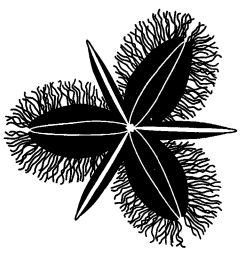
Monocots: Systematics and Evolution. (2000). Eds K.L. Wilson and D.A. Morrison. (CSIRO: Melbourne)



PHYLOGENETIC RELATIONSHIPS WITHIN THE DIURIDEAE (ORCHIDACEAE): INFERENCES FROM PLASTID *MAT*K DNA SEQUENCES

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Abstract

As delimited by Dressler (1993) Diurideae include 10 subtribes with about 43 genera. Nine subtribes are predominantly Australian whereas the tenth (Chloraeinae) is South American and New Caledonian. Dressler suggested Diurideae are allied to Orchideae and Diseae, and these three tribes constituted his subfamily Orchidoideae. Our analysis based on DNA sequence data indicates that Diurideae are monophyletic only if Chloraeinae and Pterostylidinae are excluded from the tribe. These two subtribes are better placed with spiranthoid orchids. Our data indicate that the core Diurideae are sister to Cranichideae and not to the other orchidoid taxa. Within the core Diurideae six major lineages can be recognized: Prasophyllinae, Acianthinae, core Caladeniinae, Cryptostylidinae, Diuridinae, and a combined Drakaeinae–Thelymitrinae–Caladeniinae (*pro parte*) clade. It should be noted that only groups one, two, and four have circumscriptions equivalent to those of Dressler (1993). Prasophyllinae are sister to Acianthinae, and the prasophyllid–acianthid clade is sister to the other four lineages within the tribe. Within the four-lineage clade, Caladeniinae are sister to a clade comprised of Cryptostylidinae, Diuridinae and Drakaeinae. Finally Cryptostylidinae are sister to Diuridinae and the combined cryptostylid–diurid clade is sister to Drakaeinae. *Rhizanthella* was not sampled in this study. The molecular phylogeny indicates that some morphological characters are highly homoplasious and should be used with caution for elucidating relationships within this and other groups of orchids.

Keywords: Orchidaceae, Diurideae, molecular systematics, phylogenetic relationships, *mat*K DNA sequences, monocots.

INTRODUCTION

Tribe Diurideae was established by Endlicher in 1842 to accommodate five Australian genera of orchids, and it has persisted variously circumscribed throughout many of the subsequent systematic treatments of the family (Schlechter 1926; Mansfeld 1937, 1955; Lavarack 1971, 1976; Dressler 1981, 1993; Rasmussen 1982, 1985; Burns-Balogh and Funk 1986; Clements 1995). As delimited in the most widely accepted account (Dressler 1993), Diurideae includes 10 subtribes with approximately 43 genera containing over 900 species. Nine of these subtribes (Acianthinae, Caladeniinae, Cryptostylidinae, Diuridinae, Drakaeinae, Prasophyllinae, Pterostylidinae, Rhizanthellinae and Thelymitrinae) are predominantly Australian (or Australian, New Zealand and New Caledonian), whereas the tenth (Chloraeinae) is exclusively South American and New Caledonian. The classifications of Diurideae proposed by Dressler (1993), Clements (1995) and the results presented here are summarized in Table 1.

The placement of Diurideae within Orchidaceae has also varied historically. Lavarack (1971, 1976), Garay (1972), and Rasmussen (1982, 1985, 1986) assigned Diurideae to the subfamily Neottioideae. Dressler (1981, 1993) included its constituents

Table 1. A synopsis of the classification of Diurideae in two recent treatments based on non-molecular characters (columns one and two) and the results of a molecular phylogeny based on *mat*K sequences. Genera in parentheses under Dressler (1993) represent genera not included in his treatment. Genera in parentheses under Kores *et al.* (this study) represent the inferred placement of genera not sampled in the molecular treatment.

