet cursor, the *x*,*y* coordinates were collected in a clockwise fashion, always with the blade–petiole junction as the starting point. The sample points were spaced more or less equidistantly along the outline. For the marsileaceous ferns, an average of 150 coordinate points were recorded per outline (data not shown, 97–450 points were recorded per outline, depending on its size). For *Asplenium*, an average of 307 coordinate points were recorded per outline (data not shown), with as many as 1590 points recorded for the most complex outline (leaf 59).

ANALYSIS OF LEAF SHAPE

A large number of coordinates along an outline is not a very compact or efficient way to describe a shape because such coordinates contain large amounts of redundant information (Rohlf 1990). One way to simplify the description is to transform the information in these coordinates into a more compact form. A Fourier analysis decomposes coordinate information into a weighted sum of wave terms (sines and cosines) of different frequencies. Each wave term has a corresponding scaling coefficient (i.e., weight), the Fourier coefficient, whose magnitude determines the contribution of the wave term to the overall shape. The specific Fourier coefficients thereby define the shape corresponding to the sampled coordinates, so they are mathematical descriptors of form and can be analyzed by standard statistical methods (Kuhl and Giardina 1982; Rohlf and Archie 1984). The coefficients of the lower frequency wave terms (lower order harmonics) correspond to the overall shape, and the higher order harmonics correspond to smaller details of the outline. Complex shapes with irregular outlines require more harmonics for accurate representation than do smooth, simple shapes (McLellan 1993). Fourier analysis has provided a precise, accurate, and objective description of shape over a range of size scales in other analyses of shapes, including leaf shape studies (Kincaid and Schneider 1983; Mou and Stoermer 1992; McLellan 1993; Premoli 1996; McLellan and Endler 1998; McLellan 2000; Olsson et al. 2000; Yoshioka et al. 2004; McLellan 2005; Neto et al. 2006). Ray (1990) provides alternate methods to characterize leaf shape that use a modified eigenshape technique. McLellan and Endler (1998) also survey other techniques.

An elliptic Fourier analysis was carried out on each of the 33 digitized marsileaceous leaf outlines (Fig. 5) as well as on each of the 59 digitized *Asplenium* leaf outlines (Fig. 2), using Rohlf and Ferson's EFA program, version 4/22/92 (Ferson et al. 1985),

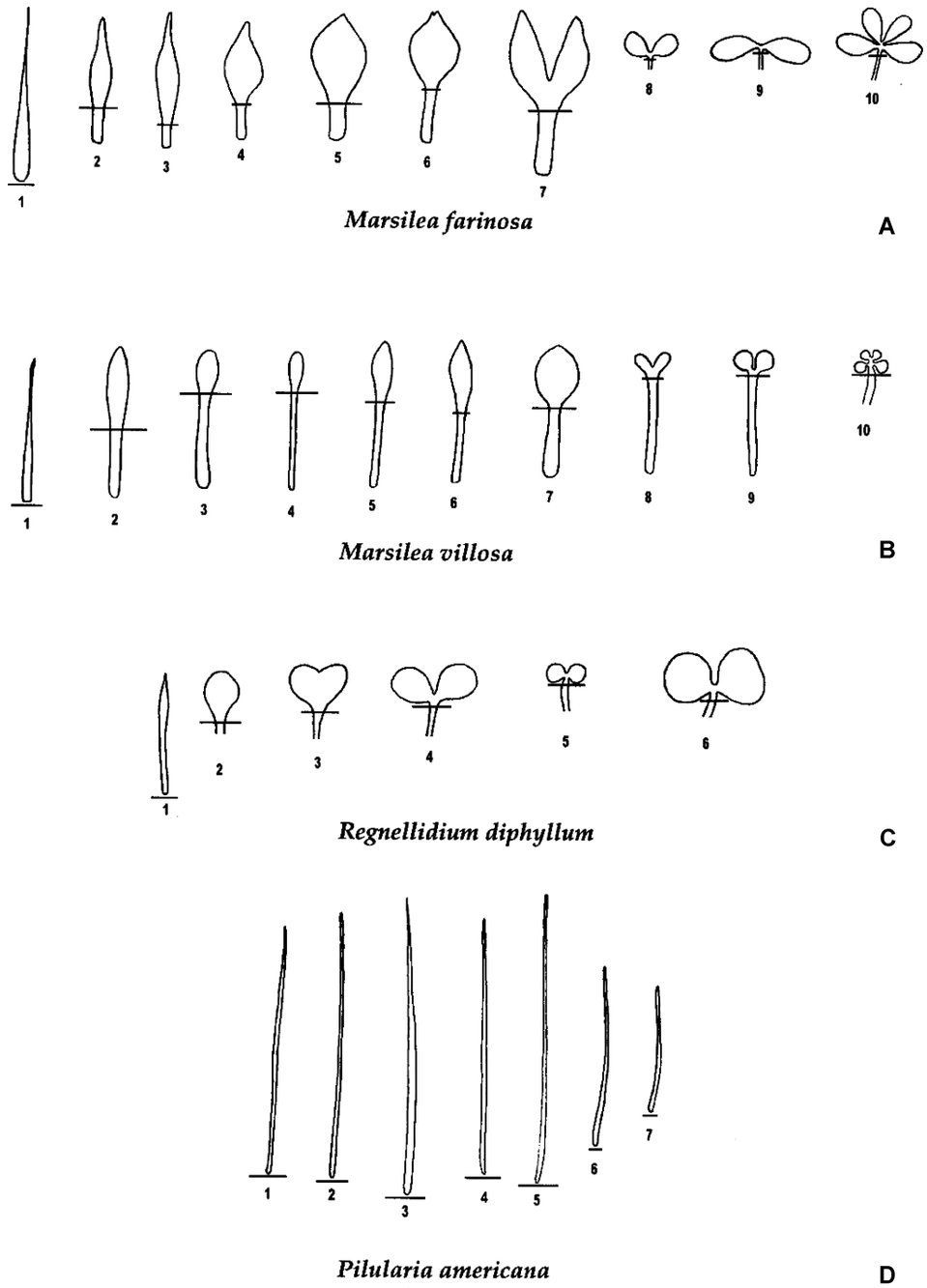

Figure 5. Ontogenetic sequences of leaf shape. (A) *Marsilea farinosa*. (B) *M. villosa*. (C) *Regnellidium diphyllum*. (D) *Pilularia americana*. Leaves are numbered according to order of appearance in sequence. Horizontal lines = 1 mm scale for individual leaves; only the blade portions above these scales were included in the digitized outline.

which computes the Fourier coefficients for outlines described by a set of *x*,*y* coordinates. The harmonics were selected to be invariant with respect to location and size, thereby removing size differences from consideration, but not invariant to starting position of the outline at the blade—petiole junction. To determine an appropriate number of harmonics to be computed, three test runs with 10, 15, and 20 harmonics were carried out with one of the more complex leaf outlines (mature leaf of *M. farinosa*,

Figure 6. Mathematically reproduced leaf outline of *Marsilea farinosa* (leaf no. 10, cf. Fig. 5A) computed by ParametricPlot in Mathematica, using 10, 15, and 20 elliptic Fourier analysis harmonics.

leaf no. 10 in Fig. 5A). Using ParametricPlot in Mathematica (Wolfram Research, Inc., 1992), the coefficients of the first 10 harmonics mathematically reproduced the actual leaf outline very closely for these three test runs (compare digitized outline in Fig. 5A with the mathematically computed outlines in Fig. 6). For accurate representation, coefficients of the first 20 harmonics were calculated for each of the 33 leaf shape outlines in Figure 5 and for each of the 59 leaves in Figure 2.

A principal components analysis (PCA) on the Fourier coefficients of the first 20 harmonics (82 coefficients total) was carried out using SYSTAT (1992). A PCA summarizes variance structure among numerous variables by reducing them to a smaller number of uncorrelated components. Plots were made of components 1×2 and 1×3 to visualize the most influential variables on those components. One of the major advantages of using Fourier coefficients as descriptors is that one can reconstruct the outline of the leaves from points along the principal component axes, and because the PCA components summarize the information in the coefficients, shape can be reconstructed on the graphs of principal components in a space of low dimensionality. This is useful as a check on the numerical results and as an aid to interpretation.

