

mination at either end. Also present on some axes are unbranched conidiophores and cube-shaped arthrospores produced by segmentation of side branches. The fossil ascomycetes provide critical information about this unique group of true fungi, and offer important characters that can be used in establishing a major lineage of Euascomycetes with perithecial ascocarps.

283 TAYLOR, WILSON A.

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Assessing the earliest putative land plant remains – Cambrian cryptospores

The pace of discovery of early land plant remains has broadened the temporal gap between the first appearance of microfossils and that of megafossils. Representatives of all major categories of cryptospores (monads, dyads and tetrads) as well as minute trilete spores have been recovered from Middle Cambrian sediments of terrestrial provenance (conservatively, a fifty million year gap). What clues do we have to the affinity of these microfossils? Those that have been examined so far with the TEM (Bright Angel Shale) possess a three-layered wall that surrounds each member of the unit. While this wall is morphologically comparable to that seen in some modern green algal cysts (e.g., *Mychonastes desiccatus*, *Chlorococcales*), the thickness of the individual sporopollenin lamellae differs by a factor of two. The presence of thinner fossil lamellae in somewhat younger specimens suggests that preservation is not a limitation. Comparisons with other fossil and modern sporopollenin-containing propagules as well as less equivocal cryptospore remains from the Middle Cambrian Rogersville Shale may provide some of the first clues to the possible identity of these earliest land plant pioneers.

284 TOMESCU, ALEXANDRU MIHAIL FLORIAN* AND GAR W. ROTHWELL

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Exploring the cladistic relationships of sphenopsids

Phylogenetic relationships of *Equisetum* are firmly established within the euphyllophytes, but more detailed resolution of sphenopsid phylogeny has not been achieved. Traditional paleobotanical studies recognize five groups of sphenopsids including Hyeniales, Pseudoborniales, Calamitales, Sphenophyllales and Equisetales, but these hypothesized relationships have not been fully tested using cladistic methodology. Sphenopsids plus cladoxylalean/zygopterid "ferns" resolved as the sister group to lignophytes in an analysis of euphyllophytes by Rothwell (1999), but *Equisetum* nested among the ferns as the sister to marattialeans in the recent analysis of living plants by Pryer et al. (2001). Relationships among sphenopsid groups are equally uncertain. Whereas Equisetales and Calamitales are widely regarded as closely related, their relationships to *Pseudobornia*, *Hyenia* and *Sphenophyllum* are far less certain. Indeed, Stewart (1980) has hypothesized that *Sphenophyllum* may be more closely related to lycophytes than to euphyllophytes. To test these competing hypotheses, we have undertaken a numerical cladistic analysis of representative rhyniophytes, zosterophylls, lycophytes, and euphyllophytes, including all of the hypothesized groups of sphenopsids. Relationships of these taxa are resolved using a matrix of more than 100 morphological characters. Principal questions addressed by this analysis include the following. Do the sphenopsids (as traditionally circumscribed) form a clade? What are the sister group relationships of the sphenopsid clade that includes *Equisetum*? And, are Sphenophyllales euphyllophytes, or do they nest within the zosterophyll/lycophyte clade?

CONTRIBUTED POSTERS

285 BOYCE, C. KEVIN^{1*}, ANDREW H. KNOLL¹, GEORGE D. CODY², MARILYN L. FOGEL², AND ROBERT M. HAZEN²

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The evolution of tracheids and lignification in early land plants

Anatomically preserved land plant fossils from the Lower Devonian Rhynie Chert contain conducting tissues that range from dark colored, elongated cells without secondary wall thickenings to tracheids similar to those of extant plants. Observed differences in conducting cell differentiation may or may not correspond to the physiological innovations of lignin biosynthesis and deposition. In order to evaluate the degree to which the morphological and physiological evolution were coupled in the tissues of early land plants, three analytical techniques were employed to study anatomically preserved fossils: electron microprobe analysis to obtain micron-scale resolution of elemental abundances, isotope ratio mass spectrometry to obtain millimeter-scale resolution of carbon isotopic abundances, and X-ray microscopy in conjunction with nuclear magnetic resonance spectroscopy to obtain micron-scale resolution of the preservation of organic molecular functionality. Studies demonstrate that original organics are not homogenized during diagenesis and are preserved in place with sufficient spatial resolution for paleobiological investigations. Tissue-specific isotopic and organic chemistry of Rhynie Chert plants suggests that the anatomical evolution of tracheid secondary thickenings and the biochemical evolution of lignin synthesis were originally independent: the earliest vascular thickenings were unligified and cell wall lignification may have first appeared in other tissues. Only later were the anatomical and biochemical characteristics coupled to produce true tracheids like those of all living vascular plants.

286 HANSEN, AMANDA, L.* AND KATHLEEN, B. PIGG

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Taxodiaceous conifers from the middle Miocene Yakima Canyon flora, Washington State, USA

Anatomically preserved taxodiaceous conifer remains are described from the middle Miocene Yakima Canyon flora of central Washington State, USA. These remains include ovulate cones and seeds, isolated cone scales, wood, foliage and twigs of possible taxodiaceous affinity. Some seeds contain mature embryos. While many specimens are contained within the chert matrix, others have been weathered out and are preserved in the round, showing external morphological features. Isolated cone scales range from 9-11 mm high x 11-12 mm wide x 4 mm thick and bear two seed depressions on their inner surface. The apophysis has an umbo that protrudes 3 mm from the outer surface of the scale and a dentate margin on the apical half. Isolated seeds are winged, elongate, 11.5-13 mm long x 6 mm wide x 3-4 mm high, and are triangular in cross section. Foliage shows prominent stomatal furrows. The taxodiaceous remains at Yakima Canyon occur commonly with the ferns *Osmunda wehrlii* Miller, *Woodwardia virginica* (L.) J. E. Smith, and a third small filicalean form and are considerably more abundant than the previously described pine from this same site, *Pinus foisyi* Miller.

287 HENRY, APRIL M.* AND KATHLEEN B. PIGG

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Hamamelidaceous infructescences from the Late Paleocene Almont, North Dakota flora

Infructescences of Hamamelidaceae are described from the Late Paleocene Almont flora of North Dakota. The study is based on forty-nine specimens that are preserved morphologically with some details of the fruit wall anatomy. The largest infructescence consists of up to ten sessile fruits attached to a peduncular axis 8 cm long x 0.5 cm thick. Biloculate fruits are roughly square in outline, around 10-12 mm in dimension, and have persistent, recurved styles on the distal faces of the carpels. Fruit walls are composed of elongate, interwoven fibrous cells similar to those seen in extant *Hamamelis*. Ellipsoid to ovoid locules are 5-10 mm long x 3-5 mm wide. Most specimens probably represent senescent fruits lacking seeds, that may have dehisced explosively like those of some modern Hamamelidaceae.

288 MCKOWN, ATHENA D.1*, RUTH A. STOCKEY2, AND CHARLES E. SCHWEGER3

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A new species of Pinus, Subgenus Pinus, Subsection Contortae based on fossil cones from Ch'ijee's Bluff, Yukon Territory, Canada

Three structurally preserved conifer ovulate cones are described from the Pliocene sediments of Ch'ijee's Bluff on the Porcupine River, near Old Crow, Yukon Territory. Cones are ovoid to conical, symmetrical, 3.4-4.4 cm and 2.8-3.4 cm wide. Ovuliferous scales are woody, 2 cm long and 1 cm wide, with a minute dorsal umbo. Bracts are 6 mm long, lack an abaxial lobe and contain a single terete trace accompanied by two resin canals. The pith is parenchymatous with few scattered sclerenchyma cells near its outer margin. Vascular tissue consists of a continuous cylinder of secondary xylem with few resin canals. The inner cortex is parenchymatous and contains between 12 - 15 resin canals of uniform diameter. The outer cortex is also parenchymatous with a thin layer of sclerenchyma at its outer edge. Vascular tissues in the ovuliferous scale and bract originate as single, continuous cylindrical strands from the axis stele and diverge in the outer cortex. The abaxially concave ovuliferous scale trace splits into separate vascular bundles that alternate with resin canals in the most distal sections. Paired ovules on the ovuliferous scales are smooth, elliptical in shape, 2.7-3.9 mm long and 2.1-2.5 mm wide. Seed wings are detachable, 16 mm long and have a basal parenchymatous pad. Some megagametophyte tissue is preserved however, no embryos were found. Fossils were compared to extant species of *Pinus* Section *Pinus* and most closely resemble those of *Pinus contorta* Dougl. ex Loud. Symmetrical cone shape, non-reflexed cone base, flattened apophyses, cone serotiny and seed wing length distinguish the fossil cones from the extant subspecies and fossils of *P. contorta*. The fossil cones will be described in a new species of *Pinus*, Subgenus *Pinus*, Subsection *Contortae*.

289 RADTKE, MEGHAN G.1*, KATHLEEN B. PIGG1, AND WESLEY WEHR2

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Fossil Corylopsis and other Hamamelidaceae-like leaves from the middle Eocene flora of Republic, Washington, USA

A fossil leaf of *Corylopsis* Sieb. & Zucc., (Hamamelidaceae; Saxafragoid clade) and several other Hamamelidaceae-like leaves are described from the middle Eocene (49-50 MA) Republic flora of eastern Washington State, USA. The fossil *Corylopsis* leaf is similar to several extant species, particularly with the presence of prominent agrophic veins and ladder-rung-like tertiaries at right angles to the secondaries. The leaf is 1.9 cm wide, of incomplete length, with an asymmetrical base. Teeth are concave apical, straight basal, with simple apices. Several other Hamamelidaceae-like leaves present in the Republic flora are simple, symmetric, range from 2.1 to 8.7 cm in length, from 0.9 to 4.3 cm in width, have a L/W ratio of 2.0 to 2.3 and are generally elliptic in shape. Bases are typically asymmetric and cordate to rounded. Leaf apices are not preserved. Venation is craspedodromous with secondary veins produced alternately from the midvein. Compound agrophic veins varying from 1 to 2 are produced by the secondaries. Ladder-rung-like tertiary veins are formed at right angles to the secondaries. Tertiaries and quaternaries comprise usually 5-sided areoles containing 5th order veins. When visible, teeth are generally concave apical, straight basal, with a simple apex. Today *Corylopsis*, an ornamental shrub commonly called "Winter Hazel", is known only from the Himalayas to Japan, but was more widely distributed in the past. The fossil record of *Corylopsis* is based primarily on seeds that occur from the Eocene throughout much of the Tertiary in Europe, the Eocene of eastern North America, and from one previous report of a leaf from the Eocene of Alaska. The presence at Republic of *Corylopsis* and other Hamamelidaceae-like leaves including the previously described *Langeria magnifica* demonstrates a high diversity in this group during the middle Eocene of western North America.

290 TCHEREPOVA, MARIA* AND KATHLEEN B. PIGG

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Permineralized Nyssa (Cornaceae) and additional endocarps of uncertain affinity from the middle Miocene Yakima Canyon flora, Washington State, USA

Permineralized endocarps of *Nyssa* (Cornaceae) and another plant of uncertain affinity are described from the middle Miocene Yakima Canyon flora of central Washington State, USA. Some specimens are preserved within the chert matrix, while others have been weathered out to show three-dimensional features. *Nyssa* endocarps are 9-14 mm long, 5-8 mm wide, elliptical in outline, and typically have 4-12 longitudinally oriented ribs extending from a somewhat pointed base to a rounded apex. They have a curved, dorsal surface and a flat to convex ventral surface with an apical germination valve and appear unilocular. The other endocarp is subglobose to somewhat flattened, sometimes showing a pointed apex, 5.5-8 mm long, and 5-6 mm in diameter. In some specimens an additional outer layer 1.5-2 mm thick is preserved that appears like a halo of either fleshy or leathery tissue, which may represent the mesocarp. The endocarp is biloculate, with a single seed in each locule except for one specimen, which has two smaller seeds in one locule. Vascular strands are present along the central septum and in some specimens strands also occur around the periphery. Seeds are pyriform and slightly asymmetrical,

4 mm long, 3 mm wide in their major plane, and 1.5 mm wide in the minor plane. The seed coat is composed of three layers: an outermost dark epidermis, a central palisade layer and an inner region of poorly preserved cells. Some features of this biloculate drupaceous fruit suggest possible affinities with Cornaceae, but the vascularization and seed structure are not typical of this family. Based on available features, another possible relationship of the Yakima endocarps may be with Rhamnaceae.

291 TIDWELL, WILLIAM D.^{1*}, LEITH S. TIDWELL², WALT WRIGHT³, AND ULRICH DERNBACH⁴

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Tempskya sp. from Neuquén, Argentina, the first Tempskya species reported from the Southern Hemisphere

Permineralized axes of a new species of *Tempskya* is described from the Cretaceous Grupo Neuquén from north-east of Zapala, Neuquén, Argentina. The species is characterized by having only inner and outer cortices that are parenchymatous. Species of *Tempskya* from the Northern Hemisphere, except for *T. reesidei* reported from New Mexico, have three layered cortices with the outer cortex being parenchymatous, the middle sclerenchymatous, and inner either sclerenchymatous or parenchymatous or both. In *T. reesidei*, the inner cortex is sclerenchymatous. *Tempskya* specimens, first described by Stokes and Webb (1824) under the name *Endogenites erosa*, have been previously reported from numerous localities in the Northern Hemisphere. Therefore, this report of *Tempskya* from Argentina and, thereby the first from the Southern Hemisphere, is both very interesting and highly significant.

PHYSIOLOGICAL SECTION, BSA

CONTRIBUTED POSTER

292 CHAPMAN, RUSSELL L.^{1*}, JUAN M. LOPEZ-BAUTISTA², SUZANNE FREDERICQ², AND DEBRA A. WATERS¹

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The biotech potential of algae colonizing oil rigs in the Gulf of Mexico: risks and rewards

In an ever-expanding quest to find treatments for diseases such as AIDS, cancer, and inflammatory or infectious diseases, government agencies as well as pharmaceutical companies are turning to the seas. More than 3,500 oil and gas platforms in the northern Gulf of Mexico provide habitats that may harbor marine organisms with pharmaceutical or other commercial usefulness. As collaborators in a project funded in part by the Minerals Management Service, we are continuing our investigations on the macroalgal epiflora of the artificial reefs created by the platform substructures. To determine if harvesting of these algae would be useful or wise, baseline information must be gathered about what organisms inhabit these artificial islands, which, if any, might be useful, and what the relative abundance of such algae is. The first step beyond our previous studies is identifying the algae collected in the spring from the six to eight platforms in our study area on the Louisiana shelf of the Gulf of Mexico. The sampling scheme allows

us to investigate near-shore to outer-shelf and along-coast variability as well as the influences of water column and currents. Once we have conducted an initial survey of designated platforms and identified the algae, we hope to be able to answer the question—Are any of the algae known to have biotechnology potential? Subsequent research should help us reach some conclusions about the environmental risks of extracting marine organisms from their steel reefs and answer the question—Is sustainable harvesting possible without disrupting biodiversity and damaging these newly created ecosystems?

PHYSIOLOGICAL SECTION, BSA

CONTRIBUTED PAPERS

293 BELL, HESTER L.^{1,2*} AND JAMES W. O'LEARY¹

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Response of Sporobolus virginicus (Poaceae) to salinity

Optimal growth of euhalophytes requires moderate concentrations of salt and, in dicots, is associated with succulence of leaves and stems and accumulation of Na⁺ in plant tissues. Relative growth rate, water and cation content were studied in *Sporobolus virginicus* (L.) Kunth, a C₄ Chlorodoid grass, grown under different concentrations of NaCl. Optimal growth occurred at 100 mmol/L NaCl and was not dependent on nitrogen levels or accompanied by accumulation of Na⁺ in leaves. Na:K ratios were lower in leaves and rhizomes than in roots indicating the possibility of discrimination in Na⁺ and K⁺ transport from roots to leaves. Water content of leaves and shoots was significantly greater in plants grown at optimal levels of salinity than in plants grown at sub or supra optimal salinity. Na⁺ and K⁺ concentrations in leaves contribute approximately 60% of osmolality. These results support the characterization of *S. virginicus* as a euhalophyte.

294 BUCK, MEGHAN*, ROBERT REINSVOLD, GERRY SAUNDERS, AND TERESA HINZ

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Effects of magnesium chloride on Pinus contorta seedlings

The objective of this study was to examine the effects of a spray application of magnesium chloride on pine seedlings. Magnesium chloride is used statewide in Colorado as a soil stabilizer on dirt roads and de-icing agent for roadways in winter. Magnesium chloride is replacing sodium chloride in these uses because of the reduced corrosive effects. However, little is known about the physiological effects of MgCl₂ on roadside vegetation. With a controlled greenhouse experiment, we examined the effects of four concentrations of reagent-grade magnesium chloride (0 M, 0.74 M, 1.48 M, and 2.22 M) and one treatment of the commercially available magnesium chloride at the concentration applied to roads (3.15 M) on aboveground organs of one-year-old *Pinus contorta* seedlings. Chlorophyll content, shoot/needle dry weight biomass, and new needle growth at the apical shoots were measured over a 60-day period. Thirty days after the first spray application, necrotic needles were apparent on seedling exposed to the three highest concentrations of MgCl₂, and lower chlorophyll contents were measured. Early results indicate that aerial drift of magnesium chloride on roadside vegetation can have an impact on pine health and vigor at the normal rates of commercial application and lower rates.

295 FOLLOSCO, MINDA P.

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Clonal propagation of Tectona philippinensis Bentham et Hooker f. ex Merrill of Family Verbenaceae

Clonal propagation was successfully achieved in *Tectona philippinensis* Bentham et Hooker f. ex Merrill (Verbenaceae). The Philippine teak is endemic and famous for its high quality timber. Its restricted distribution and its presence in highly vulnerable habitat have rendered the plant population very low, thus the World Conservation Monitoring Center in 1992 declared its global status, rare and endangered. Both untreated and treated cuttings in various concentration levels of Indolebutyric acid (IBA) and alpha-naphthaleneacetic acid (NAA) responded to rooting under misted condition in polyethylene enclosures. Response at 45 days was manifested by formation of one or more adventitious roots with or without laterals, and formation of callus and fissures near the treated base. The latter, enabled many of the cuttings to be as healthy as those that have adventitious roots. Cuttings also survived despite the absence of roots, callus or fissures but were not as healthy and vigorous as their counterparts. Percentage values for rooting, callusing and fissure formation were high and were not significantly different in all concentration levels of IBA and in the untreated treatment. The number of cuttings which did not respond in the untreated treatment, 100 and 250 ppm IBA was high and these were significantly different (5% level, F-test) with the low values obtained under 500, 750 and 1000 ppm of IBA. Survival was 95 % in untreated cuttings and ranged from 97-100% in IBA-treated cuttings. The difference was not significant. IBA- rather than NAA-treated cuttings performed better with more number of large and green leaves and with higher results in rooting, callusing and formation of fissures. High concentrations of NAA at 750 and 1000 ppm greatly reduced the survival, rooting, callusing and fissure formation in cuttings. The study shows that Philippine teak is an easy-to-root species and there is much hope for the success of re-introducing the plant in its historical ranges of habitat.

296 GREAVER, TARA LIN

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The effects of reflected light on the anatomy and photosynthesis of Ipomoea pes-caprae (L.) R. BR. (Convolvulaceae), a tropical sand dune vine

Light heterogeneity influences the anatomy and physiology of leaves. In habitats where an overhead canopy is absent (e.g. deserts and dunes) different ground covers reflect light differently, and thus create a mosaic of reflected light. I studied the effects of reflected light on the anatomy and physiology of a tropical sand dune vine, *Ipomoea pes-caprae* (Convolvulaceae). *Ipomoea pes-caprae* has a symmetric leaf anatomy, with palisade parenchyma on both the adaxial and abaxial surfaces. Because the leaf anatomy is symmetric, I tested the hypothesis that the adaxial and adaxial surfaces responded independently to local light environment. Sampling was restricted to the six youngest fully expanded leaves, and thereby removed differences in nitrogen content of leaves due to age. Two types of light environments were characterized based on ground cover substrate. In high reflected light (HRL) environments plants grew directly over sand (26%±5.2% of incident PAR reflected) and in low reflected light (LRL) environments plants grew over vegetation (6.1% ±3.0% of incident PAR reflected). Photosynthetic maxima (Amax) did not differ between the adaxial surfaces in the HRL and LRL treatments (19.0 vs. 17.5 umol photon m⁻² m⁻¹, Tukey-Kramer HSD, p

297 HACKE, UWE G.* AND JOHN S. SPERRY

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The threshold for reversal of xylem embolism in Laurus nobilis

Xylem cavitation occurs in plants as a result of drought stress and freeze-thaw cycles. In woody plants, recovery from cavitation and embolism has been shown to be associated with root pressure and the production of new vessels/tracheids. According to our current understanding of bubble dissolution, xylem pressure (Px) has to rise close to atmospheric values or above for refilling to occur. However, vessels in *Laurus nobilis* have been reported to refill under certain experimental conditions even when Px was presumably very negative (e.g., Px = -1 MPa). In order to test whether a new paradigm for xylem refilling is necessary, we tested the ability of *Laurus* plants to refill vessels after a controlled drought cycle while continuously monitoring Px a critical measurement that was not done in previous studies. Whole plants were dried in pots, and a natural "vulnerability curve" was measured, showing the percentage loss in hydraulic conductivity (PLC) caused by cavitation as a function of the xylem pressure. A subset of intact plants was dried to Px = -3 MPa, which was associated with a PLC of c. 75%. These plants were then rewatered to various Px, and the PLC was measured. If *Laurus* is able to refill its embolized vessels under negative Px, then the PLC should decrease upon rewatering, and any embolism induced during the drying cycle should be readily reversed. However, there was no embolism reversal until Px rose above -0.2 MPa. Only at Px > -0.2 MPa embolism was rapidly reversed, and refilling became apparent. Assuming the osmotic potential of the xylem sap was between -0.1 and -0.2 MPa, results are consistent with our current understanding of bubble dissolution. Results suggest that refilling of embolized vessels requires near-atmospheric xylem pressure a condition that may only occur when transpiration is minimal and soil is saturated.

298 LUTTS, S*, J P MARTINEZ, M BAJJI, AND J M KINET

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Sodium implication in the response of the halophyte species Atriplex halimus L. to various abiotic stresses

Atriplex halimus is a xero-halophyte species widespread in deserts of Mediterranean regions. The presence of this species is also reported on mining areas contaminated by heavy metals. In order to determine the physiological basis of resistance to ionic and water stresses, whole plants and calli obtained from hypocotyls, roots and shoots were exposed to various doses of NaCl, polyethyleneglycol, water shortage or heavy metals (Cd, Zn and Cu) under controlled environmental conditions. As expected NaCl, induced an increase in sodium content of both plants and cell cultures. Low NaCl doses (150 mM) clearly stimulated growth. Interestingly, plants and calli also specifically accumulated sodium in response to water stress. In vitro selection and physiological characterization of drought-sensitive and drought-resistant cell lines, as well as the poor performances of whole plants maintained in sodium-free nutrient solutions, confirmed that sodium accumulation is not a symptom of injury but may be part of an unusual physiological strategy of abiotic stress resistance. From a quantitative point of view, sodium accumulation did not significantly contribute to osmotic adjustment in water-stressed tissues. Sodium accumulation was observed on stressed calli maintained in the dark for several months. At the whole plant level, X-ray microanalysis revealed that increase in sodium concentration of cadmium-treated plants occurred mainly in leaf trichomes. These points suggest that the role of sodium in the resistance to ionic and

water stresses in *Atriplex halimus* is not necessarily linked to the well-known implication of this element in the regulation of phosphoenolpyruvate translocation in C4 plants.

299 MOHAMED, SALEH M.

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Endoproteases in Sorghum bicolor (Cv. Giza 10) during germination

The pattern of endoproteolytic activities following *Sorghum bicolor* (cv. Giza 10) grain germination was studied. The maximum activity of acidic, neutral and basic of sorghum endoproteases was observed on days 6, 4 and 8 of germination, respectively. The effect of class-specific inhibitors on the endoproteolytic activities during grain germination, assayed in solution with azocasein as a substrate, indicated that: a) Metalloendoproteases were detected from day 6 to day 10 of germination, using ethylenediaminetetraacetic acid (EDTA) as an inhibitor, b) Aspartic endoproteases were detected from day 10 to day 14 of germination, using pepstatin A as an inhibitor, c) Serine endoproteases were detected at all stages of germination, using phenylmethyl sulphonyl fluoride (PMSF) as an inhibitor, d) Thiol endoproteases were detected from day 2 to day 6 of germination, using iodoacetate (IA) and p-Hydroxymercuribenzoate (p-HMB) as inhibitors. The effect of class-specific protease inhibitors was also tested on endoprotease assays in electrophoretic gel. The endoproteolytic bands of *Sorghum bicolor* grain germination were arbitrarily classified into three groups A, B and C contained less or more than 8 bands. Group B and, to a lesser extent, group C are contained metallo-, serine and thiol endoproteases. This work shows that germinating *Sorghum bicolor* contains a complex set of endoproteases could be potentially a good starting material for preparation of different classes of endoproteases.

300 NEILL, SAMUEL O.*, KEVIN S. GOULD, PAUL A. KILMARTIN, KENNETH R. MARKHAM, AND KEVIN A. MITCHELL

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Antioxidant strategies of red versus green leaves

Anthocyanin biosynthesis increases under environmental and biotic stresses in leaves. These same factors stimulate the production of reactive oxygen species (ROS). We have tested the theory that anthocyanins protect leaves from ROS, and that red leaves are better suited to scavenge ROS than are green leaves. Total antioxidant activities of leaf extracts from red and green morphs of *Quintinia serrata* (Escalloniaceae) and *Elatostema rugosum* (Urticaceae) were quantified using (i) their abilities to scavenge the stable DPPH radical, and (ii) cyclic voltammetry. They were also analysed for their compositions of flavonoids, hydroxycinnamates and antioxidant enzymes (superoxide dismutase, catalase, and ascorbate peroxidase). Red morphs of the shade plant, *E. rugosum* were more effective at scavenging ROS than the green morphs; with anthocyanins being the most active phenolic antioxidant tested. Anthocyanins appear to be synthesised in juvenile leaves to provide protection until a sufficient pool of enzymic antioxidants can be created. In contrast, leaves from red and green morphs of the sun plant *Q. serrata* had comparable antioxidant activities. *Q. serrata* appears to employ other phenolic compounds in addition to anthocyanin to provide adequate low molecular weight antioxidant (LMWA) protection; the hydroxycinnamates were found to be the most active components.

301 OLSON, AARON

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Metabolic pathways of oats

The growth rate of a plant is determined by its genetic capabilities, and its interactions with its surroundings. Calorimetric measurements of heat and CO₂ rates were taken to determine how metabolism of oats changes with temperature. Growth rate and substrate carbon conversion efficiency are calculated from the respiration data. We plan to measure oxygen isotopes by mass spectrometry to determine the oxidative-phosphorylation pathways plants use to adapt to temperature. The results of the measurements will give a better understanding of how plants respond to stress, and what it is they do in their metabolism that allows them to make these changes.

302 RATNAYAKA, H. HARISH^{1*}, WILLIAM T. MOLIN², AND TRACY M. STERLING¹

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Oxidative stress tolerance in cotton and spurred anoda under competition and drought

Injury to plants due to environmental stress is mainly caused by the reactive oxygen species that attack biomolecules and membranes. To prevent this damage plants have evolved an antioxidant system consisting of low molecular weight antioxidants such as ascorbate, α-tocopherol, glutathione and carotenoids, and of enzymes such as catalase, ascorbate peroxidase (APX) and glutathione reductase (GR). Since competition among plants decreases the availability of moisture and nutrients, it may initiate oxidative stress in plants. We studied the effect of intraspecific and interspecific competition on oxidative stress tolerance of cotton under adequate supply of moisture and nutrients in greenhouse. Two species of cotton (*Gossypium hirsutum* L., cultivar Delta Pine 5415, and *Gossypium barbadense* L., cultivar Pima S-7) were grown alone or three plants per pot for intraspecific competition. One plant of each of cotton species was grown with two plants of spurred anoda [*Anoda cristata* (L.) Schlecht.], an important cotton weed in the southern United States, for interspecific competition. A single plant of spurred anoda was maintained per replication. These treatment combinations were replicated six times. Net photosynthesis and photosynthetic quantum yield were not influenced by planting design or species at three weeks after planting. Chlorophyll a+b content was greater in *A. cristata* and *G. hirsutum* than *G. barbadense*. Carotenoid content was highest in *A. cristata* and higher in *G. barbadense* than *G. hirsutum*. Two cotton species had constitutively greater catalase and APX activities than spurred anoda while *G. hirsutum* had greater GR activity. When half of the experimental plants were subjected to a six-day drought, APX activity increased by 62% in *G. hirsutum* and 66% in *G. barbadense*; catalase and GR were unaffected. The levels of low molecular weight antioxidants as influenced by competition and drought will be presented.

303 SMITH, BRUCE N.

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Differences in temperature dependence of respiration among subspecies and hybrid populations of big sagebrush: nature vs. nurture

Artemisia tridentata Nutt. ssp. vaseyana (mountain big sagebrush) grows at slightly higher, cooler, and drier sites than does A.t. ssp. tridentata (basin big sagebrush). Natural hybrids between the two subspecies are found on a single east-facing hillside in Salt Creek Canyon near Nephi, Utah, where the parent populations are separated by 85 m in elevation and 1.1 km linear distance along the transect. In 1993, three gardens were established with seedlings from five populations along the transect planted in each garden. Stem water potential was measured at the site using a Scholander Pressure Bomb. Gas exchange respiration was measured with an infra-red gas analyzer. Plant stress was assessed with a chlorophyll fluorescence meter and carbon isotope ratios. Mineral concentrations in soil and plant tissue were measured using Inductively Coupled Plasma spectroscopy and spectrophotometric methods. Calorimetry was used to measure rates of metabolic heat loss and respiration on the same tissue. Significant differences were observed among the gardens whatever the source of origin and among the plant sources in whichever garden they were grown. Acclimation, showing phenotypic plasticity, occurred with change of season. Both nature and nurture have an influence. Sagebrush plants from all sources grow well at cool temperatures (when moisture is abundant), but are stressed and growth ceases at temperatures much above 30 C (when moisture is scarce). Sagebrush can become dormant to withstand high summer temperatures, but may have no mechanism for slowing growth during very cold conditions, and thus may be damaged if unprotected (Nelson and Tiernan 1983). Metabolic distinctions are made among closely related populations of plants grown on a single hillside in environments with only slight differences, showing physiological adaptation to very small environmental differences.

304 STRAUB, PETER F.

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Proline catabolism in Bradyrhizobium japonicum: drought effects

Accumulation of the amino acid proline is a commonly observed phenomenon in plant roots under drought stress. The proline accumulated is believed to serve as a compatible osmolyte to balance the cytoplasm against higher exterior and vacuolar ionic concentrations. The root nodules of soybean, like other root tissues, accumulate proline under drought conditions. Unlike enteric bacteria, who are also proline accumulators under osmotic stress, the symbiotic nitrogen fixing bacteria in soybean nodules display high activity of the proline catabolic enzyme proline dehydrogenase. Previous research has shown that gene directed knock-out mutations of the proline dehydrogenase gene in the symbiotic nitrogen fixing bacteria had an effect on seed yield and number when the soybean plants nodulated with these strains were subjected to moderate water stress. The basis of the yield decrease is not known but could be due to either loss of a catabolic substrate that provides some energy during drought or due to accumulation of fixed nitrogen (proline) down-regulating the nitrogen fixation machinery. The previously described proline dehydrogenase knock-out mutations and construction of a strain containing a proline dehydrogenase promoter-reporter gene fusion (GUS) are being used to test transcriptional regulation of the proline dehydrogenase promoter with or without the presence of the working enzyme in the bacteria.