Dressler (1993)	Clements (1995)	Kores *et al.* (this study)
Orchidoideae	**Orchidoideae**	**Orchidoideae**
Orchideae	**Orchideae**	**Orchideae**
Diseae		
Diurideae	**Diurideae**	**Diurideae**
Acianthinae	Acianthinae	Acianthinae
Acianthus	*Acianthus*	*Acianthus*
	Adenochilus	
Corybas	*Corybas*	*Corybas*
Cyrtostylis	*Rhizanthella*	*Cyrtostylis*
Stigmatodactylus	*Stigmatodactylus*	*Stigmatodactylus*
Townsonia	*Townsonia*	*Townsonia*
Caladeniinae	Caladeniinae	Caladeniinae
Adenochilus		*Adenochilus*
Aporostylis	*Aporostylis*	*(Aporostylis)*
Burnettia	*Burnettia*	*(Burnettia)*
Caladenia	*Caladenia*	*Caladenia*
(Cyanicula)	*Cyanicula*	*Cyanicula*
	Cyrtostylis	
(Drakonorchis)	*Drakonorchis*	*Drakonorchis*
Elythranthera	*Elythranthera*	*Elythranthera*
Eriochilus	*Eriochilus*	*Eriochilus*
Glossodia	*Glossodia*	*Glossodia*
Leporella	*Leporella*	
Leptoceras	*Leptoceras*	*Leptoceras*
Lyperanthus	*Lyperanthus*	
(Praecoxanthus)	*Praecoxanthus*	*(Praecoxanthus)*
Rimacola	*Rimacola*	
Drakaeinae		Drakaeinae
Arthrochilus	*Arthrochilus*	*(Arthrochilus)*
Caleana	*Caleana*	*Caleana*
Chiloglottis	*Chiloglottis*	*Chiloglottis*
Drakaea	*Drakaea*	*Drakaea*
		Leporella
Spiculaea	*Spiculaea*	*Spiculaea*
Thelymitrinae		*Rimacola*
Calochilus		*Calochilus*
Thelymitra		*Thelymitra*
		Lyperanthus
Diuridinae	Diuridinae	Diuridinae
	Calochilus	
Diuris	*Diuris*	*Diuris*
Epiblema	*Epiblema*	*(Epiblema)*
Orthoceras	*Orthoceras*	*Orthoceras*
	Thelymitra	
Prasophyllinae	Prasophyllinae	Prasophyllinae
Genoplesium	*Genoplesium*	*Genoplesium*
Microtis	*Microtis*	*Microtis*

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Prasophyllum	*Prasophyllum*	*Prasophyllum*
Rhizanthellinae		(Rhizanthellinae)
Rhizanthella		*(Rhizanthella)*
Cryptostylidinae	**Cryptostylideae**	Cryptostylidinae
Coilochilus	*Coilochilus*	*Coilochilus*
Cryptostylis	*Cryptostylis*	*Cryptostylis*
	Megastylis	
	Rimacola	
	Cranichideae	**Cranichideae**
Pterostylidinae	Pterostylidinae	Pterostylidinae
Pterostylis	*Pterostylis*	*Pterostylis*
Chloraeinae	Chloraeinae	Chloraeinae
Bipinnula	*Bipinnula*	*(Bipinnula)*
Chloraea	*Chloraea*	*Chloraea*
Codonorchis	*Codonorchis?*	*(Codonorchis)*
Gavilea	*Gavilea*	*(Gavilea)*
Geoblastus	*Geoblastus*	*(Geoblastus)*
Megastylis		*Megastylis*

within Orchidoideae. Burns-Balogh and Funk (1986), in the first cladistic treatment of the family, split Diurideae into three lineages that were dispersed among Spiranthoideae, Neottioideae and Epidendroideae. Szlachetko (1991, 1995) also divided the tribe, but he included the resulting groups within Orchidoideae and a new subfamily, Thelymitroideae. Clements (1995) excluded two of the subtribes from Diurideae and placed the remainder of the tribe in Orchidoideae. Chloraeinae and Pterostylidinae, the two subtribes he excluded, were assigned to Spiranthoideae (*sensu* Dressler 1993). In addition, Clements also suggested that Diurideae and spiranthoid orchids were sister groups. This interpretation differs from that of Dressler, who proposed a relationship between Diurideae and orchidoid lineages with basitonic anthers (tribes Orchideae and Diseae), but it agrees with the findings of Kores *et al.* (1997) and Cameron *et al.* (1999) based on *rbc*L sequence data.