INFERENCE OF ANCESTRAL ONTOGENY

Using parsimony as implemented in MacClade (Maddison and Maddison 2003), we reconstructed the ontogeny of the ancestor of Marsileaceae based on the phylogeny of Pryer (1999) and using the outgroup *Asplenium*. We focused on reconstructing leaf shapes at early and late stages of ontogeny of the putative ancestor of Marsileaceae. All Marsileaceae as well as *Asplenium* begin with a simple, linear leaf, whereas mature forms are lobed to varying degrees.

Results

Marsileaceous spores germinated within an hour after they were liberated from the sorus. The development of the gametophytes and subsequent fertilization usually took place within 24 h. Embryonic sporophytes grew quickly; the first root and leaf were generally visible after 2–3 days. The ontogenetic leaf series captured for *M. farinosa*, *M. villosa*, *Regnellidium*, and *Pilularia* are shown in Figure 5. For all taxa except *Pilularia*, leaf shape changed progressively from node to node along the rhizome, until the adult leaf shape was attained.

A visual inspection of the ontogenetic sequences in Figures 2 and 5 reveals considerable qualitative similarity and overlap in early leaf-shape development in ferns. To quantify leaf-shape variation and provide an objective means of shape comparison, we represented leaf shape using coefficients of a Fourier analysis. Because a large number of Fourier coefficients is an inefficient representation of shape, we reduced dimensionality of this representation using PCA. The ontogenetic sequence of leaf shapes can then be analyzed in the PCA space. The first two principal components based on analysis of the Fourier coefficients of the first 20 harmonics are plotted in Figure 7. The plot of *Pilularia* in the upper left corner of Figure 7 shows minimal variation along both the first and second principal component axes, reflecting the uniformity of its homoblastic ontogenetic sequence (see also Fig. 5D). The plots of *Regnellidium*, *M. farinosa*, and *M. villosa* are similar to one another in that the ontogenetic sequence of leaves shows a relatively constant increase from negative to positive values along the first principal component (PC 1 describes 46.4% of the variation) and a "decrease −> increase −> decrease" pattern of variation in the second principal component (PC 2 describes 20.0% of the variation; Fig. 7). The two species of *Marsilea* differ in the relative spacing between points (i.e., the magnitude of change between successive leaves in the heteroblastic series), but the overall pattern of their "trajectories" mirrors one another and they terminate at nearly the same point. Likewise, the trajectory of *Regnellidium* differs from the other taxa in the spacing between points, but follows the same overall pattern as the *Marsilea* species (Fig. 7), stopping short of their termination points. This reflects the similarity in shape between the early leaves of *Marsilea* and the later leaves of *Regnellidium*. The trajectory for *Asplenium* is initiated close to the intersection of the PC 1 and PC 2 axes (Fig. 7), with most of the points for the early leaves (about 40) densely situated along the positive values of PC 1 and PC 2. The latter part of the *Asplenium* trajectory mostly explores negative values along PC 2 before terminating relatively close to where the trajectory was initiated.

We reconstructed leaf outlines based on the first three to five harmonics of each of the 33 marsileaceous leaves using ParametricPlot in Mathematica, and these outlines are plotted on the first two principal component axes (Fig. 8). The outlines provide a rough depiction, but they present the characteristic lobes and shapes nevertheless, indicating that at least five harmonics are sufficient to capture important aspects of leaf shape.

The loadings of the first 10 elliptic Fourier harmonics (40 coefficients) and the two zeroth harmonic coefficients on the first two principal components axes shown in Figure 7 are plotted on PC 1 and PC 2 in Figure 9; only nine of the coefficients have loadings

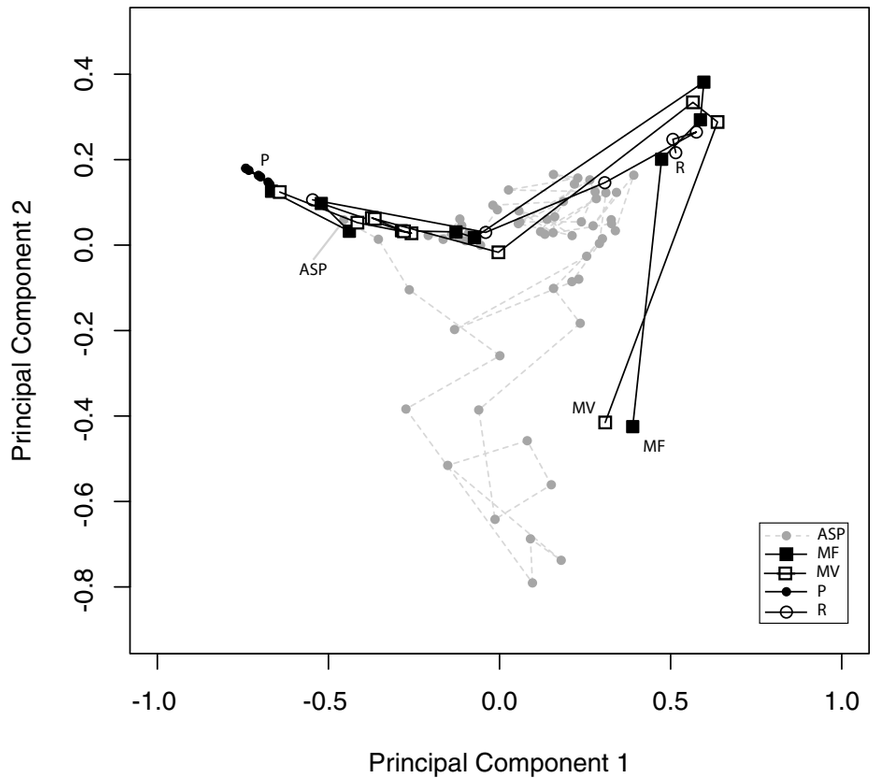

Figure 7. **Principal components analysis of elliptic Fourier coefficients for harmonics 1 through 20 for ontogenetic leaf shape sequences of *Pilularia* (P), *Regnellidium* (R), *Marsilea farinosa* (MF), *M. villosa* (MV), and *Asplenium* (ASP). The points represent 7, 6, 10, 10, and 59 leaves for each taxon, respectively, and they are connected with a line in their ontogenetic order. The first leaf of *Pilularia* is found in the center of the cluster of its points and the label "P" is next to the last leaf of the sequence. First leaves for *Regnellidium* and both species of *Marsilea* are in the upper left corner and their labels "R," "MF," and "MV" are next to the last leaf of each sequence. First leaves for *Asplenium* are clustered close to the intersection of PC 1 and PC 2 axes and the label "ASP" is next to the last leaf of that sequence. The last leaf of each sequence indicates a final or adult leaf shape; thereafter little or no changes in leaf shape occur.**

that are great enough to be visible in this plot (A0, C0, B1, C1, B2, C2, B3, C3, and C5). The coefficients of the zeroth harmonic are A0 and C0, whereas coefficients of the nth harmonic are An, Bn, Cn, and Dn. Data in Figures 8 and 9 suggest that the coefficients that contribute most to PC 1 are contained in the first three harmonics: C0, B1, C1, B2, C2, and C3. A comparison between the marsileaceous leaf outlines in Figure 5 and the trajectory points in Figures 7 and 8 demonstrates that the negative values along PC 1 correspond to unlobed leaf outlines, whereas the positive values correspond to lobed outlines. The narrower outlines have more negative values than the broader outlines, with the broadest, unlobed outlines falling at about 0. Leaves 7–10, 8–10, and 3–6 of *M. farinosa*, *M. villosa*, and *Regnellidium*, respectively, show various degrees of lobing, and have positive values on PC 1 (cf. Figs. 7 and 8). Along PC 2, the reconstructions show that the leaves with similar positive values (e.g., Figs. 7 and 8: PA1, MV1, RD5, and MF 9) have outlines that are distinctly longer on either the *x*- or *y*-axes. Leaf outlines with negative values along PC 2, are characteristically more broadly elliptical to almost round in the case of MF10 (Figs. 7 and 8).