305 THORHAUG, ANITRA^{1*} AND HAROLD WANLESS²

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The role of hurricanes, tornados and gale force winds in seagrass distribution in subtropical and tropical nearshore waters

The role of high-energy periodic wind events on seagrasses has been chiefly anecdotal. Sites in Biscayne Bay, FL and the Laguna Madre, TX are compared for 4 study sites. Two sites in Laguna Madre were investigated for seagrass test plots, sediment cores, vegetative mapping and aerial photography over a 4-year period, with a large-scale seagrass restoration (14.7 acres at one site and 60 acres at the second site) carried out in 1999. Hurricane Bret hit the 60-acre site two weeks after monitoring. A post-hurricane monitoring occurred. Aerial photos were subjected to analysis, available from 1996, 1998, 1999 post-hurricane and 2000. A second 14.7 acre Laguna Madre site was hit by a tornado after one half was restored in March 1999. Two lines of craters occurred which we documented by markers. The Biscayne Bay sites (in the Central Bay) had been mapped and ground truth data taken with sediment samples over years prior to a January 1983 hurricane force wind (off the south end of Key Biscayne). Morphological changes to the existing vegetation and corals were recorded. The second site (3 miles away) had been by examining aerial photos over a period of 20 years was restored with the seagrass Halodule wrightii during the summer, 1982, with 68% survival pre-gale in Fall 1981. Post-gale monitoring of seagrass beds showed the almost hurricane force wind caused substantial damage to the restoring seagrasses. Results of the four sites and comparisons of the vegetative cover, sediment and background vegetation will be discussed with data. Conclusions: 1.) Tornados can disrupt seagrasses leaving effects on for years; 2.) Gale force winter winds and hurricanes can alter shallow seagrass beds; 3.) Recuperation and re-growth after storm events can vary as to species and dissemule availability.

306 THORHAUG, ANITRA

658 NE 70 St., Miami, FL 33138

Test plots of seagrass species affecting long-term survival of restored seagrass

Four test plot programs were carried out for 3 species (Thalassia testudinum, Halodule wrightii, and Syringodium filiforme) and 3 techniques in Biscayne Bay, Florida over 22 years. In Laguna Madre, Texas, one test plot program with 2 species (Halodule wrightii and Halophila species), and 2 techniques were used. The result of test plot programs were used for larger scale restorations from 2 to 75 acres. The results of the larger scale plantings are judged by the investigator to have increased survival by the plantings. 1.) Some desirable sites to restore were shown to be beyond the photo compensation limits; 2.) Other barren sites were too energetic for any of the techniques to allow the development of transplant units; 3.) Some techniques originally preferred for abundance of specimens or ease of transport were not highly useful at necessary sites when tested; 4.) Some species, which would have been acceptable by the permitting agency or investigating scientist, did not demonstrate high survival. The three test plot studies in Biscayne Bay were carried out in 1976, 1982-2000. Test plots led to 6 larger plantings from 1.5 to 35 acres. The results led to separation of variables such as species, technique, site, depth, energy regime. The results of the larger scale planting were from 60 to 92% successful and led to large areas in and around the restored areas growing dense over short and long term. However, a gale-force winter storm at the 35-acre site 5 months after planting resulted in a one-third reduction in planted units. The Laguna Madre Texas sites led to a 75-acre planting. The best techniques

and species were utilized as well as having a clear concept of photo-compensation for depth which differed from that in the literature. Over 70% survival occurred despite a Hurricane and tornadoes which lowered the survival rates.

CONTRIBUTED POSTERS

307 JONES, CLAYTON A.

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Isotope ratio mass spectroscopy: a powerful tool for analyzing the respective contributions of environment and inheritance in plant growth

Artemisia tridentata Nutt. ssp. tridentata, and ssp. vaseyana, along with natural hybrids of the two, are found in Salt Creek Canyon, near Nephi, Utah. The two subspecies are separated by 1.1 km along the transect, and 85 m in elevation. Natural hybrids of the two occur on the same hill between the two parent populations. In 1993 seedlings from five populations along the transect were planted in three gardens. The upper, middle, and lower gardens allowed the five populations to grow in three different environments. Plant tissue was collected in triplicate from all five populations in each of the three gardens on 06 Nov. 1999. 5 mg (dry weight) of plant tissue were then combusted with Cu(II)O wire-form, and Cu granular at 800°C. CO₂ from the combustion of the plant tissue was then extracted using cryogenic distillation methods. The CO₂ was later analyzed in a mass spectrometer to measure the ratio of 13C/12C. This isotopic ratio yields a quantitative measurement of stress in the cumulative growth of the plant tissue. As stresses increase fractionation also increases and the ratio compared to a standard becomes more negative. The results of this analysis showed that, on average, stress/fractionation were highest in the upper garden and lowest in the lower garden. The environmental contribution to plant growth is clearly evidenced by the segregation of isotopic ratios between the gardens. The contribution of inheritance is also evident in the isotopic data. Stress/fractionation varies positively with the distance between the source location and the garden location. In addition to this variance, the hybrids exhibit lower levels of stress/fractionation in distant gardens than the parent populations. Mass spectroscopy is a powerful tool in looking at the contributions of both environment and inheritance in plant growth.

308 LOPEZ-SANTILLAN, JOSE ALBERTO¹, ALFREDO J HUERTA^{2*}, AND SERGIO CASTRO-NAVA¹

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Epicuticular leaf wax load on isogenic lines of sorghum (Sorghum bicolor L. Moench) and its contribution to water stress resistance

High concentrations of leaf epicuticular waxes are thought to enhance water stress resistance in plants because of a reduction in leaf temperature and also reduced water vapor loss from the leaves. However, even though this phenomenon has been studied for some time, much remains to be understood about the relationship between leaf wax load and leaf responses under varied environmental conditions. In this study, we report the effects of leaf epicuticular wax load on leaf temperature (LT), stomatal conductance (g), transpiration (E), and total water potential (Psi) in four isogenic lines of sorghum (obtained from the sorghum research program at Purdue University, West Lafayette,

Indiana), which varied with respect to epicuticular wax load. For this research, plants were grown in the field in northeastern Mexico (Ciudad Victoria, Tamaulipas), under both irrigated and non-irrigated conditions and sampled at 42, 58, 73, and 89 days after planting. Our results showed that LT was not affected by the amount of leaf epicuticular waxes and on average the LT was about 4 C higher under drought conditions than under irrigation. We found that g, E, and Psi were significantly correlated with leaf epicuticular wax load, but the degree of significance depended on sampling date. In general, we found that the isogenic lines with the greatest amounts of leaf epicuticular waxes showed the greatest reductions in g and E under non-irrigated conditions and also had the highest values for Psi. Under irrigated conditions, the effects of the leaf epicuticular waxes on the parameters measured were much reduced. In summary, we conclude from this study that under non-irrigated conditions, high epicuticular wax loads reduce water loss and hence allow the leaves to maintain a higher water potential.

309 MATHESON, TAINA

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Metabolic adaptation of plants to growth season temperature and temperature range along an altitudinal gradient

We are studying the metabolic adaptation of plants to temperature along an altitudinal gradient. Seven sites were selected for study, progressing from near the mouth of Provo Canyon (Utah County, UT) to just below the summit of Bald Mountain in the Uintas (Summit County, UT). We used HOBO H8 Pro-Series data loggers to record the hourly temperatures at each site throughout the 2000 growth season, from June through August. We identified two herbaceous and one woody species or congeneric species growing at every site, and collected growing tissue for calorimetric analysis. The species used were *Achillea millefolium* L., *Castilleja* spp., and *Salix* spp. Metabolic heat rates and CO₂ evolution rates were obtained at 5, 12, 19, 26, and 33 degrees C. The data show clear differences in metabolic response between plants of the same species growing at different altitudes. The hourly temperature data show that each site is characterized by a different range of temperatures experienced by plants during the growing season. We hypothesize that the mean temperature and temperature range will be correlated to the metabolic response of the plants. This hypothesis leads to a better understanding of metabolic adaptation of plants. The temperature loggers are currently in Salt Creek Canyon near Nephi (Utah County, UT), and we hope to correlate these data to previous calorimetric measurements on sagebrush grown in the canyon.

310 MCDERMOTT, MATTHEW AND DOUGLAS W. DARNOWSKI*

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Analysis of growth requirements of locally rare bladderworts (Utricularia spp.; Lentibulariaceae)

As an initial study of carnivorous plants in Great Swamp, Kent County, Maryland, a locally-rare setting, several species of bladderworts were identified and their preference for particular sites studied in the wild and in the laboratory. One species, *Utricularia geminiscapa* (Lentibulariaceae), which was not previously identified in lists for Great Swamp, grew sparsely in addition to three species (*U. macrorhiza*, *U. gibba*, *U. intermedia*) previously known from this site. These latter three occurred in abundance, though *U. intermedia* grew in highly specific locations with shallow

water and moderate shade. Conditions such as light levels and concentrations of various inorganic components of the aquatic environment were recorded over a period of six weeks, during which time the bloom of *U. macrorhiza* peaked and then waned while *U. gibba* began to bloom. Both sites favored and sites disfavored by these various species were examined for correlation with plant growth. To the same end, samples of several species were also cultivated under conditions in the laboratory chosen to mimic conditions in favored or disfavored sites in the wild. In particular, levels of [CO₂] and light were studied as they affected various growth parameters. Levels of [CO₂] and light in Great Swamp seemed to be best for either increase in length or trap production for at least two of the species studied.

311 THYGERSON, TONYA^{1*}, BRUCE N. SMITH¹, D. TERRANCE BOOTH², AND LEE D. HANSEN³

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Microcalorimetric studies on winterfat, Eurotia lanata, metabolism in response to temperature

Eurotia lanata (pursh) moq. (winterfat) is a boreal cold-desert subshrub, seldom more than 2ft. tall, and thrives in dry climates at cooler temperatures. Stem, leaves, and dispersal units called diaspores are covered with a dense mix of short and long white hairs that aid in water retention. It is excellent forage for both wildlife and domestic cattle. Diaspore collections were soaked in a tween solution, and then in dilute sodium hypochlorite and were next placed on moistened filter paper in petri dishes and germinated at room temperature. At the time of radicle emergence (ca.3mm), seeds were placed in ampules in a microcalorimeter. Heat-rate (q) was measured at a given temperature, and then a vial containing NaOH was added to measure the rate of CO₂ evolution (RCO₂) for the same tissue at the same temperature. This procedure was repeated for each of the populations at temperatures ranging from -10 to 20 degrees Celsius. From these measurements efficiency and predicted specific growth rates were calculated. Optimum temperature for germination, metabolism, and early seedling growth is about 10 degrees Celsius. Stress is noted near 20 degrees Celsius and -5 degrees Celsius. Acclimation during germination had no effect. Differences between the three populations correlated with altitude rather than latitude.

312 WALKER, JILLIAN LEIGH^{1,2*}, T. THYGERSON^{1,2}, BRUCE SMITH², LEE HANSEN³, R. CRIDDLE³, AND ROSEMARY PENDLETON⁴

¹Brigham Young University Undergraduate Student; ²Botany and Range Science Dept., BYU; ³Chemistry and Biochemistry Dept., BYU; ⁴Forestry Sciences Laboratory, 2205 Columbia SE, Albuquerque, NM 87106

Calorimetric studies of desert soil crust metabolism in response to temperature and water

Desert soil crusts are communities composed of lichens, cyanobacteria, algae, mosses, and fungi. These integrated soil crusts are susceptible to disturbance, but if intact, appear to play a role in providing essential nutrients, especially nitrogen, to higher plants. It is not currently known how or under what conditions desert crusts grow. Crust samples from localities on the Colorado Plateau, Great Basin, and Grand Junction, CO were collected and rewetted for regrowth. Both metabolic heat rate (q) and carbon dioxide evolution rate (RCO₂) were measured in microcalorimeters at temperatures from 0 to 50 degrees Celsius. The temperature dependency of different communities were evaluated, showing differences of growth at different temperatures.

PHYTOCHEMICAL SECTION, BSA

SYMPOSIUM: WHY LEAVES TURN RED: THE FUNCTION OF ANTHOCYANINS IN

VEGETATIVE ORGANS (BSA PHYTOCHEMICAL

SECTION / BSA DEVELOPMENTAL AND STRUCTURAL SECTION)

313 GOULD, KEVIN S., DAVID W. LEE*, AND JAMES WALLACE

Dept. of Biological Sciences, Florida International University, Miami, FL 33199

Why leaves turn red: the function of anthocyanins in vegetative organs

Despite a long record of research on anthocyanins in plants, their function in vegetative organs is a mystery. We have long known their pathway of synthesis and their photobiological regulation. More recently this understanding has been extended by advances in molecular genetics. Recent advances in whole plant physiology provide an opportunity for a multi-disciplinary approach to understanding the functional roles of anthocyanins in plants. This symposium assembles experts in biochemistry, molecular genetics, physiology, development and evolutionary ecology to summarize our present understanding and suggest the most promising directions for future research.

314 CHALKER-SCOTT, LINDA K.

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Do anthocyanins function as osmoregulators in leaf tissues?

Water stress can be induced in plant tissues, directly or indirectly, by a number of environmental conditions. Plants exposed to drought, heat, cold, wind, flooding, or saline conditions often synthesize foliar anthocyanins in response. Although previously thought to function as a UV screen, anthocyanins may instead serve to decrease leaf osmotic potential. This depression of leaf water potential could increase water uptake and/or reduce transpirational losses. Combined with other anti-stress activities attributed to anthocyanins (including their solar shield and antioxidative capacities), this phenomenon may allow anthocyanin-containing leaves to tolerate suboptimal water levels. Preliminary information linking leaf anthocyanin content to dehydration tolerance of several species will be presented.

315 GOULD, KEVIN S.^{1*}, SAMUEL O. NEILL¹, AND THOMAS C. VOGELMANN²

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A unified explanation for anthocyanins in leaves?

The leaves from many of New Zealand's native species are remarkably polymorphic for anthocyanin expression. Red coloration varies not only as a function of seasonal and developmental factors, but can also differ among individuals of a population, among leaves within a canopy, and even among tissues within a leaf. Moreover, the biosynthesis of anthocyanin in these leaves can be induced by a host of disparate environmental and biotic stimuli. Any unified explanation for the presence of anthocyanins in leaves must accommodate both the variability in pigmentation patterns over time and space, and the diverse range of

triggers. Our data indicate that anthocyanins confer a phytoprotective role, rather than being the default end-product of a saturated flavonoid metabolism. Anthocyanins are primarily associated with chlorophyllous tissues, and significantly modify both the quantity and quality of light incident on a chloroplast. Red leaves photosynthesize less than green leaves, but are also photoinhibited less and recover sooner following exposure to high light fluxes. Photoabatement also reduces the generation of free radicals and reactive oxygen species from photooxidation, photorespiration, and Mehler reaction activities. Anthocyanins inhibit Fenton hydroxyl radical generation by chelating to ferrous ions, and effectively scavenge superoxide and hydrogen peroxide generated by mechanical injury, sudden temperature changes, and exposures to high light. Anthocyanins are evidently versatile and highly effective phytoprotectants. However, there is probably no unified explanation for their presence in leaves. Common among the first land plants, anthocyanins have probably been hijacked over the course of evolution to perform an array of tasks.

316 HACKETT, WESLEY P.

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Differential expression and functional significance of anthocyanins in relation to phasic development

During the development of higher plants from seed, there are changes in their phenotypic characteristics. These changes in phenotypic characteristics are referred to as phasic development or phase change. Those characteristics associated with competence for reproductive development are referred to as adult or mature and those associated with vegetative growth are referred to as juvenile. The characteristics that change during phasic development are not necessarily consistent from species to species. However, phase specific accumulation of anthocyanins in stem and leaf tissue has been observed in a number of genera and species. This paper will present experimental evidence for the anatomical, biochemical and molecular bases for phase specific accumulation of anthocyanin in *Hedera helix*, a temperate zone vine species. The relationship of phase specific anthocyanin accumulation with other physiological and anatomical characteristics and the possible functional significance of accumulation will be discussed. The possible functional significance of phase specific anthocyanin accumulation in other species will also be discussed.

317 HOLBROOK, N. MICHELE¹, TAYLOR S. FEILD¹, AND DAVID W. LEE^{2*}

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Why leaves turn red in autumn: the role of anthocyanins in senescing leaves of red-osier dogwood (Cornus stolonifera)

Why the leaves of many woody species accumulate anthocyanins prior to being shed has long puzzled biologists because it is unclear what effects anthocyanins may have on leaf function. Here we provide evidence for *Cornus stolonifera* that anthocyanins form a pigment layer in the palisade mesophyll layer that decreases light capture by chloroplasts. Measurements of leaf absorbance demonstrated that red-senescing leaves absorbed more light of blue-green to orange wavelengths (495-644 nm) compared to yellow-senescing leaves. Using chlorophyll a fluorescence measurements, we observed that maximum PSII photon yield of red-senescing leaves recovered from a high light stress treatment whereas in yellow-senescing leaves failed to recover after 6 hours of dark-adaptation which suggests photo-oxidative damage.

Because no differences were observed in light response curves of effective PSII photon yield for red- and yellow-senescing leaves, differences between red- and yellow-senescing cannot be explained by differences in the capacities for photochemical and nonphotochemical light energy dissipation. A role of anthocyanins as screening pigments was explored further by measuring the responses PSII photon yield to blue light, which is preferentially absorbed by anthocyanins, versus red light which is poorly absorbed. We found that red-senescing leaves recovered rapidly to dark-adapted PSII photon yield following illumination with blue light. However, red light induced a similar, prolonged decrease in PSII photon yield in both red- and yellow-senescing leaves. We suggest that optical masking of chlorophyll by anthocyanins reduces risk of photo-oxidative damage to leaf cells as they senesce which otherwise may lower the efficiency of nutrient retrieval from senescing autumn leaves.

318 LEE, DAVID W.

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Anthocyanins in leaves: history, phylogeny and development

Anthocyanins have been intensely studied because of their expression in flowers and fruits of economic importance, yet they are also produced in vegetative organs, even in plants preceding the evolution of angiosperms. There has been much speculation on the function of this coloration, particularly by the physiological anatomists of the 19th century, but little serious research. A pre-condition to research on their function is a thorough knowledge of anthocyanin patterns in leaf tissue and their distribution among vascular plants. In a survey of 463 tropical taxa these patterns were investigated; tissue distributions in 399 taxa were mapped on the robust three-gene molecular phylogeny of Soltis *et al.* (Nature 402:402-404), pruned for these taxa with additional structure from other studies. Incidence and patterns of anthocyanin production were strongly associated with certain families, notably the Myrtaceae and Combretaceae, and were poorly represented among the Asteridae. Statistical analysis indicated that certain tissue distributions were significantly influenced by phylogenetic drift, despite many unresolved polytomies below the family level. Anthocyanin production in other plant parts was significantly associated with that in leaves in this sample of taxa. Most tissue patterns were limited to mesophyll tissue, and a smaller number limited to epidermal layers. The rarity of tissue combinations combining both germ lines (dermal and ground) suggests strong developmental constraints on locations of anthocyanin production in leaves. Only 13.5 % of these taxa produced anthocyanins during senescence, contrasting strongly with a high 70 % of 91 taxa sampled during senescence at Harvard Forest, in central Massachusetts. Similar patterns of betacyanin distribution were observed in a smaller sample of 21 taxa in the Caryophyllales. Similarities in absorbance spectra in visible wavelengths and strong *in vitro* anti-oxidant activities suggest that these two pigments may have similar physiological functions in leaves.

319 STARR, GREGORY^{1,2*} AND STEVEN F. OBERBAUER²

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The role of anthocyanins in photosynthesis of arctic evergreens during spring snow melt

In arctic ecosystems, snow cover persists into the spring long after air temperatures and light levels may be suitable for vascular plant photosynthesis. However, the general assumption is

that vascular plants under the snow show little if any photosynthetic activity. We have found that significant photosynthetic activity occurs in arctic evergreens in late spring prior to snowmelt, which in turn has implications in the ecosystem's carbon balance. This photosynthetic activity is quite variable and appears to be linked to microtopographic characteristics that affect photosynthetic photon flux densities (PPFD) reaching the plants. To protect their photosynthetic apparatus against the effects of high PPFD during spring melt, three arctic evergreens, *Ledum palustre* L., *Vaccinium vitis-idaea* L., and *Cassiope tetragona* L., were shown to produce significant concentrations of anthocyanins. The concentrations of anthocyanins were dependent on leaf positioning on individual plants and the position of individual plants within the microtopography. Plants that faced directly south tended to produce higher concentrations of anthocyanins than plants in shaded settings or northern orientations. We also demonstrate that snow load and anthocyanin concentrations are inversely correlated; as snow depth decreases and PPFD increase at the ground surface, concentrations of anthocyanins increase until moderate growing season conditions persist. These findings add additional support to the light screen hypothesis that suggests that anthocyanins protect photosynthetic tissues from photoinhibition when high light is coupled with low temperatures. This reduction in photoinhibition allows the species to increase their physiological activity prior to emerging from snow cover and allows them to reach maximum photosynthetic capacity within days of becoming snow free. This strategy is an important aspect of the acquisition of carbon reserves for these species during the short growing season.

320 WALBOT, VIRGINIA*, C. DEAN GOODMAN, AND SAVITA SHAH

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Translocation of anthocyanin from the cytoplasm to vacuole

The maize *Bronze2* (*Bz2*) gene catalyzes the last defined step in anthocyanin synthesis, acting after *Bz1*, a glucosyl transferase that catalyzes formation of cyanidin 3-glucoside (C3G). C3G is the most abundant anthocyanin "parent" molecule in maize. In *bz2* mutants, C3G accumulates in the cytoplasm, suggesting that BZ2 has a role in vacuolar sequestration. Based on similarity to a few proven plant Glutathione S-transferase (GST) genes, we predicted that BZ2 catalyzed glutathione (GSH) conjugation with anthocyanin as a pre-requisite for vacuolar compartmentation. Thus pigment synthesis would parallel steps in xenobiotic detoxification: hydroxylation by cytochrome P450 mono-oxygenases, glucosylation, glutathionation, and vacuolar sequestration. Biochemical evidence indicates that BZ2 is a carrier protein, not an enzyme. In *Petunia hybrida*, a Phi-type GST that shares only 11% amino acid identity with BZ2 performs the same role; maize GST3, a stress-induced Phi-type GST, can weakly complement *bz2* mutants. We have constructed *bz2 gst3* double mutants, and also *bz2 gst1 gst3* triple mutants; the mutants are viable, pointing out the redundancy of function within this large gene family. We propose that BZ2 has been selected for flavonoid binding to ferry cargo from synthesis sites in or associated with endomembranes to an ATP Binding Cassette transporter (ABC pump) located in the tonoplast. Anthocyanin sequestration is vanadate-sensitive, a hallmark of directly energized ABC transporters. Based on a genetic analysis of anthocyanin diffusion in tissues, we concluded that the relevant ABC transporter should be controlled by the same transcription factors that regulate the structural genes of the biosynthetic pathway. MRP29 is a maize ABC protein that is regulated by the R transcription factor, and it is highly expressed in purple tissues. Plants expressing antisense-MRP29 and *Mu*-induced mutants

are viable, and they are being used to analyze function in the anthocyanin and general flavonoid metabolism.

321 WINEFIELD, CHRISTOPHER S.^{1*}, KEVIN S. GOULD², AND KENNETH R MARKHAM³

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Flavonoid sequestration within the vacuolar compartment: a potential role for protein:anthocyanin interactions

In a wide range of species, floral pigmentation is due to the accumulation of anthocyanin pigments in the vacuoles of the flower epidermis. In most cases the accumulating anthocyanins are found evenly distributed throughout the vacuolar solution. However in a number of plants there have been reports of aggregations of anthocyanins within the vacuole. These aggregations have been variously described as "blue spherules" in epidermal cells of rose petal, "intravacuolar spherical bodies" in *Polygonum cuspidatum* seedlings, "ball-like structures" and "crystals" in stock *Matthiola incana*, petals, "blue crystals" in Larkspur, *Consolida ambigua*, and red "crystals" in mung bean hypocotyls. We have recently identified and begun characterisation of similar entities in *Eustoma grandiflorum* (lisianthus) and *Dianthus caryophyllus* (carnation). We have termed these structures Anthocyanic Vacuolar Inclusions or AVIs. They are non-membrane bound aggregations of highly pigmented material found in the vacuoles of the petal epidermis. The presence of these structures results in intensification in colour and a significant shift in the absorbance spectra of anthocyanins in these cells. We have isolated the AVIs from the inner region of lisianthus petals and have identified that they consist of a protein:anthocyanin complex to which only a subset of the anthocyanins and flavonoids produced in these tissues is bound. The ongoing characterisation of this complex and the potential implications of these interactions on flavonoid sequestration in plant tissues will be discussed.

322 WINKEL-SHIRLEY, BRENDA

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Molecular genetics and control of anthocyanin expression

The flavonoid pathway leading to anthocyanin biosynthesis has been well-characterized in a number of model systems, including maize, petunia, snapdragon and, more recently, Arabidopsis. Genetic approaches have identified many of the regulatory and structural genes required for the synthesis of these important plant pigments. In all species examined to date, control of the flavonoid pathway is known to occur, at least in part, at the level of transcription. In addition, there is evidence for regulation of this pathway at the level of the biosynthetic enzymes. Of particular interest to my research group is the possibility that flavonoid enzymes are organized as a multi-enzyme complex. This type of metabolic organization offers the potential not only for enhancing the efficiency of flavonoid biosynthesis, but also for regulating the flux of intermediates into branch pathways leading to anthocyanins and other endproducts. We have recently demonstrated specific interactions between flavonoid enzymes in Arabidopsis using co-immunoprecipitation, affinity chromatography, and two-hybrid analysis. Immunocytochemistry is providing further evidence for the co-localization of these enzymes at specific subcellular sites in root cells, consistent with biological roles of some flavonoids in auxin transport and the wound response. Additional approaches,

including the expression of scFv antibodies in transgenic plants and homology modeling of the structures of various flavonoid enzymes, are being used to identify protein domains involved in the assembly and positioning of this system within the cell. Analysis of a series of Arabidopsis mutants has also provided evidence that intermediates in flavonoid biosynthesis function in a feedback control system. Our studies indicate that plants use a diversity of mechanisms to control the synthesis of flavonoid products, including anthocyanins.

323 ZUFALL, REBECCA AND MARK D. RAUSHER*

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Diffuse coevolution and anthocyanin production

The evolution of floral displays is generally believed to be molded primarily by selection imposed by pollinators. Recently, however, it has been suggested that the evolution of floral color may also be influenced by herbivores and pathogens because several enzymes of the biosynthetic pathway that produces anthocyanin pigments are also involved in the production of various flavonoids, which can confer resistance to natural enemies. We present evidence for this type of pleiotropy in naturally occurring floral color variants of the common morning glory, *Ipomoea purpurea*. Specifically, we examined susceptibility to insect herbivores and fungal pathogens of genotypes with functional or non-functional copies of chalcone synthase, the first enzyme of the anthocyanin biosynthetic pathway. Genotypes with a non-functional CHS received approximately 25% more herbivore damage and exhibited twice the intensity of infection by *Rhizoctonia solani*. Plant fitness, as measured by seed production, was negatively correlated with amount of herbivore damage. These results indicate that the equilibrium frequencies of the CHS alleles in natural populations may be governed by diffuse selection imposed by herbivores and pathogens, as well as by pollinators.

CONTRIBUTED PAPERS

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Kaempferol rhamnoglucoside, a new flavonol from Amazonian coca (Erythroxylum coca var. ipadu)

Erythroxylum coca var. *ipadu* Plowman (*E. c.* var. *ipadu*) was harvested from cultivated fields in the Amazonian region of Colombia, South America, to determine: (a) their leaf flavonoid profile; (b) their similarity or difference to those present in our living collection of the taxon and (c) the usefulness of the flavonoids as chemotaxonomic markers for the taxon. High performance liquid chromatography was used to separate the flavonoids while spectra analyses were used for their identification. A new flavonol was found and isolated from leaf tissue of Amazonian field-grown coca (*Erythroxylum coca* var. *ipadu* Plowman) that has not been reported. The structure of the flavonol was determined to be kaempferol 4'-O-rhamnoglucoside. The array of flavonoids present in *E. c.* var. *ipadu* is indicative and consistent with ancestralship to *E. c.* var. *coca*. The new flavonol is useful as a chemotaxonomic marker for the taxon.

325 MARTIN, SUSAN S.

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Late-season accumulation of sucrose and oligosaccharides in sugarbeet (Beta vulgaris L.)

In the U.S., sugarbeet usually is planted in the spring and harvested as late as possible in the fall to allow maximum growth and yield potential. Processing companies use polarimetry as a rapid means of analysis of sucrose at the time of delivery of each harvested truckload of sugarbeets, and grower payments are based on their sugarbeets' sucrose content as well as tonnage. In some years, processing laboratory sucrose data appear to show an unexpected, rather sudden late-season increase in sucrose percentage in the roots. Much confusion has existed about this apparent late-season accumulation of sucrose, particularly with regard to the effects of temperature and precipitation. A phytochemical and physiological analysis of the potential for accumulation of sucrose and several related oligosaccharides shows that the accumulation of raffinose (galactosyl sucrose) and higher galactosyl homologs can contribute significantly to the optical rotation of sugarbeet extracts in late season. Raffinose synthesis involves the action of a cold-induced enzyme, galactinol synthetase, providing a rational basis for the widely-held (though incorrect) belief that "a light frost causes sugaring-up," or a rapid and significant accumulation of sucrose in the root. Thus, a plausible phytochemical explanation of "apparent" late-season sucrose accumulation now is possible.

326 PALMER, REID G.^{1*}, A.H.D. BROWN², MARVIN P. SCOTT¹, AND HARRY T. HORNER³

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A survey of wild perennial Glycine species to assess nectar production, composition and floral morphology

One of the limitations for the commercialization of hybrid soybean is the low level of cross-pollination in seed production fields. Insect-mediated cross-pollination is the method of choice but limited information is available in soybean on insect/flower attraction. Out-crossing in the wild perennial soybean species, *Glycine argyrea* and *G. clandestina* varied considerably, but can exceed 50% of the seed from chasmogamous fruit in some situations. Our objective was to survey the wild perennial *Glycine* species to assess nectar production and composition and floral morphology. About 70 accessions representing 19 species of the perennial soybean were studied. Measurements of the standard, wings and keel petals of two flowers per accession were taken. Nectar was collected from flowers by the use of capillary tubes. Sugar concentration was measured with a refractometer and by chemical determinations. Large differences in floral morphology of the standard, wing and keel petals were recorded. Total sugar concentrations varied among the species by 3 fold. Differences were noted among accessions of the same species and among ploidy levels within a species. The high sugar levels and floral morphology are compatible with entomophilous characteristics that favor insect visitations. The result is enhanced levels of cross-pollination.

CONTRIBUTED POSTER

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(+S)-ABA and (-R)-ABA are the final products from the oxidative cleavage of carotenoids.

The effects of diquat and ethephon (ethylene-releasing factor) on the formation of abscisic acid (ABA) were studied. Based on our data we have concluded that (+S)-ABA and (-R)-ABA are the final products from the oxidative cleavage of carotenoids. From the chemical point these products are prooxidants. Biologically active form of ABA as an antioxidant is 1',4'-diol ABA. These experiments precisely define the role of 1',4'-diol ABA as being essential for the effective defense against oxidative agents. By contrast, exogenous application of (+S)-ABA and (-R)-ABA as growth regulators demonstrated that these chemicals act as oxidative agents like as phenols. To prevent oxidative damages of the cellular biopolymers the cells increase the endogenous levels of antioxidants including 1',4'-diol ABA, prostaglandins (PGE and PGF). Hence, the increasing of endogenous concentration of (+S)-ABA and (-R)-ABA influenced by diquat (as also by another stress) is the respond of living cells on the increasing of endogenous quantities of oxidative agents. In this connection we concluded that jasmonates are also the final products from oxidative cleavage of membraneous lipids.

PTERIDOLOGICAL SECTION, BSA / AFS

SYMPOSIUM: EVOLUTION AND ADAPTATIONS OF PTERIDOPHYTES IN DRY CLIMATES

328 YATSKIEVYCH, GEORGE^{1*} AND ELISABETH A. HOOPER²

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Evolution and adaptations of pteridophytes in dry climates

A surprisingly large number of pteridophytes are able to meet the challenges of life in arid and seasonally dry habitats. This symposium will explore some of the structural, physiological, ecological, genetic, and other life-history adaptations of ferns and fern allies growing in regions where moisture deficit and high solar irradiation are part of a syndrome of factors providing strong selection pressures on evolution of the local flora. Attendant problems of taxonomic and phylogenetic interpretation that have resulted from such adaptations and directed evolution will be showcased. Studies on pteridophyte responses to aridity are still an emerging field of study, so the symposium will reflect future research needs as much as summarizing present knowledge of selected topics.