These differences in the size and placement of Diurideae stem from a lack of obvious synapomorphies for the tribe. For example, Dressler's cladogram of postulated relationships between Orchidoid tribes (Dressler 1993: Fig. 7-2) shows no synapomorphies for Diurideae. The tribe was implicitly characterized only by the absence of synapomorphies qualifying other taxa. Most Diurideae in Dressler's work have root tubers and acrotonic anthers, but only the combination of these characters is unique to the tribe. Orchideae and Diseae have well-developed root tubers but lack acrotonic anthers, whereas Spiranthoideae have acrotonic anthers but lack root tubers. Clements (1995) characterized the tribe by the annual replacement of all parts of the plant and the presence of elongate protocorms, but both of these character states undergo reversals within some clades of the tribe. Unfortunately, in a morphological context Diurideae appear to be defined largely by the absence of synapomorphies indicative of the other assemblages to which they are related.

Since there are serious differences in the accounts of the tribe based on traditional characters, a molecular treatment of Diurideae was undertaken using the plastid gene *mat*K. This gene has been utilized effectively to address systematic questions in the families Saxifragaceae (Johnson and Soltis 1994, 1995), Poaceae (Liang and Hilu 1996; Hilu and Liang 1997), Cornaceae (Xiang *et al.* 1998), Asclepiadaceae (Civeyrel *et al.* 1998), and within the orchid tribe Vandeae (Jarrell and Clegg 1995).

MATERIALS AND METHODS

Plant Accessions

A total of 70 accessions, representing 64 genera of Orchidaceae, were analyzed. This includes three genera from Cypripedioideae, 10 genera from Epidendroideae, eight genera from Spiranthoideae, and 43 genera from Orchidoideae (*sensu* Dressler 1993). Within the latter, 13 genera were included from the tribes Orchideae and Diseae and 30 genera from Diurideae. Detailed information about the taxa used in this study is available from the senior author upon request.

DNA Extractions, Amplification and Sequencing

Total genomic DNA was extracted from fresh or silica-dried plant material using a standard 2X CTAB protocol (Doyle and Doyle 1987) and further purified by ultracentrifugation utilizing a $CsCl_2$-ethidium-bromide density gradient (1.55 g ml^{-1}).

Amplification of *mat*K was carried out in a Perkin-Elmer thermal cycler utilizing 100 µl PCR reactions with 2.5 units of Taq polymerase (Promega, Inc.), 2 µl 4% bovine serum albumin, 2.8 µM $MgCl_2$ and 100 ng of the two PCR primers (*mat*K -19F

(Kores) and *trn*K2R (Johnson and Soltis 1994)). (The use of an alternative forward primer, *trn*K3914F monocot, was sometimes necessary in Orchidaceae.) The PCR profile used to amplify *mat*K was as follows: an initial premelt of 2 min. 30 sec. at 94° C; followed by 28 cycles of 1 min. denaturation (94° C), 1 min. annealing (52° C), and 2 min. 30 sec. elongation (72° C) with 8 seconds added per cycle; followed by a 7 min. final extension at 72° C. PCR products were purified using the Wizard (Promega, Inc.) purification columns following the manufacturers' protocols.

Cycle sequencing was carried out directly on the amplified product utilizing the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer), with 2.5 ng of primer in a 5 µl reaction volume. Sequencing conditions were as follows: 26 cycles of 15 sec. denaturation (96° C), 1 sec. annealing (50° C), and 4 min. elongation (60° C) utilizing a Perkin-Elmer 9600 thermal cycler. Sequencing reactions were purified by ethanol precipitation and run on an ABI Prism 377 automated sequencer. Electropherograms were analyzed with Sequencher 3.0 software (Gene Codes, Ann Arbor, Michigan, USA). Complementary strands of the complete *mat*K gene were sequenced utilizing a series of internal primers to provide complete overlap for most taxa. The specific primers utilized for PCR and sequencing are listed in Molvray *et al.* (this volume). Sequences were aligned manually. The alignment is available from the first author upon request.