The ancestry of leaf-form evolution was reconstructed on the phylogeny for juvenile leaves and for mature leaves (Fig. 10). The adult ancestor to Marsileaceae was reconstructed as having four or more lobes as well as having filiform juvenile leaves. Because leaves of all *Marsilea* investigated go through linear, bifoliate, then quadrifoliate leaf stages along the rhizome, and plants of *Regnellidium* go through filiform followed by bifoliate stages, we infer, by parsimony, that the lobed ancestor also went through these stages.

Leaf development in *Asplenium* (and very likely in most outgroup fern taxa with pinnate mature leaves) has an ontogenetic trajectory that, in its early stages, follows closely the trajectories shown for *Marsilea* in Figure 7, but then extends beyond as the leaves become more complex and assume a pinnate form. The loss of overtopping in the Marsileaceae lineage resulted in dichotomous leaf venation and a reduction to four leaflets in *Marsilea*.

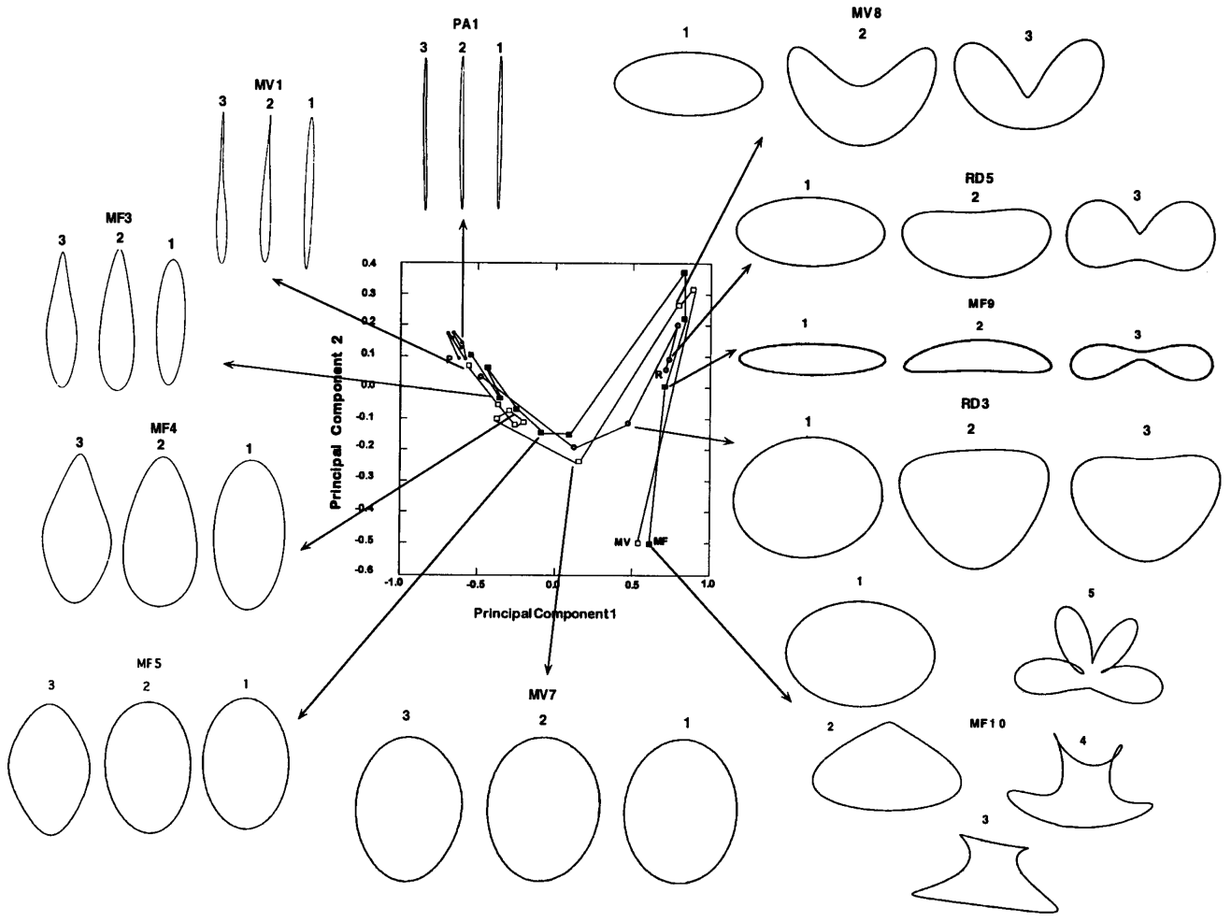

Figure 8. **Reduced reproduction of only the marsileaceous fern trajectories shown in Figure 7, with representative leaf shape outlines along the ontogenetic trajectories that were mathematically reconstructed using between three and five harmonics. The numbers above leaves indicate the number of harmonics that were used to reconstruct leaf outlines. The addition of at least five harmonics is required to approximate the quadrifoliate leaf of *Marsilea*. Key to abbreviations, e.g., MV8 = eighth leaf in the ontogenetic sequence of *Marsilea villosa* shown in Figure 5B.**

The subsequent loss of the latter portion of the ontogenetic trajectory leading to *Marsilea* (cf. Fig. 7), resulted in a reduction to two leaflets in *Regnellidium* with dichotomous venation. Finally, all heteroblastic development was lost in the lineage leading to *Pilularia* with only the earliest, filiform, juvenile leaves formed throughout the plant's life cycle.

Discussion

Our investigation on the ontogeny of leaf shape in Marsileaceae (Figs. 7 and 8) indicates that heterochrony played a crucial role in leaf-shape development. At its adult stage, *Pilularia* has the leaf shape of the most juvenile leaves of *Regnellidium* and *Marsilea*. Likewise, *Regnellidium* adult leaf shape is seen in later juvenile *Marsilea* leaves. From what is known from the ontogeny of most fern leaves (see Tuomisto and Groot 1995, and references therein), initial leaves are usually simple, whereas more complex, pinnate leaves are produced only with maturity. In Figure 2, modified from Wagner (1952c: 582), we have reproduced a representative series of juvenile leaves produced in *Asplenium*. Comparison of the early juvenile leaf-shape sequence in *Asplenium* (Fig. 2) with *Marsilea* (Fig. 5) reveals striking similarities: the first leaves are narrowly elliptical with a single vein; then leaves become broadly elliptic with dichotomous venation; eventually paired lobes, each with dichotomous venation, are formed; next, a transition to overtopping or pinnate leaf formation occurs; finally, the adult, pinnate leaf is formed.

For almost all possible outgroup members for which there is information about leaf development, the heteroblastic leaves begin simple and unlobed, and they transition through lobed and pinnate adult forms (Tuomisto and Groot 1995). Our explicit phylogenetic reconstruction of ancestral states in Marsileaceae

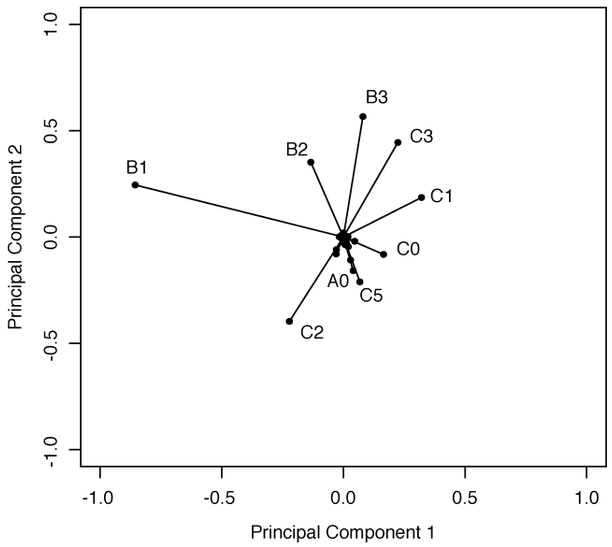

Figure 9. Plot of the loadings of elliptic Fourier harmonic coefficients onto the first two principal components axes. Lines from the origin to the loadings along PC1 and PC2 are drawn. Those with the longest lines explain the most variation in shape. Only nine of the coefficients have loadings that are great enough to allow them to be viewed in this plot (A0, C0, B1, C1, B2, C2, B3, C3, and C5).