329 BENZING, DAVID, H.

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Ferns as epiphytes

In contrast to the angiosperms where fewer than 10% of the species are epiphytic, more than half of the ferns fit this description. Moreover, the epiphytic ferns exhibit diverse structure and function and occupy everwet and shady to arid and fully exposed microsities. In fact, the pteridophytic epiphytes possess many of the same devices for accommodating scarce and unpredictable supplies of water and key mineral nutrients that their flowering plant counterparts employ to grow under the same conditions. In some other respects, the ferns exceed the angiosperms in adaptive versatility, for example, in the variety of ways that foliage is specialized to deal with drought. Solutions to aridity range from syndromes based on poikilohydry, deciduousness, and CAM photosynthesis, three conditions that do not co-occur among closely related angiosperms. Shade tolerance among the ferns also has both unusual anatomical and physiological bases. Mechanisms of mineral nutrition are also diverse and sometimes facilitated by the same kinds of mutualisms with animals that benefit certain epiphytic angiosperms. This report describes the nature of the forest canopy as habitat for vascular plants and how the pteridophytes parallel and differ from the seed plants in the ways that they use this widely available ecospace. Finally, aspects of the adaptive biology of epiphytic ferns that warrant additional study as examples of novel or extreme adaptations are mentioned.

330 CARLQUIST, SHERWIN* AND EDWARD SCHNEIDER

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SEM studies of vessels in ferns of xeric habitats

Vessels have hitherto been reported only in a few ferns (*Pteridium*, *Marsilea*) on the basis of light microscopy. These ferns occur in habitats markedly seasonal with respect to moisture availability. Light microscopy cannot adequately resolve presence of pit membranes in pits or perforation plates of vessels, but SEM reveals pit membranes in vessels prepared by means of maceration and sectioning. Care must be taken to identify any artifacts in membrane absence, tearing, and distortion. Nevertheless, we can report with certainty vessel elements in which end walls not only lack pit membranes but have perforations different in size and shape from the pits of lateral walls (*Astrolepis*, *Dicranopteris*, *Microgramma*, *Polystichum*, *Vittaria*, *Woodsia*). All of these experience prolonged drought. In addition, other fern genera of dry habitats have vessels with perforation plates morphologically like lateral wall except that pit membrane remnants (often porose, weblike, or vestigial) are present in perforations whereas nonperforated pit membranes occur on lateral walls.

331 GASTONY, GERALD J.^{1*} AND GEORGE YATSKIEVYCH²

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Morphological reassessment of molecular phylogenetic inferences in the xerically adapted cheilantheid ferns (Pteridaceae: Cheilantheoideae)

Recent molecular cladistic analyses based on *rbcL* and nuclear ribosomal DNA have provided new insights into putative evolutionary lineages within cheilantheid ferns. The molecular approach was prompted in part by Tryon and Tryon's inference that "convergence in adaptive morphology has undoubtedly been frequent among cheilantheid ferns" and their conclusion that cheilantheoids are "the most contentious group of ferns

with regard to a practical and natural generic classification." Molecular studies may provide the framework for an evolutionarily natural generic classification, but nucleotide sequences do not provide practical data in taxonomic and floristic contexts. A set of morphological characters has therefore been proposed as the basis for new cladistic analyses of cheilanthoids, with taxa selected from among those used in the molecular studies that found two major clades in the subfamily. The large clade encompassing *Pellaea* sections *Pellaea* and *Platyloma*, *Paraceterach*, *Astrolepis*, *Argyroschisma*, and American *Cheilanthes* provides an ingroup for the morphological study, with outgroup taxa drawn from the sister clade containing *Notholaena*, other American and non-American *Cheilanthes*, and *Pellaea* sections *Ormopteris* and *Holcochlaena*, plus more basal *Bommeria*. These two major clades correlate well with base chromosome numbers of $x = 29$ or 27 in subclades of the ingroup, and $x = 30$ in the outgroup, including *Bommeria*, but $x = 30$ is retained as the plesiomorphic condition in some American *Cheilanthes* in the ingroup while $x = 29$ appears to be homoplastic in some species of the outgroup clade. Whether non-homoplastic morphological data are adequate to permit rigorous morphological cladistic analysis or permit only fitting these characters to the molecular tree remains to be determined.

332 HOOPER, ELISABETH A.^{1*} AND GEORGE YATSKIEVYCH²

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Distribution of arid climates and drought-adapted pteridophytes, an introduction

Approximately one-third of the Earth's land surface is classified as arid or semi-arid. The classic 'hot' deserts of the world (e.g., Sahara) occur between 20° and 30° latitudes N and S, but arid conditions are found across the globe. Most arid areas experience high daily temperatures as well as low and unpredictable precipitation (*Selaginella lepidophylla*). It is important to note that selection pressures act on gametophytes as well as sporophytes, but little is known about gametophyte adaptations to drought. Taxonomic diversity of xerophytic pteridophytes is concentrated in relatively few families: e.g., Pteridaceae and Selaginellaceae. However, xerically-adapted species occur in most lineages. Of note are several epiphytes in the Polypodiaceae as well as some typically aquatic species whose habitats are intermittently dry (e.g., Isoetaceae and Marsileaceae). Hotspots of pteridophyte diversity can be summarized for arid regions with reasonably complete floristic data.

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Desiccation-tolerant pteridophytes: a unique position in the evolution of desiccation tolerance in land plants

Vegetative desiccation tolerance is a widespread but uncommon occurrence in plants. The majority of desiccation-tolerant plants are found in the less complex clades that constitute the algae and bryophytes. However, within the larger and more complex groups of vascular land plants there are some 120-130 species that exhibit some degree of vegetative desiccation tolerance. A phylogenetic look at vegetative desiccation tolerance reveals that this capability was lost during the time when tracheophytes first evolved and subsequently "re evolved" at least twelve separate times in various vascular plant lineages. The primitive

mechanism of vegetative desiccation tolerance in the first land plants probably involved constitutive cellular protection coupled with active and inducible cellular repair, similar to that described for modern-day desiccation-tolerant mosses. Desiccation-tolerant angiosperms utilize an inducible cellular protection mechanism of tolerance that appears to derive from the programmed cellular protection mechanism seen in seeds. Desiccation-tolerant pteridophytes appear to employ a mechanism of tolerance that appears to have characteristics of both the primitive and the more recently evolved mechanisms seen in the angiosperms. Much of our evidence for this comes from the interpretation of a wealth of physiological data derived from the ecophysiology of *Selaginella lepidophylla* and a few desiccation tolerant ferns such as *Polypodium polypodioides*. However, detailed mechanistic studies have only been attempted using the desiccation-tolerant fern *Polypodium virginianum* so much still needs to be accomplished. Pteridophytes are in a unique position for the study of desiccation tolerance and its role in the evolution of the land plants and that with the availability of the modern tools of genomics the time is right for this area to expand.

334 WINDHAM, MICHAEL D.^{1*} AND CHRISTOPHER H. HAUFLE²

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Apomixis in ferns: a consequence of marginal habitats and outcrossing?

Xeric environments impose significant constraints on plant life histories. In ferns, there is a strong correlation between the apomictic life cycle and drought-prone habitats. The life cycle of apomictic ferns involves modifications of both the sporophytic and gametophytic generations. Sporophytes have an altered sporogenetic process in which a premeiotic endomitosis results in chromosome duplication without cytokinesis. As such, spore mother cells have a doubled chromosome number, and meiosis produces spores with the same genetic composition as the original sporophyte. Gametophytes produced by these spores initiate sporophytes directly, without fertilization. The evolutionary origin of apomixis seems to be tied to forces imposed by stressful environments. Natural triploids are found at the geographical and/or physiological margins of diploid populations. Isozyme studies demonstrate that these triploids result from outcrossing between unreduced diploid gametes and "normal" haploid gametes. Such triploids are highly heterozygous, vigorous individuals, but cannot reproduce and disperse via spores. In lineages whose gametophytes seldom experience xeric conditions, these triploids occur as isolated sporophytes. However, when gametophytes regularly encounter stressful conditions, induction of sporophytes without fertilization may occur. In vitro studies show that intense insolation or high sucrose concentrations can cause gametophytes to spontaneously generate sporophytes. This process, coupled with consistent formation of unreduced gametes, has allowed ferns to proliferate in some of the driest habitats on earth. Taxon diversity is further enhanced by hybridization between apomicts and related diploid species, and this syndrome of life history features may drive adaptive radiation of desert ferns.

CONTRIBUTED PAPERS

335 AMOROSO, CECILIA BELTRAN* AND VICTOR B. AMOROSO

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Micropropagation and plantlet production of the endangered and endemic giant staghorn fern of the Philippines (Platyserium grande (Fee) Presl.)

Platyserium grande (Fee) Presl. or giant staghorn fern is a highly prized ornamental, endemic and endangered plant of the Philippines. This study was conducted to determine the appropriate culture medium and conditions for spore germination, gametophyte, and sporophyte development of *P. grande*; to find out the optimum potting medium and conditions for acclimatization of the plantlets; and to mass propagate the plantlets. Spores of *P. grande* readily germinated 11-20 days from sowing in agar culture medium with small amount of nutrients. Plantlets arose from the prothalli 7 months from spore inoculation. When the plantlets were transferred to wire baskets and clay pots containing chopped adventitious roots of *Cyathea* 12 months from inoculation, they developed bigger leaves. Many of these plantlets are growing vigorously in the greenhouse of the Department of Biology, College of Arts and Sciences, Central Mindanao University, Musuan, Bukidnon, Philippines. Thus, micropropagation (spore culture) is one way of conserving and propagating the endangered and endemic *P. grande*.

336 BARKER, MICHAEL S.* AND WARREN D. HAUKE

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An evaluation of Sceptridium dissectum (Spreng.) Lyon and S. oneidense (Gilb.) House (Ophioglossaceae) using ISSR markers: implications for Sceptridium species circumscriptions

Species of *Sceptridium* (grapeferns) are found throughout most of North America, with a continental center of diversity in the Eastern United States. The combination of few stable species-specific characters and substantial infraspecific morphological variation makes *Sceptridium* species difficult to distinguish. *Sceptridium dissectum* (Spreng.) Lyon, the most variable North American grapefern species, demonstrates variation in degree of blade dissection, pinnule shape, pinnule margins, and blade color. In 1938, Clausen recognized four varieties and one subspecies within *S. dissectum*. Of Clausen's five infraspecific taxa, only var. *obliquum* (Muhl.) Clute and var. *dissectum* Spreng. are currently retained. In 1960, Wagner raised *S. dissectum* var. *oneidense* (Gilb.) Farwell to species status citing differences in leaf color, periodicity, and root morphology. Because our observations revealed no clear morphological discontinuities among taxonomic units in *Sceptridium*, we examined the relationships among *S. oneidense*, *S. dissectum* var. *dissectum*, and *S. dissectum* var. *obliquum* using molecular markers. Preliminary collections from 17 Ohio populations included a total of 69 sporophytes: 10 *S. dissectum* var. *dissectum*, 52 *S. dissectum* var. *obliquum*, and seven *S. oneidense* individuals. We used ISSR (Inter-Simple Sequence Repeat) markers to assess the genetic distinctness of *S. oneidense*, *S. dissectum* var. *dissectum*, and *S. dissectum* var. *obliquum*. Five ISSR primers generated 69 reproducible loci. In UPGMA and AMOVA analyses, individuals identified as *S. oneidense* did not cluster with other individuals assigned to the same species. Likewise, *S. dissectum* var. *dissectum* individuals did not cluster together. Thus, our results demonstrated that the morphological features of *S. oneidense*, *S. dissectum* var. *dissectum*, and *S. dissectum* var. *obliquum* did not

correlate with ISSR banding patterns. We question recognizing *S. oneidense* as a distinct species, and the evolutionary relevance of retaining infraspecific taxa in *S. dissectum*.

337 COX, PATRICIA B.

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A re-evaluation of the pteridophyte flora in the Great Smoky Mountains National Park

As a component of the All Taxa Biodiversity Inventory in the Great Smoky Mountain National Park, a re-evaluation of the pteridophyte flora is being undertaken. In addition to the inventory of species, we are also mapping the distribution of ferns in the park. Currently there are 64 species of pteridophytes reported from the GSMP. Twenty-five (39%) are considered to be rare in the park. There are historical records for *Lycopodium annotinum*, *Thelypteris simulata*, and *T. phaegopteris*. It has been determined that *T. simulata*, had incorrect label data and was not collected inside the park. Searches for specimens of the other historical records have been made and are thought to be extirpated from this area. A tetraploid species of *Isoetes*, was recently collected in the Cade's Cove area, and is currently under investigation by both Dr. Carl Taylor and Kerry Heafner. A few of the other rare species known from the GSMNP are *Asplenium pinnatifidum*, *Botrychium lanceolatum* var. *anustisegmentum*, *B. matricarifolium*, *Lygodium palmatum*, and *Woodwardia virginica*.

338 DES MARAIS, DAVID L.^{1*}, KATHLEEN M. PRYER², AND ALAN R. SMITH³

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Phylogeny, character evolution, and biogeography of extant horsetails (Equisetum)

Equisetum is a small (15 extant species), easily recognized, and highly distinctive genus of vascular plants with a cosmopolitan distribution. Only Australia, New Zealand, and Antarctica lack native representatives. Most species are found between 40 and 60 degrees north latitude and they are generally confined to seasonally wet ground. Two subgenera have been recognized based on stomatal position and growth form: subg. *Equisetum* (8 species; superficial stomates; stems branched) and subg. *Hippochaete* (7 species; sunken stomates; stems unbranched). Fossils assigned to *Equisetum* or *Equisitites* date back to the Triassic Period or later, with possible records extending to the Carboniferous. Prior attempts at understanding *Equisetum* systematics, phylogeny, and character evolution have been hampered by the high degree of variability in the genus, as well as by rampant hybridization among members within each subgenus. Carefully avoiding hybrids, we present the first explicit phylogenetic study of *Equisetum*, including all 15 species, based on a combined analysis of two chloroplast markers, *trnL-F* and *rbcl*, and demonstrate robust support for two monophyletic groups that correspond to the two subgenera recognized by earlier workers. The species relationships resolved within each of these groups, however, are mostly in disagreement with previous views. In addition, the South American species *E. bogotense* is not a member of either of these two groups, but rather it is isolated as sister to the other 14 species. With a robust phylogeny in hand, we explore questions related to morphological character evolution and biogeography in this ancient genus. Contrary to earlier interpretations, unisexual gametophytes and a diminutive stature appear to be ancestral conditions. Several taxonomic characters such as dimorphism, previously considered to be good indicators of species relationships, are shown to be homoplastic. Using fossil evidence and geological vicariance of

continents to calibrate divergence times, we also investigate possible biogeographic hypotheses.

339 GEIGER, JENNIFER M. O.^{1*}, TOM. A. RANKER¹, AND DANIEL. D. PALMER²

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Phylogenetic systematics and biogeography of Hawaiian Dryopteris (Dryopteridaceae)

The fern genus *Dryopteris* represented worldwide by approximately 225 species. In the Hawaiian Islands, all but one species of *Dryopteris* are endemic. Past investigators have suggested that the *Dryopteris* species diversity observed in Hawaii resulted from multiple, successful colonization events to the Hawaiian archipelago. We collected DNA sequence data from two chloroplast regions, the *rbcl* gene and the *trnF-trnL* intergenic spacer. Preliminary phylogenetic analyses from *rbcl* sequence data suggest that Hawaiian *Dryopteris* is not a monophyletic group. One of two non-Hawaiian *Dryopteris* species, *D. dickinsii*, included in the analysis arises on the tree from amongst Hawaiian species, suggesting that there were at least 2 separate introductions of *Dryopteris* to Hawaii. Maximum parsimony analyses of the *trnF-trnL* intergenic spacer strongly suggest that two distinct clades exist within Hawaiian *Dryopteris*. Additionally, one clade appears to be more closely related to a non-*Dryopteris* Hawaiian species, *Nothoperanema rubiginosa*, than to other species of the same genus. This may suggest that the ancestral species that gave rise to each of these groups were themselves distantly related, that *N. rubiginosa* is simply misclassified, or that intergenic spacer sequences do not reflect species-level phylogeny.

340 GREER, GARY K.* AND DORTHY CURRY

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Pheromonal interactions among cordate gametophytes of the lady fern, Athyrium filix-femina

Pheromonal interactions between cordate gametophytes of the lady fern, *Athyrium filix-femina*, were investigated using a protocol typically used for detecting water-soluble pheromones such as antheridiogen. Three week-old, cordate gametophytes were transferred to agar containing abstracts from a previous generation of gametophytes (treatment) and to fresh nutrient agar (control). Three weeks after transfer, gametophytes were examined from treatment and control plates. Each gametophyte was measured for size (area) and shape (circularity), and scored for number of antheridia and archegonia. Treatment gametophytes were significantly smaller, less circular, had fewer archegonia, and possessed antheridia more often, than control gametophytes, a pattern consistent with known antheridiogen effects on gametophytes of transitional morphology and sensitivity. The experiment was repeated using gametophytes that were six weeks old at time of transfer. Treatment gametophytes in the second experiment did not differ in size, but were significantly more circular and possessed fewer archegonia, than control gametophytes. The second experiment revealed phytochemical interactions between cordate gametophytes. The contradictory effects of increased circularity without increased size may be due to one or more phytochemicals that accelerate anticlinal divisions in the meristem resulting in increasing circularity, at the expense of oblique or periclinal divisions that contributed to size. Likewise, the decreased production of archegonia, which can not be attributed to production of antheridia, may be a consequence of a reduced rate of oblique and periclinal divisions in the meristem, from which archegonia are ultimately derived. Antheridiogen, a water-soluble pheromone with structural affinity to gibberellins and that induces maleness in a cordate gameto-

phyte may be involved, since its production is closely associated with attainment of a cordate morphology.

341 HAUFLE, CHRISTOPHER H.^{1*}, ALAN R. SMITH², HARALD SCHNEIDER², TERRI HILDEBRAND¹, RAY CRANFILL², AND TOM A. RANKER³

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Polypodium redefined: morphology, biogeography, and DNA sequences provide clues to lineages

The ±180 species usually assigned to *Polypodium* appear to represent a polyphyletic assemblage of nearly exclusively New World species. Rooted to Old World genera, most *Polypodium* species occur in the continental upland tropics, with relatively little diversification on islands, and only the monophyletic *P. vulgare* complex found in temperate zones. Scale characters appear particularly significant for circumscribing apparently natural groups in this large genus. Glabrous and scaly-leaved species constitute separate lineages, with bicolorous, concolorous, and clathrate rhizome scales useful for further subdivisions. Other significant morphological features include complexity of vein anastomoses, numbers of soral rows, and patterns of rhizome branching. Separate and combined analyses of sequences from *rbcl*, *rps4*, and *trnL-F* yield insights for testing hypothesized subdivisions of *Polypodium* and determining interrelationships between *Polypodium* and other genera in the Polypodiaceae. *Polypodium* should be strictly applied to the glabrous-leaved species, whereas *Pleopeltis* appears to be the generic name that will be used for the scaly-leaved species. Some regularly included *Polypodium* species (e.g., *P. triseriale* and *P. ptilorhizon*) appear to have a tenuous connection to other members of the genus. *Pecluma* emerges consistently as an outgroup to the scaly polypods and not closely allied to the grammitids. Other close relatives may include *Campyloneurum* and *Niphidium*, although the precise position of these segregate genera has been difficult to determine. These analyses help provide targets for future biosystematic studies of tropical American genera and will aid in defining general patterns and processes of diversification among pteridophytes.

342 HENNEQUIN, SABINE* AND JEAN-YVES DUBUISSON

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Systematics of the fern genus Hymenophyllum s.l. (Hymenophyllaceae) inferred from rbcl and rps4 nucleotide sequences and morphology

The subcosmopolitan fern genus *Hymenophyllum* s.l. (Hymenophyllaceae) includes more than 300 species, mostly in tropical areas. Phylogenetic studies using both morphology and molecules were performed in order to clarify the still controversial systematics of this group. Previous works using *rbcl* sequences have suggested that this genus, in a broad sense, may be monophyletic, but these same studies also show a lack of resolution at the infrageneric level. This contrasts with substantial resolution in the sister genus *Trichomanes*. Another marker, the chloroplast *rps4* gene, has thus been tested. Sequences were acquired for 25 species, representing the 9 major groups of *Hymenophyllum*, including the monospecific genera *Serpillopsis*, *Hymenoglossum*, and *Rosenstockia*, as well as the peculiar *Microtrichomanes*. Five species of *Trichomanes* plus the monotypic genus *Cardiomanes* were added as outgroups. *Rps4* data provide better resolution than *rbcl* sequences by distinguishing clearly

Morton's subgenera *Sphaerocionium*, *Mecodium*, and *Hymenophyllum*. Moreover, this gene alone and in a combined analysis (*rps4* + *rbcl*) provide new insights into the relationships within *Hymenophyllum sensu lato*. Conclusions include: 1) the robustly supported inclusion of the problematic monotypic genera in *Hymenophyllum s.l.*, with *Microtrichomanes* in a basal position and closely related to subg. *Sphaerocionium*, and *Serpyllopsis* and *Rosenstockia* included in subg. *Hymenophyllum*; and 2) placement of subg. *Craspedophyllum* and subg. *Hemicyatheon* within subg. *Hymenophyllum*. In parallel, and using the same taxonomic sample, a morphological study has been initiated. Despite the paucity of informative characters for identifying natural groups, the phylogenetic trees generated from morphology reveal some relationships within *Hymenophyllum s.l.* that are consistent with those from molecules. Both approaches are discussed. In addition, the evolution of sorus shape, traditionally considered a diagnostic character in this group, is re-evaluated.

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Parental species of Isoetes allotetraploids, including "dead-beat" parents

Nucleotide sequences from the highly variable second intron of a *LEAFY* homolog (a meristem identity gene) were used to determine the parents of allotetraploid *Isoetes* species. By comparing the positions on the cladogram of the two cloned sequences from the allotetraploids with an extensive sampling of sequences from diploid species, parental species were identified. In some cases, we have been able to identify both parents with some certainty. For example, the allotetraploid *I. maritima* from the Pacific Northwest resulted from a hybridization event between *I. echinospora* (circumboreal) and *I. bolanderi* (Pacific Northwest and Rockies). *Isoetes tuckermanii* (northeastern North America) resulted from a cross between *I. engelmannii* (eastern North America) and a species with sequence similarities to *I. prototypus* (northeastern North America). In other cases, we have been able to identify only one of the parents; the other "dead-beat" parent is missing from our data set. For example, *I. appalachiana* (southeastern U.S.) is a cross between *I. engelmannii* and an unknown taxon with affinities to *I. flaccida* (Florida). *I. azorica* (Azores Islands) resulted from a hybridization event between *I. engelmannii* and a missing or undiscovered *Isoetes* species from North America or the Mediterranean region. A previous hypothesis had suggested that *I. azorica* was morphologically similar to *I. tuckermanii*. The fact that *I. azorica* and *I. tuckermanii* both have *I. engelmannii* as one of their parents may account for such an interpretation.

344 NAKAZATO, TAKUYA* AND GERALD J. GASTONY

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Molecular phylogenetics of Anogramma species and related genera (Pteridaceae: Taenitidoideae)

Anogramma Link is a genus of about five species with annual sporophytes and potentially perennating gametophytes: *A. leptophylla*, *A. chaerophylla*, *A. osteniana*, *A. lorentzii*, and *A. ascensionis*. *Anogramma caespitosa* of east Africa and *A. guatemalensis* have been segregated from nearly cosmopolitan *A. leptophylla*, and *A. novogaliciana* from Jalisco, Mexico is said to resemble *A. chaerophylla*. Nucleotide sequences of *rbcl* were determined for all of these species (except *A. ascensionis*, which is thought to be extinct, and *A. novogaliciana*, material of which is being sought), including multiple accessions of *A. leptophylla* from

diverse parts of its range. Also included were species of *Pityrogramma* (generally considered most closely related to *Anogramma*), other putatively taenitoid genera, and *Cosentinia vellea* whose spore morphology is strikingly similar to that of *A. leptophylla* and unlike that of *Cheilanthes* in which it is often subsumed. Maximum parsimony cladistic analyses were used to test 1) the monophyly of *Anogramma*, 2) support for the proposed specific distinctness of *A. guatemalensis* and *A. caespitosa*, and 3) the asserted close (sister) relationship between *Anogramma* and *Pityrogramma*, and to infer the phylogenetic relationships among these genera. Results reveal that *Anogramma* in the traditional sense is polyphyletic. *Anogramma guatemalensis* and *A. caespitosa* nest within *A. leptophylla*. *Pityrogramma* is not sister to *Anogramma* as a whole but only to *A. chaerophylla*. *Cosentinia vellea* should not be subsumed within *Cheilanthes*. *Anogramma osteniana* is well separated from its traditional congeners. Neighbor joining analysis of an AFLP (Amplified Fragment Length Polymorphism) data set inferred relationships among the *A. leptophylla* accessions, embedding *A. guatemalensis* in a New World clade and *A. caespitosa* in a separate Old World clade.

345 PETERSEN, RAYMOND L.* AND LISA FUNDERBURK

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A history of ozone pollution (1850-1990) in Eastern United States based on the use of Osmunda cinnamomea L. herbarium specimens

Over 1,000 herbarium specimens of the cinnamon fern *Osmunda cinnamomea* L. from Maryland, New Jersey, and Pennsylvania were assessed for symptoms of foliar ozone injury – interveinal bronze necrotic lesions of the pinnules. (Ozone-injury symptomatology is based on control-fumigation of live *O. cinnamomea* plants.) Herbarium sheet inventory included: locale (State and County), collection date (year and Julian day), presence or absence of ozone injury, and percent area of pinnule damaged. (Morphometric analysis of two pinnules sampled from each herbarium sheet was used to obtain estimates of percent area of pinnule damage.) From these data two measures of ozone injury were obtained: (1) % incidence of injury (i.e. number of specimen with necrotic lesions/total number of specimens/ local/decade) and (2) degree of ozone injury, expressed as % area of pinnule damaged. Based on this assessment a decade-by-decade history of ozone pollution from 1850-1990 is constructed. Herbarium specimens from the 19th century show little to no signs of ozone injury (i.e., both % incidence of injury and degree of ozone injury are less than 1%). Thereafter, an irregular but overall progressive increase in the % incidence of injury is detected. The pattern generated is somewhat reflective of historic and economic events of the 20th century – the invention of the internal-combustion engine and the automobile and the First World War, the Depression of the 1930's, the Second World War, the ending of the Second World War with the un-rationing of gasoline, the economic and industrial expansion of the 1950s and 60s, and the Clean Air Acts of the 1960s and 70s. There is a high positive regression correlation of percent area of pinnule-damage as of function of the growing season and no correlation of % pinnule area damage as a function of annual chronology (i.e., average % pinnule area damage/specimen did not change over the years). These findings demonstrate the value of herbarium specimens in the construction of environmental histories.

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Phylogenetic systematics of the Hawaiian endemic genus Adenophorus (Grammitidaceae)

A*denophorus s.l.* is one of only three endemic genera of ferns in Hawaii. It has been distinguished from other genera of Grammitidaceae primarily because of distinctive glandular paraphyses and other laminar hairs. The uniqueness of those features, however, has been questioned and other Indo-Pacific taxa may be close relatives. In particular, *Chrysogrammitis musgraviana* was proposed as being the closest Indo-Pacific relative. Hawaiian species have either been classified as a single genus with two subgenera or as two similarly circumscribed genera: *Adenophorus s.s.*, with variously deeply dissected leaves and no root buds, and *Oligadenus*, with entire or shallowly-to-deeply lobed leaves and with root buds. We investigated the phylogenetic relationships of the species of *Adenophorus s.l.* with one another and with 56 other species of the Grammitidaceae from throughout the range of the family, including other Hawaiian taxa. We obtained DNA sequences of three cpDNA regions: *atpB*, *rbcl*, and the *trnL-trnF* spacer. Phylogenetic analyses of these data yield the following hypotheses: 1) *Adenophorus s.l.* is monophyletic; 2) the Hawaiian endemic *Grammitis tenella* is the sister taxon to *Adenophorus s.l.*; 3) *Adenophorus s.s.* is monophyletic and arose from within subgenus *Oligadenus*; 4) subgenus *Oligadenus* is paraphyletic; and, 5) *C. musgraviana* is not closely related to the Hawaiian clade. The geographical origin of the group remains unresolved because the strongly supported sister clade contains species from Costa Rica, Panama, Brazil, and La Réunion.

347 SKOG, JUDITH E.^{1*}, JOHN T. MICKEL², ROBBIN MORAN², AND ELIZABETH A. ZIMMER³

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Phylogeny of the fern genus Elaphoglossum based on two chloroplast genes

Nucleotide sequences from the *trnL-F* spacer region and the *rbcl* region of the chloroplast genome were used to resolve subgeneric relationships within *Elaphoglossum*. Fifty-four representative species from the nine sections, as defined by Mickel and Atehortua, and six species from outgroup genera *Lomariopsis*, *Bolbitis*, *Rumohra* and *Athyrium* were sequenced for the *trnL* data set and about half were included in the *rbcl* data set. The genus *Elaphoglossum* is one of the largest and most complex of fern genera, composed of over 600 species which are nearly all simple-bladed with acrostichoid sori. The majority of useful taxonomic characters to date have been vegetative ones, especially those of the scales and blades. Results from this study support the use of these characters for definition of the sections and in general support the definition of the sections outlined by Mickel and Atehortua. However, new observations from this analysis indicate that there is strong support for three clades within the section *Pachyglossa* and that the subsection *Squamipedia* is more distinct than suspected. All species with resinous dots fall within the same section; there is no support for maintaining *Decorata* as a separate section; and the section *Amygdalifolia* remains unique indicating there may be at least two origins of hydathodes.

348 SMITH, ALAN R.* AND RAYMOND B. CRANFILL

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Intrafamilial relationships of the thelypteroid ferns (Thelypteridaceae)

Since its separation from the dryopteroid ferns as a distinct group, about 60 years ago, Thelypteridaceae has been treated as a natural group comprising nearly 1000, mostly tropical species. Although generally recognized as a natural monophyletic group, there is a wide divergence of views about generic circumscription. Morton placed all species in a single genus *Thelypteris*; Holttum characterized 25 genera in the Old World alone; Pichi Sermolli, largely following Holttum, accepted 32 genera; while Smith adopted an intermediate view, recognizing 5 genera. Most of the paleotropical segregates, and several of the neotropical ones, have been recently revised or monographed, making this one of the best known fern families morphologically, cytologically, and distributionally. Little, however, is known about relationships between these segregates. Data from four chloroplast genes (*rps4* + *rbcl* + *trnS* spacer, + *trnL* spacer; 2600 base pairs) for 23 of the recognized segregates show the family to be monophyletic and sister to an unresolved alliance of blechnoid, athyroid, onocleoid, and woodsioid ferns. The family itself comprises two primary lineages, one phegopterid, the other thelypterid. The phegopterid lineage includes those elements within Thelypteridaceae that are the most dissected and morphologically distinct: *Macrothelypteris*, *Pseudophegopteris*, and *Phegopteris*. Within the thelypterid lineage, three predominantly north-temperate subgroups, including *Thelypteris s.s.*, form a free-veined clade that is in turn sister to the rest of the family. All segregates possessing $x=36$ (*Cyclosorus* sensu Smith, with predominantly anastomosing veins) form a strongly supported clade. Those groups with dysploid base chromosome numbers ($x=27, 29, 30, 31, 33, 34, 35$) form a series of smaller clades basal to *Cyclosorus s.l.* Although our sampling is as yet insufficient to favor one classification over another, the present analysis suggests that recognition of an intermediate number of genera may be the most reasonable taxonomic course.