Phylogenetic Analyses

Phylogenetic analyses were performed on the *mat*K data matrix with a Macintosh PowerPC G3 computer. Initially, phylogenies were inferred from this matrix using equally weighted maximum parsimony (MP) as implemented in PAUP* (version 4.0b; Swofford 1998). Due to the large number of taxa, a heuristic search strategy was employed similar to the one proposed by Olmstead and Sweere (1994). A series of 200 searches was performed each using random taxon addition, tree bisection-reconnection swapping (TBR) with MULPARS selected, but saving no more than five of the shortest trees from each search. These equally most-parsimonious trees were then used as starting trees for TBR branch swapping (with MULPARS and STEEPEST DESCENT selected), the maximum number of trees saved was set at 1,000, and these trees were permitted to swap to completion.

Gaps in the aligned *mat*K sequences were incorporated into the parsimony analyses in the following manner. Each indel was scored as a separate presence/absence character utilizing the software PAUPGAP (developed by A. Cox, Royal Botanic Gardens, Kew). The resulting binary-coded matrix was appended to the matrix of sequence data, and the positions where the gaps occurred were treated as missing data (Swofford 1993). All characters were equally weighted in the initial analysis.

Once the initial heuristic search was completed, all the equally most-parsimonious trees were utilized for successive weighting to reduce the weighting of potentially homoplasious characters (Farris 1969). Characters were assigned new weights with the REWEIGHT CHARACTERS option in PAUP* using the Rescaled Consistency Index (RC) and a base weight of 1,000. A heuristic search was performed on the reweighted matrix using branch swapping on all trees obtained in the previous analysis. After each search the characters were reweighted again on the basis of the new trees, and this procedure was repeated until a constant length was obtained

Internal support was assessed by bootstrapping (Felsenstein 1985), utilizing character weights derived from the successive weighting. Bootstrap percentages (BP) for each node were computed after resamplings followed by a MP reconstruction (bootstrap option in PAUP 4.0b, with 1,000 replicates of heuristic search, one random sequence addition per replicate, nearest neighbor branch swapping and MAXTREES = 100).

Representatives from three genera of diandrous orchids (*Cypripedium*, *Phragmipedium* and *Selenipedium*: Cypripedioideae) were designated as outgroups for all analyses. This choice was based on the results of two other phylogenetic analyses of Orchidaceae using the plastid gene *rbc*L (Cameron *et al.* 1999) and a combined analysis of *rbc*L, *mat*K and the *trn*L–F region (Kores *et al.*, in prep.).

Results

The aligned *mat*K sequences resulted in a matrix of 1,913 characters. This included 1,808 positions representing individual nucleotides, and an additional 105 positions for binary coded indels. Indels ranged in size from 1 to 78 base pairs. Many of these indels preserved the reading frame, but there were also numerous examples of frame shifts, with premature stop codons present in many of the resulting sequences. The presence of frame shifts and stop codons indicate *mat*K has been converted to a pseudogene within many Orchidaceae, a condition not evident in the outgroups. Within Orchidaceae the substitution rate for *mat*K is approximately 2.8 times that of *rbc*L.

Considering all the characters in the data matrix, 763 were invariant, 406 were variable but uninformative, and 744 were parsimony informative. The initial analysis with all character transformations treated as equally likely and all characters equally weighted (Fitch parsimony; Fitch 1971), found a total of 96 most-parsimonious trees. Individual trees had a length of 3,785 steps, a consistency index (CI) of 0.45 and a retention index (RI) of 0.61. These trees were well resolved. However, there was a lack of resolution among the major lineages at the base of the core diurid clade and between the terminal taxa within the core *Caladenia* clade. Successive weighting, based on the initial trees, resulted in a single most-parsimonious tree with an equally weighted length of 3,787 steps (CI = 0.45, RI = 0.61, weighted length = 1,082,657 steps). This tree is shown in Fig. 1.

The resulting topology agrees with the phylogeny proposed by Cameron *et al.* (1999) based upon an extensive survey of the plastid gene *rbc*L. The cladogram presented here indicates that the monandrous orchids are composed of three major lineages: Vanilloideae, Epidendroideae and Orchidoideae. All three of these clades are strongly supported in the *mat*K analysis with bootstrap values (BP) of 100%, 100% and 99% respectively. Vanilloideae, which include the subtribe Pogoniinae, are sister to all other monandrous orchids, whereas Orchidoideae and Epidendroideae are sister to each other. This combined orchidoid–epidendroid clade contains the vast majority of genera