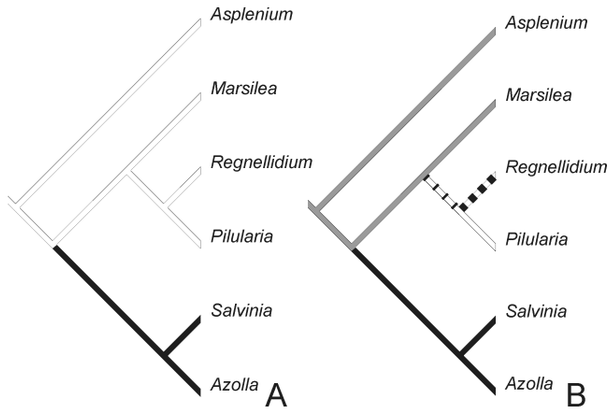

Figure 10. Reconstruction of ancestral ontogenies using parsimony. (A) Juvenile leaves. (B) Mature leaves. Branch shading: black, uncertain developmental affinities to leaf lobes of Marsileaceae; white, filiform leaves; thin stripes, two lobed leaves; dark gray, four or more lobed leaves; thick stripes, equivocal reconstruction.

(Fig. 10) is consistent with this pattern; juvenile leaves of the ancestor were reconstructed as simple and linear, whereas mature forms were reconstructed as lobed (Fig. 10), with potentially more lobes than extant *Marsilea*, because outgroup taxa have more lobes. This information suggests that the mature leaf forms of *Regnellidium* and *Pilularia* (and perhaps even *Marsilea* itself) are similar to juvenile leaf forms of the ancestor.

HETEROCHRONY IN SERIALLY HOMOLOGOUS STRUCTURES

Paedomorphosis appears to be an appropriate explanation of the patterns of ontogenetic change in Marsileaceae, as the mature leaf shapes in these taxa correspond to juvenile shapes in the ancestor. However, such an interpretation for certain structures in plants, such as leaves, requires scrutiny. The clarification of terminology related to heterochrony provided by Gould (1977) and Alberch et al. (1979) was based primarily on animals, and on a subset of animals that are noncolonial. For example, in Gould's (1977) Ontogeny and Phylogeny, Part Two is devoted to heterochrony and padomorphosis in particular, but all examples deal with animals, and the majority of these are noncolonial organisms. None deal with plants. These examples also deal with changes in sizes and shapes of a single body, bone, limb, or shell. Moreover, such examples have a sequestered germ line, so the distinction between somatic, nonreproductive growth, and reproductive maturation is often clearly delineated. The state of development at the time of reproduction serves as a useful point of comparison among related animals. Ontogenies of single structures can therefore be measured along one time axis (initiation of structure to maturation of organism).

In contrast, instead of measuring the continuous development of a single leaf (analogous to measuring a single body, bone, limb, or shell), we investigate the change in shape from one fully developed leaf to another fully developed leaf in a temporally ordered sequence of leaves along a single rhizome, which we take to be homologous from one species to the next. Does the terminology used by Alberch et al. (1979) (see Fig. 1) apply to the development of such structures in sequence? Despite its importance, serial homology of this sort has not been addressed by many investigators of heterochrony, perhaps due to the resulting conceptual challenges that we discuss below. One of the primary concerns is the assessment of homology. For example, Hufford (2001) examined changes to staminal position and number to analyze the evolution of sequences of ontogenetic states in Hydrangeaceae. Although he considered each stamen to be structurally homologous, he did not pinpoint homology from one stamen to the next, but rather, he focused on sequences of ontogenetic stages of flowers. Likewise, we do not attempt to pinpoint homology from one leaf node to the next. Rather, we examine the sequence of ontogenetic states along a developing fern rhizome and consider the shape of the most recent, fully mature leaf along the rhizome and the pattern of shape transition from one ontogenetic state (i.e., sequential addition of a leaf to the rhizome) to the next. We are no longer considering one temporal component (such as the time to maturation of a bone or a single leaf), but at least two additional temporal components: rate of mature leaf shape transition from one ontogenetic state to the next, and number of ontogenetic states prior to achieving greatest leaf complexity. These are not necessarily coupled. Both

the timing of mature leaf shape change along a rhizome and the number of ontogenetic states preceding the greatest leaf complexity may be subject to separate evolutionary-developmental influences.

Diggle (1999) and Jones (1992, 1999) focus on the issue of whole plant integration and the coupling between various temporal axes, including time to sexual maturation and heteroblastic development of modular structures (i.e., leaves) in series. Although the concepts of heteroblasty and sexual maturity have been conflated since Goebel (Jones 1999), the decoupling of leaf-shape change and attainment of sexual maturity seems apparent in cucurbits (Jones 1992), *Pisum sativum* (Wiltshire et al. 1994), and *Zea mays* (see Diggle [1999] for citations). Diggle (1999) argues that their dissociation is a prerequisite for changes in reproductive phenology to occur through heterochrony to enable vegetative and reproductive development to change relative to one another.

Reilly et al. (1997) indicate that any study of heterochrony requires (1) characterization of homologous traits, (2) an accurate and objective descriptor of these traits, (3) a relevant measure of time, (4) an ontogenetic trajectory for the traits, and (5) a polarization of the trajectories relative to ancestral ones (following Fink [1982]). Raff and Wray (1989) argue that discovering a relevant measure of time and a reference point to begin ontogenetic analysis is the most challenging aspect of this approach. The very nature of heterochrony shifts the timing and rate in development, making discovery of a common reference between two ontogenies difficult, in particular in modular organisms (Olson 2007). Traditionally, in animals, the onset of sexual maturity is used as the time reference point. However, in modular plants, sexual maturity may not be a reliable point of reference because the germ line is not sequestered and the vegetative and reproductive axes of development can be decoupled through both genetic and plastic responses (Diggle 1999, 2002). It may still be possible to distinguish between the major categories of heterochrony, i.e., pedomorphosis and peramorphosis (Olson 2007), and when points of reference can be established, finer subcategories (Fig. 1) can be distinguished.

In our study, we use the moment of spore release as one point of reference, and the attainment of the most developmentally complex leaf (during the sequence of leaf-shape transition along a single rhizome) as an additional point of reference. From the moment of spore release, there were no differences to be noted in the onset age of growth; all viable spores from each of the genera developed in a very rapid fashion, each producing the first sporophyte root and leaf after two to three days. Subsequent leaf production along a rhizome was at a parallel pace in all three genera; therefore the temporal axis we consider here is the node number. Although the onset age of spore germination is comparable to many ferns, the gametophytic phase, which can take several weeks to months in most homosporous ferns, is highly accelerated and reduced to one to two days in these heterosporous ferns (Schneider and Pryer 2002).