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Relationships among Northern Hemisphere Pteridium: some new perspectives

The systematic relationships among many *Pteridium* (bracken) taxa are still poorly understood. We sequenced several genes in an attempt to infer relationships between the Northern Hemisphere taxa. Samples were obtained from bracken in Britain, Europe, Japan, North America, and Hawaii representing the *Pteridium aquilinum* varieties *aquilinum*, *latiusculum*, *pseudocaudatum*, *pubescens*, and *decompositum*. Samples of the southern hemisphere *Pteridium esculentum* were also included as an outgroup in the study. Of particular interest were the results obtained from the chloroplast *rps4* gene sequences. In general, British and European var. *aquilinum* were united by a 5bp insertion that was not present in any of the non-*aquilinum* material. A strong similarity was observed also between *rps4* sequences from Hawaiian *decompositum* and western North American *pubescens*. Phylogenetic analyses of *rps4* sequences suggest two main clades. The first was represented by British and European *aquilinum*. The second was predominately *latiusculum*, but included the North American and Hawaiian taxa. In most analyses, the Eurasian *latiusculum* tended to form a single group. In all analyses conducted, *decompositum* and *pubescens* formed a single group. This study

provides important information needed for a global study of bracken systematics.

350 WHITTIER, DEAN P.

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Apogamy in Psilotum and Tmesipteris

Gametophytes of *Psilotum* and *Tmesipteris* grow slowly in axenic culture on a nutrient medium containing minerals and 0.5% glucose. Gametophytes grown in the dark have the typical cylindrical shape and bear antheridia and archegonia. If these gametophytes are grown in the light, they will form apogamous aerial stems from their apices. The apogamous stems have the expected morphology for aerial stems of the two genera. The stems of *Psilotum* have vascular tissue, stomata, and enations and those of *Tmesipteris* differ only in having microphylls instead of enations. If the apogamous stems of *Psilotum* are moved into the dark, their development will shift to that of rhizomes. If the nutrient medium is supplemented with 6-benzylaminopurine, gametophytes will form apogamous rhizomes in the dark. It would appear that the presence or absence of light determines whether the apogamous developments in *Psilotum* are aerial stems or rhizomes.

CONTRIBUTED POSTERS

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Integrating megaspore morphology and molecular phylogeny in Selaginella

The lycophyte genus *Selaginella* has been the subject of several taxonomic treatments in the past one hundred and fifty years. While most have considered megaspore morphology to some extent, no one has successfully reconciled this feature with a comprehensive phylogeny of the group. Recent molecular phylogenies generated using *rbcL*, ITS and 26S rDNA along with electron microscopy of the megaspores now afford the opportunity to do so, at least in part. Granules of sporopollenin on the innermost exospore surface are found in all members of the monophyletic subgenus *Tetragonostachys* and its sister taxon *S. lepidophylla*. *Tetragonostachys* includes approx. forty drought adapted species of worldwide distribution. So far, this feature has been found in thirteen species, and no where else within the genus. A highly ordered colloidal crystal-like exospore structure is found only within an as yet unnamed, well supported clade, which includes the monophyletic *Articulatae*. All examined members of the *Articulatae* have this feature. The species that possess this wall structure do not appear to form a monophyletic group, but the number of reversals and/or parallelisms involved is uncertain because of some weakly supported nodes. The species of *Selaginella* now known to possess this unusual structure throughout their exospore are: *S. articulata*, *S. diffusa*, *S. exaltata*, *S. galeottii*, *S. kraussiana*, *S. kunzeana*, *S. lingulata*, *S. lyalii*, *S. marginata*, *S. myosurus*, *S. polymorpha*, *S. remotifolia*, *S. sericea*, *S. silvestris*, *S. suavis*, *S. sulcata*. *S. pygmaea* and *S. willdenovii* show this pattern in places. A final emerging correlation involves the possession of a coarse complete reticulum with high muri by most members of this unnamed clade. The ability to recognize megaspores with particular ultrastructural features of phylogenetic significance without the use of the EM (i.e., by correlation with surface features) would allow the incorporation of dates from fossil material in the analysis.

352 PALMIERI, MARIA* AND LUCINDA J. SWATZELL

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Presence of VA mycorrhizae in the sporophytes of Cheilanthes feei and Cheilanthes lanosa

Cheilanthes is a genus of xerophytic ferns. This is somewhat of an oxymoron, since ferns typically inhabit moist environments. However, *Cheilanthes* species are apogamous. This explains how they can reproduce in dry habitats, but not how they can survive in arid settings. One possible explanation is that *Cheilanthes* species have morphological and physiological adaptations that enhance their survival. For example, *Cheilanthes feei* and *Cheilanthes lanosa*, two common and widespread North American ferns, are characterized by tomentose or hirsute trichomes beneath their pinnae. Another possible explanation for *Cheilanthes*' ability to survive dry habitats is the formation of mycorrhizal associations. In this study, we examined *C. feei* and *C. lanosa* to investigate the potential presence and distribution of mycorrhizal associations in these ferns. Without variation, VA mycorrhizal structures were present in *C. feei* and *C. lanosa* sporophyte roots. Results suggest that these common *Cheilanthes* species survive, in part, through mycorrhizal associations.

353 SWATZELL, LUCINDA J.*, SARAH L. NONDORF, AND MARIA PALMIERI

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Effects of light intensity, light quality, and temperature on Cheilanthes feei spore germination

Cheilanthes feei is a xerophytic fern that grows exclusively on dry, exposed calcareous rocks and bluffs. This is an unusual habitat for a fern. Ferns typically inhabit moist environments. They lack the evolutionary adaptations possessed by xerophytic species. *C. feei* exhibits a few characteristics of xerophytic plants (i.e. apogamy, protective trichomes). However, it is unclear how the fern survives the restrictive conditions in its ecological niche. To determine which factors play a role in the fern's survival, we tested the effects of temperature, light intensity, and light quality on spore germination. Results suggest that the optimum conditions for *C. feei* spore germination are substantially different than the environmental conditions that characterize the fern's habitat.

SYSTEMATICS SECTION, BSA / ASPT / IOPB

SYMPOSIUM: LINNAEAN TAXONOMY: A VIABLE SYSTEM FOR THE NEW MILLENNIUM?

(BSA SYSTEMATICS SECTION / ASPT)

354 DAVIS, JERROLD

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Linnaean taxonomy: a viable system for the new millenium?

The current system of biological classification and nomenclature (the "Linnaean system"), as developed over the past two and a half centuries, has been criticized of late as being inconsistent with scientific thought. Under the banner of "phylogenetic systematics," critics have claimed that the Linnaean system is incompatible with modern phylogenetics, and have proposed an

alternative, the "PhyloCode," or PC nomenclatural system, which is intended to supplant the Linnaean system. Proponents of the PC system have asserted that the Linnaean system is fatally incompatible with evolutionary theory, that it inhibits scientific inquiry, that the PC system, in contrast, is a necessary component of the ongoing evolutionary synthesis, and that it also will provide greater nomenclatural stability. In this symposium, systematists who use the Linnaean system will evaluate its philosophical underpinnings, goals, and practical utility, and compare its qualities to those of the proposed PC system. Specific arguments made by proponents of the PC system will be considered, and the relative merits of the Linnaean and PC systems will be evaluated with regard to a variety of subdisciplines within systematics, including monography, floristics, paleobotany, cladistics, and the use of nomenclatural types.

355 BARRIE, FRED R.

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Type-basis nomenclature: a precise and simple system of naming taxa

The International Code of Botanical Nomenclature is a type-basis code. The application of names of taxa at the rank of family and below is determined by nomenclatural types. A nomenclatural type is that element (specimen or illustration) to which the name of a taxon is permanently attached. The type method, first proposed over a century ago and refined since then, is a technique for affixing a label –a name– to a taxon. Gradually, and with no small amount of controversy, it replaced the old method of defining a name by identifying it with a taxon's original circumscription, a method that is inherently unstable; a circumscription may be open to interpretation or revision, but a good type specimen clearly identifies the taxon to which it belongs, irrespective of the scientific methodology used to define the taxon itself. Botanical nomenclature is not, in itself, a scientific endeavor, but a system for ordering information, i.e. taxa. Pervading the background literature for the PhyloCode is a persistent confounding of names and taxa, an insistence that they are one and the same thing. The failure to recognize the difference has led to several very tenuous positions, e.g., that names can be defined, that nomenclature itself is scientific, or that lumping and splitting –the merging and dividing of taxa– are nomenclatural, rather than taxonomic, exercises.

356 GANDOLFO, MARIA A.

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Fossils and the Linnaean system of nomenclature

For the last 90 years, since the publication of the first International Code of Botanical Nomenclature in 1906, the taxonomy of fossils has been treated with special care. Although the six principles are applied to fossils in the same way as for any other taxonomic group of plants, there are several recommendations specifically for fossil plants, among them the definition of what constitutes a taxon. Because of both consistency and flexibility, the Linnaean system of Nomenclature, based on agreed-upon rules and recommendations and modifications over the years has offered a relatively stable system to name fossils. During the last 10 years a new nomenclature system has been proposed, now dubbed the PhyloCode. The PhyloCode as posted on the web does not address numerous of the major issues regarding fossils that have been treated in the current code. The proponents of this new system also claim that the Linnaean system is archaic and insufficient for modern classification. One of their most prevalent arguments is that this new system is more stable than the Linnaean one. However, because the inclusion of fossil taxa in phylogenetic

analyses is often difficult, and their proposed system of designating groups requires phylogenetic topologies, fossil taxonomy will be inherently unstable under the new system. Other problems with the PhyloCode include the method of designating and naming of clades, since the fossil record is imperfect and as new fossils are discovered, the inclusion of these new fossils may arbitrarily change the composition of clades and therefore add more instability to the system. Examples and comparisons between both systems will be presented.

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Monographs, floras, classification, and the consumer

Nomenclatural systems are structured around classification and together they enable increasingly informed communication about biological diversity. A polemic is raging and a morass of literature is growing on the relevance of traditional approaches to classification and current nomenclatural codes, often referred to in general terms as the Linnaean system. Challengers of traditional approaches to classification and nomenclature have proposed the PhyloCode, a new set of rules which would govern the way systematists classify and name the diversity of life. Monographs and floras are two fundamental vehicles for communicating information about plant diversity. These works provide a comprehensive foundation of botanical research upon which other scientific studies are based. The information conveyed through monographs and floras is utilized directly or indirectly by a wide range of consumers both within and outside the scientific arena, such as educators, agrarians, ecologists, conservationists, amateur naturalists, and even lawmakers, to name a few. Both classification and nomenclature are essential to the process of synthesis that leads to monographic and floristic treatments. Thus, abrupt conversion to a new system of classification and nomenclature would have far-reaching consequences on the flow of information and communication from systematics, to other scientific disciplines, and to society. The purpose of this paper is to contrast the current botanical code (ICBN) and the proposed PhyloCode from the perspective of monographic and floristic research focused on Neotropical plant diversity. We weigh advantages and disadvantages of each system against a backdrop of on-going activity and communication, and draw conclusions as to which system better facilitates current needs. We suggest a pluralistic approach rooted in the stewardship of systematics, and relate this philosophical viewpoint to education, research, information synthesis, communication, the consumer, and conservation of biological diversity.

358 MOORE, GERRY

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Should taxon names be explicitly defined?

In traditional nomenclature (TRAD) a type and a rank are attached to a taxon name. In phylogenetic nomenclature (PHYLO) a name is attached to a clade by an explicit definition (changes to the definition are prohibited without the express written consent of the International Committee on Phylogenetic Nomenclature). In PHYLO, the taxonomist has no discretion in circumscribing a taxon—a name's phylogenetic definition and the accepted phylogeny determine a taxon's circumscription. Thus, under PHYLO how the name has been previously applied (e.g., circumscription, character associations) cannot be considered when applying the name under a revised phylogenetic hypothesis. Taxonomists' hands are effectively handcuffed by the explicit phy-

logenetic definitions. In TRAD the names are not shackled by explicit definitions. Thus classifications using this system are more flexible and able to accommodate new information with regards to relationships, while preserving historical usage as much as possible. Under the rankless PHYLO, the name of a taxon will not change (it may become synonymized) regardless of how shifts in hierarchical position (as a result of revised phylogenetic hypotheses) alter the content of the taxon (clade). Thus the name when used outside the context of a given phylogeny or taxonomy conveys nothing regarding set exclusivity. This contrasts sharply with TRAD where the names themselves convey broad information regarding set exclusivity. TRAD may not be perfect (no nomenclature can be) but it does an excellent job of communicating biodiversity information to the multiple constituencies that use taxon names. Why switch?

359 NIXON, KEVIN C.

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The phylocode is fatally flawed and the current Botanical Code can be easily fixed

Promoters of the so-called "phylocode" have mounted an intensive and deceptive publicity campaign. At the centerpiece of this campaign have been slogans such as that the Linnaean System will "goof you up", that the phylocode is the "greatest thing since sliced bread," and that systematists are "afraid" to propose new names because of "downstream consequences." Aside from such subscientific spin and sloganeering, proponents of the PC have offered nothing real to back up claims of greater stability for their new system. They have also misled many into believing that the PC is the only truly phylogenetic system. The confusion that has been fostered involves several discrete arguments, concerning 1) a new "method" of "designating" names; 2) rank-free taxonomy; 3) uninomial nomenclature; 4) issues of priority. Claims that the phylocode produces a more stable nomenclature are false, as shown with the example of paleoherbs. A rank-free system of naming requires an annotated reference tree for even the simplest exchanges of information, would be confusing at best, and would cripple our ability to teach, learn and use plant names in the field or in publications. We would be confronted by a mass of polynomial names, tied together only by a tree graphic, with no agreed upon name (except a uninomial, conveying no hierarchy) to use for any particular species. The separate issue of stability in reference to rules of priority and rank can be easily addressed within the current codes, by implementation of some simple changes as will be proposed here. Thus, there is no need to "scrap" the current Linnaean Code for a poorly reasoned, logically inconsistent, and fatally flawed new code that will only bring chaos.

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The Linnaean system and its 250 year persistence

The Linnaean system of nomenclature has been used and adapted by biologists over a period of almost 250 years. Under the current system of codes it is now applied to more than 2 million species of organisms. Inherent in the Linnaean system is the indication of hierarchic relationships. The Linnaean system has been justified primarily on the basis of stability. Stability can be assessed on at least two grounds: the absolute stability of names, irrespective of taxonomic concept; and the continuing application of the same names to the same concepts. More recent arguments have invoked conformity to phylogenetic methods as the primary basis for choice of nomenclatural systems, but even here stability of names as they relate to monophyletic groups is the

ultimate objective. The idea of absolute stability was wrong from the start. The reasons are several. First, taxa are concepts, no matter the frequency of assertions to the contrary; as such, they are subject to change at all levels and always will be. Second, even if the true nature of all taxa could be agreed upon, the goal would require that we discover them all and correctly recognize them for what they are. Much of biology is far from that goal at the species level and even further for generic and higher-groups. Botanical nomenclature is more stable than zoological in the spelling of species epithets. This simplifies database creation and maintenance. Yet, stability at this level is superficial. Nomenclature serves as a tool for biology. Absolute stability would hinder scientific progress rather than promote it. It can be demonstrated that the scientific goals of systematists are far from achieved. Thus, the goal of nomenclatural stability is illusory and misguided. The primary strength of the Linnaean system is its ability to portray hierarchic relationships; stability is secondary.

SYMPOSIUM: BIOGEOGRAPHY AND PHYLOGENY OF CARIBBEAN PLANTS

(BSA SYSTEMATICS SECTION / ASPT)

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Biogeography and phylogeny of Caribbean plants

The historical biogeography of the West Indies and the Caribbean region is complex, involving numerous tectonic and climatic events, as well as a meteor impact, promoting species divergence, disjunction, migration, and extinction. Further, the region is poised between the two great landmasses of the western hemisphere, for which the Antilles have simultaneously served as a refugium for once widespread taxa, a conduit for intercontinental migration, and a vehicle for adaptive radiation. The relative importance of major routes of migration, e.g., from Mesoamerica, southern Florida, and northern South America through the Lesser Antilles, has shifted over time as geohistorical events have ensued. This complexity presents a daunting yet exciting challenge for evolutionary biologists attempting to reconstruct the historical biogeography of the region and assessing the impact of this history on continental floras of the Americas and elsewhere. Considerable research has addressed Caribbean biogeography from the perspective of various animal groups, such as the insects, lizards, and fishes. There has been far less phylogenetically based research on the historical biogeography of Caribbean plant groups. The development of new molecular and analytical tools, new geological and palynological data, and ongoing research into the systematics and evolution of various Antillean plant genera provide material for a reevaluation of the historical biogeography of the Caribbean region in a phylogenetic context. This symposium will bring together researchers with phylogenetic data on various Antillean plant groups. Its goals are to further our understanding of general biogeographic patterns that underlie the distributions of Caribbean plant taxa, integrate these patterns with the biological processes thought to be involved in their development, and identify groups and methods for future research into the historical phylogeography of this biodiverse region.

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Biogeography of Acacia subgenus Acacia in the Caribbean and neotropics based on ITS nrDNA sequences and cpDNA restriction site mapping

Phylogenetic hypotheses generated from chloroplast DNA restriction site mapping and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA of *Acacia* subgenus *Acacia* from the New World and Africa indicate the importance of a group of species endemic to the West Indies as sister to the main radiation of the subgenus in the New World in North America. While the results of separate phylogenetic analyses differ in a number of important respects, the relatively basal position of the West Indian-endemic species (the *Acacia acuífera* species-group, an edaphically specialized and, for the most part, restricted endemic of serpentine and calcareous outcroppings) lends support for a boreotropical model of the historical biogeography of the subgenus. Other West Indian species not included in the *Acacia acuífera* species-group were found to share clades with mainland North, Central, and South American groups, lending support for a dispersalist model of the biogeography of these species. The results of the phylogenetic analyses indicate that Caribbean members of *Acacia* subgenus *Acacia* may display signal from early and later phases of the Caribbean flora. Weedy, widespread members in the Caribbean scarcely differentiated from mainland relatives may belong to a cohort of recently dispersed elements while the restricted serpentine- and calcareous-endemic members of the *Acacia acuífera* species-group, due to their relatively basal position within the subgenus and lack of any close relatives in mainland areas, may represent a relictual group having a relatively long history in the Caribbean. Their edaphic endemism may partially explain their ability to persist in the Caribbean as plants in these areas are likely to have been less prone to competitive exclusion by colonizing species.

363 FRITSCH, PETER W.

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The historical biogeography of Antillean Styra
(*Styracaceae*)

Morphological phylogenetic analysis of *Styrax* (120 species) has suggested that no two of the four Antillean species of this genus form sister-group relationships: one group in a clade of southern North American endemics, and the rest are each distributed among different clades of otherwise South American species. In particular, *S. obtusifolius* (Cuba and Hispaniola) has been considered the basal member in a clade of small-flowered, gynodioecious species otherwise endemic to South America. Here these relationships are tested with DNA sequence data from the ITS region. In contrast to the morphological analysis, phylogenetic analysis of the ITS data set recovers a strongly supported sister-group relationship between *S. obtusifolius* and the Puerto Rican endemic *S. porticensis*, and this clade is nested within a group of fully bisexual South American species. The other two gynodioecious species sampled form a monophyletic group that is well removed from *S. obtusifolius*. Incongruence length difference tests suggest that overall conflict between the ITS and morphological data sets of neotropical evergreen *Styrax* is mainly attributable to the conflict between the data profiles in *S. obtusifolius*. All morphological characters supporting a relationship between *S. obtusifolius* and the other gynodioecious species are associated with gynodioecy and floral reduction, suggesting that the similarity in breeding system and floral characters among distantly related Cuban and

South American species of *Styrax* represents a remarkable case of convergent evolution. This scenario is consistent with the prevalence of small-flowered species in the Cuban flora that have apparently coevolved with endemic and highly specialized microscopic insect pollinators, and with the trend toward decline on islands. Although *Styrax* is apparently boreotropical in origin, Antillean *Styrax* species have originated through dispersal from South America, as inferred from DIVA analysis. *Styrax ochraceus*, endemic to Hispaniola, still requires assessment in this context.

364 GRAHAM, ALAN

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Geohistory models and paleovegetation of the Caribbean region

The Caribbean region is tectonically one of the most complicated on Earth, as evidenced by some 13 'mobilist' models that have been proposed to explain its geohistory. Some consensus is developing around one recent model that places a Cretaceous volcanic arc of islands in the eastern Pacific that moved 1000 km between the portal formed as North and South America moved apart. The principal difference between this version and older models is that the land forming the proto-Antilles originated as islands and was never a continuous or near-continuous land bridge connecting or nearly connecting the continents. There is also uncertainty about the emergent-submergent history of the individual land fragments. The arc began colliding with the Bahamas Platform in about the middle Eocene (49 Ma), and that probably began the principal period of emergence for the Greater Antilles. The collision was at an angle (transpression) toward the east with Puerto Rico emerging last in the middle Oligocene (29 Ma). The Lesser Antilles are middle to late Eocene in age. Among recent contributions to Caribbean biohistory are a study of the middle Eocene Saramaguacan flora of Cuba, proposal of a connection (a 'landspan') between North and South America via the Antilles 35-32 Ma involving an emergent Aves Ridge, and continuing assessment of vicariance and dispersal as means of distribution of plants and animals in the Caribbean region.

365 GRAHAM, SHIRLEY A.

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Biogeographic patterns of Antillean Lythraceae

The Lythraceae are a family of 31 genera of world-wide distribution growing primarily in wet to moist habitats of the subtropics and tropics. The family is represented in the Antilles by seven genera and 39 species. Two genera, *Ginoria* and *Haitia*, and 21 species are endemic to the islands of the Caribbean. *Ginoria* with 14 species and *Cuphea* with 10-15 species are best represented. Cladistic analyses place *Ginoria* in an Afro-Asian clade as sister to *Tetraxis* of Madagascar. The ancestral route of dispersion to the Caribbean is not clear, although a basal member of the clade, *Lawsonia*, is known from the Middle Eocene of British Columbia, suggesting an ancestral boreotropical route. The center of origin for *Cuphea* is most likely eastern South America. *Cuphea* has dispersed into the Greater Antilles from Brazil a minimum of five times and into the Lesser Antilles from northeastern South America twice. *Lythrum* and *Heimia* have moved to the Antilles from North America. For *Rotala* and *Ammannia*, a boreotropical dispersion route from Asia through North America to the Caribbean, or oceanic dispersal of seeds from Africa is equally likely. Seeds of all Antillean Lythraceae are minute in size and have floats or mucilaginous hairs that enhance chances of long distance dispersal by wind and water. Vicariance, although it may have occurred, is not required to explain the distribution of Lythraceae in the Antilles.

366 HERRERA, PEDRO

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Biogeography of the Cuban Asteraceae

The biogeography of Cuban Asteraceae and their relations to the rest of Antillean genera and species are discussed. In this region, diversification and speciation in the Asteraceae has taken place mainly in the tribe Eupatorieae and a few other tribes. Evolution in these groups appears in a striking way which is also found in the rest of the Antilles, but mostly in the Greater Antilles and not in the mainland. The importance of climatic and edaphic factors on speciation, and the influence man has had on their dispersal is stressed.

367 KELCHNER, SCOT A.^{1,2*}, JUDY G. WEST¹, MIKE C. CRISP², AND ROBERT J. CHINNOCK³

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The Caribbean Bontia daphnoides and its Australian family Myoporaceae (Lamiales): evidence of an extreme dispersal event from morphological data and rpl16 intron sequences

The monotypic genus *Bontia* presents an interesting biogeographic puzzle. *Bontia daphnoides* is an endemic of the Caribbean islands, but is taxonomically classified as a member of the southern-hemisphere Old World family Myoporaceae. Ninety-five percent of the 250 species of Myoporaceae (sensu R. J. Chinnock; in review) are arid/semiarid shrubs and small trees endemic to Australia. Only six species in the family occur north of the equator, and of these all but *Bontia daphnoides* are locally distributed in the western Pacific. The supposed sister lineage to the family is the semiarid Central American tribe Leucophylleae (Scrophulariaceae), which shares anatomical similarities with Myoporaceae. Is the evolutionary history of the Caribbean *Bontia daphnoides* truly shared with Australian Myoporaceae, or has it instead descended from the geographically neighboring Leucophylleae? As part of an ongoing phylogenetic analysis of generic relationships in Myoporaceae, our first two data sets provide some evidence of historical relationships between these anatomically similar lineages. Parsimony and likelihood topologies have been constructed for morphological cladistic characters and aligned *rpl16* intron sequences, respectively. In these topologies, Leucophylleae is a lineage independent of a monophyletic Myoporaceae (sensu Chinnock), and *Bontia* is derived from within the largely arid Australian genus *Eremophila*. The results suggest that the current position of *Bontia daphnoides* in the eastern Atlantic is due to dispersal from an Australian ancestral lineage.

368 LAVIN, MATT^{1*}, MARTIN F. WOJCIECHOWSKI², ADAM RICHMAN¹, JAY ROTELLA³, MICHAEL J. SANDERSON², AND ANGELA BEYRA MATOS⁴

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Identifying Tertiary radiations of Fabaceae in the Greater Antilles: alternatives to cladistic vicariance analysis

The woody genera *Pictetia* and *Poitea* represent two of the most speciose endemic legume radiations in the Greater Antilles. Yet component, three-area-statements, and Brooks parsimony analysis suggest that Tertiary history has little influenced the distribution of these genera. Either widespread taxa and composite areas mask Early Tertiary influences or the two genera have little history on these islands. Alternatively, nucleotide diversity, evolutionary rates, and coalescent analyses of molecular phylogenies all suggest antiquity of the *Pictetia* and *Poitea* radiations. These molecular biogeographic approaches are used to identify and estimate the ages of old endemic Tethyan radiations. These results are corroborated by confirming a species-area disequilibrium among the legume floras for islands of the world. Age of island biota and area of island are both needed to most accurately predict especially endemic numbers of legume taxa. The findings presented here combined with the legume fossil record suggest that both *Pictetia* and *Poitea* stem from Early Tertiary North American boreotropical ancestors. Thus, Wolfe's hypothesis that the Greater Antilles harbor boreotropical relicts finds support.

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Exostema biogeography in light of molecular phylogenies and possible polyphyly

The genus *Exostema* (Rubiaceae) comprises 25 species of trees and shrubs with narrow tubular flowers, dehiscent capsular fruits and small, usually winged seeds. Twenty-two species are endemic to the Greater Antilles, mostly in Cuba and Hispaniola. Middle America, the Andes, western Amazonia and the Lesser Antilles each have one endemic species. Phylogenetic analyses of morphological and ITS (nuclear DNA) sequences have placed the South American species basal to the Caribbean *Exostemas*, but exact relationships among mainland and island species were uncertain. To clarify infrageneric and outgroup relationships, new *rbcl* (chloroplast DNA) sequences have been obtained for ten *Exostema* species and several closely related taxa, and extra ITS sequences added for several taxa. Both ITS and *rbcl* DNA data indicate that *Exostema* is polyphyletic with respect to various neotropical genera with funnellform flowers and capsular fruit such as *Coutarea* (mainland neotropics) and *Cubanola* (Greater Antilles). These molecular data also suggest that *Exostema* is paraphyletic with respect to the genera *Chiococca* (widespread neotropical) and *Erithalis* (circum-Caribbean & Antilles), which have indehiscent fruit quite unlike *Exostemas*. Certain morphological characters remain consistent in all species of these disparate genera: basifixed, linear anthers and spinulose, tricolporate pollen. Thus, the broadened ITS and *rbcl* analyses challenge the generic limits of *Exostema* and necessitate reexamination of relationships among various long-recognized genera. Despite these conflicts, an unambiguous biogeographic pattern is clearly supported for the clade comprising the short-flowered species of section *Parviflorum*.

The middle American *E. mexicanum* is sister to the four Cuban and Hispaniolan species, and the Andean endemic *E. corymbosum* is probably most basal among this group. Overall biogeographic trends in *Exostema s.l.* include extreme morphological divergence in both vegetative and reproductive features for island species, with species radiations in Cuba and Hispaniola on both serpentine and calcareous soils.

370 NEGRON-ORTIZ, VIVIAN* AND LINDA E. WATSON

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Molecular phylogenetic of Ernodea and Erithalis (Rubiaceae): implications for Caribbean biogeography

Two endemic Rubiaceae genera of the Caribbean, *Erithalis* P. Browne (Tribe Chiococceae) and *Ernodea* Swart (Tribe Spermacoceae), are composed of four to eight species, respectively. Each genus contains several species that appear to be single island endemics, in addition to one widespread species that is distributed throughout the Caribbean Basin. The pan-Caribbean species occur sympatrically with species of restricted distribution and/or endemics, and often are intermediate in morphology. The fruits of both genera are fleshy, potentially bird-dispersed, and float on sea water. One chloroplast intergenic spacer of *trnF/L*, and two nuclear non-coding spacer, ITS & ETS, were sequenced and analyzed phylogenetically for *Erithalis*. Analyses of the combined data set reveal two weakly supported clades: one consisting of the widespread species *E. fruticosa* and *E. harrisi*, and the other clade consisting of all remaining species. The two Jamaican endemic species, *E. harrisi* & *E. quadrangularis*, are each placed basally in these two clades. These species occur in the mountains, which suggests a migration to the coast and to other islands. The ITS were sequenced for *Ernodea*, however the data do not provide sufficient phylogenetic resolution with two exceptions: *E. cokeri* appears to be distinct and widespread *E. littoralis* appears paraphyletic. In conclusion, sequence divergence and phylogenetic resolution are low, suggesting that both genera are recently derived.

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Systematics and biogeography of Caribbean wild yams (Dioscoreaceae)

The yam family, Dioscoreaceae is represented in the Caribbean by two genera, *Rajania* L. (ca. 25 species) and *Dioscorea* L. (ca. 400 spp. worldwide). *Rajania*, a Caribbean endemic genus is distinguished from *Dioscorea* on the basis of gross fruit morphology. The fruit of *Rajania* is a samara, and that of *Dioscorea* a trilobular capsule. *Rajania* is distributed from the Lesser Antilles to the Bahamas, with species diversity concentrated in Cuba. *Dioscorea* species in the Caribbean include six endemics and four species with extra-Antillean distributions in the Americas. Recent phylogenetic studies by Caddick et al (2000) suggest that *Rajania* was derived from within *Dioscorea* but its relationship to other Caribbean *Dioscorea* species was not evaluated, nor was the monophyly of the endemic genus tested. Here we present an expanded sample of the Caddick et al. rbcL data set including Caribbean and other New World yam species not previously evaluated. In preliminary analyses *Rajania* is strongly supported as monophyletic. It is a member of a larger clade that is comprised primarily of Old World *Dioscorea* species but that also includes

Dioscorea altissima Lam. and *D. trifida* L. both of which occur in the Caribbean and in South America. The remaining Caribbean *Dioscorea* species comprise at least two South American lineages only distantly related to *Rajania*, a conclusion also supported by tuber morphology. Sequence data from the nuclear 5S-NTS are being used to evaluate relationships and biogeographic patterns within *Rajania*. In the context of the present molecular phylogeny the samara is seen as an innovation upon the *Dioscorea* ground-plan. Comparative anatomical data from developing fruits of both genera are presented and the structural changes involved in the transformation of the fruit type are characterized.

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Biogeography of the Goetzeoideae (Solanaceae)

Recent phylogenetic reconstruction of the Goetzeoideae using DNA sequence data from the chloroplast genome and the nuclear genome has provided evidence to understand its historical biogeography and present trends of morphological and ecological evolution. Our results demonstrate that the Greater Antillean genera *Coeloneurum*, *Espadaea*, *Goetzea*, and *Henoonia* belong to a clade that includes two basal lineages endemic to South America: *Duckeodendron* and *Metternichia*. This clade is one of the first lineage splits of the family Solanaceae. Pollen morphology shows congruence with the relationships outlined by the molecular phylogeny. The phylogeny indicates that in some lineages, there has been evolution towards xeric environments. In addition, there has been considerable morphological changes in reproductive structures and pollination syndromes between the continental lineages and the Greater Antillean lineages.