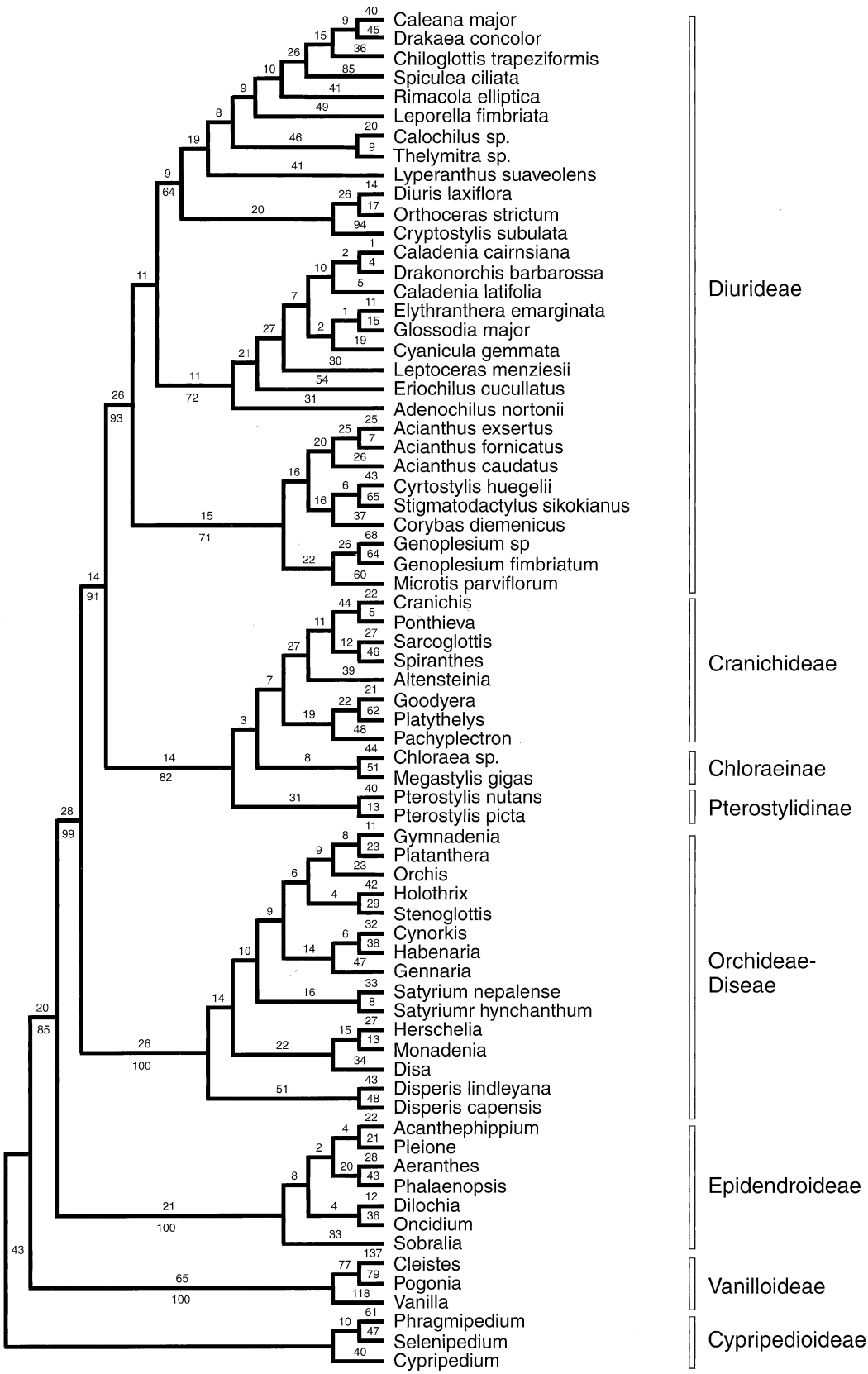

Fig. 1. The single most-parsimonious tree for the Orchidoideae *s. l.* found by successive weighting of the *mat*K sequence data. Diurideae are sister to Cranichideae. Subtribes Chloraeinae and Pterostylidinae, formerly considered Diurideae, are placed with Cranichideae. The combined diurid–cranichid lineage is sister to Orchideae–Diseae. Values above the branches are number of unweighted steps, values below the branches are bootstraps based on the successively weighted matrix. Equally weighted tree length 3,787 steps, CI = 0.454, RI = 0.611.

and species within the family, and it is moderately well supported with a bootstrap value of 85%.