PATTERNS OF LEAF-SHAPE CHANGE IN MARSILEACEAE

Not only does modularity impose difficulties in the analysis of heterochrony, but in modular, heteroblastic structures, the distinction between plasticity and genetic determinacy can be problematic. For ontogenetic comparisons of heteroblastic series to be meaningful in an evolutionary context, changes in development between series must result from genetic, rather than plastic, differences. Minimally, structural homology (cf. Hufford 2001) between metamers is required for heterochronic analysis (see the five points listed above), and morphological changes between metamers in sequence must result from alterations in the regulation of a shared developmental program responsible for metamer initiation and development. These alterations can result from preprogrammed changes in developmental regulation and consequent pleiotropic effects of developmental genes (Wagner 1996) that are independent of external environmental variation, or from regulatory changes induced by environmental variation. These separate modes of alteration can result in similar patterns of change, and they can be difficult to distinguish (Jones 1995). Diggle (2002) distinguishes these categories when she discusses differences between plasticity and heteroblasty (or, more generally, metamorphosis): plasticity is viewed as changes between metamers as a consequence of (external) environmental variation, whereas heteroblasty is apparent when metamers change shape in a stereotyped fashion under controlled (constant) environments. It is clear that both heteroblastic and plastic influences can interact during leaf development, and that both can be adaptive (Winn 1996, 1999a,b).

In marsileaceous ferns, both plastic and heteroblastic influences are apparent. Allsopp (1954, 1963, 1965, 1967) and White (1968) carried out numerous experiments to investigate the connection between nutrient availability in the environment and leaf-shape change (plasticity) in *Marsilea*. Plasticity is closely controlled by the nutritional status of the plant. The final quadrifoliate leaf of *Marsilea* can be attained only when sufficient nutrients are available; below that nutritional level, simpler leaf forms are retained or there is a reversion to such leaves if quadrifoliate leaves have already been formed. A more or less extended period of apical ontogeny and, specifically, the attainment of a certain average apical cell area is necessary before the quadrifoliate leaf can appear (Allsopp 1963, 1965, 1967; White 1968). Allsopp (1951, 1965, 1967) observed a positive correlation between levels of glucose and mannitol available to plants, the diameters of their apical meristems, and the developmental stage of leaves. When nutrients are low in the environment, leaves can shift to a prior leaf form from one node to the next, or transitions from early leaf stages to later stages can be halted. Any growth condition that inhibits or

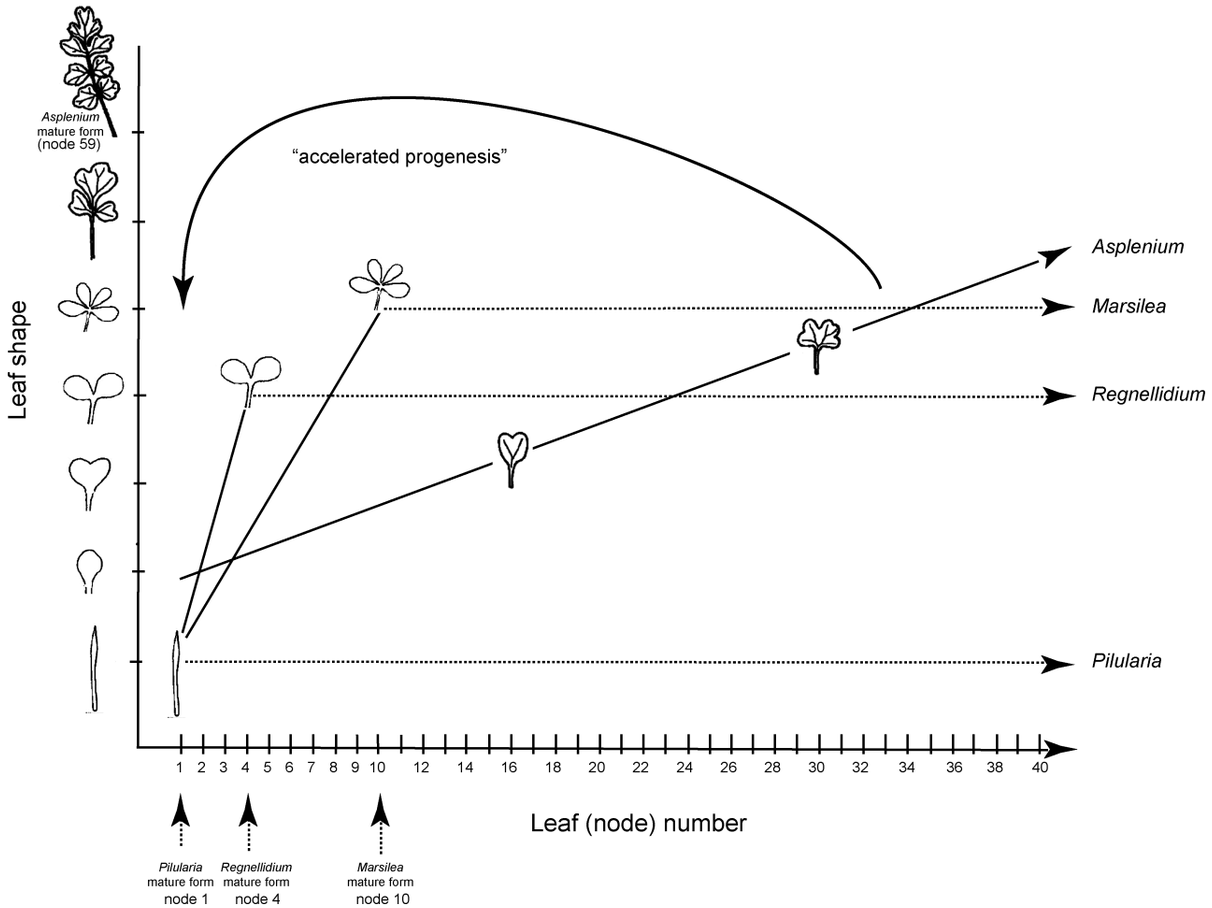

Figure 11. Schematic representation of fern ontogenetic leaf shape trajectories, sensu Alberch et al. 1979. *x*-axis, time (increments marked by successive leaf numbers along a single rhizome, for a total of 40 nodes). *y*-axis, developmental stage (shape). The onset age of ontogenetic trajectory for all taxa is at leaf (node) number 1. The trajectory is flat after the mature leaf form is achieved. Shape change is relatively rapid in Marsileaceae (*Pilularia, Regnellidium, Marsilea*), and the sequence of leaf form terminates early, compared to *Asplenium*, hence what might be referred to as "accelerated progenesis."

reverses the increase in apical cell size will inhibit or reverse the normal heteroblastic series (White 1968).

Regardless of the nutrient level of the medium, however, young *Marsilea* sporophytes never form a quadrifoliate leaf without first producing the simpler leaves, and in constant environments, the sequence of leaf shapes is completely stereotyped, starting from linear through bifoliate and then quadrifoliate leaves. This sequence of leaf-shape change was always observed in the constant environments in which our study was conducted, so differences being compared are heteroblastic, rather than plastic, according to Diggle's (2002) definition.

The ontogenetic leaf-shape sequence in *Pilularia* is homoblastic, that is, it achieves its most developmentally complex leaf form with the production of its first leaf at the first node along the rhizome (Figs. 5D and 11). No subsequent morphological change occurs in its sequence of leaves. *Regnellidium* has a heteroblastic ontogeny, and it achieves the bifoliate form at an earlier node than does *Marsilea* (Figs. 5 and 11). Thus, when *Regnellidium* produces its most developmentally complex, bifoliate leaves, it is younger than when *Marsilea* first produces bifoliate leaves (Figs. 5 and 11). The leaves of the outgroup taxon *Asplenium* appear to initiate at a more advanced stage of leaf expansion relative to marsileaceous ferns (Fig. 11), but have not yet achieved a bifoliate form by the tenth leaf (cf. Fig. 2 and 11). In fact, its ontogenetic trajectory extends well beyond the tenth node before producing adult leaf forms.

Pryer (1999) showed that the marsileaceous ferns (which are also the only ferns that are heterosporous) were a monophyletic group embedded within the paraphyletic homosporous ferns. From the evidence available, it is not unreasonable to state that the pinnate leaves of *Asplenium* and their ontogeny are representative of most homosporous ferns (and hence of most outgroup taxa); therefore, its comparatively slow ontogeny indicates that the rate of shape change (measured from node to node) in

marsileaceous ferns is accelerated compared to other ferns. Also, within Marsileaceae, the node number when mature leaves are first produced is lowest in *Pilularia*, followed by *Regnellidium*, and finally *Marsilea* (Figs. 5 and 11). All three marsileaceous genera produce their developmentally most complex leaf form at much earlier nodes relative to other ferns (e.g., *Asplenium*). Although the rate of leaf-shape change is increased relative to many other ferns, the developmental "advancement" of the mature leaf shape (as measured by the number of lobes in the leaf) is ultimately reduced relative to other ferns and their putative ancestor.