COLLOQUIUM: EVOLUTION, BIOGEOGRAPHY, AND SYSTEMATICS OF BEGONIACEAE (ASPT)

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Evolution, biogeography and systematics of Begoniaceae

The family Begoniaceae contains three genera, *Begonia*, *Hillebrandia*, and *Symbegonia*. Genus *Begonia* is pantropical and comprises roughly 1400 species divided among 63 sections. *Symbegonia* contains 12 species endemic to New Guinea and *Hillebrandia* is represented by a single species endemic to the Hawaiian archipelago. Relationships among these genera, as well as the sections of *Begonia*, are not well understood and little information pertaining to the phylogeographic history of the family exists. As the distribution of the sections of *Begonia* and the segregate genera coincide to a large degree with continental boundaries, determination of their relationships is likely to reveal the phylogeographic history of the family. This colloquium brings together several researchers from North America and Europe who have recently made significant progress in reconstructing the phylogeny of Begoniaceae using both morphology and DNA sequence data. The aim of the colloquium is to provide an overview of the advances in our understanding of Begoniaceae evolution and to foster additional collaboration among researchers working on this

large family. The initial presentations are designed to be general and introductory in scope. The succeeding talks are more focused and will consider phylogeny of specific continental groups of *Begonia*, gene flow and speciation in *Begonia*, phylogeny of *Hillebrandia* and *Symbegonia*, and the systematic and biogeographic implications of these data. The talks will consider morphological changes in the family and how these changes can be interpreted in the context of molecular-based phylogenies. The colloquium will provide the forum to more fully present the recent advances and will allow better conceptualization of the large amount of information that has been generated.

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Begoniaceae and the Cucurbitales: a morphological phylogenetic analysis

In the last 20 years, the relationships of the Begoniaceae have been redefined. Molecular phylogenies have shown that the family, along with its traditional allies Datisceae and Tetramelaceae, belongs to the Cucurbitales, with the Cucurbitaceae, a relationship already foreseen, but also with Corynocarpaceae, Coriariaceae and Anisophylleaceae, relationships that had never been predicted on the basis of non-molecular data. To illustrate potential evolutionary trends in the order, significant morpho-anatomical characters are mapped onto the rbcL and 18S molecular trees. An independent phylogenetic analysis of morphological data is also presented, as well as a combined analysis of morphological and molecular data. Features of the order that attract attention include the plagiotropic architectural habit associated with spiro-distichous leaf disposition (to distichous-opposite), and the possibility that the primitive state for the ovary may have been inferior, evolving into superior ovaries in Coriariaceae-Corynocarpaceae. Gaps in our knowledge of the group, and the significance of this analysis in terms of Begoniaceae morphology are discussed. Biogeographic ramifications will also be considered.

375 FORREST, LAURA L.* AND PETER M. HOLLINGSWORTH

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Phylogeny of Begonia

Begonia is one of the largest angiosperm genera, with 1400 species currently recognised. These were placed into 63 sections in the most recent taxonomic treatment. However, there is considerable uncertainty in both section inter-relationships and sectional composition, and there is no formalised phylogenetic hypothesis for the genus. Using the nuclear internal transcribed spacers (ITS) and partial large subunit (26S) sequences of ribosomal DNA, we have produced phylogenetic trees to form the basis of a cladistic framework for the interpretation of the evolution and sectional level systematics of *Begonia*. Cladograms were produced for 35 *Begonia*, one *Symbegonia* and two *Datisca* species, for partial 26S and ITS sequence data. Analyses suggest that African taxa are basal in *Begonia*. The New Guinea genus *Symbegonia* is nested deeply within *Begonia*. A far larger data set was constructed by sequencing the ITS region for 153 species (two *Datisca*, three *Symbegonia*, one *Hillebrandia* and 147 *Begonia*). In the resulting tree, the African species of *Begonia* resolve as paraphyletic, with both the Asian species (including Socotra) and the American species (sister to southern African species) monophyletic. Using a combined morphology ITS analysis, the fit of individual morphological characters to a tree was examined. Some of the characters which have traditionally been considered important in *Begonia* taxonomy (e.g. number of placental branches per locule) proved

highly homoplastic. The correlation between phylogenetic relationships implied by the ITS tree and the geographical distributions of species was explored. As a general rule, related species are geographically proximal, suggesting limited dispersal of lineages. In contrast, morphologically similar but geographically separated species tend to be phylogenetically disparate.

376 HUGHES, MARK*, PETER HOLLINGSWORTH, AND TONY MILLER

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Gene flow and speciation in Begonia

The genus *Begonia* is one of the six largest angiosperm genera in terms of species number. Studies of such large genera, from the phylogenetic to the population level, can give insight into the processes that generate plant biodiversity. Several properties of *Begonia* suggest that restricted capability for gene flow through pollen and seed dispersal has been important in generating its species diversity. These are: (i) Monophyletic groups in *Begonia* show strongly restricted geographical distributions, suggesting repeated allopatric speciation events. (ii) The level of narrow range endemism of *Begonia* is very high, suggesting species ranges are dispersal limited. (iii) Widespread species are rare, and tend to show marked morphological variation, suggesting low intra-specific gene flow over moderate to large scales. (iv) *Begonia* has over 1400 species, but few obvious major innovations the majority of *Begonia* appear broadly ecologically similar. Species from the Socotra archipelago (*B. socotrana* and *Begonia* sp. nov.) and South Africa (*B. sutherlandii*) are being used to investigate gene flow and population structure in *Begonia*, with the aid of microsatellite markers. A combination of population genetic, phylogenetic and distributional data represents a potential data set for understanding the processes that have led to these observed patterns of biodiversity.

377 PLANA, VANESSA

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Morphological and molecular systematics of the Afro-Madagascan fleshy-fruited Begonia

The phylogenetic relationships of African *Begonia*, in particular those of the fleshy-fruited species, were assessed by cladistic analysis of DNA sequences from the non-coding chloroplast region *trnL*. In addition, a morphological cladistic analysis of the fleshy-fruited species in sections *Baccabegonia*, *Squamibegonia*, *Tetraphila* and *Mezierea* was carried out to investigate relationships and character evolution. The *trnL* phylogeny is generally congruent with that produced from cladistic analyses of the internal transcribed spacer sequence data in a previous study, although significant incongruences between both topologies occur in the basal nodes. *trnL* and ITS data largely agree with existing sectional concepts based on morphology and there is overwhelming evidence from both data sets that the diversity of *Begonia* is geographically structured. However, sequence data has given strong evidence for some previously unsuspected relationships. Fleshy-fruits arose at least twice in Africa, refuting previous hypotheses which have suggested that fleshy-fruits represent a single evolutionary event. The position of the endemic terrestrial section from São Tomé, *Baccabegonia*, nested within an almost exclusively epiphytic clade suggests its derivation from an epiphytic ancestor. A new section to include *B. iucunda* is recommended, in light of its isolated position in the both the *trnL* and ITS phylogenies. This is supported by evidence from previous morphological studies. Similarly *B. loranthoides*, currently included in section *Tetraphila*,

but distinct from other species in the section, particularly in fruit morphology, could possibly merit a section of its own. Morphology alone in *Begonia* is considered unreliable for phylogenetic reconstruction, with most characters showing high levels of homoplasy. Although most clades in the molecular trees are supported by one or more morphological characters, a small number of cryptic clades remain for which no obvious morphological characters provide synapomorphies.

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Hillebrandia sandwichensis: evolutionary relationships and biogeography

Hillebrandia is a monotypic genus of the Begoniaceae, a family that currently includes two other genera, *Begonia* (1400 species) and *Symbegonia* (12 species). *Hillebrandia* is distinguished by its semi-inferior ovary, which is fully inferior in other Begoniaceae, and by the presence of 8-10 perianth segments compared to 2-5(8) perianth segments in other members of the family. In addition, *Hillebrandia* is the only begonia that is endemic to the Hawaiian Islands. Parsimony-based phylogenetic analyses of nuclear-encoded genes (ribosomal 18S and ITS, the internal transcribed spacer region) and chloroplast-encoded genes (rbcL and the atpB-rbcL spacer region) place *Hillebrandia* at the base of a clade that includes the remainder of family Begoniaceae. Begoniaceae are globally distributed in the tropics, but lack a fossil record to indicate its age or geographic origin. Molecular studies have indicated that *Begonia* had its origins in Africa and the African begonias are the closest species to *Hillebrandia*. By comparing rbcL sequences from *Hillebrandia* and two African begonias, and by using fossil information from close outgroup species, we have estimated the divergence time of *Hillebrandia* and *Begonia* to be approximately 40 million years ago, an age that predates the oldest of the current Hawaiian Islands. Our data suggest that *Hillebrandia* is an island relict on a relatively young archipelago. Various hypotheses as to *Hillebrandia*'s origin are considered.

379 TEBBITT, MARK C.¹, LAURA L. FORREST², W. SCOTT HOOVER³, AND SUSAN M. SWENSEN^{4*}

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Relationships of fleshy fruited Asiatic Begonia (Begoniaceae) based on ITS sequence data and restriction site mapping of PCR amplified chloroplast and mitochondrial fragments

Begonia is a large pantropical genus of herbs, shrubs and lianas. A wide variety of dispersal mechanisms have been recorded from the genus. The majority of the c. 600 Asiatic species of the genus have dehiscent, winged, capsular fruits and appear to be adapted to either wind or rain-splash seed dispersal. However, c. 25 Asiatic species have indehiscent, baccate fruits that usually lack wings and appear to be adapted for animal dispersal. This morphologically diverse group of fleshy-fruited species has traditionally been classified in the section *Sphenanthera*, which is distinguished from other Asiatic *Begonia* sections on this character alone. In order to determine whether the section *Sphenanthera* represents a monophyletic species group, nuclear, chloroplast and mitochondria markers were analysed cladistically for a representative sample of species from section *Sphenanthera* and from 11 of the 17 other Asiatic sections. *Begonia* species from Africa were used as outgroups in these analyses. *Begonia* section *Sphenanthera*

was found to be polyphyletic, indicating that baccate fruits have evolved multiple times within Asia. The evolutionary significance of animal dispersed and rain-splash dispersed seed are discussed in the light of these analyses, and changes to the sectional classification of these fleshy-fruited Asiatic species are proposed.

SYMPOSIUM: ORIGINS AND BIOLOGY OF DESERT FLORA (IOPB)

380 LOWREY, TIMOTHY K.

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Origins and biology of desert flora

This symposium deals with the origin and evolution of floristic elements and selected plant groups in deserts. Deserts cover about one fifth of the Earth's surface and occur where rainfall is less than 50 cm/year. Deserts comprise landscapes that are subjected to extremes of temperature, high evapo-transpiration, unpredictable precipitation, and complex soil types. The extreme nature of these desert environments presents numerous difficulties to the plants that inhabit them. Plants have developed a number of adaptations to cope with these harsh conditions. The symposium will address the systematics and biology of particular plant groups that are important constituents of deserts. Patterns of desert flora will be discussed as well as the hypotheses concerning the origin of particular floras.

381 CROZIER, BONNIE S.* AND ROBERT K. JANSEN

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Historical relationships in the American desert plant family Cactaceae

The study of evolution in deserts has been hindered by the lack of reliable phylogenies for major desert plant groups. Cacti are conspicuous members of the American deserts but notorious for convergent evolution of morphological characters that has thwarted phylogenetic analysis. We constructed a phylogeny of the Cactaceae based on DNA sequence comparisons from 6,000 chloroplast nucleotides and the nuclear Internal Transcribed Spacer. Sampling included more than 120 species from all currently recognized subfamilies and tribes of the family and outgroups from the Portulacaceae. Parsimony analysis resulted in a well-resolved strict consensus tree with strong to moderate bootstrap support for most branches. Some previously hypothesized relationships are supported by these results, however many others are not and provide new insights for improved taxonomy and classification in the Cactaceae. Cacti inhabit warm deserts and cold, temperate deserts and tropical, as well as other habitat types, making them uniquely useful for comparative studies, and for understanding historical connections between arid regions.

382 LANDRUM, VIC

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Four families and 40 million years of evolution and adaptation to xeric environments

Many of the eleven or so families of the Caryophyllales have been particularly successful at living in xeric environments. Four families have shown the greatest amount of adaptive changes: (1) the ice-plants (Aizoaceae), (2) the cacti (Cactaceae), (3) the Old World cacti (Didiereaceae), and the portulac

(Portulacaceae). The ice-plants adapted to the harsh conditions of the deserts of southern Africa; many evolved leaf succulence to an extreme degree, some went underground, others lost the ability to make normal wood, and all had to adapt their reproductive strategies in pollination or seed dispersal. The cacti adapted to deserts of South, Central, and North America by losing their leaves over time and evolving two types of succulent stems: columnar (ex. saguaros) and rotund (ex. peyote). Cacti adapted by greatly reducing the presence of vessels in their wood, evolving a novel tracheid type termed wide-band tracheids, and evolving CAM and C4 metabolisms. Plants of the Didiereaceae adapted to the harsh Madagascan environment by evolving both stem and leaf succulence, and evolving the columnar stem form, but these plants maintained their normal woody growth. Members of the Portulacaceae evolved in many different xeric areas of southern Africa, but all evolved leaf succulence rather than stem succulence, much the same as was found in the Aizoaceae. Each family evolved varying strategies for living in water stressed environments. Common themes among these distantly related plants are leaf and/or stem succulence, anatomical and physiological adaptations, and varying reproductive strategies.

383 LEWIS, LOUISE A.

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Green algae of desert microbiotic crusts: survey of North American taxa

Green algae are present in desert soils as components of microbiotic or cryptobiotic crust communities, along with lichens, cyanobacteria and other prokaryotes, non-lichenized fungi, and diatoms. Microbiotic crusts can be found in arid and semi-arid regions world-wide, on a large number of different soil types. Microbiotic crusts are ecologically important, playing a role in nutrient cycling and soil stabilization. There has been great interest in understanding the ecological function of and taxonomic composition of crust communities. The green algae that occur in crusts represent a diverse assemblage of taxa spanning three classes of algae, the Chlorophyceae, Trebouxiophyceae, and Charophyceae. They are genetically diverse, but are morphologically simple unicells or packets of cells. In an ongoing study of the diversity of microbiotic green algae from thirty localities in western North America, a large number of green algal taxa have been found, many of which represent new species and new genera. Some genera, such as *Bracteococcus*, are heavily represented. Phylogenetic analyses using ribosomal RNA gene sequence data have been a powerful tool in understanding the diversity and evolution of desert crust green algae. These studies indicate that microbiotic crust green algae evolved from aquatic green algae at least five independent times. In addition, desert green algae are always found to be derived from freshwater, not marine green algae. Some lineages of green algae have a high proportion of desert taxa, while other lineages of green algae have no known desert representatives. Ongoing studies are addressing the ways in which desert green algae are adapted to living in these conditions, including adaptations to high light levels.

384 MULDAVIN, ESTEBAN H.

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Some floristic characteristics of the northern Chihuahuan Desert: a search for its northern boundary

The floristic characteristics that define the northern Chihuahuan Desert are explored using indirect and direct ordinations. The floristic, geographical and environmental

data from a large releve dataset are used to evaluate species distributions within the Chihuahuan Desert and across to the adjacent Short Grass Steppe and Colorado Plateau ecoregions. The focus is on delineating more precisely the floristic boundaries between these provinces and extracting pertinent ecological and geographical information about key indicator species.

385 VERBOOM, G. ANTHONY* AND H. PETER LINDER

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Phylogenetics of the Cape grass genus Ehrharta (Ehrharteae): evidence for diversification in a summer-arid system

Despite the richness of the Cape Flora, there are relatively few published phylogenetic studies that investigate the origins of this diversity. To address this, we estimated the phylogeny of a Cape grass genus, *Ehrharta*, and used this to investigate its past diversification. With 24 species, *Ehrharta* is much larger than any other genus in its tribe, and shows a remarkable diversity of form and ecological function that suggests adaptive radiation. Our phylogenetic analyses included representatives of all four ehrharteoid genera, and used three data sources: two molecular (ITS1 and trnL-F) and one morphological. The results confirm the monophyly of Cape *Ehrharta* species and provide a framework for reconstructing historical shifts in the edaphic and climatic niches occupied by the genus in the western Cape. Habitat reconstruction suggests that the major diversification of the genus followed movement into habitats experiencing more intense and prolonged summer aridity than those inferred to be ancestral. At least some speciation within the Cape arid zone may be linked to greater edaphic diversity here.

CONTRIBUTED PAPERS

386 ALBACH, DIRK C.* AND MANFRED A. FISCHER

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Convergence and diversity in annual members of Veronica (Scrophulariaceae)

The evolution of annual lifeforms is a repeated step in the evolution of angiosperms. Despite obvious advantages of a short life cycle, the annual lifeform embodies great risks, such as necessity for rapid growth, dependence on favorable weather and pollinating agents in a single season, plus competitive disadvantages with perennial species occupying the same sites. Annual species have evolved features to circumvent these risks, many of them associated with an "annual syndrome." This includes more rapid growth to maturity and sexual reproduction, reduced stature and number and position of inflorescences, smaller genome size (associated with a shorter cell cycle, allowing faster development), increased rate of molecular evolution (probably correlated with a shorter generation time), and reduction in phytochemical arsenals (perhaps related to lower risk of herbivory during a shorter life span). Other characters not as closely associated with the annual lifeform (e.g., ultrastructure of pollen and seeds) may, therefore, provide better evidence of phylogenetic history in annual taxa. A good system in which to address questions of the evolution of annual taxa from perennial sister species is in the annual species of *Veronica* (Scrophulariaceae). These taxa previously have been classified together in section *Alsinebe* (also called *Pocilla*), but different base chromosome numbers and seed morphology have long suggested that the group is polyphyletic. Molecular data from nuclear (ribosomal DNA, ITS) and chloroplast (trnL-F) strongly support this

polyphyly. This provides the opportunity to study repeated morphological, karyological, and phytochemical trends and convergences during the repeated evolution of the annual lifeform in this genus.

387 ALICE, LAWRENCE A.* AND AMBER HOGART

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Phylogeny of Rubus (Rosaceae) based on Granule-Bound Starch Synthase I (GBSSI) gene sequences

The need for biparentally inherited, nuclear DNA markers useful at lower taxonomic levels in plant systematics research has revealed several exciting possibilities. One includes the granule-bound starch synthase I (GBSSI) gene comprising 13 translated exons and 12 introns in most species examined. *Rubus* (Rosaceae) typifies a group where GBSSI sequences may yield valuable information. Sequences of the nrDNA ITS region and, more recently, of three chloroplast DNA regions initially provided insight into the systematics of *Rubus*. However, none offers sufficient numbers of phylogenetically informative characters to generate robust hypotheses of relationship. Thus, we sequenced the 5' end of the GBSSI gene for five *Rubus* species representing three clades. GBSSI in *Rubus* and *Rosa* (both Rosoideae) occurs at two paralogous loci; GBSSI-1 and GBSSI-2. The region we sequenced includes seven complete exons, two partial exons, and eight introns. Total number of aligned nucleotides is 2157 using *Rosa* as an outgroup, with exons and introns ranging in length from 64 to 244 bp and from 64 to 477 bp, respectively. Analysis of exons and introns or introns alone recovers a single most parsimonious tree which is consistent with both ITS and chloroplast phylogenies generated previously. Although pairwise divergences for distantly related species are slightly lower for GBSSI-1 introns than for ITS 1 + ITS 2, they are notably higher for closely related species; divergence between *R. cuneifolius* and *R. hispidus* is 0.4% for ITS but 2.1% for GBSSI-1. Percent parsimony-informative characters is also higher in GBSSI-1 (6.0%) than in ITS (2.0%). Given that aligned sequences of GBSSI-1 introns are nearly 2.5-fold longer than ITS 1 + ITS 2 (1216 bp vs. 493 bp, respectively) and show higher levels of divergence, we believe the 5' region of GBSSI-1 holds great promise for phylogeny reconstruction of *Rubus* and potentially other plant groups, especially at lower taxonomic levels.

388 ALVAREZ, AIDA^{1,2*} AND KENNETH CAMERON¹

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Phylogenetic analysis of Gomphichis and the subtribe Prescottiinae (Orchidaceae)

Gomphichis contains about 23 species of terrestrial orchids. The genus has a continuous distribution from Costa Rica to Bolivia with disjunct species in the Guayana Highlands and southeastern Brazil. *Gomphichis* and its close relatives *Aa*, *Altensteinia*, *Myrosmodus*, *Porphyrostachys*, *Prescottia* and *Stenoptera* have been placed within the subtribe Prescottiinae, tribe Cranichideae (Dressler, 1993). The Prescottiinae include most of the Neotropical terrestrial orchids that flourish at high and middle elevations in the major mountain chains of Central and South America. Phylogenetic relationships within the subtribe Prescottiinae are unclear and generic limits among some of their members are questionable. In order to provide the first phylogenetic hypothesis for *Gomphichis* and the Prescottiinae, we generated a molecular phylogeny based on ITS sequence data. Ingroup

taxa included most members of the Prescottiinae, except *Stenoptera*. Outgroup taxa consisted of representatives from three of six subtribes currently recognized in Cranichideae. Preliminary analyses support the monophyly of *Gomphichis* and suggest the paraphyly of the Prescottiinae (sensu Dressler, 1993). Further molecular and morphological studies are underway to circumscribe *Gomphichis* and define an expanded concept of the subtribe Prescottiinae.

389 ANDRUS, NICOLE^{1,2*}, DAVID BOGLER², ALAN TYE³, GERALD GUALA², AND JAVIER FRANCISCO-ORTEGA^{1,2}

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Preliminary molecular evidence for the origin of Darwiniothamnus (Asteraceae)

Darwiniothamnus (Asteraceae: Astereae) is one of the seven plant genera endemic to the Galapagos Islands. Based on morphological grounds the genus, although distinct, has been suggested to be closely related to South American *Erigeron*; however this hypothesis needs to be tested by rigorous phylogenetic methods. In this study a preliminary phylogeny of the ITS region of the nuclear ribosomal DNA is presented. This phylogeny suggests that *Darwiniothamnus* is nested within the *Erigeron* clade, which is composed of *Aphanostephus*, *Conyza* and *Erigeron*. Upon further analysis it appears that *Darwiniothamnus* may be sister to *E. thrincooides* and *E. bellioides* which are both from the Antilles. Further studies are currently in progress to confirm this possible biogeographical connection between the Caribbean basin and the Galapagos Islands. These additional studies will include putative relatives from Mexico, Chile and the Revillagigedo Islands of the Pacific Ocean.

390 ARCHAMBAULT, ANNIE* AND ANNE BRUNEAU

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How useful is the LEAFY gene for the phylogeny reconstruction in the Caesalpinioideae?

We evaluated the potential of the *LEAFY* gene, a nuclear gene, as a phylogenetic marker in the subfamily Caesalpinioideae of the Leguminosae. Caesalpinioideae are large tropical trees which are extremely diverse in their floral morphology. The *LEAFY* gene has a crucial role in the transition to flowering, and in *Pisum sativum*, it also is responsible for the pinnate nature of the leaves. In all angiosperms examined to date the coding sequence is separated by two introns. We cloned and sequenced the 3' portion of the second exon, the second intron, and most of the third exon of the *LEAFY* gene for a representative sampling of species in the Caesalpinioideae and for a few other legumes. The phylogenetic analyses tell us that the gene is in a single copy. The intron is too variable to be useful for resolving relationships across the Caesalpinioideae, but the exons can be aligned and provide phylogenetic resolution. We have found a new intron inserted in the second exon, which serves as a marker for a group of genera in tribe Detarieae. The trees recovered from the phylogenetic analyses of the exons are highly resolved compared to those obtained from chloroplast sequences (*trnL* intron) and the groups are strongly supported. However, the results from the phylogenetic analysis of *LEAFY* are not congruent with those from the *trnL* intron sequences, and they differ from traditional hypotheses of relationships in the subfamily. What are the biological mechanisms that lead to this incongruence? Is *LEAFY* a poor phylogenetic

marker? Possible causes for this incongruence are explored: saturation of non-synonymous substitutions in *LEAFY*, presence of undetected multiple copies in certain taxa, differences in evolutionary rates among lineages.

391 BADR, ABDELFAHAT^{1,2*}, HANAA H. EL SHAZLY^{1,3}, HADDAD EL RABEY⁴, AND LINDA E. WATSON¹

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DNA amplified fragment length polymorphisms and the relationships of Lathyrus (Fabaceae)

DNA amplified fragment length polymorphisms in 37 taxa representing 18 species of *Lathyrus* (Fabaceae) were analyzed by distance methods and the resulting trees were used to reassess the classification of the examined species. The majority of the examined taxa represent section *Lathyrus* (27 taxa; 12 species), but ten taxa representing three other sections were also included in this study. The analysis of AFLPs demonstrated very clearly a close affinity among taxa of the same species confirming the existence of genetic basis for the taxonomic identity of the examined species. The analysis delimited the studied species in three groups, two of which may be regarded as one super group that comprised the species of section *Lathyrus*. In this section, *L. cicera*, *L. hirsutus*, *L. gorgoni* and *L. sativus* formed one group. In the second group of this section *L. annuus*, *L. blepharicarpus* and *L. hierosolymitanus* were distinguished as a subgroup from another subgroup comprised of *L. latifolius*, *L. marmoratus*, *L. sylvestris* and *L. tingitanus*. In the third group, *L. aphaca* of the monotypic section *Aphaca* was associated with two species of section *Linearicarpus*, whereas *L. shaericus* of the latter section was associated with two species of section *Clymenum*. The AFLP analysis did not support the separation of *L. gorgoni* in section *Gorgania*; it is clearly a member of section *Lathyrus* and is closely associated with *L. sativus*. AFLP data also indicated that section *Linearicarpus* seems to be an unnatural group. The relationships of the examined species, as revealed by the analysis of AFLPs, are discussed in the light of their previous classifications based on other lines of evidence.

392 BALDWIN, BRUCE G.

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Phylogeography and major range disjunctions of the rare, granite endemic Carlquistia muirii (Compositae–Madiinae): ancient long-distance dispersal or surfing on the Salinian Block?

Phylogeography of the rare, perennial, Californian tarweed *Carlquistia* [*Raillardiopsis*] *muirii* was studied to assess whether range disjunctions in the southern Sierra Nevada and between the Sierra Nevada and South Coast Ranges (Ventana Double Cone, Monterey Co.) are best explained by long-distance dispersal or vicariance. Occurrence of *C. muirii*, a granite endemic, on Ventana Double Cone in the Santa Lucia Range, ca. 240 km from the nearest Sierran population, is intriguing in part because granitic exposures are extremely limited between the southern Sierra/Tehachapi region and the northern Santa Lucia Range/Gabilan Range and unexposed granitics are covered by sediments of at least Miocene age. Vicariance could explain most of the Sierra/Santa Lucia disjunction, even without assuming past occurrence of *C. muirii* off of granitics, if the species existed ca. 5 Ma, when the Santa Lucia/Gabilan region was juxtaposed with the Sierra/Tehachapi region. Any populations then extending from the

Sierra/Tehachapi onto Santa Lucia/Gabilan granitics could have been transported in situ 240 km northwesterly by tectonic slippage of the Salinian Block along the San Andreas Fault. Phylogenetic analysis of 18S-26S rDNA ETS and ITS variation from populations throughout the distribution of *C. muirii* yielded a robust, well-resolved tree that is congruent with the area cladogram for the southern Sierra Nevada and South Coast Ranges and with the hypothesis that vicariance explains distributional patterns in *C. muirii*. However, a calibrated, rate-constant rDNA tree for perennials of the "Madi lineage" yields an estimated time of divergence between Sierran and Santa Lucian populations of only ca. 1 Ma, in conflict with a vicariance hypothesis. Patterns of ancient, long-distance dispersal mimicking vicariance may explain at least part of the fascinating phylogeography of *C. muirii*.

393 BARBER, JANET C.^{1*}, LILIANA M. GIUSSANI², AND ELIZABETH A. KELLOGG¹

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Phylogeny of Poaceae subfamily Panicoideae based on sequences of phytochrome B, a low-copy nuclear gene

We are using sequences of phytochrome B, a low-copy nuclear gene, to reconstruct phylogenetic relationships within Poaceae subfamily Panicoideae. Results corroborate relationships found by two earlier studies based on chloroplast markers (*ndhF*; combined sequences of the *trnL* intron and the *trnL-trnF* intergenic spacer regions). The Old World genus *Danthoniopsis* is excluded from the Panicoideae. Tribe Andropogoneae, including *Arundinella*, is monophyletic. Tribe Paniceae is also monophyletic, but resolution within the tribe differs from that of the chloroplast phylogenies. The chloroplast trees recovered two major clades corresponding to chromosome number, with one clade comprising the $x = 10$ taxa and a second clade composed of taxa with a base number of $x = 9$. The nuclear tree also recovers a monophyletic $x = 9$ clade but it is nested within a paraphyletic $x = 10$ group. This apparent paraphyly may be a sampling artifact, since our sampling to date within this group is minimal. Relationships within the $x = 9$ clade correspond to groups sharing the same C4 photosynthetic subtype, with one group using NAD-malic enzyme, one using PCK, and a third using NADP-malic enzyme (the "bristle" clade). All studies have demonstrated multiple origins of the C4 photosynthetic pathway within the Panicoideae, leading to the unexpected revelation that chromosome number is a better predictor of phylogenetic relationships than is photosynthetic pathway.

394 BARKMAN, TODD J.

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Angiosperm phylogenetics: genomic congruence and methodological incongruence

High confidence in angiosperm phylogenetic inference is achieved when estimates from all three genomic compartments are identical. Recent studies have shown that estimates from the nuclear, plastid, and mitochondrial genomes are largely in agreement for many higher level flowering plant relationships. In conjunction with genomic congruence, confidence is heightened in cases where different methods (e.g. parsimony, neighbor-joining, maximum likelihood) estimate identical relationships as well. In contrast to congruent genomic relationships obtained for higher level angiosperm relationships, different methods sometimes estimate contrasting but well supported phyloge-

nies for the same taxa and data. Several striking examples of method-dependent conflicting phylogenetic estimates will be discussed including the putative first branch of angiosperm phylogeny.

395 BATEMAN, RICHARD M.^{1*} AND PETER M. HOLLINGSWORTH²

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Phylogenetics of Tribe Orchideae (Orchidaceae)

Internal Transcribed Spacer (rDNA) data have been obtained from 193 terrestrial orchid species, encompassing all genera and the great majority of the widely recognized species of Orchidinae, a heterogeneous selection of species of Habenariinae, and a single Diseae outgroup. The resulting parsimony-based phylogeny reveals 11 well-resolved clades within Orchidinae and confirms the triphyly of Orchis s.l. Relationships within Habenariinae are more ambiguous; the subtribe appearing weakly monophyletic or paraphyletic according to the tree-building algorithm used, though the genus *Habenaria* is clearly highly polyphyletic. Species-level relationships are satisfactorily resolved within most of the major clades, the exceptions being widely recognized as taxonomically 'critical' groups such as *Serapias*, derived *Ophrys*, and the former genus '*Nigritella*'. Relationships among the 11 major clades are less clear, and are currently being further investigated by combining ITS with new trnL sequence data for a carefully selected subset of 63 species. Current phylogenies support the recently recognized $2n = 36$ clade; they also indicate a $2n = 40$ clade that is further diagnosed by digitate root-tubers, and is derived relative to the basally divergent clade of exclusively Asian genera. This Asian clade identifies the plesiomorphic chromosome number as $2n = 42$, and is consistent with possible southeast Asian origins of several of the more basally divergent major clades within the subtribe and of the subtribe itself, though ambiguous phylogenetic placements of the potentially pivotal African genera *Stenoglottis* and *Brachycorythis* also permit an African origin. Morphological characters informally 'mapped' across the molecular phylogeny and showing appreciable levels of homoplasy include floral and vegetative pigmentation, flower shape, leaf posture, gynostemium features, and various pollinator attractants. Brief scenarios, some adaptive and others non-adaptive, are postulated for specific evolutionary transitions.