Focusing on Orchidoideae, this clade can be further resolved into three major subclades. These include a core orchidoid subclade (tribes Orchideae and Diseae; BP = 100%); core spiranthoid subclade (Cranichideae, Pterostylidinae and Chloraeinae; BP = 82%); and a core Diurideae subclade (BP = 93%). The core spiranthoid subclade is sister to the diurid subclade (BP = 91%), and the combined spiranthoid–diurid clade is sister to the core orchidoid lineage. It should be noted that the delimitation of these subclades and their affinities in this molecular treatment differ substantially from the currently accepted phylogenies for the family based on morphology. Sampling was inadequate within Cranichideae, which resulted in a lack of resolution within this lineage, although there are a number of well-defined lineages within Diurideae.

Based on the results presented here, the core Diurideae can be subdivided into six major lineages. Four of these are equivalent to the subtribes Acianthinae (BP = 100%) Prasophyllinae (BP = 75%), Diuridinae (BP = 100%) and Cryptostylidinae (*sensu* Dressler 1993), whereas the remaining two do not correspond to any previously recognized subtribes. These include a lineage composed of the subtribes Thelymitrinae–Drakaeinae and the genus *Lyperanthus* (Caladeniinae, *sensu* Dressler 1993; BP = 100%), and a group of genera representing the core Caladeniinae (BP = 72%). The results of the successive weighting indicate that Acianthinae and Prasophyllinae are sister groups (BP = 71%), and that Cryptostylidinae and Diuridinae are sister groups (BP = 77%), but there is insufficient support to specify how these and the other groups within Diurideae are interrelated.

Two of the genera included in this analysis, *Adenochilus* and *Cryptostylis*, are somewhat problematic. Both appear to be very divergent from the other diurids, and they tend to occur as sister to each other in the general analysis. However, this relationship does not persist after successive weighting, nor is it present in preliminary results based upon other regions of the genome (Kores in prep.).

Discussion

The results of the phylogenetic analyses of *mat*K sequences do much to clarify the circumscription and phylogenetic affinities of Diurideae. According to our analyses, Diurideae as delimited by Dressler (1993) are not monophyletic. Seven of its subtribes, Acianthinae, Caladeniinae, Cryptostylidinae, Diuridinae, Drakaeinae, Prasophyllinae and Thelymitrinae, form a strongly supported, monophyletic lineage (hereafter Diurideae *s. str.*), but the remaining two subtribes sampled in this study, Chloraeinae and Pterostylidinae, are instead related to Cranichideae. This last relationship is moderately well supported by the molecular data. The Cranichideae–Chloraeinae–Pterostylidinae clade is the probable sister group to Diurideae *s. str.*

Dressler (1981, 1983) included Chloraeinae within Diurideae based on palynological studies by Ackerman and Williams (1981) and similarities in floral morphology. Previous to these treatments, Chloraeinae were generally placed as a tribe either in Acrotonae (Pfitzer 1889; Schlechter 1926) or Neottioideae (Brieger 1974–1975; Rasmussen 1982, 1985). The molecular treatment of Chloraeinae is similar to an account by Clements (1995) who recently transferred the majority of the genera within Chloraeinae to Cranichideae. This transfer was made largely on the basis of what he defined as a spiranthoid embryo pattern. Biogeographically this transfer seems reasonable since Chloraeinae, except for *Megastylis*, are exclusively South American, whereas Diurideae *s. str.* are all Australasian. In addition, the absence of root tubers in all genera except *Codonorchis*, which has stalked storage structures, also argues against placement of the subtribe with Diurideae. However, Clements (1995) included *Megastylis* within Cryptostylideae on the basis of its persistent plant habit, its *Diuris*-type embryo pattern, and its fleshy fasciculate roots, which he interpreted as storage organs. The molecular data place Chloraeinae with Cranichideae, rather than Diurideae, and except for embryology there is no morphological evidence to indicate that *Megastylis* should be excluded from the cranichid lineage. Given that *Megastylis* shows the *Diuris*-type embryo pattern and is a member of the sister group in the *mat*K analysis, the implication is that this embryo pattern may be plesiomorphic for the whole cranichid–diurid lineage.