Leaves of marsileaceous ferns therefore present an unusual situation—-leaf development is both peramorphic and paedomorphic (see Fig. 1). This pattern is explained by considering both the rate of shape transition from node to node and the ultimate complexity of the leaf. In contrast to many other ferns, marsileaceous ferns have accelerated (Fig. 1 bottom right) shape transition from node to node. Therefore, along the node number axis (Fig. 11) these ferns are peramorphic, because acceleration is a category of peramorphosis that reflects an evolved increase in parameter κ (Fig. 1 bottom right). However, their ultimate leaf complexity is progenetic (Fig. 1 top middle). Along the leaf-shape axis (Fig. 11), these ferns are paedomorphic because progenesis is a category of paedomorphosis that reflects an evolved decrease in parameter β (Fig. 1 top middle), resulting in leaves that halt development at an early point of leaf complexity relative to the ancestral phenotype (Figs. 10 and 11). These observations suggest that the two ontogenetic components considered (rate of shape transition from node to node and ultimate termination in leaf shape complexity) are at least partially decoupled during the evolution of marsileaceous ferns.

ECOLOGICAL CONSIDERATIONS

A unique innovation in the Marsileaceae is the sporocarp (Nagalingum et al. 2006), a specialized reproductive structure that encases sporangia. In contrast, other fern groups produce sporangia on mature (and more complex), photosynthetic leaf surfaces. As witnessed by the progenetic leaves of marsileaceous ferns, the evolution of the sporocarp may release them of the requirement of their ancestors and relatives to produce mature, sexual leaves. The separation of reproductive maturation and leaf-shape transition is an additional line of evidence that heteroblasty and sexual maturation are separate aspects of plant development in Marsileaceae.

Another consequence of the sporocarp is that marsileacous ferns can reach sexual maturity much sooner than other ferns. Sporocarp formation can follow swiftly in favorable growing conditions, that is, within a few weeks. Most ferns, on the other hand, require months or even years to produce leaves capable of sporangial development. The earliest possible age of reproduction in Marsileaceae greatly precedes that of most other fern taxa and their putative ancestor. Selective trade-offs between the earliest possible time of reproduction and attainment of leaves with large surface areas (that can putatively generate more photosynthates for later reproduction) are likely for marsileaceous ferns.

Stearns (1992) concluded that the benefit of early maturation is demographic: organisms occupying uncertain habitats that mature early have a higher probability of surviving to maturity than do organisms that mature late. Gould (1977) argued that progenetic species tend to live in uncertain environments characterized by ephemeral and randomly fluctuating resources. Progenetic species tend to have high reproductive rates and mature at a relatively early age, as well as having other life-history parameters traditionally associated with "*r* strategists" (Alberch et al. 1979). Organisms that respond to *r* selection for early reproduction by progenesis are likely to be very small (many of the tiniest animals are progenetic), and their contrast in size with ancestral species can be extreme (Gould 1977). Unlike many ferns that occur in relatively stable, humid environments, marsileaceous ferns inhabit seasonally dry areas where water is commonly present only during the rainy season, or where water levels fluctuate considerably from one season to another. With the arrival of rain, spores germinate, and a brief one- to two-day gametophytic phase is followed by a period of rapid rhizome growth and leaf development in the sporophyte (see fig. 9 in Schneider and Pryer 2002). These ferns produce sporocarps only when the habitat they are growing in dries; once the mud or sand is completely dry, the plant (in the form of its sporocarp) becomes dormant. It is only when the substrate becomes flooded that the sporocarps release their spores. In periods of drought, sporocarps can remain dormant and spores have been reported to remain viable for long periods of 100 or more years (Allsopp 1952; Johnson 1985).

From an ecological standpoint, these ferns possess life-history traits and thrive in habitats that are typical of progenetic species. Not only does the family as a whole exhibit reduced time to mature leaf form, but also members of the family appear to terminate development at a simplified leaf stage that is similar in complexity to juvenile stages of other ferns. Moreover, *Pilularia* and *Regnellidium* generally grow in more ephemeral and unpredictable environments than does *Marsilea* suggesting an even greater trade-off between leaf development and reproduction in these ferns. The heterochonic changes in development that characterize the Marsileaceae have made them uniquely suited to their uncertain environments (Chasan 1996).

LIFE-HISTORY EVOLUTION

Not to be neglected in a discussion of the evidence for heterochrony in the evolution of these ferns is the fact that they are heterosporous. In their paper, on the origins of heterospory and the seed habit, DiMichele et al. (1989) proposed that heterosporous

life cycles that are characterized by endosporic gametophytes (i.e., gametophytes that develop within the spore encasing) may arise through heterochronic processes, specifically by progenesis. They argue that heterospory and gametophytic unisexuality are not necessarily evolutionary antecedents of endospory; rather, these features may have arisen as a consequence of endospory. By the precocious onset of sexuality, gametophytes could reach sexual maturity while still in the early endosporic phases of development. Free-sporing heterospory with endosporic gametophytes has advantages over life histories with exosporic gametophytes in environments that require rapid completion of the sexual phase of the life cycle. Because the sporophyte and gametophyte are separate free-living organisms at maturity in marsileaceous ferns, paedomorphosis is expressed in either phase independently of the other. It appears that an evolutionary acceleration in growth rate and in the timing of meiosis (or sporangial initiation) in the sporophytic phase, and gametangial initiation in the gametophytic phase has resulted in profound ecological and morphological consequences for the entire life history of these ferns.

ACKNOWLEDGMENTS

We are thankful to J. Mercer and K. Prine for their detailed and patient lessons in morphometrics, and to the Duke Greenhouse staff for their assistance with growing young sporophytes. We are especially grateful to B. Chernoff, F. Lutzoni, L. McDade, J. Mercer, B. Mishler, S. Rice, and L. Roth for their encouragement and detailed comments on earlier drafts of this manuscript. Constructive suggestions by Associate Editor E. Conti and three anonymous reviewers were also very helpful. Financial support from the following sources is gratefully acknowledged: A.W. Mellon Dissertation Fellowship, Katherine Stern Dissertation Fellowship, National Science Foundation Dissertation Improvement Grant (DEB-9311687), Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship. Support from NSF grants DEB-0089909 and DEB-0347840 is also acknowledged. This article is part of a doctoral dissertation by KMP completed (years ago) under the direction of Brent D. Mishler, and it is dedicated to the memory of J. Klein.

LITERATURE CITED

Alberch, P. 1980. Ontogenesis and morphological diversification. Am. Zool. 20:653–667.

———. 1982. Developmental constraints in evolutionary processes. Pp. 313–332 *in* J. T. Bonner, ed. Evolution and development. Springer-Verlag, New York.

———. 1985. Developmental constraints: why St. Bernards often have an extra digit and poodles never do. Am. Nat. 126:430–433.

Alberch, P., S. J. Gould, G. F. Oster, and D. B. Wake. 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5:296–317.

Allsopp, A. 1951. *Marsilea spp*.: material for experimental study of morphogenesis. Nature 168:301.

———. 1952. Longevity of *Marsilea* sporocarps. Nature 169:79–80.

———. 1954. Juvenile stages of plants and the nutritional status of the shoot apex. Nature 173:1032–1035.

———. 1959. Effects of gibberellic acid on juvenility in *Marsilea* and certain other plants. Nature 184:1575–1576.

———. 1963. Morphogenesis in *Marsilea*. J. Linn. Soc. Bot. 58:417–427.