396 BATEMAN, RICHARD M.^{1*}, PETER M. HOLLINGSWORTH², JANE SQUIRRELL², JAMES H. DICKSON³, AND MARK TEBBITT⁴

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Population genetic structure, phylogeny, and breeding system in Epipactis (Orchidaceae: Neottieae)

Deviations from random mating are an important determinant of plant species evolution. Taxonomic complexity and controversy, and the resulting difficulties in assessing conservation priorities, are often associated with 'critical groups' incongruent with the classic Hardy-Weinberg equilibrium mode of population genetic structure. In critical genera such as *Epipactis* breeding system transitions are hypothesized to be an important mechanism underpinning speciation. We have erected a multi-gene molecular phylogenetic framework for the Neottieae before using molecular markers to investigate the amounts and partitioning of genetic variability in 26 putative species of *Epipactis*. These species have contrasting floral morphologies, geographical distributions,

habitat preferences, and propensities for vegetative spread. All species with floral morphologies consistent with self-pollination were homozygous and uniform at all examined allozyme loci. In contrast, species whose floral morphologies were consistent with allogamy differed considerably in degrees of genetic variability, with this variability typically organized according to Hardy-Weinberg expectations. Thus, the transition to autogamy has an overriding effect on the organisation of intra-specific genetic variation, but other factors determine the comparative amounts of genetic variation in allogamous species. Using a combination of isozymes, inter-SSRs, cpDNA RFLPs, and cpDNA SSRs, we determined whether autogamous taxa represent distinct lineages rather than minor mutational variants. All of the markers we employed suggest that the transition from allogamy to autogamy is highly iterative, generating multiple homozygous and uniform lineages, each distinct and separable by fixed allelic differences from other comparable lineages.

397 BATTERMAN, MCLYNDA R.W. AND THOMAS G. LAMMERS*

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Trichome morphology and its systematic implications in Centropogon and other genera of Lobelioideae (Campanulaceae)

Centropogon (Campanulaceae: Lobelioideae) includes 216 species distributed from Mexico and the Lesser Antilles to Bolivia and Brazil. The genus is characterized by solitary axillary flowers or racemose terminal inflorescences, entire corolla tube, occluded anther orifice, baccate fruit, and ellipsoidal seeds twice as long as broad or less. Five sections are currently recognized *Centropogon*, *Siphocampyloides* Benth., *Wimmeriopsis* McVaugh, *Burmeisteroides* Gleason, and *Niveopsis* Lammers. The section *Siphocampyloides*, comprising 98 species, is distinguished by the possession of branched (i.e., arbusculiform) trichomes. However, other characteristics, such as floral features, suggest this may not be the optimum classification. The species of subsection *Peruviani* McVaugh resemble section *Burmeisteroides* in corolla morphology, while those of subsection *Brevilimbati* McVaugh resemble section *Wimmeriopsis*. Furthermore, branched trichomes occur infrequently in three other genera: Neotropical *Burmeistera* and *Siphocampylus* and Hawaiian *Cyanea*. Examination of trichome morphology via scanning electron microscopy reveals considerable variation among numerous species. Some trichomes are stalked while others are sessile. Trichome branching patterns observed include dendritic, stalked-multangulate, and dendritic-echinoid. Other variation that is observable includes distance between branches, total trichome length, and total coverage of indumentum over the surface of the leaf. Preliminary evaluation of the data suggests some correlation between trichome type and other characteristics (e.g., floral features) traditionally used in the classification of the genus.

398 BAYER, RANDALL J.*, EDWARD W. CROSS, AND NEIL H. BAGNALL

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A reassessment of tribal affinities of several enigmatic genera of Australian Asteraceae, based on three chloroplast sequences

Several genera of Australian sunflowers have been doubtfully placed in various tribes throughout their taxonomic history. Our continuing studies of relationships within the Gnaphalieae have led us to investigate the tribal placement of

these enigmatic genera. Therefore, the objective of this study was to elucidate tribal relationships of *Cratystylis* S. Moore, *Isoetopsis* Turcz., and *Centipeda* Lour. by reconstructing their phylogeny within the subfamily Asteroideae. This has been accomplished through the use of sequence data from the *trnL* intron, *trnL/trnF* intergenic spacer and *matK* coding region. *Cratystylis* is a genus of four endemic Australian arid zone shrubs. It has been variously included in the Astereae, Inuleae s. *ampl.*, and even in a paraphyletic Cichorioideae. The monotypic Australian genus *Isoetopsis* has been assigned to the Anthemideae, quite often as a section within the genus *Cotula*, as *Cotula* sect. *Isoetopsis* (Turcz.) Baillon. A placement of *Isoetopsis* within the Astereae and Gnaphalieae has also been suggested. *Centipeda* is a genus of five species inhabiting Australia, South America, and New Zealand. Although traditionally a member of the informal group 'Cotuleae' in the Anthemideae, recent workers have considered it to be in Astereae or Gnaphalieae. Detailed morphological studies support neither of these placements and hence it is currently unassigned. Molecular data does not support the inclusion of the three genera in Gnaphalieae, instead there is general agreement between the tribal placement of these genera based on our molecular analysis and certain morphological and secondary chemical characters.

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Patterns of speciation and the evolution of hummingbird and self-pollination in the Erythranthe clade of Mimulus (Phrymaceae) as inferred from an AFLP phylogeny

The *Erythranthe* clade in *Mimulus* contains species that are adapted to different pollinators and one species that reproduces mainly through self-pollination. Section *Erythranthe* contains six species, five of which are hummingbird pollinated and one is bee pollinated. The estimated phylogeny of this group using data from ITS, ETS, and *trnL/F* shows no resolution for relationships among species, but does support the monophyly of the *Erythranthe* clade if the highly selfing, annual, desert-adapted *M. parishii* is included. Sister to *Erythranthe* is a clade that includes *M. bicolor*, *M. filicaulis*, and *M. rubellus*. 488 AFLP bands were scored for all of the species in *Erythranthe* and its sister clade, including multiple populations for most species. Results of analyses of AFLP fragment data indicate: 1) *Erythranthe* is monophyletic, 2) a high amount of genetic differentiation exists between *M. lewisii* populations in the Sierra Nevada and those in the Pacific Northwest and Rocky Mountains. To further investigate the relationships among species, DNA from each species (including both races of *M. lewisii*) was combined and an additional 400 AFLP fragments were collected which resulted in a phylogeny for the group. A well-resolved clade containing species in the Sierra Nevada, the Pacific Northwest and the Rockies is sister to another well-resolved clade containing species in Mexico, Arizona, and Utah. The highly selfing *M. parishii* is resolved as sister to all other species or as sister to a clade containing *M. lewisii* and *M. cardinalis*. The common ancestor of the *Erythranthe* clade is inferred to be annual, insect-pollinated and from the Sierra Nevada. Hummingbird pollination is inferred to be derived within the clade and may have arisen twice independently. Allopatric speciation is suggested to be the major mode of speciation in the clade.

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Radiation of Mimulus (Phrymaceae) in western North America: evolution of polyploidy, woodiness, and pollination syndromes

Species in *Mimulus* in western North America have become model systems for the study of evolutionary processes in nature. As currently described, *Mimulus* contains approximately 120 species, of which 75 percent occur only in western North America. *Mimulus* contains species with different ploidy levels, reproductive strategies, and pollination syndromes. Molecular phylogenetic studies in *Mimulus* have redefined Phrymaceae and indicate that at least six genera have been derived from within *Mimulus*. One clade recovered in these analyses contains all of the species of *Mimulus* in western North America, plus *Hemichaena*, *Berendtiella*, and *Leucocarpus*. A phylogenetic hypothesis for 82 species was estimated using ITS, ETS and *trnL* sequences. These analyses indicate that polyploidy has evolved independently at least six times, woody plants have evolved from herbaceous ancestors twice, self-pollination has possibly evolved 10 times and hummingbird pollination has evolved at least three times. These results also indicate that *Mimulus* has established twice in Asia and once in South America from ancestors in western North America. Within sect. *Eunanus* patterns of genetic diversification better match geographic distribution (Sierra Nevada versus the Pacific Northwest) than taxonomic circumscription. *Mimulus nanus* is not monophyletic in two distinct ways. First, individuals from the Sierra Nevada are distinct from those in the Cascade and Rocky Mountains. Second, *Mimulus nanus* in the Pacific Northwest is paraphyletic, because three currently recognized species, *M. cusickii*, *M. jepsonii*, and *M. clivicola* have been derived from within it.

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Lectotypification of the endangered species Solidago shortii (Asteraceae)

Solidago shortii (Asteraceae), Short's Goldenrod, is an endangered species native to Kentucky. This species was described by John Torrey and Asa Gray in 1842 in *A Flora of North America*. An examination of herbarium specimens of *Solidago shortii*, including all historical collections that could be located, revealed considerable nomenclatural confusion. Fourteen sheets from five herbaria exhibited six type annotations. In addition, a typification of the species had not been published. The complex nature of the protologue necessitated a consideration of Torrey and Gray's species concept and nomenclatural practices in the context of the *International Code of Botanical Nomenclature*. On the basis of these considerations, we designate one lectotype and four syntypes for *Solidago shortii*.

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Morphometrics of Solidago shortii, an endangered goldenrod from Kentucky (Asteraceae)

Solidago shortii (Asteraceae), Short's Goldenrod, is an endangered species presently known from a series of closely clustered subpopulations in the vicinity of Blue Licks, Kentucky. Short's Goldenrod was first discovered and described from a now-extirpated population at the Falls of the Ohio River, Kentucky. Our goals are to test two hypotheses: 1) Short's Goldenrod is morphologically distinct from other goldenrod species and 2) specimens from the extant Blue Licks populations are taxonomically identical with the extirpated Falls of the Ohio plants. We tested these hypotheses with a combination of field and herbarium approaches. Principal Components Analysis and Discriminant Function Analysis of measurements of 19 vegetative and reproductive characters of Short's Goldenrod and selected congeners reveal a suite of diagnostic characters for Short's Goldenrod. All specimens from both Blue Licks and Falls of the Ohio share these traits and thus appear taxonomically identical.

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Genetic variation in the renosterbos, Elytropappus rhinocerotis (Asteraceae) in the southwestern Cape, South Africa

Renosterbos is a shrub which is extremely common on the Cape coastal forelands. Together with several other fynbos species, it also occurs in isolated interior populations, confined to mesic high-altitude sites separated by large tracts of arid country. How did the outliers come to occupy such sites? Was fynbos previously more extensive, and is it currently in a contracted state due to widespread aridification since the last glacial maximum (vicariance hypothesis)? Or are the outliers the results of long-distance dispersal events (dispersal hypothesis)? These conflicting scenarios would produce different genetic signatures in extant populations, which should be detectable (in the absence of confounding factors such as subsequent gene flow) using highly polymorphic genetic markers. In the case of vicariance, long-isolated populations should have distinct genetic signatures and contain unique genotypes, and genetic difference should be correlated with duration of isolation. Neighbouring isolated populations would not necessarily be more closely related than very distant isolated populations. In the case of dispersal, colonies should consist of a subset of genotypes present in the founder population, and founder populations are likely to be those closest to colony populations. In this study we use three ISSR-PCR primers to survey the genomes of at least five individuals from each of ten populations across the entire species range, as a first step in constructing a phylogeographic hypothesis. The survey shows high levels of variation, most of which is partitioned among populations. Examination of geographic structuring in this variation may allow us to make inferences on population histories in renosterbos, which could be extrapolated to the history of fynbos as a whole.

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Simple leaves, complex leaves: which way?

The consequences of variation in leaf form (simple, complex) has long intrigued plant physiologists and ecologists. Here, phylogenetic methods (using recent molecular phylogenies of angiosperms) are used to examine patterns in the direction of change. These analyses suggest, commensurate with paleobotanical studies, that the ancestral angiosperm had simple leaves. However, contrary to the general (implicit) assumption, there is an overall trend of a lower frequency of transitions from simple to complex ('gains') than from complex to simple ('losses'). This finding has wide-ranging molecular developmental, physiological and ecological implications that are briefly discussed.

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Phylogenetic relationships in Oönoopsis (Asteraceae) based on DNA sequences from three spacer regions

Oönoopsis is a monophyletic genus consisting of five species distributed mainly in central and eastern Wyoming and Colorado. The plants are herbaceous perennials that tend to grow on alkaline soils. All members of the genus are primary selenium indicators, and appear to be obligatory seleniophiles. The purpose of this study was to determine phylogenetic relationships among species of the genus *Oönoopsis* using DNA sequence data from two chloroplast intergenic spacers (*trnL-trnF* and *psbA-trnH*) and the nuclear ribosomal internal transcribed spacers (ITS). Sequence variation was lowest in the *trnL-trnF* spacer and greatest in the *psbA-trnH* region. The total data phylogeny placed *O. multicaulis* sister to the rest of the genus. *O. foliosa* var. *foliosa* and *O. foliosa* var. *monocephala* were placed in separate clades, in disagreement with morphology-based phylogenies. Low bootstrap and decay values indicate a low amount of divergence among sequences, suggesting a relatively recent origin for the genus.

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Evolutionary relationships in Dasyliirion (Nolinaceae): an assessment using AFLP markers

Dasyliirion is a genus of prickly-leaved, dioecious rosette plants found in arid, hilly regions of the desert Southwest and Mexico. Morphological and molecular data clearly indicate that *Dasyliirion* is closely related to *Nolina*, *Beaucarnea*, and *Calibanus*, and these genera are often treated as the family Nolinaceae. *Dasyliirion* was recently monographed and 17 species were recognized. Many of these species fit the model of "semi-species" in which barriers to reproduction are weak and hybrids are common. Species relationships are poorly known, and probably reticulate. Molecular studies using several markers have generally failed to reveal much variation in *Dasyliirion*, a situation common in long-lived, woody taxa. A survey of chloroplast DNA restriction site variation indicated two major groups, one clade of species in southern and central Mexico with brushy leaf tips, and another clade containing the rest of the species. Sequencing ITS rDNA failed to provide much additional information. Recently, a study

was made using the technique known as amplified fragment length polymorphism (AFLP) to resolve species relationships in *Dasyliiron*. AFLP is widely used in population level studies, paternity analysis, cultivar identification, and crop breeding, but the limits of using the technique to compare more widely divergent taxa are uncertain. We surveyed 36 samples of *Dasyliiron* using 21 primer pairs, which generated hundreds of variable fragments. In general, the AFLP data supports the clades indicated by the chloroplast DNA restriction site data, however relationships within the major clades is highly dependent on screening the data and methods of analysis. Strengths and weaknesses of using AFLP markers in studies of this nature will be discussed.

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Conservation genetics of Microcycas calocoma, a rare cycad endemic to Cuba: preliminary data from RAPD and AFLP markers

Microcycas is a genus with one species, *M. calocoma*, endemic to Pinar del Rio Province in Cuba. Morphology and DNA sequence data indicate a close relationship between *Microcycas* and *Zamia*, although *Microcycas* has many unique molecular characters which suggests a long period of isolation. There are approximately 500-600 individuals left in the wild, occurring in 17 scattered populations. The plants have suffered a decline due to habitat clearing, overcollecting, and destruction of young plants by pigs. The natural insect pollinator may have become extinct due to fires and pesticides. There is little natural regeneration in the wild, and juveniles are uncommon. Major efforts to preserve *Microcycas* have been initiated in Cuba. Preserves have been established, plants are pollinated manually, and studies are underway to improve propagation techniques and reintroduce plants to the wild. It would be very useful to find molecular markers to measure genetic variation within and between populations. This would help establish conservation priorities and be useful in breeding and reintroduction efforts. Towards this goal, we have screened an *ex situ* population at Fairchild Tropical Garden and Montgomery Botanical Center with 80 RAPD primers and 18 AFLP primer pairs. We will eventually include samples from wild populations in Cuba. A secondary goal was to explore the possibility of using molecular techniques to screen young plants for sex determination. All cycads are dioecious, with separate sexes that can be recognized only by their reproductive structures (cones). The mechanism of sex determination control in cycads is unknown. Sex markers will be useful to determine the sex of nursery propagated *Microcycas* seedlings prior to replanting in wild populations to restore balanced sex ratios to wild populations, and may be useful in other aspects of cycad horticulture.

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Phylogeny of the Cyphomandra clade of the genus Solanum (Solanaceae)

At least 10 major clades have been identified within the genus *Solanum*. One of these, the *Cyphomandra* clade, consists of members of *Solanum* sections *Pachyphylla* (former genus *Cyphomandra*; ca. 35 spp.), *Cyphomandropsis* (13 spp.), and *Glaucophyllum* (1 sp.). Molecular data indicate that the *Cyphomandra* clade forms a well-supported monophyletic group, but its sister group is still unclear. All species of this clade investigated to date have large chromosomes and large amounts of

nuclear DNA. ITS sequence data were used to examine phylogenetic relationships among ca. 30 species of the clade. *Solanum* sections *Pachyphylla* and *Cyphomandropsis* do not appear to be monophyletic as currently circumscribed. About six discrete clades can be discerned within the *Cyphomandra* group. Many of these clades are not congruent with the species groups recognized in sections *Pachyphylla* and *Cyphomandropsis* on the basis of morphology. ITS data support the hypothesis that the Bolivian species *S. roseum*, *S. unilobum*, and *S. maternum* are the closest wild relatives of the tree tomato, *S. betaceum*.

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Phylogeny and evolution of Nymphaea: integrating evidence from different genomic regions and from paleobotany

Nymphaea is the most diverse genus (45 species) of the order Nymphaeales, which is congruently inferred as the second branch of the angiosperm tree. In a collaborative effort, several genomic regions approximately 5000 bp of the genomic regions *trnT-F*, *trnK*-intron including *matK*, and ITS1-5.8S-ITS2 were sequenced from nearly all species. In contrast to previous classifications a BAN-clade is hypothesized within *Nymphaea*, comprising the subgenera *Brachyceras* (pantropical) and *Anecephyra* (australasian) and forming a terminal lineage sister to the temperate subgen. *Nymphaea*. A second major lineage includes an assemblage of the SE-African *N. petersiana* with the subgenera *Lotos* and *Hydrocallis*. Based on a synopsis of the fossil record the earliest known appearance of *Nymphaea* is Middle Eocene (45 MYBP). Combining palaeobotanical and molecular evidence it seems that a rapid early differentiation into major clades is contrasted by comparatively recent species diversifications within these clades. Possible patterns of spatial differentiation are discussed. In the case of the temperate subgen. *Nymphaea*-clade which comprises closely related circumboreal taxa (pygmy waterlilies) as well as species confined to either North America or Europe, possible patterns of spatial differentiation are discussed.

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Phylogenetics of Prunus (Rosaceae) as determined by ITS, the chloroplast trnL-trnF spacer and s6pdh: implications for subgeneric classification, biogeography and evolution of morphology

Phylogeny of *Prunus* was reconstructed by maximum parsimony analysis of three markers, ITS, *s6pdh* and the chloroplast spacer *trnL-trnF*. Over forty species from all subgenera and most sections of *Prunus* were included in the analyses of ITS and *trnL-trnF*. ITS provided more informative sites but was highly homoplastic, whereas *trnL-trnF* was less variable and showed little homoplasy. Combined analysis of these two markers separated *Prunus* into two groups; a well supported *Cerasus* /*Padus* /*Laurocerasus* clade and a second group that contained the rest of *Prunus*. The data suggest a Eurasian origin for *Prunus* with multiple radiations into North America. A subset of the samples were selected for phylogeny reconstruction using *s6pdh*, a nuclear gene encoding an enzyme involved in sorbitol metabolism. Fourteen species of *Prunus*, representing all major clades found by the previous study using ITS and *trnL-trnF*, were included. Maximum parsimony analysis of *s6pdh* sequences agrees with results obtained with ITS and *trnL-trnF*. Some changes in the classification of *Prunus* are necessary. For example it is evident that section *Microcerasus*, previously placed in subgenus *Cerasus*, is actually more closely related to subgenus *Prunus*. Evolution of morphological traits such as dry fruits, and their ecological significance are discussed

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Ecology and evolution of Orcuttieae (Poaceae): an endangered vernal pool grass

The purpose of this project is to: 1) reconstruct a phylogeny for Orcuttieae, a small tribe of vernal pool C₄ grasses, using molecular data and 2) use the phylogeny to assess the evolutionary history and pathway modifications of C₄ photosynthesis in this group. Previous work by Keeley (1998) revealed that *Orcuttia* carries out C₄ photosynthesis in aquatic leaves but lacks Kranz anatomy while the remaining genera in the tribe, *Tuctoria* and *Neostapfia* have Kranz anatomy. Keeley (1998) proposed that *Orcuttia* is the most derived genus in the tribe followed by *Tuctoria* and the earliest derived genus *Neostapfia*. His hypothesis was based on the lack of Kranz anatomy and other aquatic adaptations. This investigation will test the hypothesis advanced by Keeley (1998) to explain the unique physiology of the Orcuttieae. A phylogeny coupled with the photosynthetic data for this group will elucidate the transition of Orcuttieae from land to an aquatic environment.

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ITS phylogeny of North American asters (Asteraceae: Astereae)

Genus *Aster* s.l. has been the object of much controversy, particularly in North America (NA). Recently, splitting the aggregate genus has been suggested, notably in the thorough morphological review of Nesom (1994, 2000), but students of the group have yet to agree on delimitation and relationships of the segregate taxa. Also, relationships with Eurasian taxa, and among them, have been controversial. Recent ITS phylogenetic analyses by Morgan (1997) and Noyes (2000, & Rieseberg 1998) provide a framework to test hypotheses of relationships among members of the *Aster* complex. In this phylogenetic analysis, we are adding more than 60 ITS sequences of many critical taxa representing all major lineages that have been recognized within *Aster* s.l., in order to intensify taxon sampling both within and among groups in the NA Astereae and in Eurasian *Aster*. All North American and derivative taxa form a monophyletic clade, while Eurasian taxa also form a single clade excluding all NA taxa. Thus, we confirm that *Aster* is polyphyletic; the name should be applied only within subtribe Asterinae (Eurasian clade); Doellingeria is strictly North American and not disjunct with east Asia. Each major clade of NA Astereae have an aster segregate at its base: *Eucephalus* and *Doellingeria* are basal to all NA Astereae; *Sericocarpus* is sister to the *Solidago* lineage within *Solidagininae*; the *Symphyotrichinae* (all asters) are sister to *Machaerantherinae*, where *Eurybia* and *Oreostemma* are basal; *Ionactis* and *Oclemea* are sister to the *Conyzinae*-*Chrysopsidinae* clade. Within the *Symphyotrichinae*, *Canadanthus*, *Ampelaster* and *A. chapmanii* form a grade basal to *Symphyotrichum* (including *Psilactis*). Biogeographically and ecologically, it appears that on both sides of North America, temperate, mesic taxa have developed first, followed by multiple radiations into more xeric habitats.

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Infrageneric relationships in the genus Pereskia (Cactaceae)

Members of the genus *Pereskia* (17 species) exhibit a number of plesiomorphic characters for the family Cactaceae such as shrubby habit, non-succulent or partially succulent leaves, and in some species, nearly superior ovaries. In addition, the members of this genus also show a transition from perigynous flowers with half-inferior ovaries to those species having true receptacular epigyny (the predominant condition in the Cactaceae). To examine this and other morphological transitions in the presumably basal cactus lineages, a well resolved phylogeny was sought to provide the basis for interpreting anatomical/structural innovations and polarizing character state changes. Interspecific relationships within *Pereskia* were inferred using combined datasets from cpDNA restriction-site data, together with sequence data from non-coding regions of the plastid genome, including the *psbA-trnH*, *trnL-trnF* and *trnT-trnL* intergenic spacers, and the *rpl16* intron. Maximum parsimony analyses netted a well-supported phylogeny for *Pereskia*, which identified three major clades, these corresponding to pre-

sumed major migration routes from a central Andean origin to two centers of diversification: one in central and eastern Brazil, and the other in southern Central America and the Caribbean. Phylogenetic evidence for the transition from perigyny in the majority of *Pereskia* species to epigyny in *P. bleo*, *P. portulacifolia*, *P. ziniiflora* and *P. quisqueyana* was observed. These taxa constitute a strongly supported monophyletic clade, suggesting that receptacular epigyny has occurred only once in the genus. The sister group relationship for the yellow flowered species of *Pereskia* (*P. aureiflora* and *P. guamacho*) was also confirmed, despite their widely disjunct distribution, further suggesting that migration has played an important role in speciation events within this genus.

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What is the sister-group of the tribe Cactaceae?
(Cactaceae: Cactoideae)

Of the various tribes described for members of the cactus subfamily Cactoideae, the tribe Cactaceae is perhaps one of the most morphologically distinguishable. The members of this tribe include the barrel cacti of North America, containing such familiar genera as *Ferocactus*, *Astrophytum*, *Lophophora*, and *Mammillaria*. One of the major phylogenetic questions that remains for this group is the assessment of the sister-group relationship, if any, to other tribes of the subfamily. Based on general stem morphology, the barrel cacti of South America, tribe Notocactaceae (e.g. *Parodia*, *Frailea*, *Blossfeldia*, *Copiapoa*, and allied genera), have been implicated as the possible sister group to the Cactaceae. Comparative sequence studies of representative members of both of these tribes, in addition to representatives from all other tribes of the subfamily Cactoideae were used to assess intertribal relationships, and to examine the phylogenetic affinities of tribe Cactaceae. Parsimony analysis of sequence data from the plastid intron *rpl16* supports the monophyly of the tribe Cactaceae, places it as a distinct clade within the subfamily, and does not infer any direct relationship to members of the tribe Notocactaceae. The *rpl16* phylogeny suggests that the Cactaceae represent an early discrete divergence from the cactoid ancestor, with no apparent directly-related sister clades. Furthermore, the data support the distinction of the Atacama endemic genus *Copiapoa* as a discrete lineage from the 'core' members of tribe Notocactaceae. Additional sequence data are being accrued to evaluate phylogenetic information from other molecular markers and to examine the robustness of the relationships supported by the *rpl16* intron phylogeny.

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An expanded phylogenetic analysis of Orchidaceae using three plastid genes: rbcL, atpB, and psaB

The most comprehensive intergeneric phylogeny of Orchidaceae to date was published two years ago by Cameron et al. This was based on 171 *rbcL* sequences, and indicated that the family would be best divided into five subfamilies: (Apostasioideae (Cypripedioideae (Vanilloideae (Orchidoideae, Epidendroideae))). However, bootstrap support for the arrangement of these subclades was poor, and there was little resolution among tribes and subtribes within Epidendroideae, the most diverse group of orchids. More recently, phylogenetic hypotheses for the family have been published or presented using smaller sets of sequences from mitochondrial and nuclear loci. These mostly agree with the *rbcL* tree, but also suffer from a gen-

eral lack of resolution and/or internal clade support. To address these issues, two additional plastid loci (*atpB* and *psaB*) have been sequenced for the most of the same genera used in the *rbcL* study. Analysed separately, they differ only slightly from the *rbcL* tree. In combination, however, the tree is well resolved, and many lineages are supported by the bootstrap. Molecular data sets for Orchidaceae will soon be as numerous as those for other large families, and will allow for a new intrafamilial classification of the family based on well supported phylogenies within the near future.

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Alternative hypotheses for the systematic placement of Mayaca

Mayaca is a monogeneric family of aquatic herbs with approximately four species in subtropical to tropical America and west Africa. The reduced size and adaptation to the aquatic habit has made the placement of *Mayaca* within the commelinid clade difficult. In traditional systems of classification it has been suggested that this genus is related to Commelinaceae based on poricidal anther dehiscence and the operculate seeds, or to Xyridaceae based on parietal placentation and orthotropous, tenuinucellate ovules. However, subsequent data has shown that seed operculum development differs between *Mayaca* and Commelinaceae, and that Xyridaceae are variable in ovule development and orientation, as well as placentation. Despite this, *Mayaca* and Xyridaceae share an exothecium, a feature not known to occur in Commelinaceae. Recent analyses of *ndhF* and *rbcL* sequence data allied *Mayaca* to Bromeliaceae and Rapateaceae; the latter also have poricidal anther dehiscence. In our on-going analysis of *rbcL*, *atpA*, and *cob* sequence data representing all orders of monocots, *Mayaca* has been placed in a variety of positions, appearing either as sister to Eriocaulaceae or to Xyridaceae. These alternative hypotheses will be evaluated with respect to morphological data.

417 CATALAN, PILAR*, PEDRO TORRECILLA, AND JOSE ANGEL LOPEZ-RODRIGUEZ

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A reclassification of within-Poaceae groups based on molecular evidence

Phylogenetic analysis have been conducted on representatives of Poaceae groups tentatively placed within subtribes Cynosurinae, Dactylinae, Loliinae, Parapholiinae, Phalaridinae, Poinae, and Sesleriinae. Sequences from the nuclear ITS region and from the chloroplast *trnL-F* region have been analyzed either separately or combinedly to obtain a reconstruction of the evolutionary relationships between these predominantly Western Mediterranean lineages. The structures shown on the resulting topologies differentiate several suprageneric groups that could be reaccommodated within Poaeae s. lat. Some of the best supported clades represent the links of: 1) *Festuca* s.s. + *Vulpia* + *Wangenheimia* + *Ctenopsis*+ *Psilurus*; 2) *Schedonorus* + *Lolium*; 3) *Dactylis* + *Lamarckia*+ *Parapholis* + *Monerma* + *Catapodium* + *Sphenopus* + *Cynosurus*. *Leucopoa*, and *Drymanthele* + *Scariosa* + *Subbulbosae* could be resolved either separated or basal to *Schedonorus* + *Lolium*. *Sesleria* and *Poa* are respectively sister lineages to the other studied taxa. Conflicts between the nuclear and the chloroplast trees are likely due to past intergroup hybridization events. Evaluation of changes of structural characters on the molecular trees confirms their phylogenetic value at some hierarchical levels.

418 CERROS-TLATILPA, ROSA* AND J. TRAVIS COLUMBUS

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Phylogenetics of Aristida (Gramineae) based on internal transcribed spacer sequences (nrDNA)

Aristida is one of three genera in the grass tribe Aristideae along with *Sartidia* and *Stipagrostis*. Composed of ca. 330–360 species, *Aristida* is distributed in the tropics and subtropics, growing mostly on grassy plains, arid lands, and open ground. Studies of this taxonomically difficult genus have relied primarily on morphology, anatomy, and karyology. Taxonomic groupings have been based principally on inflorescence architecture and awn characters. Henrard (1926, 1929) recognized seven sections within the genus. Since Henrard's contributions little revisionary work has been done. Towards an improved understanding of the relationships and evolution of *Aristida*, we are gathering data from macromorphology, lemma micromorphology, leaf blade anatomy, and the nuclear (internal transcribed spacers; ITS) and chloroplast (*trnT-L-F* region) genomes. All of the sections of *Aristida* and as well as the related genera are being sampled and, as well, one or a few of the problematic species complexes in North America (e.g., the *A. purpurea* complex) are being targeted. The overarching goal of this research is to test the existing taxonomic circumscriptions and gain insight into character evolution, phyto-geography, and ecology of this widespread and diverse genus. Sequences from the ITS region of 34 New World species of *Aristida* have proven sufficiently variable for phylogenetic inference. Three of Henrard's sections are not monophyletic. However, some species complexes centered around *A. adscensionis* and *A. purpurea* are well supported by bootstrap analysis. Additional sampling of taxa from different sections of the genus *Aristida*, as well as *Sartidia*, and *Stipagrostis*, should provide much better resolution and confidence in the phylogenies and provide a better overview of the relationships within this group.

419 CHAN, RAYMUND*, BRUCE G. BALDWIN, AND ROBERT ORNDUFF

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Cryptic diversity in the goldfield Lasthenia californica sensu lato (Compositae: Heliantheae sensu lato)

Lasthenia sect. *Baeria*, one of six sections recognized by Ornduff (1966) in *Lasthenia*, includes the most common and geographically widespread goldfield species *L. californica*. Previous biosystematic studies using morphology, flavonoids, and isozymes have not revealed any unequivocal lineages within this morphologically and ecologically highly variable taxon. Maximum parsimony analysis of DNA sequence data from the internal and external transcribed spacers of 18S-26S nuclear ribosomal DNA, and the 3' end of the *matK* coding gene and adjacent 3' *trnK* intron of chloroplast DNA from over 60 populations of *L. californica* and close relatives yielded a well-supported tree showing that *L. sect. Baeria* is monophyletic only with the inclusion of *L. leptalea*, previously placed in *L. sect. Burrielia*. Most importantly, this tree also resolves *L. californica* to be a non-monophyletic group that represents two robustly supported clades. One clade includes *L. macrantha*; the other may be sister to *L. sect. Baeria*. Each of the two groups can be diagnosed by pappus morphology (although some individuals are epappose) and to some extent by geographic distribution. We have recognized these two clades of *L. californica* as distinct taxa. The molecular results also show that the overall diversification in the clade corresponding to *L. sect. Baeria* plus *L. leptalea* has been accompanied by minimal morphological diver-

gence, which has resulted in previously underappreciated cryptic diversity.