The placement of *Pterostylis* with cranichid orchids is more unexpected. Historically *Pterostylis* has always been associated with Diurideae. The genus shares a number of characteristics with some diurid genera, such as the presence of root tubers, a long column, a hinged, insectiform labellum, and the lack of a nectary. However, it is otherwise rather aberrant in having multiple leaves per shoot, typically goodyeroid seeds (Molvray and Kores 1995), and a spiranthoid embryo pattern as defined by Clements (1995). Thus, some morphological evidence supports the inclusion of *Pterostylis* with spiranthoid orchids, as indicated by the molecular data.

The placement of the more narrowly circumscribed Diurideae in the orchid family differs substantially from the relationship suggested by Dressler (1993) and Freudenstein and Rasmussen (1999). The molecular findings indicate that Diurideae are more closely related to cranichid orchids than to other orchids with root tubers, i.e. Orchideae and Diseae. Similar results were obtained in two other molecular studies based upon *rbc*L (Kores *et al.* 1997; Cameron *et al.* 1999) and in a recent morphological treatment (Clements 1995). There appears to be no evidence to support the continued recognition of Diseae as a distinct tribe. Similar results were obtained in an analysis of Diseae based upon ITS sequence data (Douzery *et al.* 1999). Diseae appear to be a grade leading to Orchideae. The combined Orchideae–Diseae lineage is well characterized by its basitonic anthers, double viscidium, and sectile pollinia.

The tribal relationships evident in the *mat*K tree indicate that there was no single origination or loss of root tubers in Orchidoideae *s.l.* Whether tubers are postulated as the plesiomorphic condition or not, multiple gains and losses have occurred. However, based on current sampling it is more parsimonious to postulate plesiomorphy of tubers in the subfamily and their subsequent loss in *Adenochilus*, *Rimacola*, *Cryptostylis* and Cranichideae. The presence of root tubers in Diurideae and Orchideae–Diseae was the primary reason Dressler considered the two lineages related. This symplesiomorphy obscures the true relationship of Diurideae to the other lineage with acrotonic anthers, Cranichideae. The *mat*K tree also indicates that basitonic anthers, characteristic

of Orchideae–Diseae, have arisen only once and represent a derived condition.

Molecular data offer insights into why Diurideae have been a problematic taxon. Despite the fact that Diurideae have been the subject of several comprehensive studies (e.g. Lavarack 1971, 1976; Clements 1995; Szlachetko 1991, 1995) no obvious, unique synapomorphies were apparent. In effect, Diurideae were, and remain, an assemblage of taxa with an obvious Orchideae–Diseae character, the root tubers, as well as a typical spiranthoid character, their somewhat ambiguous acrotonic anthers. The resulting group has a very heterogeneous vegetative and floral morphology, a diverse set of habitat requirements and life histories, and a wide range of different pollinators (Jones 1970, 1974a, 1974b, 1981). Recently Clements (1995) has identified a number of embryological characters that delimit Diurideae *s. str.*, but these are difficult to observe without fresh material of developing ovaries and special preparation. Nevertheless, at least one macromorphological character state appears to be a synapomorphy for Diurideae, albeit a homoplasious one: the one-leaved shoot. Both of the relevant outgroups, the Diseae–Orchideae and the Cranichideae–Chloraeinae–Pterostylidinae are composed overwhelmingly of taxa with multi-leaved shoots. In contrast all Diurideae except Diuridinae, *Rimacola*, and *Arthrochilus* (unsampled in our analysis) produce one-leaved shoots. Assuming our phylogeny is accurate, the distribution of leaf number within Diurideae can be most-parsimoniously explained by postulating a reduction to one leaf in the common ancestor of the tribe and subsequent independent gains of multiple leaves in Diuridinae and *Rimacola* (and presumably also in *Arthrochilus*).