———. 1965. Heteroblastic development in cormophytes. Pp. 1172–1221 *in* W. Ruhland, ed. Encyclopedia of plant physiology, vol. 15, part 1. Springer Verlag, Berlin.

———. 1967. Heteroblastic development in vascular plants. Pp. 127–171 *in* M. Abercrombie, and J. Brachet, eds. Advances in morphogenesis, vol. 6. Academic Press, New York.

Ashby, E. 1948. Studies in the morphogenesis of leaves I. An essay on leaf shape. New Phytol. 47:152–176.

Bower, F. O. 1923. The ferns (Filicales). Volume I. Cambridge Univ. Press, Cambridge.

———. 1926. The ferns (Filicales). Volume II. Cambridge Univ. Press, Cambridge.

———. 1935. Primitive land plants. Macmillan Press, London.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, ecology, and behaviour. Univ. of Chicago Press, Chicago, IL.

Chasan, R. 1996. The advantages of being juvenile. BioScience 46:804–806.

de Beer, G. 1930. Embryology and evolution. Clarendon Press, Oxford, UK.

Deason, T. R., and H. C. Bold. 1960. Phycological studies I. Exploratory studies of Texas soil algae. Univ. Texas Publ. No. 6022. 70 pp.

Diggle, P. K. 1999. Heteroblasty and the evolution of flowering phenologies. Int. J. Plant Sci. 160:S123–S134.

———. 2002. A developmental morphologist's perspective on plasticity. Evol. Ecol. 16:267–283.

DiMichele, W. A., J. I. Davis, and R. G. Olmstead. 1989. Origins of heterospory and the seed habit: the role of heterochrony. Taxon 38:1–11.

Ferson, S. F., F. J. Rohlf, and R. K. Koehn. 1985. Measuring shape variation of two-dimensional outlines. Syst. Zool. 34:59–68.

Fink, W. L. 1982. The conceptual relationship between ontogeny and phylogeny. Paleobiology 8:254–264.

———. 1988. Phylogenetic analysis and the detection of ontogenetic patterns. Pp. 71–91 *in* M. L. McKinney, ed. Heterochrony in evolution: a multidisciplinary approach. Plenum Press, New York.

Friedman, W. E., and J. S. Carmichael. 1998. Heterochrony and developmental innovation: evolution of female gametophyte ontogeny in *Gnetum*, a highly apomorphic seed plant. Evolution 52:1016–1030.

Gallardo, R., E. Dominguez, and J. M. Muñoz. 1993. The heterochonic origin of the cleistogamous flower in *Astragalus cymbicarpos* (Fabaceae). Am. J. Bot. 80:814–823.

Goebel, K. 1900. Organography of plants. Part I. General organography. English translation by I. B. Balfour. Clarendon Press, Oxford, UK.

Gould, S. J. 1977. Ontogeny and phylogeny. Harvard Univ. Press, Cambridge, MA.

Guerrant, E. O., Jr. 1982. Neotenic evolution of *Delphinium nudicaule* (Ranunculaceae): a hummingbird-pollinated larkspur. Evolution 36:699–712.

———. 1988. Heterochrony in plants. The intersection of evolution, ecology, and ontogeny. Pp. 111–113 *in* M. L. McKinney, ed., Heterochrony in evolution: a multidisciplinary approach. Plenum Press, New York.

Gupta, K. M. 1962. *Marsilea*. Botanical monograph No. 2. Council of Scientific and Industrial Research, New Delhi.

Hennipman, E. 1977. A monograph of the fern genus *Bolbitis* (Lomariopsidaceae). Leiden Bot. Ser. 2:1–331.

Hufford, L. 1995. Patterns of ontogenetic evolution in perianth diversification of *Besseya* (Scrophulariaceae). Am. J. Bot. 82:655–680.

———. 2001. Ontogenetic sequences: homology, evolution, and the patterning of clade diversity. Pp. 27–57 *in* M. L. Zelditch, ed. Beyond heterochrony: the evolution of development. Wiley-Liss, Inc., Verdun, QC, Canada.

Imaichi, R., and M. Kato. 1993. Comparative leaf morphology of young sporophytes of rheophytic *Osmunda lancea* and dryland *O. japonica.* J. Plant Res. 106:37–45.

Jandel Scientific. 1988. Sigma scan: the scientific measurement program, version 3.90. Jandel Scientific, Corte Madera, CA.

Johnson, D. M. 1985. New records for longevity of *Marsilea* sporocarps. Am. Fern J. 75:30–31.

———. 1986. Systematics of the New World species of *Marsilea* (Marsileaceae). Syst. Bot. Monogr. 11:1–87.

Jones, C. S. 1992. Comparative ontogeny of a wild cucurbit and its derived cultivar. Evolution 46:1827–1847.

———. 1993. Heterochrony and heteroblastic leaf development in two subspecies of *Cucurbita argyrosperma* (Cucurbitaceae). Am. J. Bot. 80:778–795.

———. 1995. Does shade prolong juvenile development? A morphological analysis of leaf shape changes in *Cucurbita argyrosperma* subsp. *sororia* (Cucurbitaceae). Am. J. Bot. 82:346–359.

———. 1999. An essay on juvenility, phase change, and heteroblasty in seed plants. Int. J. Plant Sci. 190:S105–S111.

Kaplan, D. R. 1980. Heteroblastic leaf development in *Acacia*. Morphological and morphogenetic implications. La Cellule 73:136–203.

Kato, M. 1991. Further comments on an ophioglossoid archetype for the angiosperm carpel: ovular paedomorphosis. Taxon 40:189–194.

Kato, M., and K. Iwatsuki. 1985. Juvenile leaves and leaf ramification in *Phanerosorus major* (Matoniaceae). Acta Phytotax. Geobot. 36:139–147.

Kellogg, E. A. 1990. Ontogenetic studies of florets in *Poa* (Gramineae): allometry and heterochrony. Evolution 44:1978–1989.

Kincaid, D. T., and R. B. Schneider. 1983. Quantification of leaf shape with a microcomputer and Fourier transform. Can. J. Bot. 61:2333–2342.

Kluge, A. G. 1988. The characterization of ontogeny. Pp. 57–81 *in* C. J. Humphries, ed., Ontogeny and systematics. Columbia Univ. Press, New York.

Kuhl, F. P., and C. R. Giardina. 1982. Elliptic Fourier features of a closed contour. Comp. Graph. Image Process. 18:236- 258.

Lammers, T. G. 1990. Sequential paedomorphosis among the endemic Hawaiian Lobelioideae (Campanulaceae). Taxon 39:206–211.

Lord, E. M. 1982. Floral morphogenesis in *Lamium amplexicaule* L. (Labiatae) with a model for the evolution of the cleistogamous flower. Bot. Gaz. 143:63–72.

Lord, E. M., and J. P. Hill. 1987. Evidence for heterochrony in the evolution of plant form. Pp. 47–70 *in* R. A. Raff, and E. Raff, eds. Development as an evolutionary process. Alan R. Liss, New York.

Maddison, D. R., and W. P. Maddison. 2003. MacClade 4: analysis of phylogeny and character evolution. Version 4.06. Sinauer Associates, Sunderland, MA.

McKinney, M. L., ed. 1988. Heterochrony in evolution, a multidisciplinary approach. Plenum Press, New York.

McLellan, T. 1990. Development of differences in leaf shape in *Begonia dregei* (Begoniaceae). Am. J. Bot. 77:323–337.

———. 1993. The roles of heterochrony and heteroblasty in the diversification of leaf shapes in *Begonia dregei* (Begoniaceae). Am. J. Bot. 80:796–804.

———. 2000. Geographic variation and plasticity of leaf shape and size in *Begonia dregei* and *B. homonyma* (Begoniaceae). Bot. J. Linn. Soc. 132:79–95.

———. 2005. Correlated evolution of leaf shape and trichomes in *Begonia dregei* (Begoniaceae). Am. J. Bot. 92:1616–1623.