420 CHANDLER, GREGORY THOMAS^{1*}, MICHAEL DOUGLAS CRISP², AND RANDALL JAMES BAYER³

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Character evolution, toxicity, co-evolution, and relationships in the endemic Australian genus Gastrolobium (Fabaceae: Mirbelieae)

Gastrolobium (Fabaceae: Mirbelieae) is endemic throughout the south west of Western Australia, and is important both ecologically and economically. It forms a large and important part of the understorey of different ecosystems, from forest and woodland to heathland and rock outcrops. It also produces sodium monofluoroacetate, a highly toxic compound to all animals. This has led to large stock losses over time, and also to a program of eradication in many farmland areas. *Gastrolobium* has had an unstable taxonomy, and was recently expanded to include three other genera, *Brachysema*, *Jansonia* and *Nemcia*, based on two molecular phylogenetic studies. This leads us to examine and question evolutionary traits within this group, as many previously important morphological characters have been shown to be homoplastic. These include inflorescence structure, pollination syndrome, ovule number, and fluoroacetate production, which, at some point, have all been used to help define the generic limits of the genera in this group. In general, fluoroacetate levels are reduced in the more derived groups of *Gastrolobium*, and are presumed to be absent in the derived, putatively bird-pollinated lineage, which includes *Brachysema*, *Jansonia* and some species of *Nemcia*, and are highest towards the more basal, putatively bee-pollinated lineages. This provides us with a unique look into the evolution of several traits in the *Gastrolobium* group, particularly toxicity and pollination syndrome, and the apparent tradeoff that occurs between these two traits, and allows the exploration of the co-evolution of *Gastrolobium* and the development of fluoroacetate tolerance in native animals.

421 CHASE, MARK W.^{1*}, ANTONY V. COX¹, SANDY KNAPP², JEFFREY JOSEPHS¹, AND ALEX S. PAROKONNY^{3,4}

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Molecular systematics, GISH, and the origin of amphidiploids in Nicotiana (Solanaceae)

Phylogenetic relationships in the genus *Nicotiana* were investigated using parsimony analyses of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (nrDNA) and compared to previously published results from the plastid *matK* gene. In addition, origins of amphidiploid taxa in *Nicotiana* were investigated using the techniques of genomic in situ hybridization (GISH). All taxa regardless of ploidy produced clean single copies of the ITS region, even though some taxa are natural hybrids and others are artificial hybrids (allopolyploids). The patterns of relationships in *Nicotiana* are largely congruent with previous evolutionary ideas of Goodspeed, who studied their morphology and cytology, but some important differences are apparent. None of the subgenera of *Nicotiana* is monophyletic, and although most of the currently recognized sections are coherent, others are clearly polyphyletic. Relying solely upon ITS nrDNA and *mat*

Kanalysis to reveal phylogenetic patterns in a complex genus such as *Nicotianais* insufficient, and it is clear that conventional analysis of single data sets, such as ITS, is likely to be misleading in at least some respects about evolutionary history. ITS sequences of artificial as well as natural and well-documented amphidiploids are similar to one of their two parents, usually the maternal (but not always) and not in any sense themselves "hybrid". Knowing how ITS evolves in artificial amphidiploids gives insight into what the ITS analysis might reveal about naturally occurring amphidiploids for which little of their origin is known, and it is in this perspective that analysis of ITS sequences is highly informative.

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Phylogeny of extant Cycadales inferred from chloroplast matK gene

Cycadales, the cycad order, has approximately 185 extant species in eleven genera and three families (Cycadaceae, Stangeriaceae and Zamiaceae) (Jones 1993). Cycads are confined to the tropical and subtropical regions of both hemispheres. They were shown to be the basal-most clade of living gymnosperms based on multiple gene analyses (Chaw et al. 2000; Bowe et al. 2000), but relationship of their families and genera are not completely known. In this report chloroplast matK gene is used to infer the phylogeny among the genera and higher taxa of cycads. Ginkgo is employed as the outgroup. Analysis using either the non-synonymous sites or the first two position of matK gene identifies *Cycas* as the basal-most group of cycads, *Stangeria* as the next basal, and the rest genera form a well supported group, in which three subfamilies, Bowenioideae, Encephalartoideae, and Zamioideae as defined by Stevenson (1992), are each resolved as monophyletic. Traditionally the latter two subfamilies constitute the family Zamaiaceae, which, however, is not sustained by the matK data. In contrast to morphological and cytological studies, our data do not support inclusion of *Bowenia* in the family Stangeriaceae. In addition, *Lepidozamia* is shown to be sister to *Encephalartos* rather than to *Macrozamia*. The nonsynonymous sites of matK gene in cycads (except *Lepidozamia*) have evolved in a relatively clock-like manner.

423 CIMINO, M. T.*, L. M. BRINKAC, M. E. HOPKINS, N. A. GROSS, AND R. A. BEVER

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Molecular systematic analysis of botanical trace evidence

Molecular analysis of trace botanical evidence is a developing forensic discipline. Traditionally, the morphology of plant debris has been examined for useful forensic data. The composition of a trace evidence sample may provide leads as to the geographic location or general environmental conditions of the sample origin. However, the identification of forensic botanicals is often confounded by the lack of morphological characters. The use of genetic markers has the potential to overcome shortcomings associated with traditional botanical forensics. Depending on the forensic sample's composition, a single DNA sequence or numerous DNA sequences can be determined from a single collection of trace evidence. Using sequences in public databases, taxonomic placement of the sequences determined from the forensic material is often possible. The distribution and ecology of the identified taxa can then be used to infer information about the geographic origin of the forensic evidence.

424 CIOFANI, KRISTEN, LYDIA MIRAMONTES, AND RICHARD JENSEN*

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Morphometric analyses of red maple (Acer rubrum L.), silver maple (Acer saccharinum L.), and their hybrid (Acer X freemanii E. Murray)

The phenomenon of morphological intermediacy often can be used to identify individuals of hybrid origin. However, morphological intermediacy is not always apparent in individual characters. Our experience is that multivariate analyses are very likely to reveal hybridity because the combinations of characters in the hybrids disrupt the patterns of covariance that allow recognition of the parental taxa. For this study, we sampled six trees (two each from three accessions) of *Acer X freemanii* from the Morton Arboretum, along with 40 trees from northern Indiana field identified as either *A. rubrum* or *A. saccharinum*. Leaves were pressed and dried and leaf blade outline and landmark data were captured using MorphoSys. A data set of linear and angular characters was used for input to cluster analysis and principal components analysis. The same multivariate tools were used to analyze elliptic Fourier coefficients derived from leaf outlines and thin-plate spline weights derived from landmark configurations. All three approaches provide views in which some or all of the hybrids are intermediate between the parental taxa and in which some field-collected trees also appear to be of hybrid origin. But, there are differences with respect to which trees may be of hybrid origin. These differences reflect the different aspects of leaf blade shape derived from each set of data.

425 CLARK, JOHN L.

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Preliminary phylogeny of Alloplectus (Gesneriaceae) based on morphology and ITS sequence data

The genus *Alloplectus* (Gesneriaceae) ranges from Central America to Peru with a few species in the Lesser Antilles, but is most diverse in western Ecuador and Colombia. Members of this genus are herbaceous perennials that are an important component of the under story vegetation in lowland and transitional forests throughout the northern Andes, Central America, and lowlands of northwestern South America. The last detailed treatment of *Alloplectus* was in 1865 and current studies have been limited to species descriptions. Estimates of total diversity in *Alloplectus* range from 35 to 70 species. A preliminary molecular analysis utilizing ITS indicates that *Alloplectus* as currently circumscribed is not monophyletic. The currently recognized species of *Alloplectus* are resolved in two distinct clades. One clade of *Alloplectus* species is resolved as sister-group to *Columnnea* and is characterized by the presence of resupinate flowers. The other clade of *Alloplectus* species is non-resupinate and groups with *Drymonia*. Resupination of flowers is an important morphological synapomorphy for *Alloplectus* sensu stricto that has not been previously recognized. The non-resupinate species of *Alloplectus* are most closely related to *Drymonia*, but taxon sampling within *Drymonia* is insufficient at present to determine whether they should be included in *Drymonia* or treated as a new genus.

426 CLINEBELL II, RICHARD R. AND PETER C. HOCH*

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Pollination of Gaura villosa (Onagraceae) by antlions (Neuroptera)

The genus *Gaura* (Onagraceae) comprises 21 species native to North America, ranging from central and southwestern United States to Mexico and Guatemala, centering on Texas. Five species are self-pollinating, and of the 16 outcrossing species, 13 are reported to be pollinated by small moths, one by hawk-moths, and two by bees, butterflies and flies. As part of an analysis of the evolution of pollinating mechanisms in the genus, we studied *G. villosa*, a species characteristic of sandy soils, at the Monahans Sandhills and nearby sites in western Texas, with repeated visits throughout the daily and seasonal flowering cycle. The pattern that emerged was unexpectedly complex: a total of 505 individuals of at least 34 species of insects were collected while visiting the flowers, including beetles, flies, bees, butterflies, moths, and antlions. Five species were overwhelmingly important as floral visitors and pollen vectors: two species of halictid bees (*Sphécodogastra*), two noctuid moths (species of *Bulia* and *Melipotis*), and the antlion *Scotoleon minusculus*. At least four other species of antlions visit the flowers and carry pollen; all visits by antlions occurred between midnight and 5:00am, which may explain why this phenomenon has not been reported before. This is the first documentation of pollination by antlions, and may be the first for any member of the insect order Neuroptera.

427 COLUMBUS, J. TRAVIS* AND ERIC H. ROALSON

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Phylogenetics of Hilaria and Pleuraphis (Gramineae: Chloridoideae)

Cladistic parsimony analysis of internal transcribed spacer (ITS) region nrDNA sequences indicates a sister relationship between the North American chloridoid grass genera *Hilaria* (8 or 9 spp.) and *Pleuraphis* (3 spp.). Introgression between *P. jameisii* and *P. rigida*, previously inferred from morphology, is evidenced in the ITS phylogeny. *Hilaria* is composed of three main lineages: (A) *H. semplei*, (B) *H. belangeri*, *H. cenchroides*, and *H. swallenii*, and (C) *H. annua*, *H. ciliata*, *H. hintonii*, and one, possibly two, undescribed species. Characters from morphology and leaf blade transectional anatomy support the *Hilaria* and *Pleuraphis* clades and the three lineages within *Hilaria*. However, species circumscription within *Hilaria* clade B remains uncertain. Much variation exists in the leaf blade structure of these genera. *Pleuraphis* possesses a suite of anatomical and cytological features that suggests the NAD malic enzyme (NAD-ME) photosynthetic pathway. *Hilaria* species in clade C exhibit a blade structure indicative of the PEP carboxykinase (PCK) pathway, whereas *H. semplei* and species in clade B are intermediate between clade C and *Pleuraphis* with respect to the anatomical/cytological characters that predict the subtype of C₄ photosynthesis.

428 CONSAUL, LAURIE L.* AND LYNN J. GILLESPIE

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Preliminary morphological and molecular analyses on alkali grasses (Puccinellia, Poaceae) in the Canadian arctic archipelago

Alkali grasses (*Puccinellia*, Poaceae) have traditionally posed problems for identification, and the species in the Canadian Arctic archipelago (CAA) are no exception. Although there is

considerable morphological variation within the genus, circumscription of taxa is problematical because of overlap of character states and many apparently intermediate specimens. While two species are quite distinct, nine other traditionally recognized species of the CAA have poorly defined species boundaries. The aim of this study was to define the taxonomic limits of species or species groups based on morphological data. In the first part of the investigation, we redefined the character states of several floret and spikelet characters that had been difficult to interpret in previous keys and descriptions. The second part of the study involved analyses on a morphological character set that included these revised characters plus other characters previously shown to have low plasticity in this genus. These analyses showed that *P. vahliana* was distinct based in part on glume lengths, *P. phryganodes* was a distinct stoloniferous species, and *P. langeana* was readily distinguished by its small size and lack of hairs. The remaining species were less clearly distinguished in the ordination analyses performed. The *P. arctica* aggregate separated from the other taxa, but there was no resolution of species within this group. Hypotheses of species boundaries in the arctic taxa, based on current evidence, are presented. Our present aim is to test these hypotheses with more detailed morphological, anatomical, and molecular analyses. Initial screening of the above taxa for 16 enzymes revealed very low levels of allozyme variation within the genus, with potential markers for *P. langeana* and *P. vahliana* in one enzyme system. Restriction site analysis of PCR amplified DNA of eight species showed only minor variation for *P. vahliana* and *P. phryganodes*, and none for six other species analyzed.

429 COSKUN, FATIH* AND CLIFFORD R. PARKS

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Biogeography and phylogeny of the genus Osmanthus (Oleaceae): evidence from nuclear ribosomal ITS DNA Sequences

The classic biogeographic disjunct pattern, known as "the Tertiary relict disjunction", has been of interest to botanists for a long time, since the time of Linnaeus. The genus *Osmanthus*, with its more than 25 species, demonstrates this classic disjunct pattern, with a majority of the species of this genus represented in eastern Asia, and the others in northern Turkey and in south eastern United States. Green (1958) examined this genus thoroughly in his monograph primarily based on morphological evidence. In our study, we conducted a phylogenetic analysis to infer relationships among the species of *Osmanthus* by using nuclear ribosomal ITS DNA sequences. We sampled taxa from all the sections recognized by Green (1958). After constructing the phylogeny of the genus based on ITS DNA sequences, we compared current distribution of the genus with inference from the DNA molecules and generated biogeographic patterns for *Osmanthus* species.

430 COSKUN, FATIH*¹, JIANHUA LI², AND CLIFFORD R. PARKS¹¹Dept. of Biology, CB# 3280, Coker Hall, UNC-Chapel Hill, NC 27599-3280; ²Arnold Arboretum of Harvard University, 125 Arborway, Jamaica Plain, MA 02130*Molecular systematics and biogeography of the genus Tilia (Tiliaceae)*

The similarity between the forests of eastern North America and eastern Asia has intrigued botanists since the time of Linnaeus. However, scientific attention has been directed onto this classic disjunction pattern, now known as "the Tertiary relict disjunction", after Gray's 1859 treatment. Showing this classic

pattern, the genus *Tilia*, the limes or lindens, includes more than 45 species in the Northern Temperate zone with a majority of the species in eastern Asia. Since the publication of the first monograph of this genus by Ventenat in 1803, many articles have discussed the relationships of *Tilia* species. These have been primarily based on morphological evidence. In this study, an effort is made to understand the relationships among the species of this genus using nuclear ribosomal ITS DNA sequences. Taxa are sampled from all the sections and subsections of the genus based on Engler's (1909) treatment, with representatives from North America, Europe, and western, central and eastern Asia. Phylogenetic trees are constructed based on the ITS sequences; and finally, phylogeographic patterns are generated for this genus and interpreted in the light of the fossil record.

431 COSTELLO, ANNEMARIE^{1,2*} AND TIMOTHY J. MOTLEY²

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Systematics of the Tetraplasandra group (Araliaceae) using molecular and morphological data

Tetraplasandra is part of a generic complex that includes *Munroidendron*, *Reynoldsia*, and *Gastonia*. Phylogenetic relationships and generic delimitations of the *Tetraplasandra* group have been historically problematic. Our previous molecular analyses based on independent and combined ITS and 5S-NTS data sets revealed that the Hawaiian taxa of the *Tetraplasandra* group (*Tetraplasandra*, *Munroidendron*, and *Reynoldsia sandwicensis*) are monophyletic and *Reynoldsia* is paraphyletic. In *Tetraplasandra*, superior ovaries are derived from inferior ovaries. The shift to superior ovaries in *Tetraplasandra* is associated with a reduction in floral parts and may be related to a change in pollination syndrome. A cladistic analysis of the *Tetraplasandra* group was generated based on floral and fruit morphology and was combined with the ITS and 5S-NTS data. Our results suggest that plesiomorphic floral characters of the family, along with superior ovaries, are derived characters within the *Tetraplasandra* group.

432 CRAWFORD, DANIEL J.* , REBECCA T. KIMBALL, AND MESFIN TADESSE

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Relationships in subtribe Coreopsidinae (Asteraceae: Heliantheae): insights from ITS sequences

Amolecular phylogenetic study of subtribe Coreopsidinae was conducted using ITS sequences from 18 genera traditionally placed in the subtribe. The two largest genera *Bidens* and *Coreopsis* do not appear monophyletic, and occur instead in several strongly supported lineages. The small genus *Coreocarpus* is resolved into three distinct lineages. Although based on somewhat limited taxon sampling, both *Cosmos* and *Dahlia* occur as monophyletic groups, with *Dahlia* a basal lineage within the subtribe. The ITS phylogeny does not support the inclusion of the two island genera *Petrobium* and *Oparanthus* in *Bidens*, as has been suggested. Strong support is provided for inclusion of the insular genus *Fitchia* in Coreopsidinae, with *Oparanthus* appearing sister to it. Prior investigations of the subtribe have included grouping genera to reflect relationships and cladistic analyses of morphological characters; certain results of the present study are concordant with previous studies whereas in other instances sharply different relationships are portrayed. The molecular phylogeny resolves a number of strongly supported lineages within Coreopsidinae, but there is not always clear resolution of relationships among the lineages.

433 CRAWFORD, PHILLIP T.* AND WAYNE J. ELISENS

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Systematics of native North American Linaria (Scrophulariaceae)

The native North American toadflaxes (*Linaria* section *Leptoplectron*) comprise a morphologically-variable complex of taxa that have been collectively recognized as a distinct genus (*Nuttallanthus* D. Sutton) and individually assigned to specific and varietal rank. *Linaria canadensis* and *L. texana* are locally abundant in sandy soils throughout much of temperate North America; *L. floridana* is confined primarily to Atlantic and Gulf coastal areas where all three taxa occur occasionally in dense, mixed populations. These plants produce cleistogamous flowers early in the growing season; showy, fragrant chasmogamous flowers appear later and attract numerous Lepidopteran and Hemipteran visitors. Chromosome counts reveal no variation in ploidy level within the complex. Field and greenhouse breeding-system studies indicate high levels of self-fertilization: all individuals studied were entirely self-compatible and many "chasmogamous" flowers selfed prior to anthesis; pollen is required for seed production. While hand-performed cross pollinations between plants within each taxonomic group resulted in reduced seed set (possibly due to limited pollen transfer), no intertaxon hand-pollinations produced viable seeds. Field tests conducted in several populations of *L. texana* involved five treatments: open pollination, pollinator exclusion, floral emasculation and pollinator exclusion, emasculation and open-pollination, and hand/self-pollination of emasculated flowers. These tests indicate *L. texana* is facultatively xenogamous; while many potential pollinators were observed visiting successive flowers, successful pollen transfer and seed set was rare. These results are consistent with an analysis of isozyme variation, which revealed low levels of observed heterozygosity and intrapopulation genetic polymorphism throughout the complex. Analyses of morphological and genetic variation within and among 50 populations of *Linaria canadensis*, *L. texana* and *L. floridana* yield broadly consistent results and suggest that these taxa should be recognized as distinct species. Phenetic analyses identify numerous quantitative and qualitative morphological differences among these taxa; isozyme data indicate that these taxa are reproductively isolated in areas of sympatry.

434 CROSS, HUGH B.^{1,2*}, RAFAEL LIRA SAADE³, ISELA RODRIGUEZ AREVALO³, AND TIMOTHY J. MOTLEY¹

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Molecular phylogenetics of the tribe Sicyeae (Cucurbitaceae)

The tribe Sicyeae (Cucurbitaceae) is a large group of vigorously climbing vines found mostly in the New World tropics, and is composed of two subtribes, the Sicyinae and Cyclantherinae. The Sicyinae are characterized by single seeded ovules and include the important vegetable crop chayote (*Sechium edule*). Recent morphological studies have identified major relationships within the tribe. However, the group has not yet been subjected to molecular analysis. In this study the phylogenetic relationships of the tribe Sicyeae were investigated using sequences of the ITS nuclear ribosomal and the chloroplast *trnL-F* regions (spacer

+ intron). All genera in the tribe were sampled; the genus *Cucurbita* was used as the outgroup. Nucleotide sequences of each region were analyzed independently and in combination using parsimony. Sequence data from the ITS region were informative at the species level, and could distinguish between wild and domesticated *Sechium edule*. The *trnL-F* sequence data was informative principally at the generic level. *Rytidostylis*, and *Cyclanthera* were found to be monophyletic genera. The genera *Sicyos* and *Sechium* appear to be paraphyletic. Additionally, sectional relationships within *Sechium* do not seem to conform to earlier hypotheses based solely on morphology. Within *Sechium*, the Central American species form a well-defined monophyletic clade, and include members of both sections *Sechium* and *Frantzia*. The single species of section *Frantzia* in Mexico, *Sechium mexicanum*, appears to be more closely related to the genera *Sicyos* and *Sechiopsis*.

435 DAVIS, CHARLES C.

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Madagasikaria (*Malpighiaceae*): an exciting new discovery from Madagascar, with important implications for floral evolution within *Malpighiaceae*

The *Malpighiaceae* comprise approximately 1250 species in 65 genera, with approximately 100 species belonging to the 15 Old World genera. Of the 100 species distributed across the Old World, 71 of them are endemic to Madagascar and belong to nine genera. These Malagasy taxa represent most of the major phylogenetic lineages within the family and are represented in as few as six and as many as nine independent clades. On a recent expedition to Madagascar I discovered a new genus and species of *Malpighiaceae*. *Madagasikaria* has a schizocarpic fruit with distinctive winged mericarps that possess a lateral wing, which completely encircles the nut, and a peculiar dorsal wing, which is folded over on itself. On the basis of *ndhF* and *trnL-F* chloroplast data, *Madagasikaria* is closely related to the Malagasy endemic genera *Rhynchophora* and *Microsteira*. These plants all appear to be functionally dioecious, and some of these appear morphologically to be androdioecious (which is a very rare breeding system in flowering plants). This has important implications for floral evolution within the family. Neotropical *Malpighiaceae* are pollinated by specialized oil-collecting bees and consequently exhibit highly conserved floral morphology despite tremendous diversity in fruit morphology and habit. These oil-collecting bees are absent from the paleotropics, where lineages lack the oil glands and typical flower orientation crucial to pollination by neotropical oil collecting bees. Based on my molecular systematic studies of *Malpighiaceae* the shift from the neotropical pollination syndrome has apparently taken place a number of times independently.

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Phylogeny of Malpighiaceae: evidence from chloroplast ndhF and trnL-F nucleotide sequences

The *Malpighiaceae* are a family of approximately 1250 species of predominantly New World tropical flowering plants. Intrafamilial classification has long been based on fruit characters. Phylogenetic analyses of chloroplast DNA nucleotide sequences were analyzed to help resolve the phylogeny of *Malpighiaceae*. A total of 79 species, representing 58 of the 65

currently recognized genera, were studied. The 3' region of the gene *ndhF* was sequenced for 77 species and the noncoding intergenic spacer region *trnL-F* was sequenced for 65 species; both sequences were obtained for the outgroup, *Humiria* (*Humiriaceae*). Phylogenetic relationships inferred from these data sets are largely congruent with one another and with results from combined analyses. The family is divided into two major clades, recognized here as the subfamilies *Byrsonimoideae* (New World only) and *Malpighioideae* (New World and Old World). Niedenzu's tribes are all polyphyletic, suggesting extensive convergence on similar fruit types; only Jussieu's tribe *Gaudichaudieae* and Anderson's tribes *Acmanthereae* and *Galphimieae* are monophyletic. Fleshy fruits evolved three times in the family and bristly fruits at least three times. Among the wing-fruited vines, which constitute more than half the diversity in the family, genera with dorsal-winged samaras are fairly well resolved, while the resolution of taxa with lateral-winged samaras is poor. The trees suggest a shift from radially symmetrical pollen arrangement to globally symmetrical pollen at the base of one of the clades within the *Malpighioideae*. The Old World taxa fall into at least six and as many as nine clades.

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Phylogenetic relationships among the monocots, as inferred from morphology and nucleotide sequence variation in three genes

A phylogenetic analysis of the monocotyledons was conducted, using four character sets (morphology plus nucleotide sequences of the plastid-encoded gene *rbcl* and the mitochondrion-encoded genes *atpA* and *cob*). The taxon set comprises 58 dicot and gymnosperm outgroups, and 155 extant monocots, representing all major lineages of the monocots. The deepest branch within the monocots is between a clade that includes *Acorus* and all sampled elements of *Alismatanae* and *Aranae*, and a second clade that includes all other monocots. Within the latter group there are four lineages of *Lilioids*, plus a *Commelinid* lineage that includes all remaining monocots. Within the *Commelinids*, *Arecales* are sister of a clade that includes all other members of the group. Morphological characters that contribute substantially to the overall phylogenetic structure include presence/absence of oxalate raphides, vessel elements in leaves, and cell wall ferulates, plus variation in phyllotaxis, leaf venation, stomatal type, microsporogenesis, placentation, endosperm storage constituents, embryo form, and modification of the first tepal as a bracteole.

438 DELPRETE, PIERO G.^{1*} AND TIMOTHY J. MOTLEY²

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Molecular systematics of the Chiococceae-Catesbaeeae Complex (Rubiaceae): flower and fruit evolution and systematic implications

Fruit features have historically been used as one of the cardinal characters for subfamilial, tribal and generic classification in the family *Rubiaceae*. Recent phylogenetic studies using *rbcl*, *trnL-F*, *rps16* and ITS1&2 and detailed palynological analyses have shown that the *Catesbaeeae-Chiococceae* Complex (CCC) is a group that includes approximately 27 genera and 196 species primarily concentrated in the Greater Antilles (nearly 70% of the taxa),

Central and South America, and with 3 genera occurring in Malesia and the South Pacific. These molecular analyses showed that utility of fruit characters must be carefully reconsidered, and demonstrated that fleshiness of the mesocarp, placentation, ovule number and insertion are variable within monophyletic groups. Based on these phylogenies, it was possible to detect pattern of flower and fruit evolution within this group. All the analyses clearly indicate that the ancestral fruit type of the CCC is a multi-seeded capsule, from which were apparently derived leathery berries and drupaceous fruits in several, separate, evolutionary events. In addition, the molecular phylogenies prompted a reevaluation of the generic delimitations within the CCC, which will be used for the ongoing monographic treatment of this group.

439 DEVORE, MELANIE L.^{1*}, JOHN J. SKVARLA², ZAIMING ZHAO³, AND ROBERT K. JANSEN³

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Comparisons of Tubuliforidites and pollen types within Asteraceae and related families: how do you recognize Asteraceae in the fossil record?

A number of dispersed Cretaceous and Tertiary pollen grains with exine morphologies suggestive of Asteraceae have been assigned to the form genus *Tubuliforidites*. Zavada and de Villiers (2000) carefully examined the ultrastructure of two species of *Tubuliforidites* with Anthemoid type morphology from the Paleocene-Eocene of South Africa. The fossil grains, as well as Calyceraceae, Barnadesioideae, and Mutisieae, have a reticulate wall layer. Unlike *Tubuliforidites*, members of Barnadesioideae have thick columellae that extend through the exine. In Mutisieae a distinct intertectum is present. Like Barnadesioideae, some members of Mutisieae have basal columellae that extend through the exine. The sister group to Asteraceae, Calyceraceae, and a second family Valerianaceae, exhibit wall structure types most similar to that of *Tubuliforidites*. It is possible that the reticulate layer in Barnadesioideae and Mutisieae is not homologous to the condition found in Calyceraceae, Valerianaceae, and *Tubuliforidites*. Grains of Calyceraceae, Valerianaceae, and *Tubuliforidites* appear to have a reticulate exine layer that is derived from divisions of the upper portion of columellae. In considering both wall structure and phylogenies of Asteraceae and closely related families, it appears that the Anthemoid pattern has evolved multiple times both within Asteraceae and in related families. The presence of this pattern in fossil grains is not a good character to use to recognize Asteraceae in the fossil record. The challenge for the paleopalynologist interpreting the ultrastructure of grains believed to have affinities with Asteraceae is to determine if the reticulate layers are not derived from distal divisions of the columellae. Also, the presence of columellae extending through the wall would also suggest an affinity to Asteraceae.

440 DICKINSON, TIMOTHY A.

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What IS Suksdorf's hawthorn?

In 1907 Sargent published his *Crataegus douglasii* var. *suksdorfii*, based on *Crataegus* collections sent him by Wilhelm Suksdorf. Suksdorf had noted variation in the number of stamens in the black-fruited specimens and Sargent replied that he was surprised to learn of a 10-stamen form with black fruit. Sargent

then ascertained that the type of Lindley's *C. douglasii* (grown from seed collected by David Douglas in the Columbia River basin) in fact corresponded to Suksdorf's 10-stamen form, and named the new 20-stamen variety in Suksdorf's honor. Although Sargent referred to *C. punctata* var. *brevispina* Douglas (published by Hooker in 1832) in his treatment of *C. douglasii* for *Sylva of North America* (1892) he apparently neglected Hooker's comment that Douglas' specimens comprised two varieties. Moreover, in his 1907 paper he mentioned that Heller had published *C. gaylussacia* based on black-fruited specimens from California. What then is Sargent's *C. suksdorfii*? Recent opinion has recognized only Lindley's and Sargent's taxa, contrasting them on the basis of correlated variation in leaf shape, thorn size, and floral architecture. In seeking to understand the variation in floral architecture within these black-fruited hawthorns I have attempted to resolve what names should be applied to what phenotypes. Suksdorf provided collections of southern Washington plants to many herbaria, so that there are several specimens available from the individuals referred to in the protologue for var. *suksdorfii*. Leaf, thorn, and floral measurements, including Fourier analyses of leaf outlines, reveal that this type material is distinct from both varieties collected by Douglas, leading to a proposal for restricting the application of Sargent's name, and resurrecting one or both of the names proposed by Douglas and Heller to account for the phenotypes associated with *C. suksdorfii* up to now.

441 DILLON, MICHAEL O.

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Biogeography and diversity of the Solanaceae in the lomas formations of Chile and Peru

The western coast of South America [5-30°S lat] is dominated by desert conditions that form a continuous, hyper-arid belt, broken only by occasional river valleys. The native, non-riparian vegetation of these deserts is largely confined to localities where recurring fogs meet the near shore terrain. The fogs supply moisture for the development of unique plant communities termed lomas formations. Over 100 of these communities have been identified and they exist as terrestrial islands within the ocean of arid habitat ranging over 3500 kms from northern Peru to north-central Chile. Endemism reaches over 40% at some localities. The combined vascular flora of the lomas formations contains over 1400 species and the Solanaceae is represented by 19 genera and 129 species arrayed in five subfamilies: Cestroioideae, Nicotianoideae, Petunioideae, Schizanthoideae, and Solanoideae. *Lomas* endemics are found in the following 11 genera: *Exodeconus*, *Grabowskia*, *Leptoglossis*, *Lycopersicon*, *Lycium*, *Nolana*, *Nicotiana*, *Reyesia*, *Salpiglossis*, *Schizanthus* and *Solanum*. *Nolana*, with 71 endemic lomas species, stands out as the largest and most wide-ranging genus of the lomas flora and the only one to be encountered in nearly all lomas formations. A suite of *Nolana* species have been investigated using ITS and *matK* sequence data to reconstruct phylogenies and these have yielded conflicting results. Morphological and physiological specializations that have allowed species diversification were examined in relation to suggested phylogenies. The biogeographic patterns within the Solanaceae lomas endemics reflect different arrival times and origins for its members. Short-term climatic fluctuations, such as El Niño events, and longer-term climatic change associated with glacial cycles, are believed to have been influential in expansions and contractions in the flora of Andean Cordillera and the coastal deserts and have helped shape present-day distributional patterns.