Clements (1995) identified two unique embryo patterns within the orchidoid lineage, which he referred to as type II and type III embryo patterns. The type II pattern is characterized by the absence of a suspensor and an embryo that projects outside the micropylar end of the inner integument during development ('spiranthoid pattern'). In the type III pattern a one- to twelve-celled suspensor develops and grows into the inner integument space and occasionally into the inter-ovarian cavity via the micropyle. However, the embryo proper remains entirely within the inner integument, which expands to accommodate the embryo's development ('orchidoid pattern'). Clements further distinguishes three subtypes within the orchidoid pattern, based on the number of cells in the suspensor. *Orchis* type has suspensors with 4–12 cells, *Diuris* type has one or rarely two cells, and *Townsonia* has no suspensor. The latter type would be equivalent to the spiranthoid pattern, except that the embryo does not project beyond the inner integument.

Clements concluded that the *Diuris*-type development pattern is a unique synapomorphy for Diurideae; however, it too is somewhat problematic. *Megastylis*, which molecular data places in the spiranthoid clade, has diurid-type embryology. Considering the suspensor alone, the molecular topology indicates that character evolution proceeds from a multicellular suspensor in Orchideae and Diseae, through a one (or rarely two)-celled suspensor in Diurideae, culminating in the absence of a suspensor in spiranthoids. However, the absence of a suspensor in *Townsonia* must then be an independent loss of this structure. According to Clements, the 'spiranthoid' embryo pattern is derived from the 'orchidoid' pattern, and this hypothesis is consistent with the topology provided by *mat*K. However, the presence of a typical 'diurid' pattern within *Megastylis* is more problematic. If *Megastylis* is sister to the remaining taxa of Cranichideae, then the transition to the 'spiranthoid' pattern could have occurred after this taxon diverged. However, if *Megastylis* occupies a more derived position among Cranichideae, then the presence of its *Diuris*-type pattern would have to be considered homoplasious. Despite this possibility, embryological patterns appear to be largely congruent with the molecular topology and may prove a valuable source of new characters for phylogenetic analysis once more work is done on other groups within Cranichideae.

Patterns of homoplasy are pervasive in the orchid family in general, as well as in diurids in particular. Some noteworthy examples are sectile pollen, which appear to have arisen at least four times (Freudenstein and Rasmussen 1997). Obligate mycoheterotrophy has arisen some twenty times, possibly more, in the family (Molvray *et al.* this volume). Homoplasy in root tubers has been mentioned earlier. Two other examples within Diurideae are the rhizomatous habit and wasp pollination. The former is a particularly striking example. Five genera within the tribe have rhizomes: *Adenostylis, Cryptostylis, Rhizanthella, Rimacola,* and *Townsonia*. The molecular phylogeny places four of these genera in different, well-supported lineages. (*Rhizanthella* has not yet been sampled.) This topology implies that rhizomes have arisen (or been lost) at least four times within Diurideae. A similar conclusion can be inferred for wasp pollination. This pollination syndrome is well developed within the more derived members of Drakaeineae, but it is also represented in the genus *Drakonorchis*, which the molecular phylogeny places within *Caladenia*. Hence, wasp pollination has arisen on at least two occasions in the tribe, probably from ancestors that employed bee pollination. These two examples illustrate that even well-defined, complex characters may be convergent within Diurideae, which has compounded the problem of phylogenetic reconstruction. Nevertheless, several macromorphological characters appear to be uncontradicted synapomorphies for suprageneric clades in Diurideae. Possession of lateral sepals much longer and narrower than the dorsal sepal characterizes the *Diuris–Orthoceras* clade. The presence of a mitra surrounding the column uniquely marks the *Thelymitra–Calochilus* clade. The tubular leaf of Prasophyllinae is also an unequivocal synapomorphy.

Given the patterns of homoplasy present in Orchidaceae, the particular value of molecular trees is that they allow the independent assessment of non-molecular character evolution. Molecular approaches have proven extremely useful in Orchidaceae in general, and Diurideae in particular, for clarifying phylogeny and character evolution in an extremely complex family.

ACKNOWLEDGEMENTS

The authors wish to thank the following individuals and institutions for help in obtaining the numerous collections used as sources of DNA for this study and for providing access to their facilities: the staff of the Molecular Systematics Section, Jodrell Laboratory, Royal Botanic Gardens Kew; Steve Hopper and Kingsley Dixon, Kings Park Botanic Garden, Perth, Australia; Andrew Brown, Division of Conservation and Land Management,

Western Australia; and the members of the Native Orchid Society of Western Australia. This study was funded by NSF Postdoctoral Fellowship BIR-9508358.

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