McLellan, T., and J. A. Endler. 1998. The relative success of some methods for measuring and describing the shape of complex objects. Syst. Biol. 47:264–281.

Mishler, B. D. 1986. Ontogeny and phylogeny in *Tortula* (Musci: Pottiaceae). Syst. Bot. 11:189–208.

———. 1987. Leaf development in *Tortula papillosissima* (Pottiaceae). Mem. New York Bot. Garden 45:38–54.

———. 1988. Relationships between ontogeny and phylogeny, with reference to bryophytes. Pp. 117–136 *in* C. J. Humphries, ed. Ontogeny and systematics. Columbia Univ. Press, New York.

Mishler, B. D., and E. De Luna. 1991. The use of ontogenetic data in phylogenetic analyses of mosses. Adv. Bryol. 4:121–167.

Mou, D., and E. F. Stoermer. 1992. Separating *Tabellaria* (Bacillariophyceae) shape groups based on Fourier descriptors. J. Phycol. 28:386–395.

Nagalingum, N. S., H. Schneider, and K. M. Pryer. 2006. Comparative morphology of reproductive structures in heterosporous water ferns and a reevaluation of the sporocarp. Int. J. Plant Sci. 167;805–815.

———. 2007. Molecular phylogenetic relationships and morphological evolution in the heterosporous fern genus *Marsilea*. Syst. Bot. 32:16–25.

Nagalingum, N. S., M. D. Nowak, and K. M. Pryer. 2008. Assessing phylogenetic relationships in extant heterosporous ferns (Salviniales), with a focus on *Pilularia* and *Salvinia*. Bot. J. Linn. Soc.. 157:673–685.

Neto, J. C., G. E. Meyer, D. D. Jones, and A. K. Samal. 2006. Plant species identification using elliptic Fourier leaf shape analysis. Comput. Electron. Agr. 50:121–134.

Olson, M. E. 2007. Wood ontogeny as a model for studying heterochrony, with an example of paedomorphosis in *Moringa* (Moringaceae). Syst. Bio. Div. 5:145–158.

Olson, M. E., and J. A. Rosell. 2006. Using heterochrony to detect modularity in the evolution of stem diversity in the plant family Moringaceae. Evolution 60:724–734.

Olsson, A., H. Nybom, and H. C. Prentice. 2000. Relationships between Nordic dogroses (*Rosa* L. sect. Caninae, Rosaceae). Syst. Bot. 25:511–521.

Premoli, A. C. 1996. Leaf architecture of South American *Nothofagus* (Nothofagaceae) using traditional and new methods in morphometrics. Bot. J. Linn. Soc. 121:25–40.

Pryer, K. M. 1999. Phylogeny of marsileaceous ferns and relationships of the fossil *Hydropteris pinnata* reconsidered. Int. J. Plant Sci. 160:931–954.

Raff, R. A., and T. C. Kaufman. 1983. Embryos, genes, and evolution. MacMillan, New York.

Raff, R. A., and G. A. Wray. 1989. Heterochrony: developmental mechanisms and evolutionary results. J. Evol. Biol. 2:409–434.

Ray, T. S. 1990. Application of eigenshape analysis to second order leaf shape ontogeny in *Syngonium podophyllum* (Araceae). Pp. 201–213 *in* F. J. Rohlf and F. L. Bookstein, eds. Proceedings of the Michigan morphometrics workshop. Special publication number 2. The Univ. of Michigan Museum of Zoology, Ann Arbor.

———. 1992. Landmark eigenshape analysis: homologous contours: leaf shape in *Syngonium* (Araceae). Am. J. Bot. 79:69–76.

Reilly, S. M., E. O. Wiley, and D. J. Meinhardt. 1997. An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. Biol. J. Linn. Soc. 60:119–143.

Robson, K. A., J. Maze, R. K. Scagel, and S. Banerjee. 1993. Ontogeny, phylogeny and intraspecific variation in North American *Abies* Mill. (Pinaceae): an empirical approach to organization and evolution. Taxon 42:17–34.

Rohlf, F. J. 1990. Fitting curves to outlines. Pp. 167–177 *in* F. J. Rohlf, and F. L. Bookstein, eds. Proceedings of the Michigan morphometrics workshop. Special publication number 2. The Univ. of Michigan Museum of Zoology, Ann Arbor.

Rohlf, F. J., and J. W. Archie. 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). Syst. Zool. 33:302–317.

Sahni, B. 1925. The ontogeny of vascular plants and the theory of recapitulation. J. Ind. Bot. Soc. 4:202–216.

Schmidt, K. D. 1973. Morphologie, Anatomie und Entwicklung von *Marsilea* L. Oberhess. Naturwiss. Zeit. Bd. 39/40:11–28.

———. 1978. Ein Beitrag zum Verständnis von Morphologie und Anatomie der Marsileaceae. Beitr. Biol. Pflanzen 54:41–91.

Schneider, H., and K. M. Pryer. 2002. Structure and function of spores in the aquatic heterosporous fern family Marsileaceae. Int. J. Plant Sci. 163:485–805.

Smith, K. K. 2001. Heterochrony revisited: the evolution of developmental sequences. Biol. J. Linn. Soc. 73:169–186.

Stearns, S. C. 1992. The evolution of life histories. Oxford Univ. Press, New York.

SYSTAT. 1992. SYSTAT, version 5.2. SYSTAT, Inc., Evanston, IL.

Takhtajan, A. L. 1953. Phylogenetic principles of the system of higher plants. Bot. Rev. 19:1–45.

Tryon, A. F. 1960. Observations on the juvenile leaves of *Pellaea andromedifolia*. Contr. Gray Herb. 187:61–68.

Tryon, R. M., and A. Tryon. 1982. Ferns and allied plants, with special reference to tropical America. Springer-Verlag, New York.

Tuomisto, H., and A. T. Groot. 1995. Identification of the juveniles of some ferns from western Amazonia. Am. Fern J. 85:1–28.

Wagner, W. H., Jr. 1952a. The fern genus *Diella*, its structure, affinities and taxonomy. Univ. California Publ. Bot. 26:1–212.

———. 1952b. Juvenile leaves of two polypodies. Am. Fern J. 42:81–85.

———. 1952c. Types of foliar dichotomy in living ferns. Am. J. Bot. 39:578–592.

———. 1957. Heteroblastic leaf morphology in juvenile plants of *Dicranopteris linearis* (Gleicheniaceae). Phytomorphology 7:1–6.

Wagner, G. P. 1996. Homologues, natural kinds and the evolution of modularity. Am. Zool. 36:36–43.

White, R. A. 1966. The morphogenetic effects of protein synthesis inhibition in *Marsilea*. Am. J. Bot. 53:158–165.

———. 1968. A correlation between the apical cell and the heteroblastic leaf series in *Marsilea*. Am. J. Bot. 55:485–493.

———. 1971. Experimental and developmental studies of the fern sporophyte. Bot. Rev. 37:509–540.

Wiltshire, R. J. E., I. C. Murfet, and J. B. Reid. 1994. The genetic control of heterochrony: evidence from developmental mutants of *Pisum sativum* L. J. Evol. Biol. 7:447–465.

Winn, A. A. 1996. The contributions of programmed developmental change and phenotypic plasticity to within-individual variation in leaf traits in *Dicerandra linearifolia*. J. Evol. Biol. 9:737–752.

———. 1999a. Is seasonal variation in leaf traits adaptive for the annual plant *Dicerandra linearifolia?* J. Evol. Biol. 12:306–313.

———. 1999b. The functional significance and fitness consequences of heterophylly. Int. J. Plant Sci. 160:S113-S121.

Wolfram Research, Inc. 1992. Mathematica, version 2.1. Wolfram Research, Inc., Champaign, IL.

Yoshioka, Y., H. Iwata, R. Ohsawa, and S. Ninomiya. 2004. Analysis of petal shape variation of *Primula sieboldii* by elliptic Fourier descriptors and principal components analysis. Ann. Bot. 94:657–664.

Associate Editor: E. Conti