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Dissecting an extensive polyploid complex in Glycine subgenus Glycine (Leguminosae) using low copy nuclear genes, nuclear ribosomal genes, and chloroplast sequences

Of the two major polyploid complexes in *Glycine* subgenus *Glycine*, the *G. tomentella* complex is the largest. *G. tomentella* is polymorphic-and polyphyletic-at the diploid level, with several clades well-defined in gene trees using 18S-26S nuclear ribosomal gene (nrDNA) ITS and histone H3-D sequences. Polyploids also called *G. tomentella* combine genomes of these and other *Glycine* taxa. The diversity of the *G. tomentella* complex includes one diploid genome that is also a donor for an allotraploid race of the *G. tabacina* complex, the other major polyploid complex of the subgenus. This means that, in effect, a single large complex accounts for most neopolyploidy in *Glycine*. Some of the combinations hypothesized from DNA sequence data were predicted based on other data, such as isozymes and 5S rRNA gene repeat sizes, but other polyploid genome constitutions were unexpected, and indicate that there is more complexity in the group than was suspected. In most polyploid races, both of the predicted homoeologous nrDNA repeat classes are present but often in very unequal amounts. Polymorphism for chloroplast sequences in accessions from some *G. tomentella* allopolyploids suggests that in some cases both diploid progenitors may have served as maternal parents in the original cross that led to a given allopolyploid. This is in marked contrast to the *G. tabacina* complex, where crosses appear always to have involved the same taxa as chloroplast genome donors even when those crosses occurred several times.

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Phylogenetics of Paniceae (Poaceae)

The phylogenetics of Poaceae is generally well-studied with the exception of the large and variable Paniceae within which intergeneric relationships are uncertain. Paniceae demonstrate unique variability of photosynthetic physiology and anatomy including both non-kranz and kranz species and all subtypes of the latter. This variability suggests both hypotheses of independent origin and reversals (e.g. from kranz to non-kranz). These hypotheses can be tested by phylogenetic analysis of independent molecular characters. The molecular phylogeny of 60 species of Paniceae was explored using sequences from the grass-specific insert found in the plastid locus *rpoC2*. Phylogenetic analyses confirmed some long recognized alliances in Paniceae, some recent molecular phylogenetic results, and suggested new relationships as well. Broadly, Paniceae were found to be paraphyletic with Andropogoneae, *Panicum* was found to be polyphyletic, and *Oplismenus hirtellus* was resolved as the sister group to the remaining ingroup species. A particularly well supported clade in the *rpoC2* tree included four genera with non-kranz species and three with distinctively keeled paleas. As previously suggested, the PEP-ck subtype arose once within Paniceae. All clades with non-kranz species had kranz ancestors or sister taxa suggesting repeated loss of the kranz syndrome. Other correlations between photosynthetic physiology and molecular phylogeny await further study.

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Some observations on the reproductive morphology of the wahlenbergioid genera of the family Campanulaceae s.str. from the fynbos vegetation of South Africa

The morphology of the capsule, particularly the mode of dehiscence has traditionally been an important character for the classification of the Campanulaceae s. str., of which there are approximately ten endemic genera recognised in the South African region. Most of these are confined to the fynbos vegetation of the Western Cape Province. It is hypothesised that the edaphic conditions of the Western Cape, combined with very rugged topography and selection for highly specialised pollinators has contributed significantly to local speciation events and ultimately the evolution of these unique genera.

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Reconstruction of the phylogeny of the Campanulaceae s.str. using ITS sequences of nuclear ribosomal DNA

The phylogeny of the Campanulaceae as inferred from ITS sequences of almost 100 taxa comprising the majority of genera reveals a division of the family into two major clades. The basal clade consists of tropical and Asian taxa of the platycodonoid alliance. The other clade consists of wahlenbergioid taxa of the southern hemisphere and campanuloid taxa of the northern hemisphere. These results are consistent with pollen data and are remarkably congruent with data from genome rearrangements and sequences from several chloroplast genes. The topology of the campanuloid clade suggests that large genera such as Campanula are not monophyletic.

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Phylogenetic insights into the Maloideae (Rosaceae) from the granule-bound starch synthase I (GBSSI) gene

The nuclear GBSSI gene is duplicated in all Rosaceae, and there is a second duplication in all members of subfamily Maloideae, including three genera with dry fruits that have not traditionally been included in the subfamily, *Kageneckia*, *Lindleya*, and *Vauquelinia*. We obtained sequence for about 1800 nucleotide sites, including seven complete exons and eight introns (only seven in two of the four copies of the gene) plus parts of the first and ninth exons at the 5' end of the gene. In a sample that includes all but two of the 32 genera in the family, we have sequences for all four copies of the gene for 14 genera, for three copies for 6 genera, for two copies for 9 genera, and for just one copy for *Pseudocycdonia*. That we are readily able to align introns within each copy of the gene is compatible with the low sequence divergence in this gene (about 2 to 5% between Maloideae genera other than *Kageneckia*, *Lindleya*, and *Vauquelinia*) and in other molecular data. Analysis of GBSSI sequence data for our sample

plus the sister group of Maloideae (*Porteranthus*) supports a sister-group relationship between *Kageneckia*, *Lindleya*, plus *Vauquelinia* and the remainder (core) of the Maloideae. We therefore use these three genera as outgroups for analyses of core Maloideae. The data give strong support for monophyly of core Maloideae and varying levels of support for several groups of two to three genera, namely *Amelanchier*, *Malacomeles*, plus *Peraphyllum*; *Aria* plus *Chamaemespilus*; *Cotoneaster* plus *Malus*; and *Dichotomanthes* plus *Photinia*. Short branch lengths characterize much of the most parsimonious trees, suggesting rapid diversification and frustrating our efforts to understand higher-level relationships in the subfamily.

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Systematic affinities of two enigmatic African families, Grubbiaceae and Hydrostachyaceae evidence from nuclear 26S rDNA sequence data

Various chloroplast DNA data suggested Cornales including two enigmatic monotypic African families Grubbiaceae and Hydrostachyaceae. Forty species representing all major groups of Cornales, including two of three species Grubbia, and four samples representing three of 22 species Hydrostachys, were sequenced for 26S rDNA containing approximate 3300bp in the present study. Phylogenetic analyses using both parsimony and maximum likelihood methods identified Grubbia as the sister of another African genus Curtisia with high bootstrap support congruent with previous findings based on rbcL & matK sequence data. These two genera were allied with the nyssoids in the parsimony analysis, but were recognized as sister of a monophyletic group consisting of nyssoids, matixioids, Cornus, and Alangium in the ML analysis. The four sequences of Hydrostachys were missing approximate 1000 bp near the 5' end. Those four samples of Hydrostachys formed a strongly supported, long-branched monophyletic group sister to the remainder of Cornales. However, this placement was not strongly supported in the parsimony tree. Moreover, this 26S rDNA-based relationship of Hydrostachys differs from that suggested by some earlier analyses of chloroplast genes.

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Phylogenetics of the genus Tulipa (Liliaceae): evidence from five plastid DNA regions

Sequence analyses of five plastid DNA regions (part of the *matK* exon, the *trnL* intron, the *trnL*-*trnI* intergenic spacer, and the *rpl12* and *rps16* introns, totalling more than 3700 characters in the aligned matrix) were used to investigate the monophyly of *Tulipa* and its relationships with other genera of Liliaceae. If the genus *Amanais* recognised for *T. edulis* and allies, the remainder of *Tulipa* is strongly supported as monophyletic. *Amanais* and *Erythronium* are the closest relatives of *Tulipa*. In *Tulipa*, two major clades, more or less corresponding to *Tulipa* sections *Tulipa* (*Leiostemones*) and *Eriostemones* as recognised by Hall (1940), are strongly supported, but resolution within these clades is poor, due to low levels of sequence divergence. *Tulipa sprengeri*, treated as an isolated species by Hall, is shown to be a member of section *Eriostemones*, and at least some members of subsection *Clusianae*, section *Tulipa*, fall in an isolated position closer to *T. uniflora* (thought by Hall to be a member of *Tulipa* section *Orithyia*) than to members of section *Tulipa*. These results will be discussed in terms of the morphology and ability of the different taxa to hybridize with each other.

449 FAYETTE, KIM* AND LEO P. BRUEDERLE

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Morphometric analyses support specific status for Eutrema penlandii Rollins (Brassicaceae)

Eutrema penlandii Rollins (Brassicaceae) was originally described in 1950 from material collected in 1935 at Hoosier Pass in Park County, Colorado. *Penland's* alpine fen mustard is a globally rare species restricted to continuously wet, alpine areas along a 25 mile stretch of the Continental Divide in the Mosquito Range. The closest relative to *E. penlandii* is the circum-boreal *E. edwardsii* R. Brown, with the two species being disjunct by over 2000 km. In 1987, W.A. Weber subsumed *E. penlandii* into its North American congener, publishing the name *E. edwardsii* ssp. *penlandii* (Rollins) W.A. Weber. Since then, there has been considerable interest and debate among taxonomists and conservation biologists regarding the systematic relationship between the two taxa. Population genetic data revealed the two to be distinct ($I = 0.914$), but provided little further insight into the level at which *E. penlandii* should be recognized. In order to provide a clearer understanding of the taxonomic relationship of these taxa, morphometric data were collected for 34 continuous, quantitative reproductive and vegetative characters from 136 herbarium accessions of *E. penlandii* and *E. edwardsii*. Although the ranges for all 34 characters overlapped, Mann-Whitney tests revealed significant differences among the medians in 24 of the 34 characters, while t-tests revealed significant differences among the means in 19 of 26 normally distributed characters. Multivariate analyses provided further evidence with regard to the distinct nature of these taxa. The first three principal components deriving from a principal components analysis explained 61% of the variation in the data, with PCA scatterplots revealing discrete groupings corresponding to species boundaries. Similarly, discriminant functions analysis correctly classified the taxa 100% of the time. These data reveal the two taxa to be morphologically distinct and, coupled with the population genetic data, further support recognition of *E. penlandii* at the species level.

450 FISHBEIN, MARK^{1*}, ROBERTA J. MASON-GAMER¹, DEBORAH J. HOPP¹, ERIN DOUTHIT¹, AND STEVEN P. LYNCH²

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Phylogeny of North American Asclepias estimated from non-coding chloroplast (rpl16 intron and trnC-rpoB spacer) DNA sequences

Asclepias is a large genus with about 120 species native to North America. The genus has been of great interest to systematists, ecologists, and evolutionary biologists because of complex floral morphology, specialized interactions with insect herbivores, and low fruit set suggestive of sexual selection. Current understanding of phylogenetic relationships within *Asclepias* is based on the last comprehensive monograph of the genus and cladistic analysis of morphological data. To further explore these relationships, DNA sequences from two non-coding regions of the chloroplast genome were analyzed. Sequences were obtained from the *rpl16* intron (c. 1200 bp) and the *trnC-rpoB* spacer (c. 1175 bp) regions for samples representing 100 species. Analyses of these sequences resulted in phylogenetic trees in which several small clades were strongly supported, but for which relationships among these clades and remaining species were supported quite weakly. The strongly supported clades correspond to several of the smaller infrageneric groupings (e.g., subg. *Podostemma*, ser.

Incarnatae) identified in the most recent generic revision. Well supported relationships conflicting with that revision suggest that earlier classification relied too strongly on a small set of floral characters (e.g., shape of corona segments), to the exclusion of vegetative characters (e.g., growth form). Our results are consistent with a rapid radiation of *Asclepias* in North America.

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Artifacts of coding amino acids and other composite characters for phylogenetic analysis

A phylogenetic analysis can be no better than the homology hypotheses on which it is based. Care must be taken both in formulating these hypotheses and in their formalization i.e., character coding. Just as it is inappropriate to code character states of individual characters as separate presence/absence characters, it is inappropriate to combine independent characters because not all information in the data is being utilized. Composite characters link otherwise-discernible states from different characters together to form new character states. There are two related problems with this coding. First, there is a loss of hierarchic information between the reductive and composite characters when unordered states are used. Second, the linking of independent characters that occurs during the construction of composite-character states creates putative synapomorphies that were not present in the independent characters. For codon and amino-acid characters, the problem may occur whenever more than one position of a codon is variable among the terminals sampled. Groups that are resolved as paraphyletic using reductive coding may be resolved as monophyletic using composite coding. In addition to the problem with artificial resolution caused by the use of composite characters, amino-acid characters are subject to loss of information and convergence caused by different codons specifying the same amino acid. The artificial character states indicated by the amino-acid characters are unlikely to be congruent with the true gene tree, and therefore, these artificial character states are likely to be homoplasious. Amino-acid characters have been considered to be more conservative than nucleotide characters. While the intent may be one of conservatism, the actual effect, with the complications caused by the use of composite characters, is not.

452 FREUDENSTEIN, JOHN V.^{1*}, CASSIO VAN DEN BERGH², W. MARK WHITTEN³, KENNETH M. CAMERON⁴, DOUGLAS H. GOLDMAN⁵, AND MARK W. CHASE²

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A multi-locus combined analysis of Epidendroideae (Orchidaceae)

Epidendroideae comprise approximately 80% of the species of Orchidaceae. The subfamily includes much of the variation that is found in anther characters, traditionally the most important in orchid classification. Simultaneous parsimony analysis of plastid *rbcL*, *matK*, *trnL-F* spacer and intron, nuclear 26S and ITS, and mitochondrial *nad1b-c* intron reveals support for many tribal and subtribal groups within the family. The terrestrial tribe

Neottieae is strongly supported as sister to the remainder of the subfamily. The next resolved clades include soft-pollinia groups, including Triphoreae, Tropidieae, Nervilieae, and Sobraliinae, some of which have at times been associated with other subfamilies. The "upper" epidendroids form a monophyletic group that comprises largely epiphytic plants with more specialized modes of pollen delivery, the pollinia often being associated with stalks of various types. The non-vandoid groups include many traditionally recognized subtribes, such as Epidendreae, comprising Laeliinae + Pleurothallidinae, and a narrowly defined Arethuseae + Coelogyninae. The higher level associations of many of these groups are still unclear because of the large number of short branches at the base of this clade. The majority of vandoid orchids, those possessing an early-bending incumbent anther, superposed pollinia, and cellular pollinium stalk, form a well-supported clade that comprises Dressler's Maxillarieae (monophyletic) and Cymbidieae (paraphyletic). Other vandoids comprise the largely Old World Vandae, sister to Polystachyinae, and Calypsoeae. Character transformations confirmed by these patterns include a progression from soft to hard pollinia, changes from four to either two or eight pollinia, and shifts from terminal to lateral inflorescences. In order to resolve the relationships of the major clades at the base of the subfamily, which are confounded by short branch lengths that may reflect rapid radiation, additional focused sampling is needed.

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*The phylogeny and historical biogeography of Cercis: evidence from ITS and *ndhF* DNA sequences*

The genus *Cercis* (Fabaceae: Caesalpinioideae: Cercidae) consists of approximately ten species distributed in mesic to arid climates across the warm-temperate regions of both North America and Eurasia. Variation in the shape, thickness, and upper surface of the leaves of *Cercis* species is correlated with climate type, and several biogeographers have invoked this pattern to support or refute hypotheses bearing on the evolution of arid biomes across the Northern Hemisphere. To employ *Cercis* in general biogeographical models, however, presupposes that a rigorous phylogenetic estimate of the genus exists, which has not been the case. We estimated the phylogeny of *Cercis* with DNA sequences of the nuclear ribosomal ITS region and a portion of the chloroplast gene *ndhF*. The phylogenetic relationships inferred from each region accord with one another and with results from the analysis of the combined data. The combined analysis recovered a topology in which a well supported clade of North American and western Eurasian species is nested within a paraphyletic group of Chinese species. *Cercis canadensis* from eastern North America is more closely related to *C. siliquastrum* from western Eurasia than to *C. occidentalis* from western North America. From DIVA and character optimization analyses, we inferred that the initial intercontinental divergence event in *Cercis* involved mesophytic ancestors. Subsequent inferred intercontinental divergence events involving xerophytic ancestors imply a Tertiary floristic connection between the arid regions of western North America and western Eurasia, and secondary migration to mesophytic habitats in eastern North America. Calibration of branch lengths with the fossil record suggests that the North American and western Eurasian lineages

diverged between 9.1 and 32 million years ago. The oldest of these values is consistent with more or less direct trans-Atlantic dispersion across a North Atlantic land bridge (>13 million years ago), whereas the youngest requires an explanation involving long distance dispersal.

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An evaluation of the proposed sister group relationships of the tribe Liabeae (Compositae)

Historically the members of the Liabeae (Compositae) were placed in the tribes Vernoniaeae, Heliantheae, Helenieae, Senecioneae and/or Mutisieae. In the 1970's Robinson brought the genera together into one tribe and the most recent cladogram based on morphology weakly grouped the Liabeae with the Vernoniaeae when compared with the Lactuceae. Early molecular work in the family showed the Arctoteae, Liabeae, Lactuceae, and Vernoniaeae in a variety of placements and resulted in an unresolved polytomy or grade located between the tribe Mutisieae and the subfamily Asteroideae. Recent Mutisieae and Liabeae DNA sequence data have been combined with previously published sequence data to address the placement of the Liabeae; the outgroups were a clade of Asian and African Mutisieae, the core Mutisieae, and the Cardueae. The results show that the four tribes form a monophyletic group whose sister group is the Asteroideae. Within this four taxon clade the Vernoniaeae, Liabeae, and Arctoteae form an unresolved monophyletic group with the Lactuceae as its sister group. The distribution of the tribes in the four taxon clade is interesting because the members of the Liabeae are primarily Andean, those of the Vernoniaeae are mostly found in Brazil and tropical Africa, and the Arctoteae taxa are confined to southern Africa, so that the three form a southern hemisphere clade, while the distribution of the Lactuceae is Laurasian.

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Evolutionary reduction of inner pollen sacs (microsporangia) within the asteracean genus Microseris: a model system for the evolution of diagnostic characters

Reduction from the normal 4 pollen sacs (microsporangia) to 2 microsporangia (MS) per anther has occurred independently in about 50 angiosperm families and thus is a prime example for the parallel evolution of a potentially diagnostic character (homoplasy). Within the asteracean genus *Microseris* the reduction of the inner (adaxial) MS is a synapomorphy for three diploid annual species. The cosegregation of MS numbers with genetically mapped molecular markers (AFLPs) was analyzed in the F6 inbred population of an interspecific cross between tetrasporangiate (4MS) *M. douglasii* and disporangiate (2MS) *M. bigelovii*. One major gene and four modifying genes specifically affecting the expression of the inner MS were detected. The dominant (4 MS) allele of the major gene was epistatic over three of the modifiers. The modifiers could stabilize a 2 MS phenotype only in the homozygous recessive (2 MS) genotype of the major gene. 2 MS were produced if at least 5 alleles of those modifying genes determined the 2 MS phenotype. Homozygosity for the 4 MS allele in only one of the modifying genes in an otherwise 2MS genetic background produced an unstable phenotype with variable average MS numbers in different plants. However more than two alleles from *M. douglasii* (4 MS) led to the production of 4 MS in most flowers of one capitulum. This non-linear ("canalized") relationship between gene dosis and expression of MS and the observation of

genotypes with variable expression of inner MS suggests how the disporangiate phenotype has evolved from a stable tetrasporangiate phenotype with a minimum of potentially maladapted intermediate phenotypes.

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Phylogenetic relationships of the Australian tribe Anthocercideae (Solanaceae) based on chloroplast DNA sequences

Tribe Anthocercideae (Solanaceae) consists of seven genera with 31 species endemic to Australia. Past classification of the group resulted in the placement of the seven genera into several tribes. The group was later unified into tribe Anthocercideae, and is now placed as sister to *Nicotiana*, but phylogenetic work on Anthocercideae remains limited. A more extensive study encompassing all genera was needed to discover lower level relationships within the tribe. A phylogeny of the group was created by studying variation in two chloroplast DNA regions, *ndhF* and *trnL-trnF*. This phylogeny allowed us to look at floral character evolution and rates of gene evolution. Tribe Anthocercideae, without genus *Symonanthus* forms a well-supported, monophyletic group. Each genus in the tribe also forms a monophyletic group, except for *Cyphanthera*, which appears to be paraphyletic. *Symonanthus* is found to be more closely related to *Nicotiana* than to the rest of the tribe. It was found that bilocular stamens is the ancestral condition, and unilocular stamens are evolutionarily derived. Floral character evolution and rates of gene evolution will be discussed in more detail.

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Characteristics from living flowers improve classification of Tillandsia (Bromeliaceae)

Today, relationships among species are assessed by molecular techniques, including dna sequencing. *Tillandsia*, a large, morphologically diverse and mostly epiphytic neotropical genus that includes nearly one quarter of the species in the entire family, is, however, still in need of alpha-taxonomic investigation. Previous treatments of the 400+ species of *Tillandsia* were based on characters found on herbarium specimens. Since dried flowers in these species preserve poorly and are sometimes absent, floral characters are difficult to observe in this manner. Smith and Downs were able to find only one character by which to distinguish subgenus *Allardtia* from subgenus *Tillandsia* (together 269 species) in their treatment of the Tillandsioideae for Flora Neotropica—whether the stamens were included (*Allardtia*) or exerted (*Tillandsia*). Previous field studies of 79 species of subgenus *Tillandsia* and 12 species of subgenus *Allardtia* in Mexico showed that rather than two natural groups, five distinct groups could be circumscribed by suites of characters from fresh flowers, and that these groups cut across current taxonomic lines. Subgenus *Tillandsia* has its center of distribution in Mexico, while subgenus *Allardtia* is largely centered in the Andes of South America. Current field studies in Ecuador are expanding the number of species examined and support the hypothesis that a thorough examination of flowering specimens in vivo of the majority of the species in these two subgenera can result in a more natural classification for this difficult group.

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A phylogenetic analysis of Poa (Poaceae) based on cpDNA restriction site data: DNA variation versus morphological divergence

Poa (at ca. 500 species perhaps the largest grass genus) occurs throughout temperate and boreal regions of the globe. A new phylogenetic study of Poa, based on data from restriction site analysis of PCR amplified regions of chloroplast DNA (cpDNA) (trnT-trnF, trnF-trnV, trnV-rbcL, rbcL-ORF106, trnH-trnK), expands sampling to where about 1/6 of the species have been characterized for cpDNA types. A broad phylogenetic structure detected in an earlier study (Soreng, AJB 77: 1383-1400. 1990) gained additional and robust support. Accounting for wide extragenic sampling, Poa still remains monophyletic if *Austrofestuca* is included within it. The new analysis suggests that divergence in morphology and ecology is often decoupled from the frequency of fixation of phylogenetically informative cpDNA variation. Some species groups are widely distributed with strikingly disparate morphology and ecology, yet have diverged little in their cpDNA profiles, whereas the opposite pattern occurs in other groups. One of the largest and most derived clades with morphologically and ecologically diverse species that show little cpDNA divergence includes: *Austrofestuca*, several *P.* subg. *Poa* sections (*Dasympoa*, *Dioicopoa*, *Homalopoa*, *Madropoa*) and other informal groups, with species endemic to Europe, China, Australia, North America, and South America. One small, early diverging cpDNA lineage includes two morphologically and ecologically unrelated subgroups (*Poa* subg. *Arctopoa* of alkaline seeps of the high Tibetan Plateau to sub-arctic salt marshes; and *P.* subg. *Poa* sect. *Sylvestres* of forests of the Appalachian Mts.), but few cpDNA characters distinguish them. In contrast, in another large, derived clade (*P.* sects. *Abbriviateae*, *Oreinos*, *Pandemos*, *Secundae*, and *Stenopoa*) cpDNA variation is extensive and phylogenetically informative variation has been detected even within species. Morphological and ecological divergence, however, is not as great as in the previous two clades.

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Chloroplast DNA restriction site variation in North American Poa section Poa

Poa sect. *Poa* comprises three species and numerous infraspecific taxa in North America. *Poa pratensis* and *P. arctica* are widespread and morphologically highly polymorphic with numerous infraspecific taxa, while *P. macrocalyx* is restricted to coastal Alaska in North America. We used restriction site analysis of PCR amplified chloroplast DNA (cpDNA) to examine variation both among species and at the infraspecific level in *P. pratensis* and *P. arctica*. Based on our larger phylogenetic analysis of *Poa* the section was determined to be a strongly supported monophyletic group. Variation within the section was detected in only one of the five cpDNA regions examined (trnT-trnF, trnF-trnV, trnV-rbcL, rbcL-ORF106, trnH-trnK). All infraspecific taxa of *P. arctica* examined shared an identical restriction site haplotype, differing from the other two species in two or three restriction sites in the rbcL-ORF106 region. In contrast, *P. pratensis* exhibited infraspecific cpDNA variation in the same region, with two cpDNA groups: an indigenous arctic/alpine complex comprising *P. pratensis* subsp.

alpigena and *colpodea*, and an introduced non-arctic/alpine complex. These two cpDNA groups differ in three restriction sites, a level of variation greater than is generally found within species complexes in *Poa*. They also differ considerably in length of the rbcL-ORF106 region, suggesting that these restriction site differences may be the result of an insertion-deletion event, specifically a deletion in arctic/alpine *P. pratensis*. While most *P. pratensis* subsp. *pratensis* had the non-arctic/alpine haplotype, Low Arctic plants were found to have the arctic/alpine haplotype and are intermediate in overall morphology, suggesting they may be the result of hybridization between subspecies. *Poa macrocalyx* was found to share an identical haplotype with arctic/alpine *P. pratensis*. We hypothesize that the two cpDNA groups in the *P. pratensis/P. macrocalyx* complex represent a fundamental division between an indigenous (sub)arctic/alpine complex and an introduced non-arctic/alpine complex.

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ITS secondary structure derived from comparative analysis: implications for sequence alignment and phylogeny in the Asteraceae

We present a secondary structure model for the nuclear rDNA ITS region based on statistical analyses of covariation in 340 Asteraceae ITS sequences. The model includes novel structural motifs for ITS1 and ITS2 and supports some of the structural proposals of previous studies using thermodynamic criteria. The positional homology implied by the model provides a framework for molecular evolutionary studies and the first family-level phylogeny independent of chloroplast DNA data. The highly resolved ITS tree shows considerable congruence with the relationships among major lineages of the Asteraceae suggested by previous cpDNA studies. Combined analyses provide additional resolution and support for relationships in the family.

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Phylogenetic relationships of endemic species of Crambe sect. Dendrocrambe (Brassicaceae) in Macaronesia based on chloroplast DNA restriction site data

Fourteen species of *Crambe* sect. *Dendrocrambe* (Brassicaceae) are endemic to the Macaronesian islands and are found in a variety of habitats. A previous study using ITS sequence data indicated that the island endemics form a monophyletic group comprised of three major lineages. Although interspecific relationships were resolved in some cases, relationships among the three major groups and among other species were not sufficiently resolved. We are performing a cpDNA restriction site study to provide additional resolution and to have an independent molecular marker to test previous hypotheses of the evolution of *Crambe* in Macaronesia. Our preliminary cpDNA results are congruent with the previous ITS study and suggest that inter-island colonization and subsequent differentiation is the major pattern of evolution in this group.

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Commelinoid monocot phylogeny revisited, using a large chloroplast data set

The commelinoid monocots (*sensu* APG 1998) comprise four large orders (Arecales, Commelinales, Poales and Zingiberales) and several taxa of uncertain affinity. They include some of the largest flowering-plant families and encompass an incredible array of ecological and morphological diversity. Recent taxonomic treatments and molecular studies have greatly clarified the circumscription of the commelinoids and constituent orders, but many aspects of relationship in the group remain poorly resolved at the family level and above. We present results from an ongoing study of higher-order relationships in the commelinoids, based on analysis of ~13.5 kb (unaligned) of DNA sequence data per taxon, for 13 taxa in ten families. The regions sampled include *atpB*, *rbcl*, *ndhF*, ten photosystem II genes, two *ndh* genes, three ribosomal protein genes, three introns and two intergenic spacer regions. Parsimony-based bootstrap analyses (using the Fitch parsimony criterion) robustly support commelinoid monocot monophyly, although several major taxa have not yet been sampled, including Arecales. The position of the family Dasygongonaceae was not fully resolved, but it is clearly isolated from Commelinales, Zingiberales and Poales. Zingiberales is strongly supported as the sister-group of the Commelinales. Our results support the inclusion of Hanguanaceae in Commelinales, as was recently proposed by Chase et al. 2000, although the monophyly of the redefined Commelinales (Commelinaceae, Hanguanaceae, Haemodoraceae, Philydraceae and Pontederiaceae) was only weakly supported. Within the Commelinales, Haemodoraceae and Pontederiaceae were strongly supported as sister taxa, as were Commelinaceae and Hanguanaceae. Philydraceae was moderately well supported as the sister-group of Haemodoraceae-Pontederiaceae. We also address the identity of the sister-group of the commelinoids, and discuss various aspects of the molecular evolution of the regions examined.

463 GRIFFITH, M. PATRICK

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*Natural interspecific hybridization in *Opuntia* of the northern Chihuahuan Desert region*

Possible natural interspecific hybridization among taxa of *Opuntia* subgenus *Opuntia* was investigated in the northern Chihuahuan Desert region. The investigation employed experimental hybridization and random amplified polymorphic DNA (RAPD) data. Plants in the Sul Ross State University Garden representing three ploidy levels, ten species, and three varieties were used for all experiments. Reciprocal crosses were made between putative parental taxa, and each cross was analyzed for fruit and seed set. For each taxon, tests were performed to control for possible apomictic, autogamous, and geitonogamous seed set. Data gathered from the tests provided basic information regarding the breeding systems of the taxa investigated. Reciprocal crosses between ploidy levels set fruit and seed unidirectionally. Seed set data suggest that *O. engelmannii* possibly spreads primarily through vegetative means, or by dispersal of self-fertilized seed. RAPD banding pattern data suggested that at least one *Opuntia* population was of hybrid origin.

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*Polyphyly in the Brazilian bamboo genus *Apoclada* (Poaceae: Bambusoideae)*

The Brazilian bamboo genus *Apoclada* has been extensively studied using both morphological and molecular techniques and has been found to be polyphyletic. The *ndhF* gene of the chloroplast DNA from all three species of *Apoclada* was sequenced and compared with existing sequences for several of its relatives. The data show strong evidence for polyphyly in *Apoclada* as it has been traditionally described. The two cerrado species form a clade but *Apoclada simplex* is more closely allied with the genus *Guadua*. Several morphological characters were also considered and give a congruent answer. Habitat preferences were modelled as well and show discrete spatial environmental characteristics for each species.

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*Diploid hybrid speciation in *Ceanothus*: morphomolecular analysis of two putative hybrid species and their proposed parent species*

Several species of the North American shrub genus *Ceanothus* have been proposed as exemplars of diploid hybrid species. Based on observations of natural hybrids, synthesis of garden hybrids, cytology, distribution, and perceived morphological intermediacy, Nobs (1963) concluded that *C. masonii* and *C. sonomensis* (among others) are diploid hybrid species, derived from separate hybridization events between *C. cuneatus* and *C. gloriosus*. We tested Nobs' proposal for the origins of *C. masonii* and *C. sonomensis* using molecular (i.e., nuclear ribosomal and chloroplast DNA sequences and allozyme diversity) and morphological (i.e., foliar characteristics) data. Results of our molecular analyses provided no unequivocal support for the proposed hybrid origin of either *C. masonii* or *C. sonomensis*. Additionally, statistical and multivariate analyses of foliar characteristics indicated that the proposed hybrid species possess character states more extreme than either parent. Recent studies of character inheritance and expression in hybrids suggest that the assumption of morphological intermediacy in hybrids is unwarranted, therefore, our morphological results are not inconsistent with possible hybrid origins. However, our results do call into question at least one of Nobs' original premises. As an alternative to the hybrid origin scenario, we propose that *C. masonii* and *C. sonomensis* are the products of divergent speciation, driven by recent climatic and geological changes effecting the western edge of the North American continent.